

Effect of α -tocopherol on LDL oxidation and glycation: in vitro and in vivo studies

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Abstract Much data support a role for both low density lipoprotein (LDL) oxidation and glycation in atherogenesis. While α -tocopherol decreases the oxidative susceptibility of LDL, its role in decreasing LDL glycation is unclear. Hence we tested the effect of α -tocopherol both in vitro and in vivo on LDL oxidation and glycation. LDL was isolated after enrichment of plasma with α -tocopherol. This resulted in a 2-fold increase in α -tocopherol in LDL (AT-LDL). During a 6-day incubation of control LDL (C-LDL) and AT-LDL with 25 mM glucose, there were no significant differences in the degree of glycation on days 1, 3, and 6. Also, apoB advanced glycosylation end product levels were not significantly different between C-LDL and AT-LDL. There was a progressive increase in the susceptibility of LDL to oxidation with increasing LDL glycation as evidenced by reduced lag time of copper-catalyzed LDL oxidation. However, AT-LDL was more resistant to copper-catalyzed oxidation. Similar findings were observed when the LDLs were incubated with endothelial cells. ■■ The data from the α -tocopherol supplementation study confirmed our in vitro findings that α -tocopherol significantly decreases oxidative susceptibility of LDL, but does not affect its glycation. Therefore, while glycation increases LDL oxidative susceptibility, α -tocopherol decreases the oxidation of glycated LDL but not LDL glycation.—Li, D., S. Devaraj, C. Fuller, R. Bucala, and I. Jialal. Effect of α -tocopherol on LDL oxidation and glycation: in vitro and in vivo studies. *J. Lipid Res.* 1996. **37**: 1978–1986.

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Plausible modifications of lipoproteins, such as LDL, include non-enzymatic glycation and oxidation (1). Several lines of evidence support a role for glycated LDL and advanced glycosylation end products (AGE) in the genesis of the atherosclerotic lesion (2, 3). Furthermore, numerous laboratories have now documented that oxidatively modified LDL is pro-atherogenic and exists in vivo (4–6). α -Tocopherol, a potent, lipid soluble antioxidant, has been shown to decrease the susceptibility of LDL to oxidation both in vitro and after in vivo supplementation (7, 8). Furthermore, some studies have indicated that α -tocopherol might decrease protein glycation. Ceriello et al. (9, 10) have shown that in addition

to α -tocopherol decreasing the glycation of albumin in vitro, supplementation with α -tocopherol decreased protein glycation in insulin-dependent diabetics (IDDM) in a dose-dependent fashion. Furthermore, Aoki et al. (11) showed in diabetic rats that vitamin E supplementation decreased AGE formation as evidenced by a decrease in the thermal rupture time of collagen. However, Paolisso et al. (12, 13) have shown that while α -tocopherol supplementation in non-insulin-dependent diabetics (NIDDM) decreased protein glycation, this effect is possibly mediated by an improvement in insulin action resulting in a decrease in plasma glucose levels. In addition, two recent in vivo supplementation studies with α -tocopherol showed decreased LDL oxidative susceptibility, but no effect on protein glycation (14, 15). However, in both these studies the number of subjects was small and other factors in the in vivo environment could have obscured the effect of α -tocopherol on protein glycation.

Furthermore, these studies examined the oxidative susceptibility of LDL without separating the glycated and non-glycated fractions. Thus, while α -tocopherol appears to have a clear role in decreasing the oxidative susceptibility of LDL, the evidence regarding its effect on protein glycation is unclear. The present study was undertaken to gain a better appreciation of the role of α -tocopherol on LDL oxidation and glycation. Accordingly, we tested the effect of α -tocopherol on LDL oxidative susceptibility and glycation, including AGE

Abbreviations: LDL, low density lipoprotein; AGE, advanced glycosylation end products; IDDM, insulin-dependent diabetes mellitus; NIDDM, non-insulin-dependent diabetes mellitus; AT-LDL, α -tocopherol-enriched LDL; C-LDL, control LDL.

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modification, after enrichment of plasma with α -tocopherol in vitro, and also after in vivo supplementation in diabetic subjects.

MATERIALS AND METHODS

Subjects

Blood samples were collected for the study from healthy, non-smoking male or female subjects if they fulfilled the following criteria: not on any vitamin supplements for at least 6 months before entry; alcohol intake < 1 oz/day; normal fasting plasma glucose, hepatic and renal function tests; no evidence of malabsorption, pancreatic, or biliary diseases; not on estrogen, thyroxine, or non-steroidal anti-inflammatory drugs, and no acute medical condition for at least 3 months prior to participating in the study.

Enrichment of LDL with α -tocopherol

After obtaining informed consent, fasting blood (180 ml) was obtained for LDL isolation, determination of glycated plasma proteins, and HbA_{1c}. The samples for LDL isolation were collected into tubes containing ethylenediamine tetraacetic acid (EDTA) (1 mg/ml). All blood samples were collected on ice and the plasma was separated by centrifugation at 4°C at 2000 g for 20 min.

Freshly isolated plasma was spiked with α -tocopherol (1 μ M in dimethylsulfoxide-DMSO) or DMSO (vehicle control) as described by Esterbauer et al. (7). Also, a dose-response study was undertaken in which plasma was spiked with α -tocopherol in concentrations of 1.0, 2.0, and 4.0 μ M. After an incubation of 3.5–4 h at 37°C, LDL was isolated by sequential ultracentrifugation as previously described (16). The isolated LDL was extensively dialyzed against three exchanges (4 liters, 4 liters, and 2 liters) of 150 mmol/L sodium chloride (NaCl)–1 mM EDTA, pH 7.4, at 4°C for 24 h, and filtered through a 0.22- μ m filter. Thereafter, the LDL was stored at 4°C under nitrogen until protein was measured by the method of Lowry et al. (17). LDL was then diluted to a concentration of 3 mg/ml. The enrichment of LDL with α -tocopherol was quantitated by reversed phase HPLC after ethanol precipitation and hexane extraction as described previously (18).

In vitro glycation of LDL

LDL (3 mg/ml) was incubated with 0, 25, 50, and 100 mM glucose in PBS (0.01 mol/L phosphate, 0.15 mol/L NaCl, pH 7.4) at 37°C for 6 days under sterile conditions in 6-well Primaria plates. Sodium azide (0.02%) was added to prevent bacterial growth. Throughout, EDTA was present at a concentration > 0.5 mM to minimize LDL oxidation. On days 1, 3, and 6, the incubated LDL

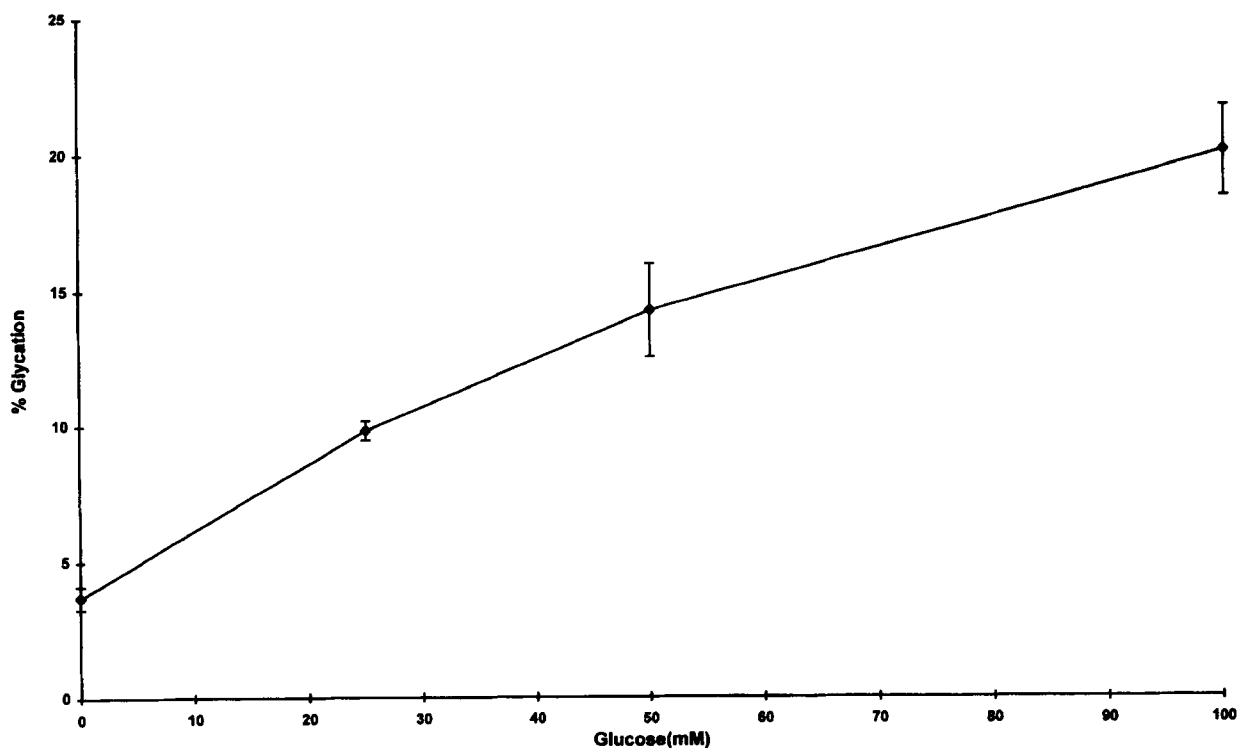


Fig. 1. Dose response of LDL glycation. LDL was incubated with 0, 25, 50, and 100 mM glucose for 6 days. The percent glycation was determined by a modified method of m-aminophenyl boronate affinity chromatography. Data are mean of 3 experiments.

was removed and dialyzed against 1 L of PBS at 4°C in the dark. The protein concentration was again determined as described (17) and percent glycation was measured by m-aminophenyl boronate affinity chromatography (19, 20) using Glycogel II test columns (Pierce Chemical Company, Rockford, IL). Briefly, 1.2 mg LDL (400 µL) was loaded on the column and non-glycated LDL was eluted with 5.5 ml of the reagent wash buffer containing ammonium acetate, magnesium chloride, and 0.5% phenoxyethanol, pH 8.05. Glycated LDL was eluted with 2 ml of sorbitol elution buffer, pH 10.25, as described by Panteghini, Bonora, and Pagani (20). Protein content of non-glycated and glycated eluates were measured by the BioRad assay (21) and % glycation was arrived from the formula: % LDL glycation = (OD of glycated fraction × 2/[OD of glycated fraction × 2 + OD of nonglycated fraction × 5.9]) × 100. We obtained an intra- and inter-assay C.V. of 3.9% and 7.4%, respectively, for this assay.

In order to determine whether glycation interferes with the dye binding assay, LDL was incubated with 0, 25, 50, and 100 mM glucose for 6 days at 37°C. After dialysis, the protein concentration of the different LDLs was determined by the dye binding assay. The protein concentration of the LDL incubated in PBS and the LDL incubated with 100 mM glucose for 6 days differed by only 2.7% (n = 3 experiments).

For measurement of apoB-AGE levels, C-LDL and AT-LDL at a concentration of 3 mg/ml were incubated with 25 mM glucose for 6 days. Baseline and day 6 samples were dialyzed and assayed for apoB-AGE levels. AGE-modified apoB (apoB-AGE) levels were measured by Dr. H. Founds at Alteon, Inc. (Ramsey, NJ) by a sandwich ELISA method using an AGE-specific capture antibody and an anti-apoB detector antibody (22, 23). AGE units were defined relative to a standardized preparation of AGE-modified BSA as previously described (22).

Oxidative susceptibility of LDL was assayed by two methods: *i*) copper-catalyzed LDL oxidation and *ii*) cell-mediated LDL oxidation. In copper-catalyzed LDL oxidation, C-LDL and AT-LDL (100 µg protein/ml) were oxidized in a cell-free system using 5 µmol/L copper in PBS at 37°C (24). The 8-h time course of oxidation was studied by continuous measurement of conjugated dienes at 234 nm at 10-min intervals (25). The rate of LDL oxidation was determined from the propagation phase of the time course curve using a spline function, and the lag phase was obtained by drawing a tangent to the slope of the propagation phase and extrapolating it to the x-axis (8).

The lag time constitutes the interval from zero time to the intersection point. Also, the time course of oxidative modification by endothelial cells of C-LDL and AT-LDL at baseline and after a 6-day incubation with 25

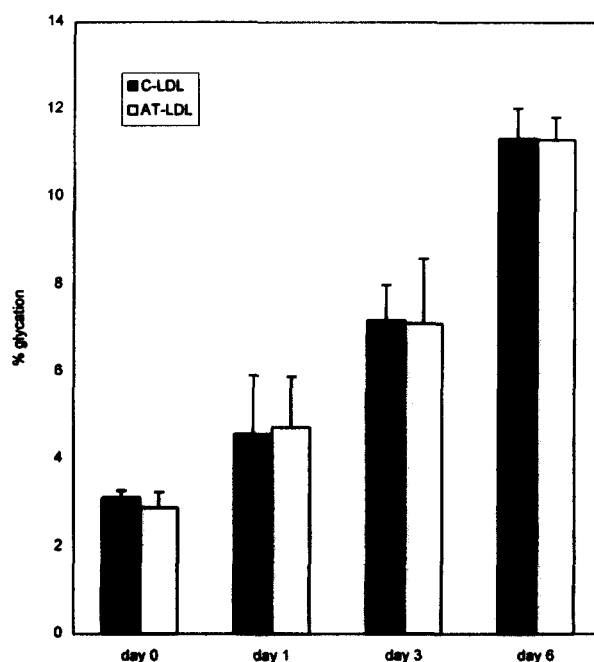


Fig. 2. LDL glycation in the presence and absence of α -tocopherol. AT-LDL (α -tocopherol = 23.6 nmol/mg protein) and C-LDL (α -tocopherol = 11.9 nmol/mg protein) were incubated with 25 mM glucose for 6 days. The percentage glycation was determined at baseline (day 0), day 1, day 3, and day 6. At days 0, 1, 3, and 6 there were no significant differences in percent glycation between C-LDL and AT-LDL ($P > 0.05$).

mM glucose was studied. In cell-mediated LDL oxidation, human umbilical vein endothelial cells (HUVEC) were isolated and cultured as described previously (26). After three washes with Ham's F-10 medium (GIBCO-BRL), C-LDL and AT-LDL (100 µg/ml in Ham's F-10 medium) were added to the confluent cultures of HUVEC in 16-mm wells in triplicate and incubated for 0, 6, 12, and 24 h at 37°C in a 5% CO₂ incubator. At each time point, the reaction was stopped by adding EDTA and BHT as previously described (16). Oxidative modification was quantitated by determining the amount of thiobarbituric acid-reactive substances (TBARS) formed in wells containing cells and cell-free controls by a modified TBARS assay (27). TBARS activity was expressed in terms of malondialdehyde (MDA) equivalents (nmol/mg LDL protein) after subtracting the cell-free control values.

To test the effect of α -tocopherol supplementation in vivo on LDL glycation and oxidation, LDL glycation and oxidation were determined in 10 NIDDM subjects, 5 of whom were given 1200 IU/day *d*- α -tocopherol or placebo for 8 weeks. LDL oxidation, glycated plasma proteins, HbA1C, glycated LDL, and α -tocopherol levels in plasma and LDL were measured at baseline and after supplementation. The details of this in vivo supplement-

TABLE 1. Dose-response effect of α -tocopherol (AT) on LDL glycation

AT μM	AT in LDL <i>nmol/mg/protein</i>	% Glycation		
		Day 0	Day 3	Day 6
0	28.33 \pm 6.51	3.23 \pm 0.57	6.95 \pm 2.32	12.0 \pm 1.89
1	53.13 \pm 5.06 ^a	3.45 \pm 0.58	6.2 \pm 1.9	11.58 \pm 1.33
2	63.17 \pm 12.5 ^a	3.28 \pm 0.52	6.68 \pm 1.58	12.63 \pm 2.60
4	68.34 \pm 17.00 ^a	3.25 \pm 0.68	6.53 \pm 1.77	11.48 \pm 0.95

Plasma was incubated with 1, 2, and 4 $\mu\text{mol/L}$ α -tocopherol for 4 h after which LDL was isolated. Percent glycation was determined on C-LDL and AT-LDL after 0, 3, and 6 days of incubation with 25 mM glucose as described in Methods.

^a $P < 0.01$ versus control LDL; $n = 4$ experiments.

tation study in 28 diabetics (both NIDDM and IDDM) have been reported previously (15). In the present report, we have focused on LDL glycation and oxidation as LDL glycation was not reported in the previous study.

Statistical analysis

Data are expressed as mean \pm SD of at least three separate experiments. Statistical analysis was performed by paired Student's *t*-test to determine differences be-

tween C-LDL and AT-LDL for the in vitro study and baseline and supplemented phases for the in vivo study (Microsoft Excel). Repeated measures analysis of variance were used to assess changes in glycation and oxidation over the 6-day period. Significance was defined at the 5% level. Spearman rank correlation was used to assess the association between LDL glycation and oxidation parameters. Statistical analysis was performed using BMDP Statistical Software (SPSS Inc., Chicago, IL).

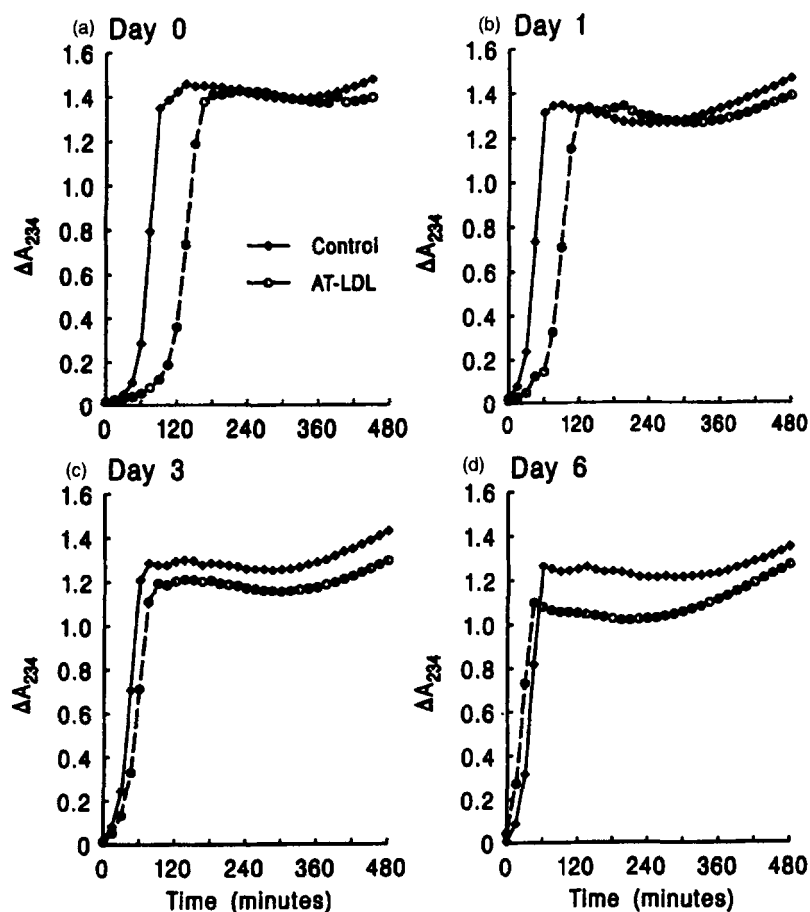


Fig. 3. Copper-catalyzed oxidation of glycated LDL. A typical time course of oxidation of LDL at baseline (a); day 1 (b); day 3 (c), and day 6 (d) incubation with 25 mM glucose. The diamond represents C-LDL and the circle represents AT-LDL. α -Tocopherol significantly delayed copper-catalyzed oxidation in AT-LDL, as evidenced by a longer lag time of oxidation. The difference in lag time between C-LDL and AT-LDL disappeared at approximately day 6 of glycation. Percent glycation of C-LDL and AT-LDL at baseline (day 0) are 3.3% and 3.1%; at day 1, 5.9% and 6.0%; at day 3, 7.2% and 6.2%; at day 6, 12% and 11.6%, respectively.

RESULTS

α -Tocopherol is a potent lipid-soluble antioxidant (7). It has been shown previously that α -tocopherol-enriched LDL displays decreased oxidative susceptibility. We used LDL from healthy donors to see whether α -tocopherol had any beneficial effect on the glycation of a lipoprotein. Incubation of LDL with increasing concentrations of glucose (up to 100 mM) for 6 days produced a dose-dependent increase in LDL glycation (Fig. 1). For subsequent experiments, a concentration of 25 mM glucose was chosen as it is more representative of the diabetic milieu and this concentration has been shown to result in maximum LDL oxidation in vitro (28). Increasing glycation of LDL was also observed as the duration of incubation with 25 mM glucose was increased from 0 to 6 days (Fig. 2). In spite of a 2-fold increase of α -tocopherol in LDL after enrichment (23.6 vs. 11.9 nmol/mg protein), the glycation of C-LDL and AT-LDL was not significantly different over the 6-day time course (day 6: 11.0% \pm 0.65 vs. 10.5% \pm 1.56, p = 0.45). In addition, a dose-response study enriching plasma with α -tocopherol in concentrations of 1.0, 2.0, and 4.0 μ M while significantly increasing LDL α -tocopherol levels had no significant effect on LDL glycation (Table 1). α -Tocopherol enrichment also had no effect on apoB AGE levels after 6 days of glycation (n = 4 experiments, AT-LDL 624.7 \pm 114 vs. C-LDL 725.4 \pm 115.5 U/mg protein, P = 0.11).

Typical time-course curves observed during copper-catalyzed LDL oxidation on days 0, 1, 3, and 6 of glycation are shown in Fig. 3a–d. Lag phase of oxidation was assessed in LDL with and without AT enrichment (Table 2). Compared to C-LDL, the lag time of AT-LDL was prolonged significantly on day 0 (P < 0.005), day 1 (P < 0.05), and day 3 (P < 0.05) during glycation as shown in Table 2. There was no significant increase in the oxidation of non-glycated LDL over 6 days. In both groups there were progressive increases in the susceptibility of LDL to oxidation from day 0 to day 6 as evidenced by a reduction in the lag phase (P < 0.05) (Table 2). The glycation of LDL was significantly correlated with the decrease in lag phase (r = -0.65, P < 0.001). Furthermore, the oxidative modification of C-LDL and AT-LDL by human endothelial cells was studied at baseline and after a 6-day incubation with 25 mM glucose. Both at baseline and after 6 days of glycation, the C-LDL was more prone to oxidation compared to AT-LDL as evidenced by the time course curves (Fig. 4).

In addition to our in vitro experiments, we tested the effect of α -tocopherol supplementation in diabetic subjects on LDL oxidation and glycation as shown in Table 3. After α -tocopherol supplementation, there was a 2.5-fold increase in α -tocopherol in LDL at 8 weeks

compared to placebo (16.11 \pm 5.02 vs. 40.11 \pm 10.13 nmol/mg protein, P < 0.001). Compared to the placebo, the lag phase of oxidation was significantly prolonged in the AT-LDL group at 8 weeks (P < 0.02). However, there were no significant differences in HbA_{1c} levels (P = 0.40) and degree of glycation of LDL (P = 0.39) at 8 weeks between the two groups.

DISCUSSION

Diabetic patients have an increased propensity to develop premature atherosclerosis. However, the mechanisms underlying this remain to be elucidated. Two plausible mechanisms are LDL glycation and oxidation (1, 2). Oxidation of LDL has been proposed as a key early step in the pathogenesis of atherosclerosis (4). Oxidatively modified LDL has been shown to induce endothelial cell dysfunction and to stimulate foam cell formation and endothelial-leukocyte adhesion and migration (4–6). It appears that hyperglycemia could also lead to an increase in oxidative stress in uncontrolled diabetics. Several mechanisms may be responsible for increased oxidation with high glucose levels. Reactive oxygen species can be generated during glucose autooxidation (29, 30) and during glycation (31, 32). Although the relationship between glycated LDL and oxidized LDL is unclear at present, it seems that glycated LDL has some characteristics of oxidized LDL. For instance, there is increased uptake of glycated LDL by monocyte/macrophages (33); glycated LDL can stimulate platelet aggregation (34) as well as promote covalent binding to structural proteins in the vascular wall (35), which may contribute to the early fatty streak lesion. Furthermore, advanced glycosylated end products could promote atherosclerosis via numerous mechanisms (3). Binding of AGE to endothelial cell through the cell surface receptor could increase vascular permeability which may promote intimal lipoprotein deposition (36). AGE could cross-link collagen in connective tissues which may serve as reactive loci trapping circu-

TABLE 2. Effect of in vitro α -tocopherol enrichment on LDL oxidation

	Lag Time			
	Day 0	Day 1	Day 3	Day 6
C-LDL	43.8 \pm 28.8	28.2 \pm 11.4	22.2 \pm 2.4	16.8 \pm 7.8
AT-LDL	96.0 \pm 33.6 ^a	59.4 \pm 33.0 ^b	30.0 \pm 6.0 ^b	20.4 \pm 4.8

In both groups there were progressive increases in the susceptibility of LDL to oxidation from day 0 to day 6 (P < 0.05). Values given as mean \pm SD, n = 5 experiments. C-LDL, control low density lipoprotein; AT-LDL, α -tocopherol-enriched LDL; days 0, 1, 3, 6 indicate days of incubation with 25 mM glucose at 37°C.

^a P < 0.005 (AT-LDL vs. C-LDL).

^b P < 0.05 (AT-LDL vs. C-LDL).

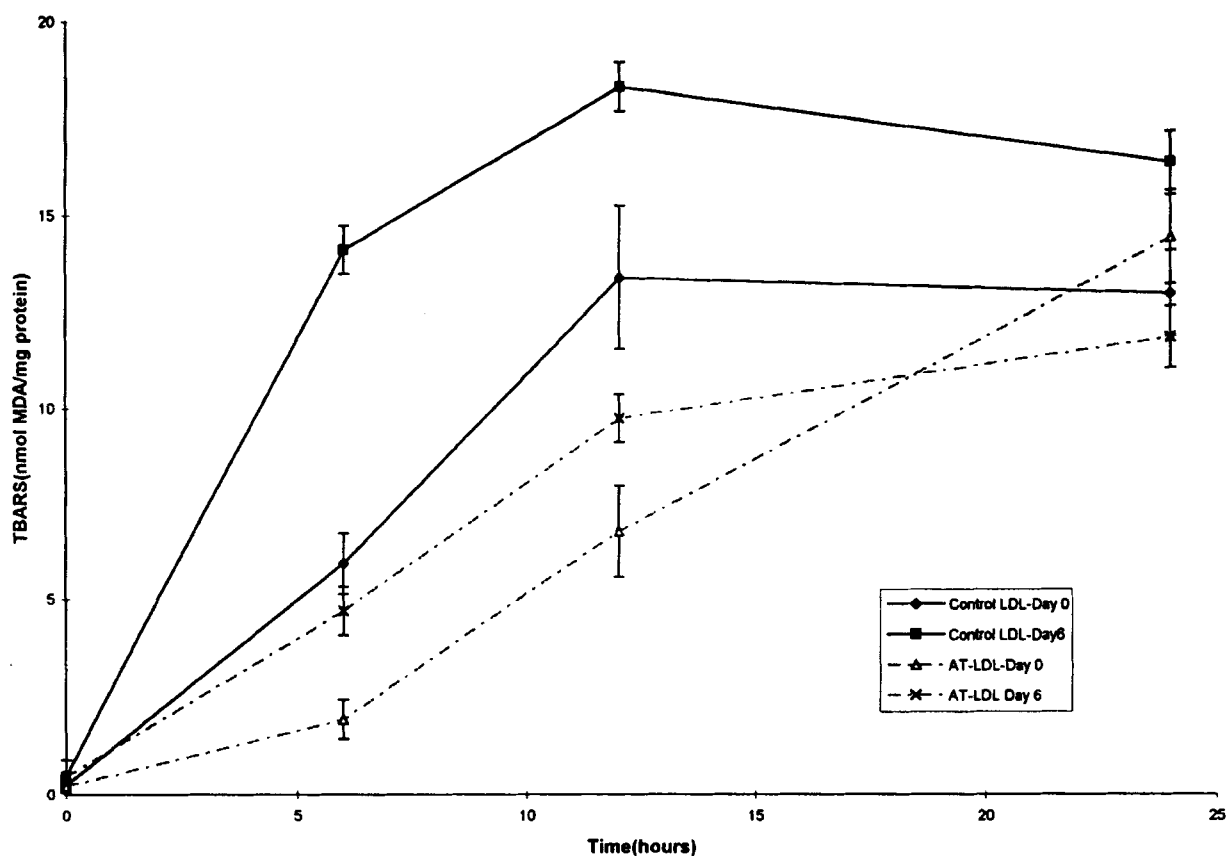


Fig. 4. Endothelial cell-modification of LDL. C-LDL and AT-LDL were incubated with 25 mM glucose for 6 days. On day 0 and day 6, C-LDL and AT-LDL (100 $\mu\text{g}/\text{ml}$) were incubated for 0, 6, 12, and 24 h with confluent monolayers of HUVEC at 37°C in Ham's F-10 medium and Ham's F-10 medium alone (cell-free controls). At each time point supernatants were harvested and assayed for TBARS activity (nmol MDA equivalents/mg protein). Percent glycation of C-LDL and AT-LDL at day 0 was 3.23 ± 0.57 and 3.45 ± 0.58 ; at day 6 was 12.00 ± 0.89 and 11.57 ± 1.33 , respectively. Data are mean of 4 experiments.

lating serum protein such as lipoprotein and immunoglobulin (37–39). AGE are chemotactic for monocytes and could stimulate IL-1 release from macrophages. AGE also promotes atherogenesis by their procoagulant activity such as decreasing cell surface anticoagulant thrombomodulin and increasing tissue factor expression. Thus, LDL glycation and oxidation are hypothesized to be mutually reinforcing in the pathogenesis of macrovascular complications in diabetes (2). α -Tocopherol, a potent lipophilic antioxidant, is the most abundant antioxidant in LDL. In vitro and supplementation studies have clearly shown that α -tocopherol decreases the susceptibility of LDL to oxidation (7, 8). However, it is not clear whether α -tocopherol protects LDL from glycation or AGE modification. Although several studies have shown that glycated LDL is more sensitive to oxidation (40–42), there are no previous studies relating increased glycation to LDL oxidation kinetics. Also, there appear to be no studies examining the effect of α -tocopherol on the oxidative susceptibility of glycated LDL. In order to gain more insight on the effect of

α -tocopherol on both these processes, we have studied the oxidation of glycated LDL by both a transition metal and human endothelial cells.

The reported effect of α -tocopherol on protein glycation by Ceriello et al. (9, 10) was potentially important, because, if confirmed, it would provide another plausible reason for antioxidant supplementation in diabetes. We studied in vitro serum albumin glycation according to the protocol of Ceriello et al. (9) in which they demonstrated that α -tocopherol decreased albumin glycation. We did not observe any effect of α -tocopherol on albumin glycation (data not shown). To determine whether α -tocopherol had an effect on LDL glycation, we isolated LDL from healthy donors as an alternative protein for our study. The intrinsic affinity of LDL for α -tocopherol allowed us to enrich α -tocopherol in LDL by incubating plasma with α -tocopherol and then isolating LDL. Furthermore, as many of the confounding factors present in in vivo studies are removed in the in vitro setting, this allowed us a better appreciation of the effect of α -tocopherol on LDL glycation and oxidation.

There was no significant difference between the C-LDL and AT-LDL in terms of protein glycation. In this regard, our data on LDL glycation are at variance with that of Ceriello et al. In our study, LDL was used for glycation which is a relatively short-lived plasma protein. However, Fu et al. (43) using antioxidants other than α -tocopherol also failed to show an effect on the glycation of tendon collagen. α -Tocopherol clearly decreased the oxidative susceptibility of LDL as evidenced by a prolongation of the lag phase of LDL oxidation at baseline and during the early time course of glycation. Aoki et al. (11) also did not find an effect on protein glycation with α -tocopherol supplementation in diabetic rats; however, they showed that α -tocopherol supplementation partially inhibited AGE formation in which thermal rupture time of tail tendon collagen shortened significantly with the α -tocopherol-supplemented diet in diabetic rats; but another index of AGE formation, collagen-linked fluorescence, did not change. We were also interested to determine whether α -tocopherol affected LDL-AGE formation. We assayed apoB AGE levels in the samples after 6 days of glycation. α -Tocopherol had no significant effect on apoB AGE levels after this short period of glycation.

Glycation of LDL has been shown to increase the susceptibility of LDL to oxidation (23, 40–42). However, in the majority of these studies, the kinetics of LDL oxidation was not reported. We used 25 mM glucose for LDL glycation, which has been shown to cause maximum LDL oxidation by Hunt et al. (28), to study oxidative kinetics of the glycated LDL. The lag time of oxidation of both C-LDL and AT-LDL were decreased with increasing LDL glycation, indicating that the susceptibility of LDL to oxidation was increased. There are several mechanisms that could account for the increased oxidative susceptibility of LDL. Previous investigators have shown that autooxidation of glucose itself could generate reactive oxygen species (32, 44). Kawamura, Heinecke, and Chait (30) have shown that incubation of LDL with glucose increases LDL oxidation via a superoxide-mediated mechanism. Also, early glycation products result in free radical generation such as superoxide which could accelerate lipoprotein oxidation (31).

A variety of cells in the vascular wall have been shown to be involved in atherogenesis by cell–cell interactions (45) or secretion of factors such as cytokines and growth factors. Monocyte/macrophages can be converted to foam cells by uptake of oxidized LDL via the scavenger receptor (4, 5). In addition, endothelial cells and smooth muscle cells in the vascular wall are capable of oxidizing LDL (4–6). Therefore it is desirable to study LDL oxidation by human endothelial cells, which may play an important role in early development of the atherosclerotic lesion and may better reflect the in vivo situation. It was not surprising that α -tocopherol decreased LDL oxidation mediated by endothelial cells as previous studies have shown that α -tocopherol co-incubation decreases LDL oxidation by endothelial cells incubated with physiological concentrations of glucose (46, 47). Our data further demonstrated that α -tocopherol enrichment also decreases the oxidative susceptibility of glycated LDL by endothelial cells. Thus, this study demonstrates that in addition to α -tocopherol decreasing the susceptibility of LDL to oxidation (7, 8), it also has a beneficial effect on the oxidation of glycated LDL.

As no observable changes in LDL glycation were seen in our in vitro study, we also wished to determine whether α -tocopherol has any effect on LDL oxidation and glycation in diabetic patients. We supplemented a group of diabetic patients with either a relatively high dose of α -tocopherol (1200 IU/day) or placebo for 8 weeks. The oxidizability of LDL from the subjects was reduced as shown by increase in lag time of LDL oxidation after α -tocopherol supplementation. There were no significant differences in HbA_{1C} and glycated LDL between the α -tocopherol and placebo groups after 8 weeks of α -tocopherol supplementation. This is in agreement with the findings of Aoki et al. (11) that supplementation of α -tocopherol to a group of diabetic rats for 4 weeks had no significant effect on serum glucose and glycated hemoglobin levels. Shoff et al. (48) found that there was no relationship between α -tocopherol intake and glycated hemoglobin A_{1C} (HbA_{1C}) in patients with diabetes, although the α -tocopherol levels in that study were non-pharmacologic. Ceriello et al. (9) demonstrated that serum albumin glycation can be inhibited

TABLE 3. Effect of in vivo α -tocopherol supplementation on LDL oxidation and glycation

Diabetic Group	Lag Time		G-LDL		HbA _{1C}	
	0 Week	8 Weeks	0 Week	8 Weeks	0 Week	8 Weeks
	<i>min</i>		<i>%</i>		<i>%</i>	
Placebo (n = 5)	93.0 ± 30.0	93.0 ± 21.0	4.79 ± 0.47	5.15 ± 0.90	6.93 ± 0.57	7.17 ± 1.22
AT (n = 5)	80.4 ± 16.2	148.8 ± 45.6 ^a	4.64 ± 1.37	5.78 ± 2.71	7.35 ± 1.65	6.60 ± 1.70

Data are expressed as mean ± SD; AT, α -tocopherol supplement; G-LDL, glycated low density lipoprotein; HbA_{1C}, hemoglobin A_{1C}. α -Tocopherol levels at baseline and 8 weeks were 15.3 and 15.9 nmol/mg protein in the placebo group and 16.1 and 40.1 nmol/mg protein in the AT-supplemented group, respectively.

^a*P* < 0.02.

with co-incubation of pharmacologic dose of α -tocopherol and that the glycation of hemoglobin was inhibited by administration of α -tocopherol in diabetic patients (10). However, it was suggested by Paolisso et al. (12, 13) that the beneficial effect of α -tocopherol on protein glycation after supplementation with 600 to 1200 mg/day of α -tocopherol was due to improvement in insulin action with a consequent decrease in the glucose levels of the subjects. It is interesting to note that Reaven et al. (14) showed that while α -tocopherol significantly reduced LDL oxidizability, it had no effect on protein glycation in well-controlled male NIDDM patients. The same conclusion was also derived from a more recent in vivo study by Fuller et al. (15) which included IDDM and NIDDM patients of both sexes with varying degrees of glycemic control. While in both these studies there were a limited number of subjects, they arrived at the same conclusion as the present report.

In summary, while the present report confirms the previous studies that glycation of LDL increases oxidative susceptibility, this is carefully quantitated by the measurement of lag phase. In addition, this study shows that α -tocopherol has no effect on LDL glycation and AGE formation but decreases the oxidative susceptibility of glycated LDL. ■■

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