α-Tocopherol supplementation decreases production of superoxide and cytokines by leukocytes ex vivo in both normolipidemic and hypertriglyceridemic individuals[1,2]

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ABSTRACT

Background: α-Tocopherol plays an important role in protecting LDL against oxidation. However, additional effects of α-tocopherol at the intracellular level may contribute to the clinical outcome of intervention studies.

Objective: We investigated whether α-tocopherol influences the inflammatory responses of immune cells in normolipidemic and hypertriglyceridemic subjects.

Design: *RRR*-α-Tocopherol was administered for 6 wk at a dose of 600 IU (402 mg)/d to 12 primary hypertriglyceridemic and 8 normolipidemic (fasting triacylglycerol >3.0 and <2.0 mmol/L, respectively) subjects. Cytokine production [tumor necrosis factor α (TNF-α), interleukin (IL)-1β, and IL-8] by mononuclear cells and superoxide production by polymorphonuclear cells and in diluted whole blood were determined before and after the intervention.

Results: Cytokine and superoxide production did not differ significantly between hypertriglyceridemic and normolipidemic subjects. α-Tocopherol supplementation resulted in a 2- to 3-fold increase in the concentration of α-tocopherol in plasma and LDL. Whereas superoxide production in response to phorbol 12-myristate 13-acetate decreased in all subjects, response to oxidized LDL increased in 19 of 20 subjects. Response to opsonized zymosan before α-tocopherol supplementation was not significantly different from that after supplementation. Lipopolysaccharide-induced cytokine production by mononuclear cells decreased after supplementation with α-tocopherol.

Conclusions: α-Tocopherol differentially influences inflammatory responses of immune cells. These effects of α-tocopherol may be relevant in chronic inflammatory processes such as atherogenesis. *Am J Clin Nutr* 2000;71:458–64.

KEY WORDS α-Tocopherol, superoxide, chemiluminescence, cytokines, oxidized LDL, hypertriglyceridemia, men, reactive oxygen species, antioxidants

INTRODUCTION

A considerable body of evidence supports a causal role of oxidatively modified LDL in the atherosclerotic process (1, 2). LDL is protected from oxidation by antioxidants and the most important lipophilic antioxidant is α-tocopherol (3, 4). In animal models, antioxidants have been shown to inhibit the progression of fatty streaks (for review *see* reference 5) and prospective epidemiologic studies suggest an inverse correlation between the incidence of coronary artery disease and α-tocopherol concentrations (6–8). Moreover, results of the Cambridge Heart Antioxidant Study (CHAOS) suggest that even patients with established atherosclerosis may benefit from α-tocopherol therapy (9). However, it is not clear whether the decrease in morbidity and mortality found was related to its antioxidant capacity because α-tocopherol has additional biological effects, including effects at the intracellular level. It has been reported to inhibit protein kinase C (10) and it may also play a role in preventing activation of intracellular redox-sensitive signal transduction pathways such as nuclear transcription factor κB (NF-κB) (11). Via these mechanisms, α-tocopherol may influence cellular functions such as cell proliferation, platelet aggregation, cellular superoxide and cytokine production, and adhesion molecule expression (12–18). Devaraj et al (14) showed decreased monocyte function after α-tocopherol supplementation, and we showed decreased production of tumor necrosis factor α (TNF-α) in smokers and of interleukin (IL) 1 receptor antagonist and TNF-α in smokers and nonsmokers after α-tocopherol treatment (19).

The first goal of the current study was to investigate the effects of α-tocopherol on superoxide production by polymorphonuclear cells (PMNs) as well as in whole blood, and cytokine production by peripheral blood mononuclear cells (PBMCs) in normolipidemic subjects. In addition to the more commonly used stimuli, such as phorbol ester, opsonized zymosan, and lipopolysaccharide, we also used native and oxidized LDL to stimulate the cells ex vivo.

In patients with hypertriglyceridemia plus hypercholesterolemia, a higher release of reactive oxygen species (ROS) was found by PMNs in response to phorbol ester (20). Therefore, as the second goal of the present work, we assessed the above-mentioned cellular measures in both hypertriglyceridemic and normolipidemic subjects.

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SUBJECTS AND METHODS

Subjects

Twelve male patients with primary hypertriglyceridemia and 8 normolipidemic male volunteers (control subjects), recruited from an outpatient clinic and from the healthy population participated in the study after providing informed written consent. The protocol of the study was approved by the Comissie Experimenteel Onderzoek met Mensen medical ethical committee. Inclusion criteria for fasting triacylglycerol were concentrations of >3.0 mmol/L for hypertriglyceridemia and <2.0 mmol/L for normolipidemia. Individuals with secondary causes of hyperlipoproteinemia, including liver diseases, renal impairment, hypothyroidism, diabetes mellitus, gross obesity, or a history of excess alcohol intake were excluded. A history of cardiovascular disease, current smoking, and infectious or inflammatory diseases were also exclusion criteria. Subjects were not to use antilipidemic drugs for ≥4 wk before blood sampling and had not been using any antioxidant supplements.

After subjects had fasted overnight, blood samples were obtained by venipuncture into vacuum tubes (Monoject; Sherwood Medical, Ballymoney, United Kingdom) containing tripotassium EDTA or lithium heparin. Two baseline samples were taken, one sample 1 wk before and one sample at the start of α-tocopherol supplementation ($t = -1$ and $t = 0$, respectively). α-Tocopherol was administered at a daily dose of 600 IU as *RRR*-α-tocopherol in soybean oil (corresponding to 402 mg, as checked by HPLC) for 6 wk. Two blood samples were again taken in the fifth and sixth weeks of supplementation ($t = 5$ and $t = 6$, respectively). Compliance with α-tocopherol supplementation was checked by capsule counting and was on average 93.3%.

Preparation of LDL and opsonized zymosan for in vitro assays

To obtain a large batch of LDL for stimulation of isolated leukocytes (as described below) sterile LDL from 50 mL EDTA-treated blood, donated by a healthy volunteer, was isolated by single-spin density-gradient ultracentrifugation (21). Disposable supplies and solutions were endotoxin-free or were autoclaved for 5 h at 180°C before used. After dialysis against 10 mmol phosphate buffer/L, containing 0.9% NaCl, pH 7.4, EDTA (final concentration: 100 μmol/L) and saccharose (10%) were added and LDL was frozen in aliquots at −80°C (22). On the day before each experiment, LDL was thawed and dialyzed for 7 h against an identical buffer without EDTA and saccharose (refreshed after 1 and 3 h) in a Slide-A-Lyzer cassette (Pierce Chemical Company, Rockford, IL).

Subsequently, some of the LDL was incubated with 20 μmol $CuSO_4$/L for 15 h at 37°C in a shaking water bath (LDL_{ox}). The remaining LDL was kept at 4°C in the dark (LDL_{nat}). Oxidative modification of LDL was assessed by its conjugated diene content and electrophoretic mobility on agarose gel. Conjugated dienes increased reproducibly from 174 ± 11 nmol/mg protein ($n = 12$) in LDL_{nat} to 856 ± 35 nmol/mg protein ($n = 12$) in LDL_{ox}. Accordingly, electrophoretic mobility in agarose gel increased markedly and consistently with oxidation, whereas electrophoretic mobility of LDL_{nat} was unaltered compared with that in fresh plasma (data not shown). Protein content was measured by the method of Lowry et al (23) using bovine serum albumin as a standard and with chloroform extraction of the color solution to remove turbidity. Protein amounted to 33.6 ± 3.0 and 31.3 ± 1.3 mg/L for LDL_{nat} and LDL_{ox}, respectively.

Zymosan A (Sigma-Aldrich, Deisenhofen, Germany) was suspended in phosphate-buffered saline (PBS; 250 mg in 30 mL) and ultrasonicated for 1 h. After centrifugation for 5 min at 690 × *g*, the pellet was suspended in 10 mL human serum and incubated for 45 min at 37°C in a shaking water bath. Subsequently, the opsonized zymosan suspension was centrifuged as above, washed 3 times in PBS, and finally taken up in 25 mL PBS and frozen in aliquots at −80°C until used for chemiluminescence measurements.

Isolation of leukocytes

Production of cytokines and of superoxide was assessed in PBMCs and PMNs, respectively. PBMCs were prepared from heparin-treated whole blood by Ficoll-Paque (Pharmacia Biotech AB, Uppsala, Sweden) gradient centrifugation according to the method of Boyum (24), washed 3 times with PBS, and finally resuspended in RPMI 1640 (Dutch modification; ICN Biomedicals Inc, Aurora, OH) at 1×10^9 cells/L. PMNs were isolated from the pellet fraction of the Ficoll-Paque gradient by lysis of the erythrocytes in buffer with 155 mmol NH_4Cl/L and 10 mmol $KHCO_3$/L (25). PMNs were washed twice in PBS and suspended in Hanks balanced salt solution (HBSS; Gibco Life Technologies, Paisley, Scotland) supplemented with 0.25% human serum albumin (HSA; Behringwerke AG, Marburg, Germany). PMN suspensions had a purity of >99%. Cell concentration was assessed by using a cell counter (Coulter Electronics, Luton, United Kingdom).

Chemiluminescence measurements

Production of superoxide by isolated PMNs and in diluted whole blood was assessed by monitoring luminol-enhanced peroxidase-catalyzed chemiluminescence. The method was specific for cellular superoxide production because, in the absence of cells, no chemiluminescence could be detected. Chemiluminescence of PMNs was measured on a Victor 1420 multilabel counter (Wallac, Turku, Finland) at room temperature by using white 96-well microplates (catalogue no. 3096; Corning Costar Corporation, Cambridge, MA). Each well contained 2×10^5 cells, 50 μmol luminol/L, 4500 U horseradish peroxidase/L (Sigma-Aldrich), and stimulus [50 μg phorbol 12-myristate 13-acetate (PMA)/L, 25 μL opsonized zymosan, 25 μL LDL_{nat}, 25 μL LDL_{ox}, or buffer] in 200 μL HBSS-HSA (0.25%). Chemiluminescence of heparin-treated whole blood [final dilution: 3000× in HBSS-HSA (0.25%)] in response to PMA was measured identically in a microplate luminometer (MicroLumatPlus LB96V; Berthold Co, Wildbad, Germany) at 37°C. Reactions were started by adding the stimulus. The kinetic data that were obtained and the processing that was used are illustrated in **Figure 1**. For spontaneous luminol-enhanced chemiluminescence of PMNs, activity at $t = 60$ min was noted; for PMA and opsonized zymosan, peak values ($\approx t = 5$ and $t = 15$ min, respectively) were noted; for LDL_{nat} and LDL_{ox}, chemiluminescent activity at 75 min was measured. For PMA stimulation of diluted whole blood, integrated chemiluminescence at 20 min was calculated.

Horseradish peroxidase was added to the system to overcome peroxidase deficiency extracellularly (26). In previous experiments we found that the addition of extra peroxidase did not affect superoxide production (measured as reduction of cytochrome *c*) of PMN stimulated with PMA, but increased luminol-enhanced chemiluminescence 3–4-fold. Hence, only the detection of superoxide is enhanced in the presence of extra peroxidase.

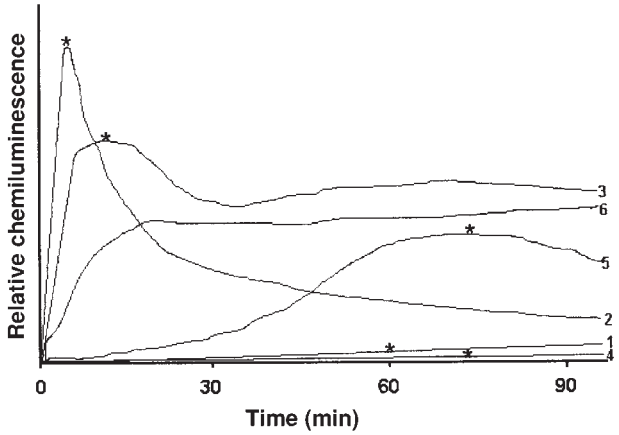

FIGURE 1. Kinetics of luminol-enhanced chemiluminescence by polymorphonuclear cells (PMNs) and diluted whole blood. For PMNs, spontaneous chemiluminescence (line 1) and responses to phorbol 12-myristate 13-acetate (line 2), opsonized zymosan (line 3), native LDL (line 4), and oxidized LDL (line 5) were recorded. Asterisks indicate the chemiluminescent activity of PMNs that were noted for the different curves. For diluted whole blood, the response to phorbol 12-myristate 13-acetate (line 6) was recorded and the area under the curve for 20 min after stimulation determined.

Culture of PBMCs

PBMCs were cultured for 16 h at 37°C in 24-well plates in the presence and absence of lipopolysaccharide (LPS; *Escherichia coli* serotype O55:B5; Sigma-Aldrich; 0.08 and 8 μg/L final concentrations), LDL_{nat}, and LDL_{ox} (both at 80× and 40× final dilution, corresponding to final concentrations of 0.4 and 0.8 mg/L, respectively). Each well contained 2×10^5 cells in RPMI 1640 (610 μL final volume) supplemented with 2 mmol L-glutamine/L, 50 mg garamycin/L, 1% pyruvate, 5% heat-inactivated human plasma, and stimuli as indicated. After culture, medium was centrifuged in a microcentrifuge and supernates were frozen at −80°C until cytokine analysis. Concentrations of TNF-α and IL-1β in cell supernates were determined by radioimmunoassay as described by Drenth et al (27). IL-8 was measured by sandwich enzyme-linked immunosorbent assay (Pelikine Compact, Amsterdam).

Other methods

Cholesterol and triacylglycerol concentrations in serum were determined by enzymatic methods (Boehringer-Mannheim, Mannheim, Germany) on a Hitachi 747 analyzer (Hitachi, Tokyo). Serum HDL-cholesterol concentrations were determined after precipitation of LDL, VLDL, and chylomicrons by using phosphotungstate-Mg^{2+} (28). LDL-cholesterol concentrations in serum were calculated by using the Friedewald formula (29).

Statistical analysis

The computer program ASTUTE (Microsoft Ink, Redmond, WA) was used for the analysis. For each subject and each variable, the mean before ($t = -1$ and $t = 0$) and the mean after ($t = 5$ and $t = 6$) α-tocopherol supplementation were calculated. Group results are expressed as means ± SDs. The data were evaluated by using Student's *t* test for paired and unpaired data. A two-tailed *P* value < 0.01 was considered to be statistically significant. Wilcoxon's signed-rank test was also used to compare pre- and postsupplementation data within each group. Both tests yielded strikingly similar results and levels of significance were not different.

RESULTS

Subjects

Characteristics of the studied groups are given in **Table 1**. Mean age was not significantly different between groups; BMI was significantly higher in the hypertriglyceridemic patients than in the normolipidemic subjects. Basal triacylglycerol concentrations were significantly higher in these patients. Whereas total cholesterol was slightly but significantly higher, HDL cholesterol was significantly lower in the patients than in the control subjects. The LDL-cholesterol concentrations of normolipidemic and hypertriglyceridemic subjects were not significantly different. Lipid and lipoprotein concentrations were unchanged after α-tocopherol treatment.

Effects of α-tocopherol supplementation on the α-tocopherol content of plasma and LDL

Baseline concentrations of α-tocopherol in the plasma of patients were significantly higher than those of control subjects but the α-tocopherol content of LDL was not different between groups (Table 1). Supplementation with α-tocopherol resulted in a 2–3-fold increase in the concentration of α-tocopherol in plasma and a 2-fold increase in that in LDL, both in patients and in control subjects (Table 1).

Superoxide production

Spontaneous chemiluminescence by PMNs of control subjects and hypertriglyceridemic patients was not significantly different but the SD in the patients was larger than that in the control subjects (2371 ± 1882 and 1750 ± 265 relative light units, respectively). Also, the SD of LDL_{ox}-induced chemiluminescence was larger in the patients than in the control subjects (**Figure 2**). This was because the PMNs of some patients consistently showed high chemiluminescence in response to this stimulus. These were not the patients with the highest lipid concentrations. The chemiluminescence of PMNs in response to opsonized zymosan and of PMNs and whole blood in response to PMA were not significantly different between the 2 groups (Figure 2 and **Figure 3**). In response to stimulation with LDL_{nat}, the chemiluminescence of PMNs was not higher than the spontaneous chemiluminescent activity of PMNs (data not shown).

The kinetics of the chemiluminescent responses had not changed after α-tocopherol supplementation, but α-tocopherol differentially changed the height of the peaks and plateau levels of the curves. Whereas the chemiluminescence of PMNs in response to opsonized zymosan was unaltered after α-tocopherol supplementation, chemiluminescence in response to PMA was significantly lower after α-tocopherol supplementation than at baseline (Figure 2). The effect of α-tocopherol was comparable in control and hypertriglyceridemic subjects and was also found to be similar for whole blood chemiluminescence (Figure 3). In contrast with PMA-induced chemiluminescence, the chemiluminescence of PMNs in response to LDL_{ox} was significantly higher after α-tocopherol supplementation than at baseline in both control subjects and in patients (Figure 2). Spontaneous chemilumi-

TABLE 1
Subject characteristics, plasma lipids, and α-tocopherol concentrations before and after α-tocopherol supplementation[1]

	Before		After	
	NL (n = 8)	HTG (n = 12)	NL (n = 8)	HTG (n = 12)
Age (y)	49.5 ± 9.6	55.8 ± 12.1	—	—
BMI (kg/m^2)	25.1 ± 1.8	28.6 ± 3.4[2]	—	—
Total cholesterol (mmol/L)	5.46 ± 1.33	6.65 ± 1.00[2]	5.54 ± 1.36	6.88 ± 1.39
Total triacylglycerol (mmol/L)	1.18 ± 0.61	5.81 ± 2.55[3]	1.15 ± 0.34	6.29 ± 2.90
HDL cholesterol (mmol/L)	1.29 ± 0.29	0.80 ± 0.13[4]	1.23 ± 0.31	0.78 ± 0.13
LDL cholesterol (mmol/L)	3.68 ± 1.09	3.72 ± 0.90	3.83 ± 1.12	3.87 ± 1.09
α-Tocopherol				
(mg/L plasma)	26.3 ± 3.6	49.8 ± 12.1[5]	47.7 ± 9.5[6]	125.0 ± 43.8[6]
(mg/g LDL protein)	14.1 ± 1.2	13.2 ± 2.0	25.3 ± 3.2[6]	25.5 ± 5.5[6]

[1] $\bar{x}$ ± SD.
[2–5] Significantly different from NL (t test): [2] $P < 0.05$, [3] $P < 0.0001$, [4] $P < 0.01$, [5] $P < 0.001$.
[6] Significantly different from before supplementation, $P < 0.001$ (paired t test).

nescence by PMNs was not affected by α-tocopherol supplementation (data not shown).

Cytokine production of PBMCs

In supernates of PBMCs cultured in vitro without stimuli, no IL-1β (detection limit: 80 ng/L) could be detected and TNF-α was detectable in 10 subjects (4 control subjects and 6 patients) only (range: 0.10–0.36 μg/L). IL-8 was present in all cultures of all subjects and did not differ significantly between control subjects and patients (9.1 ± 6.4 and 9.3 ± 3.2 μg/L, respectively). Stimulation of PBMCs with LPS resulted in a concentration-dependent induction of TNF-α and IL-1β and increase of IL-8 (**Figure 4**). After α-tocopherol supplementation, cytokine production by PBMCs in response to LPS was significantly lower than at baseline. The decrease was comparable in control subjects and in patients. Neither LDL_{nat} nor LDL_{ox}, at final concentrations of 0.8 and 0.4 mg/L

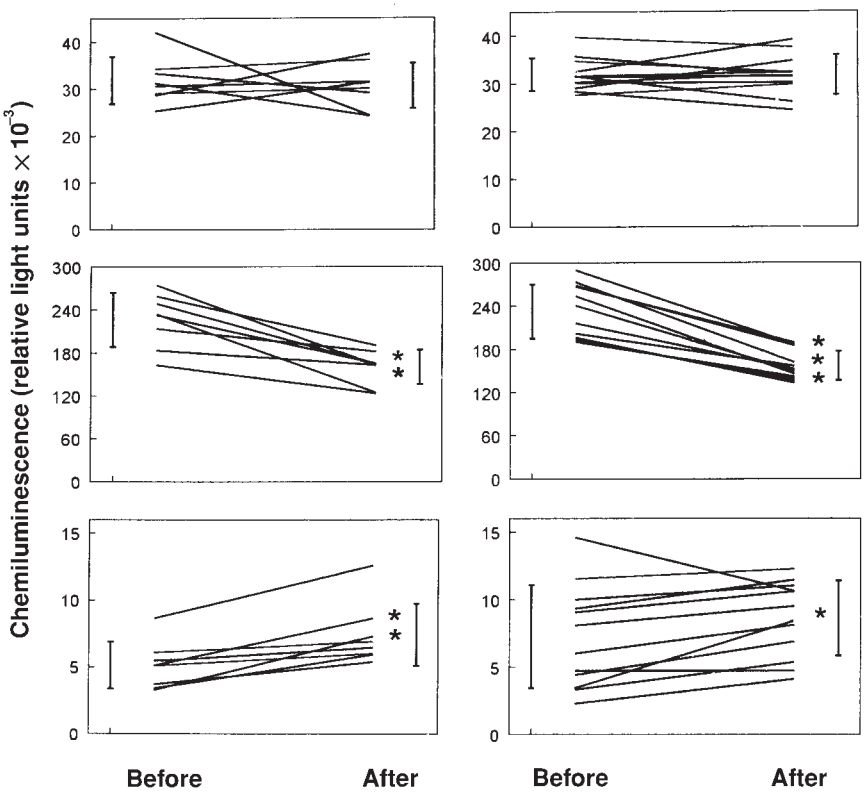

FIGURE 2. Luminol-enhanced chemiluminescence of polymorphonuclear cells isolated before and after α-tocopherol supplementation in 8 normolipidemic (left panels) and 12 hypertriglyceridemic (right panels) subjects. Cells were stimulated with opsonized zymosan (top), phorbol 12-myristate 13-acetate (middle), or oxidized LDL (bottom). Chemiluminescence was measured at time points indicated in Methods. *,**,***Significantly different from before α-tocopherol supplementation (paired t test): *$P < 0.05$, **$P < 0.01$, ***$P < 0.0001$.

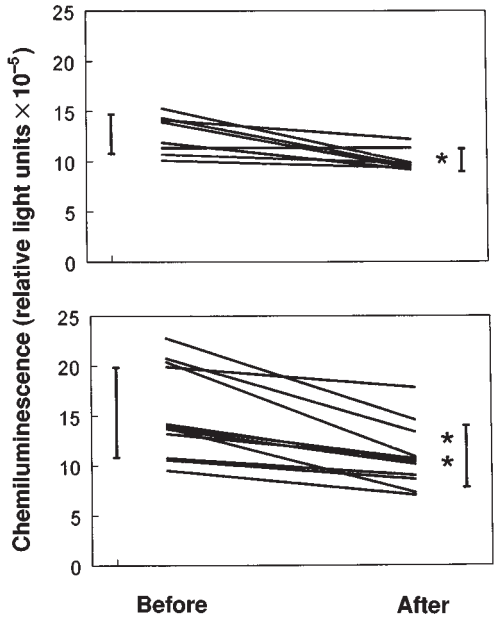

FIGURE 3. Phorbol 12-myristate 13-acetate (PMA)–induced, luminol-enhanced chemiluminescence of diluted whole blood of 8 normolipidemic (top) and 12 hypertriglyceridemic subjects (bottom) subjects before and after α-tocopherol supplementation. Integrated chemiluminescence was measured for 20 min after addition of PMA, as described in Methods. *,** Significantly different from before α-tocopherol supplementation (paired *t* test): *$P = 0.01$, **$P < 0.01$.

induced IL-1β or TNF-α and neither affected spontaneous IL-8 release (data not shown).

DISCUSSION

Triacylglycerol-rich lipoproteins have been suggested to play a crucial role in leukocyte activation. Pronai et al (30) reported increased production of superoxide by monocytes in exclusively hypertriglyceridemic patients. Araujo et al (20) found a positive correlation between ROS production by PMNs and plasma total triacylglycerol, VLDL-triacylglycerol, and LDL-triacylglycerol in patients with hypertriglyceridemia plus hypercholesterolemia. In the present study, we measured superoxide production by PMNs and in whole blood of hypertriglyceridemic patients and healthy control subjects. Production of superoxide by PMNs and whole blood in response to different stimuli was not significantly different between the groups. Spontaneous generation of superoxide by PMNs was low, indicating minimal activation by the isolation procedure; stimulation with LDL_{ox}, opsonized zymosan, and PMA caused 3-, 20-, and 200-fold increases in superoxide production, respectively. However, we could not confirm the data of Araujo et al, who found a greater release of ROS by PMNs in hyperlipoproteinemic subjects (20). This discrepancy may be explained by methodologic differences. Our chemiluminescence assay included extra peroxidase to ensure optimal detection of superoxide. PMA causes only minimal degranulation of azurophilic granules containing myeloperoxidase; therefore, peroxidase activity might be suboptimal for detection of superoxide. Because Araujo et al did not add extra peroxidase to the cells, differences in chemiluminescence between control subjects and hyperlipoproteinemic patients may reflect differences in peroxidase activity.

We did not find increased production of superoxide in hypertriglyceridemic patients, but some patients consistently showed high spontaneous production of superoxide by PMNs and a high response to LDL_{ox}, leading to a large variation in these variables in the patients. Extreme production of superoxide, however, was not related to plasma triacylglycerol concentrations.

After supplementation with α-tocopherol, we found a decreased capacity of PMNs to generate superoxide in response to PMA. The concentration of α-tocopherol in plasma and LDL had increased 2–3-fold and the α-tocopherol content of leukocytes may have increased to the same extent. In a study by Devaraj et al (14), a similar increase in plasma α-tocopherol led to a 2.5-fold increase in monocyte α-tocopherol content. Concomitantly, release of ROS by monocytes in response to LPS was reduced. Indirect evidence from

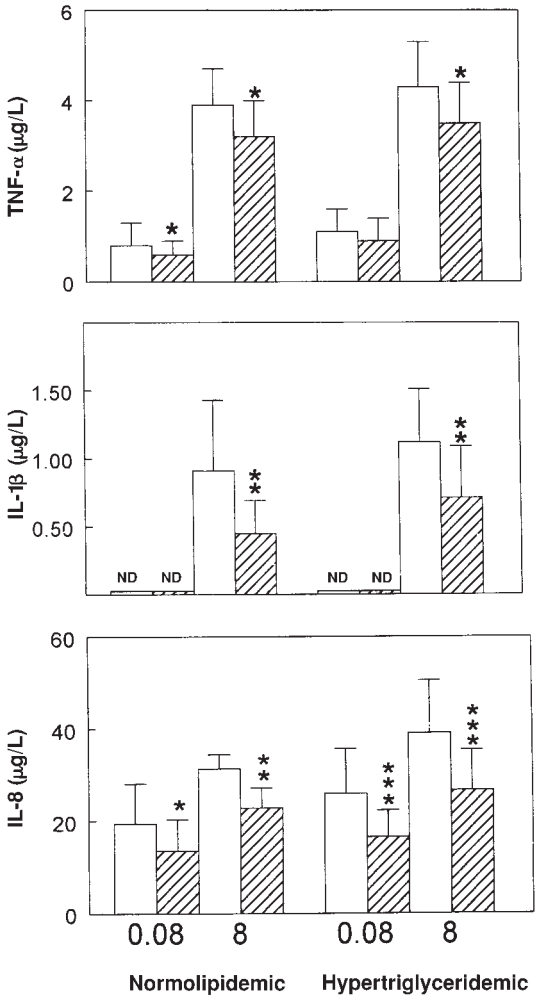

FIGURE 4. Lipopolysaccharide (LPS)-induced production of tumor necrosis factor α (TNF-α), interleukin (IL)-1β, and IL-8 by mononuclear cells of 8 normolipidemic and 12 hypertriglyceridemic subjects before (open bars) and after (hatched bars) α-tocopherol supplementation. Two different concentrations of LPS (0.08 and 8 μg/L) were used to stimulate the cells. ND, not detectable. *,**,***Significantly different from before α-tocopherol supplementation (paired *t* test): *$P < 0.05$, **$P < 0.01$, ***$P < 0.0001$.

the use of the protein kinase C inhibitor calphostin C suggested that the inhibition was due to an inhibition of protein kinase C activity by α-tocopherol. In other studies, using rat peritoneal neutrophils enriched in vitro with α-tocopherol, a stimulus-specific inhibition of superoxide generation was found. Protein kinase C–dependent generation of superoxide (eg, induced by PMA, dioctanoylglycerol, or calciumionophore A23187) but not protein kinase C–independent generation of superoxide (eg, induced by opsonized zymosan, sodium dodecyl sulfate, or formylmethionyl-leucyl-phenylalanine) was inhibited by addition of α-tocopherol to the cell suspension (15). The results of the present study reinforce and expand the in vitro data from rat PMNs by showing identical effects using PMNs of human subjects supplemented with α-tocopherol. Moreover, assessment of superoxide production using the diluted whole-blood assay yielded similar data. Recently, Cachia et al (31) suggested that in human monocytes preincubated with α-tocopherol, reduced protein kinase C activity results in impaired assembly of NADPH-oxidase.

In an attempt to meet pathophysiologic conditions present subendothelially at sites of atherogenesis, we also used LDL as a stimulus for PMN superoxide generation. Whereas LDL_{nat} did not induce superoxide production by PMNs, LDL_{ox} induced an increase in superoxide production to peak activity ≈75 min after stimulation. In contrast with PMA-induced generation of superoxide by PMNs, LDL_{ox}-induced generation of superoxide was significantly higher after supplementation with α-tocopherol than at baseline. Maeba et al (32) showed that LDL_{ox} needs to be phagocytosed before inducing superoxide production by PMNs and animal studies indicated stimulatory effects of α-tocopherol on phagocytic activity of leukocytes (33, 34). The net effect of α-tocopherol on cellular superoxide production is likely to be the result of its effects on the different components involved in the mechanism of superoxide production in response to a particular stimulus.

Reportedly, LDL_{ox} can induce activation of immune competent cells. However, experimental conditions may be crucial for the outcome (35, 36). The extent of oxidation of LDL as well as the concentration of LDL used determine whether it is proinflammatory or cytotoxic. In the present study we stimulated PBMCs ex vivo with LDL_{nat} and LDL_{ox} at final concentrations of 0.4 and 0.8 mg/L. Great care was taken to prevent any contamination with LPS. It was found that LDL, independent of whether it was native or oxidized, did not induce release of TNF-α, IL-1β, or IL-8 by PBMCs. On the other hand, LPS induced production of these cytokines in a dose-dependent manner. Possibly, the concentration of LDL used to stimulate the cells was too low because at 4 mg/L, LDL_{ox} did induce superoxide production by PMNs.

Production of cytokines by PBMCs of hypertriglyceridemic and control subjects in response to LPS was not significantly different. After supplementation with α-tocopherol, in vitro cytokine production of PBMCs in response to LPS decreased significantly. Several reports in the literature describe the effects of α-tocopherol on cytokines. In vitro α-tocopherol inhibited PMA-induced IL-1β expression in the human monocytic leukemia cell line THP-1 (18) and LPS-induced activation of rat Kupffer cells (17). Furthermore, endotoxin-induced production of IL-6 was enhanced by α-tocopherol deficiency in rats (37). In normal healthy volunteers, Devaraj et al (14) found decreased production of IL-1β by monocytes after α-tocopherol supplementation, and we observed decreased cytokine production in whole blood after α-tocopherol supplementation in smokers and nonsmokers (19). Inhibition of cytokine production by α-tocopherol might be related to the antioxidant capacity of α-tocopherol to moderate intracellular oxidative status and thereby influence activation of redox-sensitive NF-κB (11). Islam et al (12) showed that pretreatment of U937 cells with α-tocopherol significantly decreased the LPS-induced activation of NF-κB. However, other mechanisms cannot be excluded. Recently, Devaraj and Jialal (38) suggested involvement of 5-lipoxygenase in the α-tocopherol–mediated inhibition of IL-1β release from human monocytes. Further studies are necessary to elucidate the mechanism or mechanisms by which α-tocopherol modulates cytokine production.

In conclusion, in the present study no indications of abnormal superoxide or cytokine production were found in hypertriglyceridemic subjects. Furthermore, α-tocopherol was shown to have differential effects at the cellular level, depending on the stimulus used. Superoxide production capacity of PMNs, assessed in response to PMA, was inhibited, but the response to opsonized zymosan was not changed and the response to LDL_{ox} was enhanced. In addition, cytokine production capacity of PBMCs, assessed in response to LPS, was inhibited. Via these mechanisms, α-tocopherol may influence the inflammatory responses of immune cells infiltrating subendothelial spaces.

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