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Effect of Antioxidant Vitamins on Low Density Lipoprotein Oxidation and Impaired Endothelium-Dependent Vasodilation in Patients With Hypercholesterolemia

DAVID M. GILLIGAN, MD, MICHAEL N. SACK, MD, VICTOR GUETTA, MD, PHILIP R. CASINO, MD, ARSHED A. QUYYUMI, MD, FACC, DANIEL J. RADER, MD, JULIO A. PANZA, MD, RICHARD O. CANNON III, MD, FACC

Bethesda, Maryland

Objectives. The aims of this study were to determine whether antioxidant vitamins could reduce the susceptibility of low density lipoprotein (LDL) to oxidation and improve endothelium-dependent vasodilator responsiveness in patients with hypercholesterolemia.

Background. Animals and humans with hypercholesterolemia have exhibited impaired endothelium-dependent vasodilation. In vitro studies suggest that oxidatively modified LDL can impair nitric oxide production.

Methods. Forearm blood flow was measured with strain gauge plethysmography and brachial artery drug infusions in 19 patients, aged 52 ± 9 years, with hypercholesterolemia (mean ± SD total cholesterol 283 ± 22 mg/dl, LDL 197 ± 31 mg/dl) and in 14 subjects, aged 48 ± 8 years, with normal cholesterol levels (total cholesterol 169 ± 20 mg/dl, LDL 102 ± 23 mg/dl). Acetylcholine (7.5, 15 and 30 μg/min) was utilized as an endothelium-dependent vasodilator, and sodium nitroprusside (0.8, 1.6 and 3.2 μg/min) was used to test endothelium-independent vasodilation. Oxidative susceptibility of LDL was measured by a spectrophotometric assay of conjugated diene production after the addition of copper chloride. Hypercholesterolemic patients then received daily antioxidant vitamin supplements (beta-carotene [30 mg], ascorbic acid [vitamin C] [1,000 mg], vitamin E [800 IU]) for 1 month, with repeat measurement of both forearm blood flow responsiveness to the same agonists and LDL oxidizability.

Results. The maximal flow in response to acetylcholine was impaired in patients compared with that in normal subjects (9.8 ± 7.8 vs. 15.9 ± 8.1 ml/min per 100 ml, p = 0.03), with similar maximal flow responses to sodium nitroprusside (9.5 ± 4.2 vs. 9.0 ± 2.8 ml/min per 100 ml, p = 0.72). After 1 month of vitamin therapy, the onset of LDL oxidation was prolonged over baseline measurements by 71 ± 67%, and the maximal rate of oxidation was decreased by 26 ± 25% (both p < 0.001). However, the maximal forearm blood flow response to acetylcholine remained unchanged from baseline values (maximal flow after acetylcholine 9.0 ± 6.2 vs. 9.8 ± 7.8 ml/min per 100 ml, p = 0.57). This study had 80% power (alpha = 0.05) to exclude a 45% increase over baseline value in acetylcholine-stimulated flow during vitamin therapy.

Conclusions. Although 1 month of administration of antioxidant vitamin supplements in hypercholesterolemic patients reduced the susceptibility of LDL to oxidation, impairment in endothelial function remained unaltered. The use of nonvitamin antioxidants or concomitant reduction in LDL levels, as well as more sensitive techniques for measuring vascular responsiveness, may be required to show a beneficial effect on endothelial vasodilator function.

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The role of the endothelium in controlling vascular tone has been increasingly recognized over the past decade (1,2). Impaired endothelium-dependent vasodilation has been demonstrated in patients with atherosclerosis (3) and in conditions predisposing to the development of atherosclerosis, such as hypercholesterolemia, even before structural vascular disease is established (4–9). This impaired endothelium-dependent vasodilator responsiveness in hypercholesterolemic patients is likely a consequence of reduced bioavailability of nitric oxide (9).

Oxidative modification of low density lipoprotein (LDL) may be important in the development of atherosclerosis in hypercholesterolemic animals and humans (10). Oxidatively modified LDL may also impair the function of signal transduction pathways that link endothelial cell surface receptors to stimulation of nitric oxide production (11,12). Recent interest in antioxidant therapy has primarily focused on its antiatherogenic effects, whose mechanism is believed to be the incorporation of lipophilic antioxidant vitamins into the lipoprotein particle, thus protecting LDL from the pro-oxidant environment of the arterial wall. This effect could reduce the formation of foam cells and retard the development or progression of atherosclerosis (13). However, protection of LDL from oxidative modification by antioxidant vitamins could also improve endothelial production of nitric oxide. Thus, the aim of this study was to determine whether antioxidant vitamins could

From the Cardiology Branch, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, Maryland.

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Address for correspondence: Dr. Richard O. Cannon III, Building 10, Room 7B15, National Institutes of Health, Bethesda, Maryland 20892.

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reverse abnormal endothelial function in hypercholesterolemic patients over a period of time too brief to be likely to affect atherogenesis.

Methods

Study patients. Hypercholesterolemic patients 18 to 75 years old with fasting total cholesterol levels ≥250 mg/dl were eligible for study. Nineteen asymptomatic hypercholesterolemic patients, 9 men and 10 women, aged 52 ± 9 years, without known atherosclerotic cardiovascular disease, were enrolled. Their lipid profile showed the following values: total cholesterol 283 ± 22 mg/dl, triglycerides 172 ± 79 mg/dl, LDL 197 ± 31 mg/dl and high-density lipoprotein (HDL) 48 ± 13 mg/dl. All patients were free from hypertension, diabetes or other systemic disease and were not receiving medication or hormone replacement therapy. Three were cigarette smokers. No patient had taken any cholesterol-lowering agents in the previous 2 months or any antioxidant vitamin supplements in the preceding 6 months. All patients had normal findings on physical examination, rest electrocardiogram (ECG), chest X-ray study and symptom-limited treadmill exercise test performed with the standard Bruce protocol. No patient experienced angina pectoris or claudication during exercise. Fourteen healthy volunteer subjects, 6 men and 8 women, aged 48 ± 8 years, were also studied. Their lipid profile showed the following values: total cholesterol 169 ± 20 mg/dl, triglycerides 83 ± 31 mg/dl, LDL 102 ± 25 mg/dl and HDL 50 ± 13 mg/dl. These subjects were screened by clinical history, ECG and blood chemistry studies to ensure the absence of cardiovascular or other systemic disease, and they were not receiving medications, vitamin supplements or hormone replacement therapy.

Protocol. This study was approved by the National Heart, Lung, and Blood Institute Review Board, and all participants gave written informed consent. Alcohol, caffeine and smoking were prohibited for 24 h before the study. A cannula (1.75 in. [4.45 cm], 20 gauge, Arrow) was inserted into the brachial artery of the nondominant arm. A blood sample for lipid profile was obtained. Forearm blood flow studies were performed by using strain gauge plethysmography, as previously described for our laboratory (14). Briefly, a mercury-filled Silastic strain gauge connected to a plethysmograph (model EC4, DE Hokanson), in turn connected to a chart recorder (Pharmacia LKB, Biotechnology, Sweden), was calibrated to measure forearm volume changes. A rapid cuff inflator (model E10, DE Hokanson) was used to occlude venous outflow from the extremity and a wrist cuff was inflated to 50 mm Hg over systolic pressure 1 min before each measurement to exclude the hand circulation. Forearm blood flow was expressed as ml/min per 100 ml of forearm volume. Brachial artery pressure was measured directly from the intraarterial catheter (Spacelabs model 90308). Forearm vascular resistance was calculated as the mean arterial pressure divided by the forearm blood flow and is expressed as units.

An intraarterial infusion of 5% dextrose solution was begun at 1 ml/min and continued throughout drug infusions. Basal measurements were obtained 3 min after the start of the infusion. Forearm blood flow was then measured during intraarterial infusions of acetylcholine chloride (Sigma Chemical) at 7.5, 15 and 30 μg/min, and sodium nitroprusside at 0.8, 1.6 and 3.2 μg/min. Infusion rates (0.25, 0.5 and 1 ml/min) were identical for each drug. Each dose of drug was infused for 5 min and forearm blood flow was measured in the last 2 min of each dose. The order of administration of these drugs was randomized and a 30-min rest period ensued between infusions.

Isolation of LDL. In 14 hypercholesterolemic patients and 8 normal subjects, plasma was separated by low speed centrifugation. To inhibit auto-oxidation, ethylenediaminetetraacetic acid (EDTA) (1 mg/ml) and butylated hydroxytoluene (BHT) (4.4 μg/ml) were added to the samples as soon as the plasma was separated from the blood cells. To isolate LDL from plasma, by sequential ultracentrifugation between densities of 1.006 and 1.063 g/ml was performed, as previously described (15). Before the oxidation assay was begun, individual samples of LDL were dialyzed in a 400-fold volume of 0.01 mol/liter phosphate buffer 0.16 mol/liter sodium chloride (NaCl) solution (PBS), pH 7.4, with 10 μmol/liter EDTA (PBS-EDTA) for 24 h, with a change of the dialysis solution after 12 h. LDL protein determination was performed before the oxidation assay by using the BCA method (Pierce) (16).

LDL oxidation assay. Oxidizability of LDL was assessed using a spectrophotometric technique similar to that described by Esterbauer et al (17). LDL samples were diluted to a final protein concentration of 50 μg/ml in 1 μmol/liter EDTA-PBS. Ten μl of a 0.5 mmol/liter $CuCl_2$ solution was added to 1 ml of LDL (final concentration 5 μmol/liter copper chloride [$CuCl_2$]). Peroxidation of LDL was assessed by using ultraviolet spectrophotometric absorbance of 234 nm as an index of conjugated diene formation after the initiation of oxidation by $CuCl_2$. Absorbance was measured at 10-min intervals for 240 min and half-hourly thereafter for a total of 7 h. The change in absorbance with respect to time was divided into three consecutive phases, as previously described: lag time, propagation phase and decompensation phase. The lag time was defined as the interval between initiation of oxidation by the addition of $CuCl_2$ (time 0) and the intercept of the tangent of the slope of the absorbance curve during the propagation phase. The propagation phase, in which polyunsaturated fatty acids of LDL are rapidly converted into conjugated hydroperoxides, is represented by the rapid increase in absorbance with respect to the plateau of the lag time. The decompensation phase is the time period after the point of maximal absorbance and is characterized by an initial decrease in the absorbance for approximately 2 h, followed by a gradual increase in absorbance, due to decompensation of lipid hydroperoxides.

Analytic methods. Plasma cholesterol and triglycerides were quantitated by automated enzymatic techniques on an Abbott Laboratories V™SE analyzer. HDL cholesterol was quantified in plasma after dextran sulfate precipitation. Plasma beta-carotene and vitamin E levels were measured by high

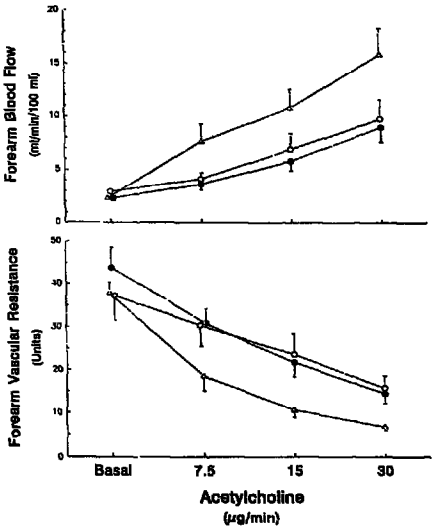

Figure 1. Forearm blood flow (**upper panel**) and resistance (**lower panel**) in response to serial doses of acetylcholine in 14 normal subjects (**open triangles**) and in 19 hypercholesterolemic patients at baseline (**open circles**) and 1 month after administration of daily antioxidant vitamin supplements (beta-carotene [30 mg], vitamin C [1,000 mg], vitamin E [800 IU]) (**closed circles**). The baseline acetylcholine response of hypercholesterolemic patients was lower than that of normal subjects ($p < 0.01$). The acetylcholine response of hypercholesterolemic patients was not altered by 1 month of antioxidant vitamin therapy ($p = 0.26$ for flow, $p = 0.58$ for resistance). Values are mean value ± SEM. All p values were obtained by analysis of variance for repeated measures.

performance liquid chromatography with fluorometric detection (18). Vitamin C levels were measured by the spectrophotometric measure of the 2,4-dinitro phenylhydrazine derivative of dehydroascorbic acid (19).

Vitamin supplement study. After performance of the baseline LDL oxidation assay and forearm flow study, hypercholesterolemic patients received daily treatment with beta-carotene (30 mg), vitamin C (ascorbate) (1,000 mg) and vitamin E (*dl*-alpha-tocopherol acetate) (800 IU) for 4 weeks. At the end of 4 weeks, patients returned to the Clinical Center for blood sampling for repeat studies: LDL isolation for oxidation assay, measurement of plasma lipid and vitamin levels and forearm flow study identical to that performed at baseline.

Statistical analysis. Differences between two means of continuous data (ages, lipid levels, vitamin levels, LDL oxidation data) were compared by paired or unpaired Student *t* test as appropriate. The forearm flow responses to acetylcholine and sodium nitroprusside in the two groups at baseline and in the hypercholesterolemic patients before and after vitamin treatment were compared by analysis of variance for repeated measures using a multiple linear regression model that included dummy variables to correct for variability between subjects (20). Correlations were performed by linear regression analysis. Data are expressed as mean value ±1 SD. Error bars on the figures represent ±1 SEM. A two-tailed p value < 0.05 was accepted as indicating statistical significance.

Results

Baseline responses to acetylcholine and sodium nitroprusside. The forearm vasodilator response to acetylcholine was significantly lower in hypercholesterolemic patients than in normal subjects ($p < 0.01$) (Fig. 1), with a maximal forearm flow of 9.8 ± 7.8 ml/min per 100 ml in patients compared to 15.9 ± 8.1 ml/min per 100 ml in normal subjects ($p = 0.03$). The relative decrease in forearm vascular resistance with acetylcholine from a baseline of 37.2 ± 24.9 U for patients and 37.9 ± 9.0 U for normal subjects was much lower in patients than in normal subjects ($p < 0.01$) (Fig. 1). Because hypercholesterolemic patients were slightly older than normal subjects (52 ± 9 vs. 48 ± 8 years, $p = 0.08$), associations between age and the maximal vasodilator response to acetylcholine were sought. An inverse correlation between age and the maximal flow response to acetylcholine was present for normal subjects ($r = -0.49$, $p = 0.07$) but not for hypercholesterolemic patients ($r = 0.005$). The maximal flow in response to acetylcholine was lower in male than in female hypercholesterolemic patients (5.9 ± 2.9 vs. 13.4 ± 9.3 ml/min per 100 ml, $p = 0.03$),

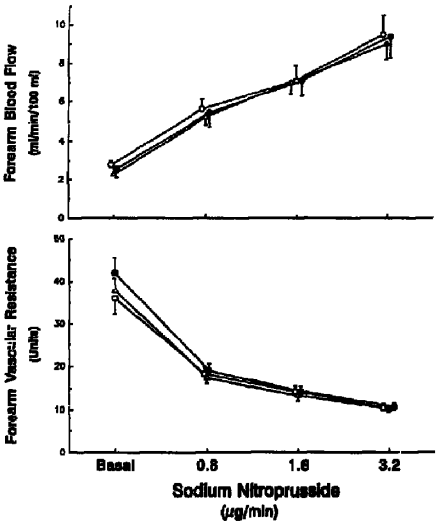

Figure 2. Forearm blood flow (**upper panel**) and resistance (**lower panel**) in response to serial doses of sodium nitroprusside in 14 normal subjects (**open triangles**) and in 19 hypercholesterolemic patients at baseline (open circles) and 1 month after daily administration of antioxidant vitamin supplements (**closed circles**). Values are mean value ± SEM.

whereas it was similar for male and female normal subjects (15.4 ± 10.0 vs. 15.5 ± 10.0, p = 0.28). The response to sodium nitroprusside was the same in both groups (Fig. 2), with a peak flow of 9.5 ± 4.2 ml/min per 100 ml in patients compared to 9.6 ± 2.8 ml/min per 100 ml in normal subjects (p = 0.72).

Baseline measurement of LDL oxidation. The lag times to onset of LDL oxidation were similar in patients and normal subjects (114 ± 41 vs. 119 ± 16 min, p = 0.68), as were the maximal oxidation rates (0.66 vs. 0.60 absorbance/h, p = 0.18). For normal subjects, the lag time to onset of LDL oxidation correlated marginally with the plasma triglyceride level (r = 0.65, p = 0.07) but not with total cholesterol (r = 0.00), very low density lipoprotein (VLDL) (r = 0.14), LDL (r = 0.24) or HDL (r = 0.50) levels. For patients, the lag time of LDL oxidation correlated marginally with the plasma triglyceride levels (r = 0.52, p = 0.054) and significantly with the plasma VLDL (r = 0.67, p = 0.008) and LDL (r = 0.56, p = 0.035) levels. There was no correlation with the total cholesterol (r = 0.07) or HDL (r = 0.21) levels. Further, there was no correlation between the lag time to onset of LDL oxidation in hypercholesterolemic patients and their plasma beta-carotene (r = 0.01), vitamin C (r = 0.40) or vitamin E (r = 0.38) levels. For both study groups, there was no correlation between the lag time to onset of LDL oxidation and the maximal forearm flow response to acetylcholine (r = −0.15 for normal subjects and r = −0.41 for patients).

Vitamin supplements in hypercholesterolemic patients. After 1 month of treatment with beta-carotene, vitamin C and vitamin E supplements, plasma levels of all three vitamins were increased over baseline values: beta-carotene 0.21 ± 0.07 to 0.34 ± 0.12 mg/dl (p < 0.001); vitamin C 1.0 ± 0.3 to 1.9 ± 0.7 mg/dl (p < 0.001); and vitamin E 2.2 ± 0.7 to 3.5 ± 1.9 mg/dl (p = 0.01). The plasma lipid levels (total cholesterol 280 ± 43 mg/dl, triglycerides 182 ± 114 mg/dl, LDL 192 ± 41 mg/dl, HDL 46 ± 12 mg/dl) were unchanged from baseline values.

Effect of vitamin supplements on LDL oxidation. The oxidizability of plasma LDL was reduced after 1 month of vitamin supplement therapy, with a 71 ± 67% increase in the lag time to onset of LDL oxidation (p < 0.001) and a 26 ± 25% decrease in the maximal rate of oxidation (p < 0.001) compared to baseline measurements (Fig. 3). The lag time to onset of LDL oxidation on vitamin supplements correlated strongly with the plasma vitamin E level (r = 0.72, p = 0.003) but not with the plasma beta-carotene (r = 0.13) or the vitamin C (r = 0.10) level. The lag time to onset of LDL oxidation correlated significantly with plasma triglyceride (r = 0.73, p = 0.002), VLDL (r = 0.66, p = 0.009) and LDL (r = 0.54, p = 0.04)

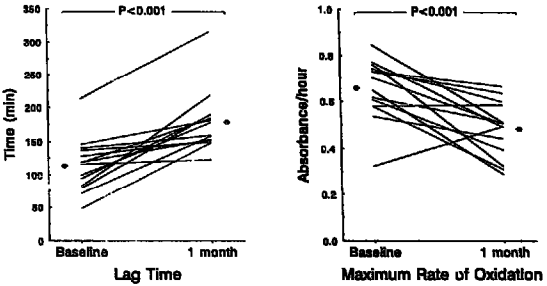

Figure 3. Individual lag times to onset of low density lipoprotein (LDL) oxidation after in vitro addition of copper chloride, as assessed by spectrophotometric absorbance at 234-nm wavelength (**left panel**) and maximal rates of oxidation (**right panel**) for LDL from 19 hypercholesterolemic patients at baseline and 1 month after daily administration of antioxidant vitamin supplements.

levels and marginally with the total cholesterol level ($r = 0.49$, $p = 0.06$) but not with the HDL level ($r = 0.15$).

Effect of vitamin supplements on forearm vascular responses. At the 1-month follow-up forearm flow study during vitamin therapy, basal blood flow was lower (2.2 ± 0.7 vs. 2.7 ± 0.8 ml/min per 100 ml, $p < 0.001$) and basal forearm vascular resistance higher (45 ± 17 vs. 36 ± 18 U, $p < 0.001$) than in the baseline study. Mean arterial blood pressure was similar for both studies (86 ± 9 at baseline vs. 88 ± 9 mm Hg with vitamins, $p = 0.23$). Despite the significant effect of vitamin therapy on oxidizability of LDL, the vasodilator response to acetylcholine remained depressed (Fig. 1), with the peak forearm blood flow of 9.0 ± 6.2 ml/min per 100 ml with vitamin therapy compared to 9.8 ± 7.8 ml/min per 100 ml at baseline ($p = 0.57$). The peak forearm flow response to acetylcholine was similar between baseline and vitamin studies for men (5.9 ± 2.9 vs. 6.8 ± 3.2 ml/min per 100 ml, $p = 0.43$) and women (13.4 ± 9.3 vs. 11.0 ± 6.0 ml/min per 100 min, $p = 0.14$). The vasodilator response to sodium nitroprusside was unchanged by vitamin supplements (Fig. 2), with peak flows of 9.4 ± 4.7 ml/min per 100 ml with vitamin therapy compared to 9.5 ± 4.2 ml/min per 100 ml at baseline ($p = 0.81$). There was no correlation between the time to onset of LDL oxidation and the maximal forearm flow response to acetylcholine ($r = 0.06$).

The variances for the change in acetylcholine-stimulated forearm flow were 23.7 for the average acetylcholine response (7.5, 15 and 30 μg/min) and 51.5 for the peak acetylcholine response. Given the sample size of 19 patients, our study has 80% power (alpha = 0.05) to exclude with respect to baseline values an average increase ≥45% and a peak increase ≥47% in acetylcholine-stimulated forearm flow with vitamin therapy.

Discussion

Consistent with previous reports from our laboratory (9) and others (6,8), patients with hypercholesterolemia in the present study exhibited an impaired forearm blood flow response to the endothelium-dependent vasodilator acetylcholine compared with that of normal subjects, whereas forearm flow responses to the endothelium-independent vasodilator sodium nitroprusside were similar in the two groups. This impairment in acetylcholine-stimulated flow was more pronounced in male than in female hypercholesterolemic patients. Because oxidatively modified LDL may inhibit the production of nitric oxide (11,12), we chose to measure the oxidative susceptibility of LDL from hypercholesterolemic patients and then assess the impact of antioxidant vitamin supplementation on both oxidative susceptibility of LDL and endothelial vasodilator function. The susceptibility of LDL to oxidation at baseline was similar in our hypercholesterolemic patients and normal subjects, a finding that probably reflects the protective effect of vitamins from dietary sources that become incorporated into circulating lipoproteins (17,21–24). However, the heightened release and activity of oxygen free radicals in the vessel wall of hypercholesterolemic patients might rapidly exhaust the protective effect of dietary antioxidant vitamins incorporated into LDL particles. We hypothesized that antioxidant vitamin supplements might protect LDL from oxidation and improve endothelial production of nitric oxide, leading to improved vasorelaxation in response to endothelium-dependent vasodilator agonists.

Vasomotor function in hypercholesterolemia. Previous studies performed in hypercholesterolemic patients (4–9) have shown impaired endothelium-dependent vasodilation and nitric oxide bioavailability. Studies performed on animal arterial tissue in vitro have provided insights into the mechanisms of this abnormal endothelial function in hypercholesterolemia. Mangin et al. (11) showed that oxidatively modified LDL, but not native LDL, impairs the vasorelaxant response of arterial rings to acetylcholine. Shimokawa et al. (12) showed that the pertussis toxin-sensitive Gi protein-dependent signal transduction pathway linked to adenylyl cyclase (utilized by acetylcholine and other agonists) is impaired in tissue from hypercholesterolemic animals with early atherosclerosis. Keaney et al. (25) recently reported that vitamin E (approximately 3,000 IU/day) fed to hypercholesterolemic rabbits for 28 days, increasing plasma levels by >600% and aortic tissue levels by

10-fold with respect to control levels, prolonged the lag time of LDL oxidation and improved acetylcholine-induced relaxation of arterial rings from these animals.

Antioxidant vitamins in hypercholesterolemia. These observations provided incentive for our study investigating the effect of a combination of antioxidant vitamins previously shown to reduce the susceptibility of LDL from humans to oxidation. We found that 1 month of vitamin supplementation with relatively high doses of beta-carotene, vitamin C and vitamin E increased plasma levels of these vitamins and prolonged the onset of oxidation of LDL and decreased the maximal rate of LDL oxidation from hypercholesterolemic patients, responses similar to those reported in normal subjects (26–28) and cigarette smokers (29). The prolongation of time to onset of LDL oxidation in hypercholesterolemic patients correlated strongly with the plasma level of vitamin E after 1 month of administration, but not with plasma levels of the other vitamins, suggesting that the major antioxidant effect of the vitamins we administered was probably rendered by vitamin E. The strong correlation of the lag time of LDL oxidation with triglycerides and VLDL levels in hypercholesterolemic patients, both before and after vitamin supplementation, may indicate the importance of triglycerides in determining plasma vitamin E levels or in the incorporation of vitamin E into the LDL particle (30). However, despite the beneficial effects of vitamins on oxidizability of plasma LDL from hypercholesterolemic patients in our study, the forearm flow response to the endothelium-dependent vasodilator acetylcholine was not improved after 1 month of vitamin supplementation.

Antioxidant vitamins and vasomotor responsiveness. There are several potential reasons why we did not see an improvement in endothelial responsiveness despite an antioxidant effect of vitamins on plasma LDL. First, with our sample size of 19 patients and the variance of blood flow measurements in response to acetylcholine, our study rules out a ≥45% increase in flow with vitamin therapy over baseline values. Accordingly, a lesser improvement in acetylcholine-stimulated flow could have been missed by our study. Second, the defect in endothelial function in hypercholesterolemic patients may be irreversible. This possibility is somewhat unlikely as recent studies have indicated that impaired endothelium-dependent vasodilator responses in hypercholesterolemic patients can be improved by administration of L-arginine, the substrate for nitric oxide production by way of nitric oxide synthase in the endothelium (31–33), although a recent study (34) did not confirm this finding. Further, several studies (35–38) indicate that lipid reduction therapy improves endothelium-dependent vasodilator function in hypercholesterolemic patients. Third, a beneficial effect of vitamin supplementation may require higher doses of vitamins or longer duration of administration to permit LDL with greater antioxidant vitamin content to penetrate the vessel wall and accumulate in sufficient quantity to affect endothelial nitric oxide production or bioavailability. In this regard, Keaney et al. (25) showed in hypercholesterolemic rabbits weighing ~3 kg vascular benefit of a total daily dose of vitamin E almost fourfold higher than that used in our study.

Finally, the lack of enhancement of acetylcholine-stimulated forearm blood flow after antioxidant vitamin supplementation in our study raises the possibility that oxidized LDL is not entirely responsible for depressed endothelium-dependent vasodilation in hypercholesterolemic humans. Superoxide anions and other reactive oxygen species produced within the vessel wall in hypercholesterolemia (39) may be unaffected by antioxidant vitamins, resulting in persistent smooth muscle constriction (40) or degradation of nitric oxide to biologically inactive nitrogen oxide compounds (41). Non-vitamin antioxidants or antioxidant enzymes or concomitant reduction in LDL levels may be required to show improvement in endothelium-dependent vasodilation in hypercholesterolemic patients. Additionally, more sensitive techniques may be required to show long-term changes in forearm vascular responsiveness.

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