Effects of Vitamin E on Chronic and Acute Endothelial Dysfunction in Smokers

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OBJECTIVES The aims of this study were to determine whether chronic or acute impairment of flow mediated vasodilation (FMD) in the brachial artery of smokers can be restored or preserved by the antioxidant vitamin E.

BACKGROUND Transient impairment of endothelial function after heavy cigarette smoking and chronic endothelial dysfunction in smokers result at least in part from increased oxidative stress.

METHODS We studied 22 healthy male smokers (mean ± SD, 23 ± 9 cigarettes per day) randomly assigned to receive either 600 IU vitamin E per day (n = 11, age 28 ± 6 years) or placebo (n = 11, age 27 ± 6 years) for four weeks and 11 age-matched healthy male nonsmokers. Flow mediated vasodilation and endothelium-independent, nitroglycerin-induced dilation were assessed in the brachial artery using high resolution ultrasound (7.5 MHz) at baseline and after therapy. Subjects stopped smoking 2 h before the ultrasound examinations. At the end of the treatment period, a third scan was obtained 20 min after smoking a cigarette (0.6 mg nicotine, 7 mg tar) to estimate transient impairment of FMD.

RESULTS Flow mediated vasodilation at baseline was abnormal in the vitamin E (5.3 ± 3.8, p < 0.01) and in the placebo group (6.4 ± 3.5, p < 0.05) compared with nonsmoking controls (11.6 ± 4.7). Using a two-way repeated measures analysis of variance (ANOVA) to examine the effects of vitamin E on FMD, we found no effect for the grouping factor (p = 0.5834) in the ANOVA over time but a highly significant difference with respect to time (p = 0.0065). The interaction of the time factor and the grouping factor also proved to be significant (p = 0.0318). Flow mediated vasodilation values remained similar after treatment for four weeks in both groups but declined faster after smoking a cigarette in subjects taking placebo compared with those receiving vitamin E (p values from successive differences for the time/group factor: 0.0001/0.0017). The transient attenuation of FMD (calculated as the percent change in FMD) was related to the improvement of the antioxidant status, estimated as percent changes in thiobarbituric acid-reactive substances (r = −0.67, p = 0.0024). Nitroglycerin-induced dilation did not differ between study groups at baseline or after therapy.

CONCLUSIONS These results demonstrate that oral supplementation of vitamin E can attenuate transient impairment of endothelial function after heavy smoking due to an improvement of the oxidative status but cannot restore chronic endothelial dysfunction within four weeks in healthy male smokers. (J Am Coll Cardiol 2000;35:277–83) © 2000 by the American College of Cardiology

Endothelial dysfunction occurs at an early stage of atherosclerosis and has been observed in coronary and peripheral arteries of long-term smokers (1,2) as well as after heavy cigarette smoking (3,4). The precise mechanism of smoking-related endothelial dysfunction is not well understood and very likely multifactorial. However, recent clinical and experimental observations strongly suggest a role of oxygen-derived free radicals (5–7). Cigarette smoke contains large amounts of free radicals, which may degrade nitric oxide released from the endothelium and also produce highly reactive intermediates, resulting in endothelial injury.

Epidemiologic studies have found an inverse association between the frequency of coronary artery disease (CAD) and dietary intake of antioxidant vitamins (8,9). However, a possible cause and effect relation between the intake of antioxidants and a reduction in the complications of CAD has thus far only been shown for vitamin E (10).
**Abbreviations and Acronyms**

ANOVA = analysis of variance  
CAD = coronary artery disease  
FMD = flow-mediated dilation  
LDL-C = low-density lipoprotein cholesterol  
NMD = nitroglycerin-induced dilation  
TBA = thiobarbituric acid  
TBARS = thiobarbituric acid–reactive substances

With regard to the effects of antioxidant vitamins on endothelial function, controversial results have been obtained. Improvement of endothelial dysfunction in smokers was noticed in the forearm vasculature after intraarterial infusion of the watersoluble antioxidant vitamin C (11) and in the brachial artery after intravenous vitamin C infusion (12). The beneficial effect of vitamin C supplementation was associated with a decrease in thiobarbituric acid–reactive substances (TBARS) as an index of oxidative stress. In contrast, oral application of vitamin C for one month showed no beneficial effect on endothelial function in the forearm vessels of patients with hypercholesterolemia (13) but resulted in acute improvement of endothelium-dependent vasodilation in the brachial artery of patients with CAD when applied at a higher dose (14).

Recently, we demonstrated that the oral intake of vitamin E, one of the main lipid-soluble antioxidants in human plasma lipoproteins, in addition to lipid lowering therapy, restored vascular reactivity in the brachial artery of hypercholesterolemic men (15). In contrast, vitamin E alone failed to improve endothelial function in forearm circulation of patients with hypercholesterolemia (13). However, the effect of vitamin E supplementation on endothelial function in the brachial artery of smokers is unknown.

In this study, we hypothesized that vitamin E supplementation, through an antioxidant effect, may restore chronic or preserve acute impairment of endothelium-dependent vasodilation in the brachial artery.

**METHODS**

**Subjects and treatment.** In a randomized, double-blind, placebo-controlled trial, we studied 22 young, healthy male smokers (mean ± SD, 23 ± 9 cigarettes per day) randomly assigned to receive either all-racemic alpha-tocopherol (Roche Austria, Vienna), the most active form of vitamin E, at a dosage of 600 IU (n = 11, age 28 ± 6 years) or placebo (n = 11, age 27 ± 6 years) for four weeks. Both substances were encapsulated without any visible difference and the ultrasound operator was blinded to the group assignment. The reliability of medication intake was controlled by taking pill counts, which revealed no essential irregularities. Two probands, one in each group, did not complete the study (withdrawal of consent). Thus, 22 smokers (11 probands in each group) could be statistically analyzed after conclusion of the study. Both study groups were compared with an age-matched control group of 11 male nonsmokers to demonstrate that flow-mediated dilation (FMD) at baseline was abnormal among the smokers. No participant had a history of hypercholesterolemia, hypertension or diabetes mellitus. None of the participants took antioxidative drugs before this study. The investigation conformed with the principles outlined in the Declaration of Helsinki. Written, informed consent was obtained from all participants.

**Assessment of vasodilation and blood flow.** Endothelium-dependent FMD following reactive hyperemia and endothelium-independent nitroglycerin-induced dilation (NMD) were examined in the brachial artery according to the method described by Celermajer et al. (16). Using high resolution ultrasound (7.5 MHz linear array transducer), measurements of the right brachial artery were taken at rest after lying quietly for at least 10 min, after cuff deflation completing suprasystolic compression (250 mm Hg for 4.5 min) of the right upper arm and after sublingual application of 0.8 mg nitroglycerin. Scans of the brachial artery were taken proximal to the bifurcation of the radial and the ulnar artery above the antecestitial fossa at end diastole, incident with the R wave on a continuously recorded electrogram by the same ultrasound operator. Diameter measurements were taken from one media-adventitia interface to the other for at least three times at baseline and every 30 s following reactive hyperemia and after administration of nitroglycerin. The maximum FMD and NMD diameters were calculated as the average of the three consecutive maximum diameter measurements following hyperemia and nitroglycerin, respectively. Vasodilation was then calculated as the percent change in diameter compared with baseline. The impairment of FMD after heavy smoking (delta FMD) was estimated as the percent change in FMD values compared with baseline.

In all subjects, blood flow was calculated at rest and within 15 s after cuff deflation by multiplying the velocity time integral of the flow signal by the heart rate and the vessel cross-sectional area (3.14 × D^2/4). Reactive hyperemia was then calculated as percent change in blood flow during reactive hyperemia compared with baseline.

Ultrasound measurements were performed at baseline and after therapy for four weeks. Subjects stopped smoking 2 h before the examinations. At the end of the treatment period, a third scan was obtained 20 min after smoking a cigarette (0.6 mg nicotine, 7 mg tare) to estimate transient impairment of FMD.

The mean range of intraobserver difference for measurements of baseline diameter was 0.10 ± 0.07%, that of %FMD 2.4 ± 2.2% and that of %NMD 2.7 ± 1.9% as reported elsewhere (17).

**Determination of lipids and lipoproteins.** Cholesterol and triglycerides were measured enzymatically using assay kits from Boehringer Mannheim (Mannheim, Germany). High-density lipoprotein cholesterol was measured from the...
supernatant after precipitation with polyethylene glycol (Reagent A from Immuno A.G., Vienna, Austria). Low-density lipoprotein cholesterol (LDL-C) was calculated according to the Friedewald equation.

**Alpha-tocopherol measurements in human plasma samples.** The neutral lipid fraction (200 μl) including alpha-tocopherol, the most active form of vitamin E, was extracted in a basic extraction system consisting of 200 μl ethanol, 200 μl H2O and 200 μl hexane. The upper organic layer (20 μl) containing alpha-tocopherol was analyzed by high-performance liquid chromatography using an ExSil 100 20 × 0.46 cm silica column (mobile phase: hexane/1% ethanol, 1 ml/min) and fluorescence detection (Hitachi, E: 295 nm/Em: 390 nm) (18). Quantitation was performed by peak area comparison with external alpha-tocopherol standards of known concentrations. The alpha-tocopherol serum level was then normalized to LDL-C because there is evidence in the literature that the vitamin E content in LDL-C and not in the whole plasma correlates with lipid peroxidation (19,20). The increase of alpha-tocopherol/LDL-C (delta alpha-tocopherol/LDL-C) under therapy was calculated as the percent change in alpha-tocopherol/LDL-C.

**Determination of malondialdehyde with thiobarbituric acid.** The measurements of TBARS are based on the reaction of malondialdehyde, a secondary breakdown product of lipid hydroperoxides, with thiobarbituric acid (TBA). The assay was performed as described previously (21). The plasma sample was mixed with two volumes of cold 10% trichloroacetic acid for protein precipitation. Following centrifugation, the supernatant was mixed with an equal volume of 0.67% TBA in a boiling water bath for 10 min. After cooling, the absorbency was measured at 532 nm, and concentration of malondialdehyde was calculated from epsilon = 153 000 M−1 cm−1 (21). Measurements were performed in duplicate. The decrease of TBARS (delta TBARS) under therapy was calculated as the percent change in plasma TBARS levels.

**Statistical analysis.** Results are expressed as mean ± standard deviation (SD). Differences between groups were analyzed using one factor analysis of variance (ANOVA) followed by Scheffé’s test, when comparing all three study groups (including nonsmoking controls) and Student t test, when comparing the vitamin E with the placebo group only. To examine whether FMD can be restored or preserved by vitamin E, a two-way repeated measures ANOVA with the group factor vitamin E/placebo and the repeated factor time (three levels: baseline/after four weeks/after heavy cigarette smoking) was performed. Successive differences contrasts between neighboring points in time were calculated as supportive statistical analyses. Univariate analyses of the effects of delta alpha-tocopherol/LDL and delta TBARS on delta FMD as well as of cigarettes smoked per day on TBARS levels were performed with linear regression. Differences were considered significant at p < 0.05.

**RESULTS**

**Clinical and biochemical parameters.** No differences were found among all three study groups with regard to age, lipids and lipoproteins serum levels (Table 1). Alpha-tocopherol and TBARS plasma levels at baseline were similar in the placebo and the vitamin E group. Vitamin E supplementation for four weeks led to significant increases of alpha-tocopherol serum levels and alpha-tocopherol serum levels normalized to LDL-C as well as of delta alpha-tocopherol and delta alpha-tocopherol/LDL-C. The percent changes of TBARS under therapy were also different between study groups (Table 2).

**Vasodilation and blood flow responses.** Baseline diameter and increases in blood flow during reactive hyperemia were similar among the study groups at baseline (Table 1) and after treatment for four weeks, respectively. Thus, it can be assumed that the stimulus for endothelium dependent vasodilation was similar in all study groups and that it was not influenced by the study medication. Smoking a cigarette did not effect brachial artery diameter and blood flow.

Comparing the two study groups consisting of male
3.5 vs. 11.6
6
smokers, FMD was impaired in both the vitamin E (5.3
6
smokers with an age-matched control group of male non-
FMD
5
Table 2. Oxidation Parameters and Results of Ultrasound Measurements in the Brachial Artery

<table>
<thead>
<tr>
<th></th>
<th>Placebo (n = 11)</th>
<th>Vitamin E (n = 11)</th>
<th>p</th>
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<tr>
<td>FMD (%)</td>
<td></td>
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<tr>
<td>baseline</td>
<td>6.4 ± 3.8 (0.0–10.9)</td>
<td>5.3 ± 3.8 (0.2–13.9)</td>
<td>0.51</td>
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<tr>
<td>after therapy</td>
<td>6.9 ± 3.5 (2.7–13.9)</td>
<td>6.9 ± 4.0 (0.8–12.4)</td>
<td>0.98</td>
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<tr>
<td>after therapy and acute smoking</td>
<td>2.7 ± 2.8 (0.5–9.1)</td>
<td>5.8 ± 3.2 (0.4–11.3)</td>
<td>0.028</td>
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<tr>
<td>delta FMD (%)</td>
<td></td>
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<tr>
<td>after acute smoking</td>
<td>−0.77 ± 0.31 (−1.51–0.34)</td>
<td>−0.12 ± 0.37 (−0.83–0.44)</td>
<td>0.0002</td>
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<tr>
<td>TBARS (μmol/L)</td>
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<tr>
<td>baseline</td>
<td>0.10 ± 0.03 (0.06–0.15)</td>
<td>0.11 ± 0.04 (0.06–0.19)</td>
<td>0.66</td>
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<tr>
<td>after therapy</td>
<td>0.13 ± 0.03 (0.08–0.17)</td>
<td>0.10 ± 0.03 (0.06–0.16)</td>
<td>0.056</td>
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<tr>
<td>delta TBARS (%)</td>
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<tr>
<td>after therapy</td>
<td>0.36 ± 0.42 (−0.13–1.08)</td>
<td>−0.06 ± 0.35 (−0.62–0.48)</td>
<td>0.033</td>
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<tr>
<td>alpha-tocopherol (μg/ml)</td>
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<tr>
<td>baseline</td>
<td>12.2 ± 3.8 (4.5–19.6)</td>
<td>14.5 ± 3.6 (9.6–20.7)</td>
<td>0.17</td>
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<tr>
<td>after therapy</td>
<td>13.3 ± 3.8 (6.4–20.2)</td>
<td>23.5 ± 6.6 (13.0–32.6)</td>
<td>0.0005</td>
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<tr>
<td>delta alpha-tocopherol (%)</td>
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<tr>
<td>after therapy</td>
<td>0.07 ± 0.24 (−0.34–0.37)</td>
<td>0.62 ± 0.27 (0.18–1.07)</td>
<td>0.0002</td>
</tr>
<tr>
<td>alpha-tocopherol/LDL-C (μg/100 mg% LDL)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>baseline</td>
<td>10.2 ± 3.4 (4.5–19.6)</td>
<td>10.7 ± 4.3 (9.6–20.7)</td>
<td>0.75</td>
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<tr>
<td>after therapy</td>
<td>11.0 ± 2.9 (6.6–14.8)</td>
<td>16.7 ± 6.3 (7.6–28.4)</td>
<td>0.018</td>
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<tr>
<td>delta alpha-tocopherol/LDL-C (%)</td>
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<tr>
<td>after therapy</td>
<td>0.17 ± 0.40 (−0.44–0.96)</td>
<td>0.60 ± 0.42 (0.08–1.35)</td>
<td>0.035</td>
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</table>

FMD = flow-mediated dilation; LDL-C = low-density lipoprotein; TBARS = thiobarbituric acid–reactive substances.  
delta TBARS, delta alpha-tocopherol and delta FMD = percent change after therapy and heavy smoking, respectively.  
Values are mean ± SD (range); p = for comparison of subjects receiving placebo and subjects receiving vitamin E (600 IU).  

By performing a two-way repeated measures ANOVA, we found no effect for the grouping factor (group factor p value = 0.5834) in the ANOVA over time. With respect to time, there was a highly significant difference (time factor p value = 0.0065), and the interaction of the time factor and the grouping factor also proved to be significant (p = 0.0318), thus confirming the beneficial effect of vitamin E supplementation (Fig. 1).

Supporting statistical analyses revealed a stronger decline of FMD values after acute cigarette smoking in subjects taking placebo compared with those receiving vitamin E (p values from successive differences for the time/group factor: 0.0010/0.0017) (Fig. 2). However, FMD values did not change, neither after vitamin E nor after placebo intake for four weeks, when subjects stopped smoking at least 2 h before the ultrasound examination (p values from successive differences for the time/group factor: 0.3099/0.5756).

Nitroglycerin-induced vasodilation was similar in all study groups at baseline and after the treatment period.

Relationship between oxidative stress, smoking habits and FMD. Univariate analyses over both study groups revealed significant inverse correlations between the transient impairment of FMD after acute smoking (delta FMD) and the improvement of the antioxidant status (delta TBARS, r = −0.67, p = 0.0024; delta alpha-tocopherol/LDL, r = 0.51, p = 0.016; Fig. 3). Alpha-tocopherol serum levels or delta alpha-tocopherol were not related to the impairment of FMD. The number of cigarettes smoked per day correlated with TBARS at baseline (r = 0.49, p = 0.025). No significant correlation was found between pack-years and TBARS levels. The close correlation between delta FMD and delta TBARS (r = −0.71, p = 0.031) also remained following exclusion of the placebo group.

DISCUSSION

This study demonstrates a partially beneficial effect of vitamin E supplementation on endothelium–dependent vasodilation in the brachial artery of male smokers. Vitamin E supplementation for four weeks prevented the transient further impairment of endothelium–dependent vasodilation after acute smoking, while it showed no effect on chronic

FMD (%) baseline 6.4 ± 3.8 (0.0–10.9) 5.3 ± 3.8 (0.2–13.9) 0.51 after therapy 6.9 ± 3.5 (2.7–13.9) 6.9 ± 4.0 (0.8–12.4) 0.98 after therapy and acute smoking 2.7 ± 2.8 (0.5–9.1) 5.8 ± 3.2 (0.4–11.3) 0.028

Figure 1. Bar graph showing FMD values of subjects receiving placebo and of subjects receiving vitamin E (600 IU/day) at baseline, after therapy for four weeks and after heavy smoking at the end of the treatment period. Time factor p value = 0.0065. Group factor p value = 0.5834. Interaction of the time factor and the grouping factor: p = 0.0318. FMD = flow-mediated vasodilation. Open square = placebo; closed square = Vitamin E.
endothelial dysfunction in these subjects. In addition, the attenuation of transient endothelial dysfunction after acute smoking correlated with an improvement of the antioxidant status under vitamin E supplementation.

Effects of chronic smoking on endothelial dysfunction. Endothelial dysfunction has been observed in both peripheral and coronary arteries of long-term smokers (1,2). The precise mechanism of this impairment by chronic smoking has not yet been elucidated but may be related to oxidative stress. In support of this, cigarette smoke has been shown to contain large amounts of free radicals such as superoxide anion and hydroxyl radicals (11,22), agents known to degrade endothelium derived relaxing factor and to impair FMD (6). Superoxide anion, in turn, can rapidly react with nitric oxide to form peroxynitrite, a molecule with high cytotoxic potency (23,24). Moreover, it has been reported that cigarette smokers have higher rates of in vivo and in vitro lipid peroxidation (25). Thus, atherogenic effects of smoking may be mediated in part by free radical damage to lipids, in particular oxidative modification of LDL, which promotes foam cell formation (26,27), monocyte adhesion (28,29) and is cytotoxic to vascular cells (30).

Role of antioxidants in smokers. Epidemiologic studies have shown that plasma levels of antioxidant nutrients, such as vitamin C and E, are significantly lower in smokers compared with nonsmokers (31). In the Health Professionals’ follow-up study, a reduction in major coronary events among healthy male subjects taking 100 to 250 IU supplementation of vitamin E per day, with little further benefit at higher doses, was reported (8). In contrast, in a randomized therapeutic trial among Finnish smokers, no beneficial effect on cardiovascular events was observed for either vitamin E or beta-carotene intake (32). However, the dose of vitamin E applied in this study (50 mg per day) was below the protective range suggested in the Health Professionals’ follow-up study. Therefore, a cause-and-effect relation between vitamin E intake and a reduction in cardiovascular events has thus far been suggested only for vitamin E supplementation in patients with angiographically proven coronary atherosclerosis (10).

Effects of antioxidant vitamins on endothelial function in smokers. Endothelium-dependent vasodilation in the forearm circulation of chronic smokers can be improved by acute intraarterial administration of vitamin C (11). Moreover, Motoyama et al. (12) demonstrated that an acute impairment of FMD in the brachial artery after heavy smoking is caused by an increase of oxidative stress within 10 min. This transient impairment of endothelial function

Figure 2. FMD values before and after heavy smoking at the end of the treatment period for subjects receiving placebo as well as for subjects receiving vitamin E (600 IU/day). P values for the time factor = 0.0001; p value for the group factor = 0.0017. FMD = flow-mediated vasodilation.

Figure 3. Transient impairment of FMD (delta FMD) after heavy smoking vs. improvement of the antioxidant status (delta TBARS) under vitamin E supplementation for four weeks. FMD = flow-mediated dilation; TBARS = thiobarbituric acid-reactive substances.
lasts for ≤90 min (4) and can be attenuated by intravenous infusion of vitamin C (12). In contrast, oral vitamin E supplementation did not improve endothelial function in the forearm circulation of normocholesterolemic chronic smokers (33). Our data suggest that the oral intake of vitamin E for four weeks is not able to reverse chronic endothelial dysfunction of smokers but does prevent the transient further impairment of endothelium-dependent vasodilation in the brachial artery after acute smoking. This acute effect is likely due to the antioxidant effect of vitamin E which is supported by its close correlation with

1) the decrease in TBARS, reflecting attenuation of oxidative stress under vitamin E supplementation and

2) the increase of alpha-tocopherol plasma levels normalized to LDL-C, suggesting reduced lipid peroxidation under therapy (19, 20). Since TBARS are used to measure actual oxidative stress, it was not surprising that TBARS levels correlated with the total number of cigarettes smoked per day and not with the number of pack-years.

Interestingly, no improvement of chronic endothelial dysfunction was found, although the oxidative status was improved. These results are in accordance with previous observations in the forearm vasculature, in which vitamin E supplementation failed to improve endothelium-dependent vasodilation, although the susceptibility of LDL to oxidation could be reduced (34). However, the beneficial effect of vitamin E supplementation on the impairment of endothelial function after heavy smoking reported in this study is unlikely due to increased resistance of LDL-C to oxidation, as this process would last longer than the time elapsed between heavy smoking and the ultrasound examination (20). Thus, other mechanisms must be considered, such as the scavenging of superoxide or an improvement of the intracellular antioxidant status due to the accumulation of alpha-tocopherol in the vascular wall and in endothelial cells in particular (35).

A possible explanation for the discrepancy between the lack of effect on chronic endothelial dysfunction after vitamin E supplementation and its beneficial effect on transient endothelial dysfunction in our study may be the relatively low degree of oxidative stress while subjects refrained from smoking before the examination, compared with the burst of oxidative stress after heavy smoking. Recently, Heitzer et al. (33) revealed a beneficial effect of vitamin E supplementation on chronic endothelial dysfunction selectively in hypercholesterolemic smokers which was associated with a reduction of elevated autoantibody titers against oxidized LDL. Alternatively, chronic endothelial dysfunction caused by smoking may be due to other mechanisms not affected by the antioxidant or other properties of vitamin E. These mechanisms may include an increase in asymmetric dimethylarginine, an endogenous nitric oxide synthase inhibitor (36), as well as more long-lasting endothelial cell damage, due to cytotoxic intermediates (23, 24).

**Study limitations.** A possible drawback of this study may be that we did not measure TBARS after heavy smoking. However, an acute increase of TBARS after heavy smoking as well as an attenuation of this increase by an antioxidant has been demonstrated previously (12). In this study, TBARS levels and alpha-tocopherol serum levels normalized to LDL-C were measured to estimate the attenuation of oxidative stress under vitamin E supplementation. The fact that TBARS levels were not significantly different in both study groups (p = 0.06) is most likely due to the high SD of this parameter (19). However, differences in delta TBARS, alpha-tocopherol/LDL-C and delta alpha-tocopherol/LDL-C strongly suggest an improvement in the antioxidant status of subjects taking vitamin E. Finally, we cannot exclude that the duration of vitamin E treatment may have been insufficient. However, long term vitamin E supplementation for four months also failed to restore endothelial function in long-term smokers with a lipid profile similar to our study subjects (33).

**Conclusions.** This study demonstrates that oral vitamin E supplementation can attenuate the transient impairment of endothelial function after heavy smoking due to an improvement of the antioxidant status, but cannot restore chronic endothelial dysfunction within four weeks. These data are consistent with the concept that vitamin E may in part be beneficial due to direct tissue effects that are different from the prevention of the formation of oxidized LDL-C.

**References**

9. Stampfer MJ, Hennekens CH, Manson JE, et al. Vitamin E consump-