Effect of Vitamin E on Endothelial Vasodilator Function in Patients With Hypercholesterolemia, Chronic Smoking or Both

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OBJECTIVES The purpose of this study was to test the hypothesis that long-term supplementation with Vitamin E improves endothelium-dependent relaxation in hypercholesterolemia patients and/or chronic smoking, two risk factors that have been shown to be associated with increased radical formation.

BACKGROUND Experimental evidence suggests that oxidized low density lipoprotein (LDL) impairs endothelium-dependent relaxation, and vitamin E, a lipid-soluble antioxidant, reduces the oxidation of LDL.

METHODS Thirteen subjects with hypercholesterolemia, 14 smokers and 15 hypercholesterolemic smokers were enrolled in a double-blind, placebo-controlled study. After baseline measurements of plasma autoantibodies against oxidized LDL and assessment of endothelium-dependent relaxation using intra-arterial forearm infusions of acetylcholine, participants within each group were randomly assigned in a 1:2 fashion to receive either placebo or vitamin E for 4 months, when plasma levels of autoantibodies against oxidized LDL and vascular function were reassessed.

RESULTS Vitamin E significantly augmented endothelium-dependent relaxation in hypercholesterolemic smokers but not in patients with either hypercholesterolemia or chronic smoking. At baseline, hypercholesterolemic smokers had significantly higher autoantibody levels against oxidized LDL (compared with the other two groups), which were significantly reduced after 4 months of vitamin E supplementation. There was a significant relationship between improvement in acetylcholine-induced vasodilation and the change in autoantibody titer against oxidized LDL ($r = -0.59; p = 0.002$).

CONCLUSIONS Long-term vitamin E supplementation improves endothelium-dependent relaxation in forearm resistance vessels of hypercholesterolemic smokers, which are characterized by increased levels of autoantibodies against oxidized LDL. These findings may suggest that the beneficial effect of vitamin E is confined to subjects with increased exposure to oxidized LDL.

(J Am Coll Cardiol 1999;33:499–505) © 1999 by the American College of Cardiology

The endothelium plays an integral role in the regulation of vascular tone, platelet activity and vascular thrombosis and is intimately involved in the pathogenesis of atherosclerosis (1). Hypercholesterolemia and smoking are two major risk factors for the development of atherosclerosis, and both have been shown to be associated with impaired endothelial function (2–4). Oxidative modification of low density lipoprotein (LDL) cholesterol is thought to be an important step in promoting endothelial dysfunction and ultimately atherosclerosis (5,6). Indeed, we have recently shown a close relationship between plasma levels of autoantibody against oxidized LDL cholesterol and endothelium-dependent relaxation in human forearm circulation (3).

Antioxidants such as vitamin E have been shown to reduce the oxidative susceptibility of lipoproteins (7–9) and may have antiatherosclerotic effects (10). In hypercholesterolemic animal models, vitamin E increased resistance to lipoprotein oxidation and preserved normal endothelial function (11,12). Epidemiologic studies also indicate an inverse association between the intake of vitamin E and coronary heart disease (13–15). Yet the data from clinical trials concerning the beneficial effect of vitamin E supplements in protecting from coronary artery disease and cardiovascular mortality have been inconsistent. Whereas some trials found no effect on mortality from cardiovascular disease among those receiving 50 mg of vitamin E (15,16), another trial has suggested a reduction in the incidence of nonfatal myocardial infarction with larger doses of supple-
ments (17). Conceivably, vitamin E may not prevent cardiovascular disease and ischemic events in the general population but rather in susceptible individuals, that is, those with limited or impaired defense mechanisms against oxidative stress such as hypercholesterolemic patients (18) and/or chronic smokers (19). Since endothelial dysfunction may represent an early step in the development of cardiovascular disease and trigger ischemic events, the present trial was designed to investigate the effect of vitamin E supplementation on endothelium-dependent vasodilation and LDL oxidation in subjects with hypercholesterolemia and/or chronic smoking.

METHODS

Subjects and study design. Subjects were eligible for the treatment trial if they had a serum LDL cholesterol level, measured after a 12-h fasting period, of more than the 75th percentile for age and gender and were not receiving cholesterol-lowering medication (lowest cutoff 155 mg%). An age-matched group of normal individuals (control group) without risk factors was assessed for endothelial function and LDL values for comparison but was not enrolled in the long-term treatment protocol. Long-term smokers were included if they had a history of >20 pack-years (1 pack-year defined as smoking 20 cigarettes per day for 1 year or the equivalent). No participant had a history of diabetes mellitus, arterial hypertension, heart failure or any other systemic disease predisposing them to endothelial dysfunction. Nonsmokers were not exposed to smoking at their homes, leisure or work and therefore, exposure to environmental tobacco smoke was minimal or absent. Further exclusion criteria were current use of antioxidants or vasoactive medication.

After an initial screening period to confirm eligibility requirements, 42 subjects were included in this prospective, randomized, double-blind, placebo-controlled study: 13 subjects with hypercholesterolemia, 14 long-term smokers and 15 hypercholesterolemic subjects who smoked. Participants within each group were randomly assigned in a 1:2 fashion to receive either placebo or vitamin E in order to treat approximately one third of each group with placebo and the other two thirds of each group with vitamin E. This randomization procedure was used to minimize studies in patients on placebo. After the initial study of vascular function, the subjects received either placebo or vitamin E (d-alpha-tocopherol acetate 544 IU per day) for four months. The hypercholesterolemic subjects received a dietary counseling but no lipid-lowering medication. At the four-month follow-up serum lipid concentrations and vascular function were measured using the same protocol. This study was approved by the Ethics Committee of the University of Freiburg, and each subject gave written informed consent.

Vascular function studies. Studies took place in a 23°C temperature-controlled laboratory in the postprandial state. Under local anesthesia and sterile conditions, a 20-gauge polyethylene catheter was inserted into the brachial artery of the nondominant arm for infusion of drugs. Forearm blood flow was measured by venous occlusion plethysmography as recently described (3). Systolic, diastolic and mean arterial pressures and heart rate were determined at the contralateral arm with a Dinamap recorder.

Baseline measurements of forearm blood flow were obtained during intra-arterial infusion of 0.9% saline at a rate of 1.66 ml/min. To assess endothelium-dependent vasodilation, acetylcholine chloride was administered at increasing concentrations (7.5, 15, 30 and 60 μg/min). Then, sodium nitroprusside was infused as an endothelium-independent vasodilator (1, 3 and 10 μg/min). Each dose of drug was infused for five min, with measurements of forearm blood flow being made over the final two min of each infusion period.

Measurement of autoantibody against oxidized LDL. Blood samples were obtained after a 12-h fasting period on the same day as the flow measurements. Serum was separated from blood elements by centrifugation, and aliquots were stored at −80°C. Immunoglobulin G autoantibodies against copper-oxidized LDL were measured in a subset of patients as previously described (3). All measurements were done in duplicate without knowledge of the forearm blood flow measurements and were performed in a single assay (S.Y.H. and J.L.). Results were expressed as ratio of oxidized LDL to native LDL at 1:50 dilution.

Statistical analysis. All data are expressed as mean ± SEM. Statistical analysis was performed by analysis of variance for repeated measurements followed by Tukey’s Studentized Range Test. Associations between the effects of vitamin E and clinical parameters were examined by regression analysis.

RESULTS

Patient characteristics. The treatment study population consisted of 42 subjects. Baseline characteristics and lipid profiles of subjects are shown in Table 1. Total cholesterol and LDL cholesterol plasma levels were lower in chronic smokers by definition as compared to hypercholesterolemic subjects. There were no differences in HDL cholesterol and triglyceride levels between the groups. The lipid measurements at follow-up showed no significant changes in any of the groups. A normal control (nonsmoking) group (n = 10) was studied in the same manner and had normal cholesterol, LDL and high density lipoprotein levels.
Forearm vasomotor function. Intra-arterial infusion of acetylcholine caused a dose-dependent and significant increase in forearm blood flow in all groups. The acetylcholine-induced increase in forearm blood flow was significantly impaired in all three treatment groups (hypercholesterolemia, smoking, both risk factors) as compared to the age-matched control group (p < 0.05). In the control group, acetylcholine (7.5, 15, 30, 60 μg/min) increased forearm blood flow from 3.0 ± 0.3 to 7.1 ± 0.5, 10.6 ± 0.8, 17.0 ± 0.6 and 21.9 ± 0.7 ml/min/100 g. Sodium nitroprusside (1, 3, 10 μg/min) increased forearm blood flow from 3.1 ± 0.2 to 7.1 ± 0.3, 11.1 ± 0.8 and 14.9 ± 0.5 ml/min/100 g.

Vitamin E treatment did not affect the acetylcholine-induced increase in forearm blood flow in hypercholesterolemic patients (Fig. 1). In chronic smokers, vitamin E therapy tended to cause a mild augmentation in blood flow (Fig. 1), but the study did not have enough power to detect a significant difference. However, in hypercholesterolemic smokers (Fig. 1), the maximal flow in response to acetylcholine increased from 7.8 ± 2.4 ml/min/100 ml at baseline increased forearm blood flow from 3.1 ± 0.2 to 7.1 ± 0.3, 11.1 ± 0.8 and 14.9 ± 0.5 ml/min/100 g.

Figure 1. Mean (±SEM) responses of forearm blood flow to intra-arterial acetylcholine in hypercholesterolemic subjects (left panel), chronic smokers (middle panel) and hypercholesterolemic smokers (right panel) at baseline and after 4 months of treatment with either placebo or vitamin E. Vitamin E therapy improved the acetylcholine-induced increase in forearm blood flow (reflecting endothelium-dependent vasodilation) only in hypercholesterolemic smokers (*p < 0.01 as compared to the corresponding placebo group).

**Table 1.** Characteristics and Lipid Profiles of Study Groups

<table>
<thead>
<tr>
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<th>Hypercholesterolemia</th>
<th>Chronic Smoking</th>
<th>Hypercholesterolemia and Smoking</th>
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<td></td>
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<td>Vitamin E (n = 10) Placebo (n = 4)</td>
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<td>Cholesterol (mg/dl)</td>
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<td></td>
<td>Follow-up</td>
<td>292 ± 15</td>
<td>268 ± 10</td>
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<td>LDL cholesterol (mg/dl)</td>
<td>Baseline</td>
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<td>216 ± 5</td>
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<tr>
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<td>Follow-up</td>
<td>211 ± 7</td>
<td>194 ± 5</td>
</tr>
<tr>
<td>HDL cholesterol (mg/dl)</td>
<td>Baseline</td>
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*p < 0.05 versus hypercholesterolemia, and smoking and hypercholesterolemia. Values are given as mean ± SEM. HDL = high density lipoprotein; LDL = low density lipoprotein; MAP = mean arterial pressure. 
to 15.7 ± 3.2 ml/min/100 ml after 4 months of vitamin E. This difference between the acetylcholine-induced vasodilator response at baseline and that at follow-up was statistically significant (p < 0.003).

Infusion of sodium nitroprusside increased forearm blood flow in all groups. There was no significant change between baseline and follow-up in any of the groups (data not shown).

**Autoantibody titer against oxidized LDL.** Autoantibody levels against oxidized LDL averaged 1.12 ± 0.16 in the control group and were not significantly increased in the group with hypercholesterolemia or chronic smoking. However, hypercholesterolemic smokers had significantly higher autoantibody titer levels against oxidized LDL compared with the other groups (p < 0.03; Fig. 2). The elevated autoantibody titer level was reduced after 4 months of vitamin E supplementation and did not differ from the other two groups at follow-up. Vitamin E did not affect the autoantibody titer of oxidized LDL in the smoking and the hypercholesterolemic group. Autoantibody titer against oxidized LDL did not change in placebo-treated patients, indicating that storage of plasma did not affect autoantibody levels.

**Relation between autoantibody titer against oxidized LDL and changes in endothelial function.** There was a significant relationship between improvement in acetylcholine-induced vasodilation and autoantibody titer against oxidized LDL at baseline (r = 0.50; r = 0.013). Patients with a higher degree of autoantibodies against oxidized LDL at baseline were more likely to have improvement by vitamin E supplementation. Furthermore, the change in autoantibody titer against oxidized LDL correlated significantly with the change in endothelial function (r = −0.59; p = 0.002; Fig. 3).

**DISCUSSION**

Both hypercholesterolemia and chronic smoking have been shown to impair endothelial vasodilator function in humans (2–4). These risk factors synergistically reduce endothelial-dependent relaxation, and their combined presence is associated with increased plasma levels of auto-antibodies against oxidized LDL (3). Similar to these previous observations, endothelial vasodilator function was impaired in patients with hypercholesterolemia or chronic smoking (as compared to age-matched normal individuals [3]); however, the most severe impairment of endothelium-dependent relaxation was noted in patients with both risk factors, confirming our previous finding. Moreover, the combined presence of chronic smoking and hypercholesterolemia was associated with increased levels of autoantibodies against oxidized LDL. The present study demonstrated a beneficial effect of vitamin E supplementation on endothelial vasodilator dysfunction selectively in hypercholesterolemic smokers, and this effect on vascular function was associated with a reduction of the elevated autoantibody titer against oxidized LDL in these patients.

**Potential mechanisms.** The endothelium is an important modulator of vasomotor tone through the release of endothelium-derived relaxing factors such as nitric oxide. Impairment of the endothelium-dependent release of nitric oxide (NO) has been demonstrated in patients with risk factors for atherosclerosis including hypercholesterolemia and chronic smoking (2–4). The underlying mechanisms are not clearly identified, but increased vascular production of superoxide anion has been implicated as contributing to impaired endothelium-dependent vascular relaxation in animal models of hypercholesterolemia (20). In fact, there is some evidence that LDL uncouples L-arginine metabolism.
from NO release to increase production of superoxide anions by endothelial NO synthase (21), supporting the concept in concert with other studies (22) that a dysfunctional endothelial NO synthase represents one source of reactive oxygen metabolites.

Recent evidence also suggests that increased degradation of NO by oxygen-derived free radicals may be an important mechanism for endothelial dysfunction in chronic smoking (23,24). In addition, reactive oxygen species may promote oxidative modification of LDL within the vascular wall, which is thought to be a key process in the development of endothelial dysfunction and atherosclerosis (5,6).

We have recently shown that cigarette smoking potentiates endothelial dysfunction of forearm resistance vessels in subjects with hypercholesterolemia, possibly due to enhanced oxidation of LDL (3) as suggested by a close inverse relationship between plasma levels of autoantibody titer to oxidized LDL and acetylcholine-induced blood flow responses.

In the present study we tested the hypothesis that this study population benefits from antioxidant therapy. There are several important differences between the naturally occurring antioxidants ascorbic acid, vitamin E and 

Vitamin E and Endothelial Function

Heitzer et al.

February 1999:499–505

JACC Vol. 33, No. 2, 1999

Limitations of the study. It should be noted that similar doses of vitamin E as used in the present study have been shown to affect diene lag time (7,8), suggesting that the beneficial effect of vitamin E is due to reduced oxidation of LDL (assuming that the decrease in the levels of autoantibodies against oxidized LDL reflect reduced exposure to oxidized LDL). However, the present study cannot definitively prove that the beneficial effect of vitamin E on endothelial vasodilator function is attributed to the reduction of autoantibodies against oxidized LDL. There is experimental evidence that NO can prevent LDL oxidation by endothelial cells (32). Thus, the reduced levels of autoantibodies against LDL may be secondary to increased availability of endothelium-derived NO. Furthermore, improvement of endothelial vasodilator function may be attributed to increased release of prostaglandin from the endothelium, endothelium-derived hyperpolarizing factor or a reduced production of a vasoconstrictor prostanoid. Notably, patients with either hypercholesterolemia or chronic smoking had impaired endothelium-dependent relaxation, which was not affected by vitamin E treatment. The selective beneficial effect of vitamin E in patients with both risk factors and increased levels of autoantibodies against LDL suggests that only the synergistic adverse effect
of these risk factors on endothelial vasodilator function is prevented by vitamin E, consistent with recent observations in hypercholesterolemic rabbits exposed to tobacco smoke (33). Notably, a small improvement in endothelial function was observed in smokers; however, our study did not have enough power to conclude that this difference was significant.

Autoantibody levels were not measured in all patients due to technical reasons. However, the patients without these measurements did not differ in clinical characteristics or endothelial function tests from those individuals with autoantibody measurements. Moreover, treatment effects were observed with vitamin E but not placebo and were related to the baseline values, indicating a true treatment effect of vitamin E.

In conclusion, the present study demonstrates that vitamin E supplementation improves endothelial vasomotor function in subjects with increased autoantibody titer against oxidized LDL. The beneficial effect of vitamin E selectively in these “high risk” patients may represent one explanation for the failure of recent large-scale trials to demonstrate a preventive effect of vitamin E in the general population.

Acknowledgment
We would like to thank Manfred Olschewski for valuable advice and additional statistical analysis. Furthermore, we are grateful to Wyeth-Pharma GmbH, Germany, for providing vitamin E and placebo capsules.

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