

## THE EFFECT OF CHOLESTEROL-LOWERING AND ANTIOXIDANT THERAPY ON ENDOTHELIUM-DEPENDENT CORONARY VASOMOTION

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**Abstract Background.** Patients with coronary artery disease and abnormalities of serum lipid levels often have endothelial vasodilator dysfunction, which may contribute to ischemic cardiac events. Whether cholesterol-lowering or antioxidant therapy can restore endothelium-dependent coronary vasodilatation is unknown.

**Methods.** We randomly assigned 49 patients (mean [ $\pm$ SD] serum cholesterol level,  $209\pm 33$  mg per deciliter [ $5.40\pm 0.85$  mmol per liter]) to receive one of three treatments: an American Heart Association Step 1 diet (the diet group, 11 patients); lovastatin and cholestyramine (the low-density lipoprotein [LDL]-lowering group, 21 patients); or lovastatin and probucol (the LDL-lowering-antioxidant group, 17 patients). Endothelium-dependent coronary-artery vasomotion in response to an intracoronary infusion of acetylcholine ( $10^{-8}$  to  $10^{-6}$  M) was assessed at base line and after one year of therapy. Vasoconstrictor responses to these doses of acetylcholine are considered to be abnormal.

**Results.** Treatment resulted in significant reductions

in LDL cholesterol levels of  $41\pm 22$  percent in the LDL-lowering-antioxidant group and  $38\pm 20$  percent in the LDL-lowering group ( $P<0.001$  vs. the diet group). The maximal changes in coronary-artery diameter with acetylcholine at base line and at follow-up were  $-19$  and  $-2$  percent, respectively, in the LDL-lowering-antioxidant group,  $-15$  and  $-6$  percent in the LDL-lowering group, and  $-14$  and  $-19$  percent in the diet group ( $P<0.01$  for the LDL-lowering-antioxidant group vs. the diet group;  $P=0.08$  for the LDL-lowering group vs. the diet group). (The negative numbers indicate vasoconstriction.) Thus, the greatest improvement in the vasoconstrictor response was seen in the LDL-lowering-antioxidant group.

**Conclusions.** The improvement in endothelium-dependent vasomotion with cholesterol-lowering and antioxidant therapy may have important implications for the activity of myocardial ischemia and may explain in part the reduced incidence of adverse coronary events that is known to result from cholesterol-lowering therapy. (N Engl J Med 1995;332:488-93.)

**H**YPERCHOLESTEROLEMIA is a health risk, and epidemiologic studies have shown a link between total cholesterol levels and the risk of cardiac events.<sup>1,2</sup> Studies have shown that lowering the levels of total and low-density lipoprotein (LDL) cholesterol can result in a decrease in cardiac morbidity and mortality.<sup>3,4</sup> Angiographic studies of coronary arteries have demonstrated a disparity between the decrease in cardiac events and the extent of regression of coronary-artery lesions. Mechanisms other than the regression of coronary stenoses may therefore be important in the beneficial effect of cholesterol lowering.<sup>5,6</sup>

The healthy endothelium, in part by the release of paracrine factors such as nitric oxide, has an important role in maintaining vascular integrity.<sup>7,8</sup> In vitro studies have shown that LDL cholesterol and, in particular, its oxidized derivative are injurious to the endothelium.<sup>9</sup> Abnormalities in the coronary-artery vasomotor response to acetylcholine, an endothelium-dependent vasodilator, can be observed in patients with atherosclerosis.<sup>10</sup> A close relation between abnormalities in lipids and coronary-artery endothelial dysfunction has also been demonstrated.<sup>11,12</sup> However, the benefits of reducing the levels of LDL cholesterol and of

antioxidant therapy in restoring endothelial function have not been examined in the clinical setting. In a controlled clinical trial, we sought to test the hypothesis that a reduction in LDL cholesterol levels or the combination of such reduction with antioxidant therapy would improve the impaired endothelium-dependent vasodilator responses seen in the coronary arteries of patients with atherosclerosis.

### METHODS

#### Patient Population

Patients were eligible for the study if they had a total serum cholesterol level before catheterization of 180 to 280 mg per deciliter (4.7 to 7.2 mmol per liter) and were not receiving cholesterol-lowering medication. Patients were excluded if they had uncontrolled hypertension, diabetes mellitus, or heart failure; if they were cigarette smokers; or if they had recently had a myocardial infarction.

Sixty-seven patients were recruited and randomly assigned in approximately equal numbers to the three treatment groups, after which they underwent base-line evaluation. Complete data, including the results of coronary angiography and testing of coronary-artery vasomotion after one year of follow-up, were available for 49 patients, who formed the study population. There was no difference in demographic characteristics, the degree of atherosclerosis, or the base-line vasomotor response between the study population and the 18 patients who did not complete the protocol.

#### Study Design

##### Assessment of Endothelium-Dependent Vasomotion

Written informed consent was obtained from the patients before catheterization in accordance with the guidelines established by the Committee for the Protection of Human Subjects. Long-acting vasoactive medications taken by the patients, including calcium-channel blockers, beta-blockers, nitrates, and angiotensin-converting-enzyme inhibitors, were discontinued for at least 18 hours before catheterization.

Endothelium-dependent vasomotion was assessed by serial intracoronary infusions of acetylcholine (Miochol, Iolab Pharmaceuticals, Claremont, Calif.), with final estimated intracoronary concentrations of  $10^{-8}$  to  $10^{-6}$  M. Endothelium-independent vasomotion was as-

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essed by an infusion of nitroglycerin at 16  $\mu\text{g}$  per minute, according to an established protocol.<sup>10</sup> Serum lipid concentrations were measured in the cardiac catheterization laboratory for all patients at base line and follow-up, with the patient fasting. Quantitative coronary angiographic images were obtained after each intervention with a previously validated method, and at the follow-up study the patients were studied in the same catheterization laboratory with the same imaging protocol.

### Treatment

After the initial study of coronary vasomotion, the patients were randomly assigned to receive one of three treatments: an American Heart Association Step 1 diet (the diet group); the same diet plus lovastatin and cholestyramine (the LDL-lowering group); or the same diet plus lovastatin and probucol (the LDL-lowering-antioxidant group). There was no blinding with respect to treatment assignments. All the patients received dietary instruction from a registered dietitian. The medications were titrated over the first two months to the maximal tolerable doses.

### Quantitative Coronary Angiography

Technically suitable single-plane angiograms were selected for computer analysis on the basis of a previously described method.<sup>10</sup> An automated edge-detection program was used to search densities and seek inflection points, measuring the diameter of the vessel along the 8-to-10-mm length of the selected segment (Quantum IC software, ImageComm, Sunnyvale, Calif.). The percentage of change in diameter was determined for each dose of acetylcholine, and the maximal constriction was also noted. In the analysis, the average of the responses in two coronary-artery segments was calculated for each patient in order to determine an average percentage of change in diameter in response to acetylcholine and nitroglycerin. The segments were matched in the follow-up study with respect to anatomical determinants, such as side branches. The angiograms were coded and digitized by the investigators and were analyzed by a single technician who was unaware of the patients' treatment assignments and the phase of the study.

### Statistical Analysis

The differences between treatment groups in clinical characteristics and lipid profiles were analyzed by a two-way repeated-measures analysis of variance. The primary end point, the average change in the maximal coronary vasomotor response to acetylcholine in two coronary-artery segments, was analyzed by a two-way repeated-measures analysis of variance with treatment group and time taken into account. Testing included a Bonferroni correction as appropriate. Linear regression analysis was used to compare the continuous relation between changes in the response to acetylcholine and changes in lipid measurements and clinical characteristics. A two-sided P value of less than 0.05 was considered to indicate statistical significance. Data are expressed as means  $\pm$ SD unless otherwise specified.

## RESULTS

### Characteristics of the Patients

The study population consisted of 49 patients, 37 men and 12 women, with a mean age of  $56 \pm 9$  years. Eleven patients were randomly assigned to the diet group, 21 to lovastatin and cholestyramine (the LDL-lowering group), and 17 to lovastatin and probucol (the LDL-lowering-antioxidant group). The base-line characteristics of the patients are shown in Table 1.

Ninety-two percent of the patients had angiographic evidence of atherosclerosis. Only 4 patients (8 percent) had smooth coronary arteries, whereas 2 (4 percent) had irregularities, 24 (49 percent) had single-vessel coronary disease, and 19 (39 percent) had double-vessel disease. Atherosclerosis was equally distributed among the three groups. Coronary angioplasty of a coronary vessel other than the study vessel was per-

Table 1. Clinical Characteristics of the Study Patients.\*

CHARACTERISTIC	DIET GROUP (N = 11)	LDL-LOWERING GROUP (N = 21)	LDL-LOWERING- ANTIOXIDANT GROUP (N = 17)
Age — yr	55 $\pm$ 7	59 $\pm$ 9	52 $\pm$ 9
Sex — M/F	8/3	18/3	11/6
Hypertension — no. (%)	8 (73)	7 (33)	6 (35)
Cigarette smoking — no. (%)	1 (9)	2 (10)	6 (35)
Base-line lipids — mg/dl			
Total cholesterol	196 $\pm$ 24	209 $\pm$ 36	217 $\pm$ 32
LDL cholesterol	125 $\pm$ 24	139 $\pm$ 38	145 $\pm$ 37
HDL cholesterol	40 $\pm$ 13	41 $\pm$ 12	39 $\pm$ 11
Triglycerides	155 $\pm$ 88	163 $\pm$ 90	167 $\pm$ 96
Apolipoprotein A1	127 $\pm$ 30	122 $\pm$ 31	120 $\pm$ 31
Apolipoprotein B	125 $\pm$ 20	133 $\pm$ 34	132 $\pm$ 29
Total no. of risk factors	2.1 $\pm$ 0.7	1.8 $\pm$ 0.6	2.0 $\pm$ 0.8
Previous MI — no. (%)	5 (45)	6 (29)	7 (39)

\*Plus-minus values are means  $\pm$ SD. LDL denotes low-density lipoprotein, HDL high-density lipoprotein, and MI myocardial infarction. To convert values for total, LDL, and HDL cholesterol to millimoles per liter, multiply by 0.02586; to convert values for triglycerides to millimoles per liter, multiply by 0.01129; to convert values for apolipoprotein A1 to micromoles per liter, multiply by 0.357; to convert values for apolipoprotein B to micromoles per liter, multiply by 0.182.

formed in 35 patients (71 percent) at the time of the base-line vasomotion study.

### Clinical Follow-up

During the treatment phase and before the completion of the one-year follow-up study, 11 patients presented with symptoms suggestive of recurrent ischemia and underwent diagnostic catheterization (3 in the diet group, 2 in the LDL-lowering group, and 6 in the LDL-lowering-antioxidant group). There was no statistical difference among the groups with respect to the proportion requiring treatment.

Adverse effects attributable to the study medications occurred only in the patients receiving cholestyramine. Constipation or gastrointestinal upset was severe enough to cause the discontinuation of cholestyramine therapy in four patients, and to cause a decrease in the dose in three. The mean dose of cholestyramine at the follow-up study was 12 g per day. There were no adverse effects of lovastatin therapy. The mean doses were 62 mg per day in the LDL-lowering group and 61 mg per day in the LDL-lowering-antioxidant group. The patients in the LDL-lowering-antioxidant group received 500 mg of probucol twice a day without side effects.

### Changes in Lipid Profiles

The lipid profiles in the three treatment groups at base line and at the one-year follow-up are shown in Figure 1. There were no differences among the three groups in lipid measurements at base line. The mean decreases in total and LDL cholesterol levels in the LDL-lowering group ( $23 \pm 13$  percent and  $38 \pm 20$  percent, respectively) and in the LDL-lowering-antioxidant group ( $33 \pm 15$  percent and  $41 \pm 22$  percent) were significantly different from that in the diet group ( $P < 0.001$ ), but not from each other. The mean decrease in high-density lipoprotein (HDL) cholesterol in the LDL-lowering-antioxidant group ( $21 \pm 14$  per-

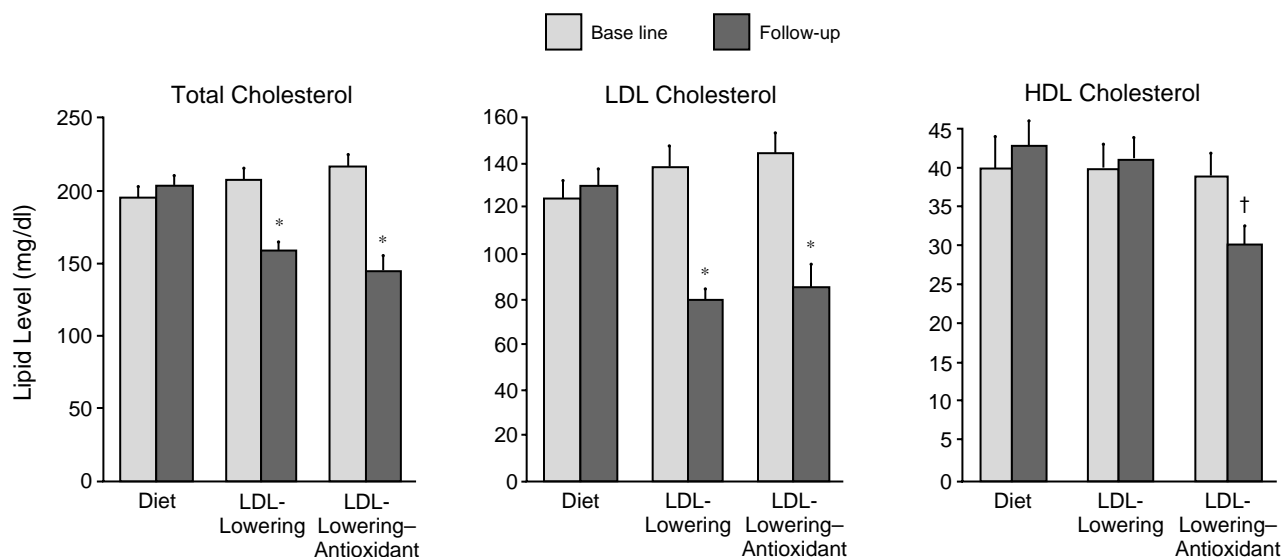


Figure 1. Mean ( $\pm$ SE) Lipid Levels in the Three Study Groups at Base Line and after One Year of Therapy.

To convert values to millimoles per liter, multiply by 0.02586. Asterisks indicate  $P < 0.001$  for the comparison with the diet group; the dagger indicates  $P < 0.001$  for the comparisons with the diet group and the LDL-lowering group.

cent) was significantly different from the changes in both the diet group and the LDL-lowering group ( $P < 0.001$ ). There was no significant change in triglyceride levels from base line to follow-up in any of the groups.

#### Coronary-Artery Vasomotor Function

At base line there was no significant difference among the three treatment groups in the vasomotor response of epicardial coronary arteries to either acetylcholine or nitroglycerin.

For the patients in the LDL-lowering-antioxidant group, the mean maximal change in coronary-artery diameter in response to acetylcholine was  $-19 \pm 19$  percent at base line, as compared with  $-2 \pm 18$  percent at follow-up one year later (negative values indicate coronary vasoconstriction). LDL lowering also reduced the constrictive response to acetylcholine, from  $-15 \pm 19$  percent at base line to  $-6 \pm 18$  percent at follow-up. There was no change in the vasomotor response in the diet group ( $-14 \pm 19$  at base line vs.  $-19 \pm 18$  percent at follow-up). The improvement in the vasomotor response to acetylcholine was significantly greater in the LDL-lowering-antioxidant group than in the diet group ( $P < 0.01$ ). However, the improvement in vasomotor response in the LDL-lowering group did not differ from that in the diet group ( $P = 0.08$ ).

When the analysis incorporated the entire dose-response curve (Fig. 2 and 3), there was a significant difference among the three groups with regard to the change in response to acetylcholine ( $P = 0.02$ ). There was more improvement in the dose-response curve to acetylcholine in the LDL-lowering-antioxidant group than in the diet group ( $P = 0.01$ ), but the improvement in the LDL-lowering group did not differ from that in the diet group.

The vasodilator response to nitroglycerin, the endothelium-independent dilator, did not differ among the three groups at base line or after one year of therapy.

#### Predictors of Improvement in the Response to Acetylcholine

The strongest univariate predictor of improvement in acetylcholine-induced vasoconstriction was the degree of constriction at base line ( $r = 0.52$ ,  $P < 0.001$ ). Patients whose coronary arteries had more constriction in response to acetylcholine at base line were more likely to have improvement during the study period. The treatment group was also a strong univariate predictor of change in endothelial vasodilator function ( $r = 0.45$ ,  $P = 0.006$ ). There was a significant, but moderate, relation between improvement in acetylcholine-induced vasoconstriction and the degree of reduction in the levels of both total and LDL cholesterol ( $r = 0.31$ ,  $P = 0.04$ ).

When stepwise multiple linear regression analysis was used, only the treatment group ( $P = 0.004$ ) and the response to acetylcholine at base line ( $P < 0.001$ ) were significant predictors in a multivariate analysis.

#### DISCUSSION

This randomized, controlled study demonstrated that coronary-artery endothelial dysfunction, which is characteristic of patients with hypercholesterolemia and atherosclerosis, can be significantly improved by a combination of LDL-lowering and antioxidant therapy.

The endothelium is an important modulator of coronary vasodilation through the release of endothelium-derived nitric oxide, and the response to acetylcholine depends on the integrity of this tissue.<sup>8</sup> This endothelium-dependent pathway for nitric oxide is impaired in patients with atherosclerosis,<sup>10</sup> probably because of the direct injurious effects of elevated levels of LDL cholest-

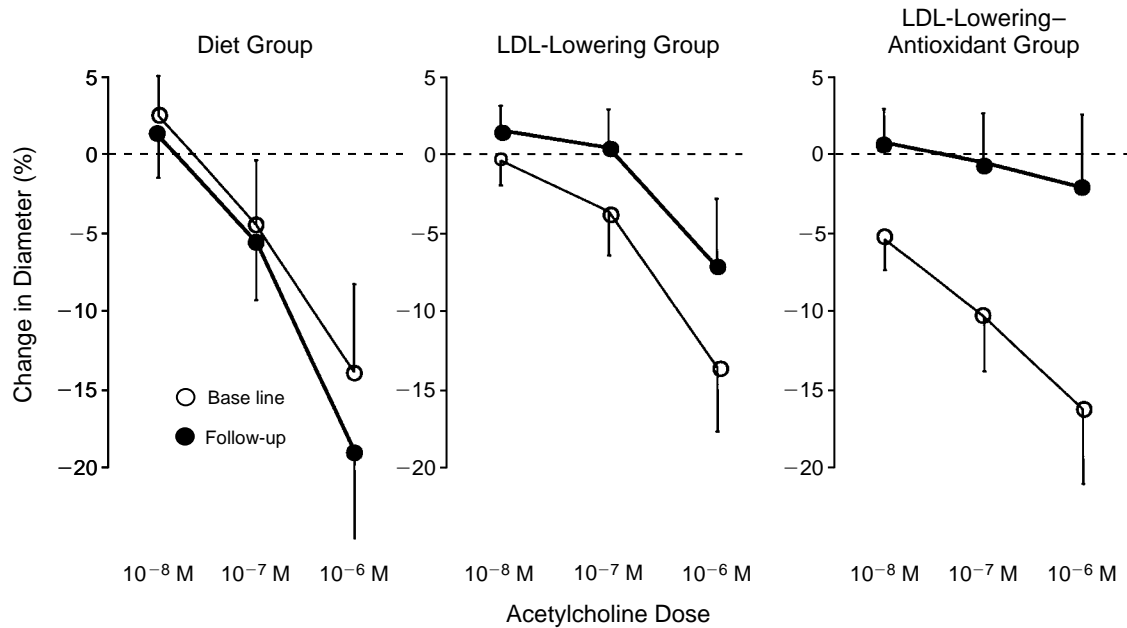


Figure 2. Mean ( $\pm$ SE) Change in Coronary-Artery Diameter in Response to Serial Infusions of Acetylcholine at Base Line and after One Year of Therapy in the Three Study Groups.

The improvement in the response from base line to follow-up in the LDL-lowering-antioxidant group was significantly greater than that in the diet group ( $P < 0.05$ ). Negative numbers indicate vasoconstriction.

terol on the endothelium.<sup>11,12</sup> In patients with coronary artery disease, vasoconstrictor responses may play a part in the pathogenesis of ischemia.<sup>13</sup> Stenotic lesions have been shown to constrict in response to stimuli such as exercise or mental stress.<sup>14,15</sup> The responses observed to these stimuli parallel the vasoconstriction observed in response to acetylcholine in the same patients, suggesting the presence of an impairment in endothelium-dependent vasodilatation. In atherosclerosis induced by dietary hypercholesterolemia, cholesterol lowering improves vasodilator responses to acetylcholine both in vitro and in vivo.<sup>16,17</sup> In addition, functional improvement has been shown to precede structural regression.<sup>18</sup> Recently, Leung et al. demonstrated in an uncontrolled study that coronary-artery endothelial function could be improved after six months of therapy with cholestyramine in patients with hypercholesterolemia but with no angiographic evidence of atherosclerosis.<sup>19</sup>

The present study has shown that a medical regime combining aggressive lowering of LDL cholesterol levels with antioxidant therapy over a one-year period results in significant improvement in endothelium-dependent vasomotor responses to acetylcholine. Complete normalization of the response to acetylcholine was not seen, suggesting an attenuation rather than a normalization of endothelial dysfunction. Whether a longer period of therapy would result in more improvement in the dilator response needs to be tested. It is encouraging to find that endothelial function can be significantly improved in patients with only mild hypercholesterolemia.

There is accumulating evidence to suggest that the

oxidation of LDL particles is important in the pathogenesis of atherosclerosis and endothelial dysfunction.<sup>20</sup> Experimental studies suggest two distinct mechanisms that link oxidative stress with impairment in endothelium-dependent vasodilatation. First, an oxidized LDL particle is markedly more effective than native LDL in

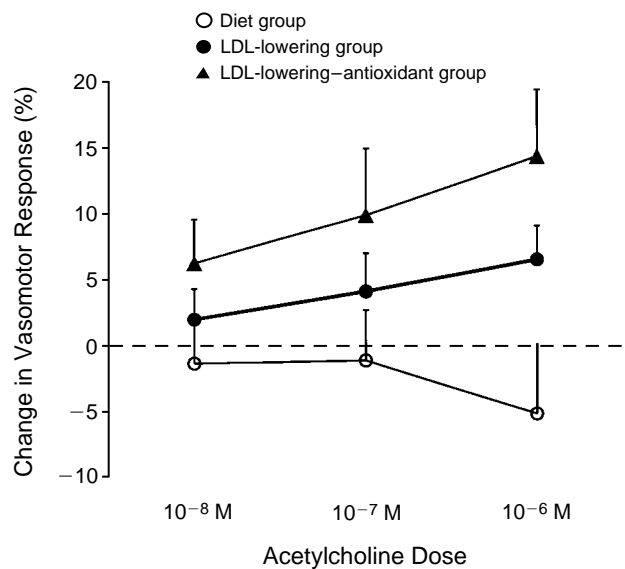


Figure 3. Mean ( $\pm$ SE) Change in the Vasomotor Response to Acetylcholine after One Year of Therapy in the Three Study Groups.

$P < 0.05$  for the comparison of the three groups and for the comparison of the LDL-lowering-antioxidant group with the diet group. Negative numbers indicate vasoconstriction.

impairing the vasodilator response to acetylcholine.<sup>9,21</sup> Lysolecithin, a product formed as a consequence of lipid peroxidation of LDL particles, may be involved in the development of abnormal arterial vasomotion.<sup>22</sup> Probucol is transported in lipoproteins, where it acts as a chain-breaking antioxidant and inhibits lipid peroxidation and oxidative modification of LDL particles *in vitro*.<sup>23,24</sup> Simon et al. administered probucol to cholesterol-fed rabbits and demonstrated that its antioxidant effect improved endothelium-dependent responses.<sup>25</sup> Second, studies by Ohara and colleagues have suggested that hypercholesterolemia is a stimulus to the augmented generation of superoxide radicals by the endothelium.<sup>26</sup> Superoxide directly inactivates nitric oxide and may also increase the subsequent oxidation of LDL particles by the formation of peroxynitrite.<sup>27</sup> A recent *in vitro* study using isolated rabbit arteries has suggested that probucol improves endothelium-dependent vasodilator responses in the presence of LDL, not only by protecting the LDL particle against oxidation, but also perhaps by scavenging oxygen free radicals in the arterial wall.<sup>28</sup>

In our study, the patients randomly assigned to the LDL-lowering-antioxidant group had significantly better vasomotor responses to acetylcholine after therapy than did those in the diet group or those treated with LDL lowering alone. The relative contributions of the lowering of LDL cholesterol levels and antioxidant therapy cannot be separated on the basis of our results.

After adjustment for assignment to the LDL-lowering-antioxidant group, the remaining predictor of improvement in endothelial function was the base-line vasomotor response to acetylcholine. The patients with the most abnormal endothelial function at base line were likely to show the greatest improvement. Improvement in levels of total or LDL cholesterol was related to improvement in endothelial function in a univariate analysis, but not in the multivariate model after adjustment for assignment to treatment group. This further suggests that other factors besides cholesterol lowering, such as the antioxidant effect of probucol, may be partly responsible for the improvement in endothelial vasodilator responses.

The clinical improvement seen with cholesterol-lowering therapy seems to be disproportionate to the small degree of anatomical regression of atherosclerotic stenoses that can be achieved by this therapy.<sup>5,6</sup> In plaques with large cholesterol pools, the resorption of cholesterol may diminish the propensity of the plaque to rupture.<sup>6</sup> We suggest that, in addition to this mechanism, a beneficial effect on endothelial function may contribute to the clinically important benefit seen with cholesterol lowering.

About 25 percent of our patients did not complete the study, and there were more dropouts in the diet group than in the other two groups. However, there were no significant differences in the demographic variables, the angiographic burden of atherosclerosis, or the vasomotor responses between these patients and the overall study population.

The LDL-lowering-antioxidant strategy was more

effective in restoring endothelial function than was the dietary treatment, which alone had little or no effect on lipid levels. The LDL-lowering regime improved vasomotor responses from base line, but there was only a trend toward improvement in the LDL-lowering group as compared with the diet group ( $P=0.08$ ). Although the magnitude of improvement was less in the LDL-lowering group than in the LDL-lowering-antioxidant group, the two results were not significantly different. In clinical trials, it is often difficult to prove that one highly effective treatment is superior to another, given the inherent limitations of the sample in studies requiring catheterization and populations less homogeneous than those in experimental studies. The relative contributions of cholesterol lowering and antioxidant effects cannot be ascertained from this study, but the results suggest that the beneficial effects of probucol outweigh its potentially detrimental HDL-lowering effects.

In conclusion, a combination of LDL cholesterol-lowering and antioxidant therapy improved endothelium-dependent vasodilator responses to acetylcholine in patients with coronary atherosclerosis. This observation may have important clinical implications for the activity of myocardial ischemia and the reduction of adverse coronary events that is known to occur with cholesterol-lowering therapy.

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