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Rapid Improvement of Nitric Oxide Bioavailability After Lipid-Lowering Therapy With Cerivastatin Within Two Weeks

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OBJECTIVES We investigated whether improvement of endothelial dysfunction in hypercholesterolemia can be achieved with short-term lipid-lowering therapy.

BACKGROUND Impaired endothelium-dependent vasodilation plays a pivotal role in the pathogenesis of atherosclerosis and acute coronary syndromes.

METHODS In a randomized, double-blind, placebo-controlled trial, we studied 37 patients (52 ± 11 yrs) with low density lipoprotein cholesterol ≥160 mg/dl (196 ± 44 mg/dl) randomly assigned to either cerivastatin (0.4 mg/d) or placebo. Endothelium-dependent vasodilation of the forearm vasculature was measured by plethysmography and intra-arterial infusion of acetylcholine (ACh 12, 48 μg/min) and endothelium-independent vasodilation by intra-arterial infusion of nitroprusside (3.2, 12.8 μg/min).

RESULTS Low density lipoprotein cholesterol decreased after two weeks of treatment (cerivastatin −33 ± 4% vs. placebo + 2 ± 4%, x ± SEM, $p < 0.001$). Endothelium-dependent vasodilation improved after two weeks of therapy with cerivastatin compared with baseline (ACh 12 μg/min: + 22.3 ± 5.2 vs. + 11.2 ± 1.9 ml/min/100 ml, $p < 0.01$; ACh 48 μg/min: +31.2 ± 6.3 vs. +19.1 ± 3.1 ml/min/100 ml, $p < 0.05$). In contrast, changes in forearm blood flow to ACh were similar before and after therapy in the placebo group (ACh 12 μg/min: +12.9 ± 3.6 vs. +9.0 ± 1.9 ml/min/100 ml, NS; ACh 48 μg/min: +20.7 ± 3.7 vs. 19.4 ± 2.9 ml/min/100 ml, NS). Endothelium-dependent vasodilation improved in comparison with placebo (ACh 48 μg/min: +203 ± 85% [cerivastatin] vs. −26 ± 71% [placebo], $p < 0.05$). This improvement in endothelium-dependent vasodilation was no longer observed when the nitric oxide-synthase inhibitor N(G)-monomethyl-L-arginine was coinfused (ACh 48 μg/min + N(G)-monomethyl-L-arginine 4 μmol/min −48 ± 85% [cerivastatin]).

CONCLUSIONS Short-term lipid-lowering therapy with cerivastatin can improve endothelial function and NO bioavailability after two weeks in patients with hypercholesterolemia. (J Am Coll Cardiol 2001;37:1351–8) © 2001 by the American College of Cardiology

Recent studies have shown that cholesterol-lowering therapy improves endothelium-dependent vasodilation in coronary (1,2) and systemic (3) arteries due to an increased bioavailability of nitric oxide (NO) (4), the most important endothelium-derived vasodilating substance. Apart from vasodilating effects, NO has been found to be a principal factor involved in the antiatherosclerotic properties of the endothelium (5). Nitric oxide interferes with key events in the development of atherosclerotic plaques, such as monocyte and leukocyte adhesion to the endothelium (6,7) and platelet aggregation and adhesion (8). Endothelial expression of adhesion molecules and proinflammatory cytokines involved in active inflammation and accumulation of macrophages at sites of plaque rupture is selectively reduced by NO (6,9). Nitric oxide also decreases endothelial permeability (9), and inhibits vascular smooth muscle cell proliferation (10). In accordance with these findings, inhibition of the NO-producing enzyme, NO synthase causes accelerated atherosclerosis in experimental models (11). Thus, improvement of the bioavailability of NO seems to play a pivotal role in the regression and delayed progression of coronary atherosclerosis induced by lipid-lowering therapy (12).

Increased bioavailability of NO has been demonstrated after six months of lipid-lowering therapy (4). Improvement of endothelial function can occur after four (13) to six (14) weeks of oral pharmacologic therapy with different HMG-CoA reductase inhibitors. In addition, improved endothelial function has been observed even after a single low density lipoprotein (LDL) apheresis within hours (15,16). Rapid improvement of NO bioavailability is most attractive, since it offers an early and new therapeutic approach in acute coronary syndromes. Nevertheless, lipid-lowering therapy is often delayed after acute coronary syndromes because of the usual practice of initially starting cholesterol lowering with dietary therapy (17). The objective of this study was to determine whether lipid-lowering therapy with a statin can rapidly improve endothelial function via improved bioavailability of NO in the forearm vasculature in patients with hypercholesterolemia.

METHODS

Study population. In a randomized, placebo-controlled, double-blind study, patients were randomly assigned to either placebo or cerivastatin for a period of 14 days.

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Abbreviations and Acronyms

ACh	= acetylcholine
ANOVA	= analysis of variance
FBF	= forearm blood flow
HDL	= high density lipoprotein
LDL	= low density lipoprotein
L-NMMA	= N(G)-monomethyl-L-arginine
NO	= nitric oxide
PAI-1	= plasminogen activator inhibitor type 1
t-PA	= tissue-type plasminogen activator

Inclusion criteria were: age 20 to 65 years, increased serum LDL cholesterol level of ≥160 mg/dl and serum triglyceride level ≤400 mg/dl while not receiving cholesterol-lowering medication. Exclusion criteria were: patients with active smoking habits and nonsmokers with <1 year of cessation, diabetes mellitus (HbA1c > 7% or fasting blood glucose > 120 mg/dl or antidiabetic medication), arterial hypertension (diastolic blood pressure ≥ 95 mm Hg or systolic blood pressure ≥ 160 mm Hg) or presently taking any blood pressure-lowering agent, any cardiovascular or cerebrovascular event within the last three months, overt peripheral vascular disease, severe cardiac pathologies (uncontrolled arrythmias, atrial fibrillation, unstable angina, congestive heart failure, New York Heart Association classification II–IV, coronary artery bypass grafting or percutaneous transluminal coronary angioplasty within the previous six months), known intolerance or hypersensitivity to statins, severe disorders of the gastrointestinal tract (chronic diarrhea, ulcerative colitis, etc.), pregnant or lactating women, familial monogenic hypercholesterolemia, secondary hyperlipoproteinemia, vascular abnormalities in the forearm vasculature, liver or kidney disease (AST and ALT levels > 200% of upper normal limit, alkaline phosphatase, bilirubin and serum creatinine > 150% of upper normal limit), patients regularly or occasionally practicing weightlifting or body building, patients who work on night shifts, patients who are taking any lipid-lowering medication or patients on steroids or immunosuppressive agents.

Thirty-seven patients were included in this randomized, double-blind, placebo-controlled trial. They were randomly assigned by a randomization list in a 1:1 fashion to the treatment (n = 19) or the placebo group (n = 18). One patient in the cerivastatin group did not complete the study (withdrawal of consent). Another patient in the treatment group took the medication for 20 days instead of 14 days and was, therefore, excluded from per protocol analysis. Thus, 17 patients in the cerivastatin group and 18 patients in the placebo group entered statistical analysis. The baseline characteristics of these patients were not different between the treatment and placebo group, except for differences in body weight (Table 1). In addition to the abovementioned study population, 19 healthy subjects (age 51 ± 6 years), all nonsmokers with normal total cholesterol levels (189 ± 31 mg/dl), LDL cholesterol levels (95 ± 24 mg/dl), blood pressure and fasting blood glucose, were

Table 1. Baseline Characteristics and Lipid Profiles of Patients With Hypercholesterolemia (x ± SD, n = 35) in the Two Treatment Groups

	Cerivastatin n = 17	Placebo n = 18	p Value
Gender (m/f)	9/8	13/5	NS
Age (yrs)	51.3 ± 11	52.7 ± 10	NS
Height (m)	1.69 ± 0.09	1.74 ± 0.08	NS
Weight (kg)	72 ± 12	78 ± 10	0.025
Body mass index (kg/m^2)	24.5 ± 2.2	25.7 ± 2.3	NS
Blood pressure			
Systolic (mm Hg)	127 ± 12	132 ± 10	NS
Diastolic (mm Hg)	81 ± 8	83 ± 5	NS
Active smoker (yes/no)	0/17	0/18	NS
Hypertensives (yes/no)	0/17	0/18	NS
Total cholesterol (mg/dl)	286 ± 54	283 ± 46	NS
HDL cholesterol (mg/dl)	51 ± 16	54 ± 11	NS
LDL cholesterol (mg/dl)	198 ± 45	196 ± 42	NS
LDL/HDL ratio	4.2 ± 2.0	3.8 ± 1.1	NS
Triglycerides (mg/dl)	174 ± 103	167 ± 68	NS
VLDL (mg/dl)	46 ± 51	37 ± 25	NS
Fibrinogen (mg/dl)	317 ± 17	299 ± 16	NS
Apolipoprotein B (mg/dl)	143 ± 30	139 ± 23	NS
Apolipoprotein E (mg/dl)	5.1 ± 1.6	3.9 ± 1.0	NS
Lipoprotein (a) (mg/dl)	39 ± 45	20 ± 26	NS
Homocysteine (μmol/l)	9.5 ± 2.6	11.7 ± 4.8	NS
t-PA (μg/ml)	7.9 ± 3.7	8.9 ± 4.6	NS
PAI-1 (U/ml)	3.7 ± 2.1	3.7 ± 1.9	NS
von Willebrand factor-antigen (%)	170 ± 80	163 ± 88	NS
Thrombomodulin (ng/ml)	3.5 ± 1.4	3.5 ± 1.1	NS

HDL = high density lipoprotein; LDL = low density lipoprotein; PAI-1 = plasminogen activator inhibitor type 1; t-PA = tissue-type plasminogen activator; VLDL = very low density lipoprotein.

examined at baseline and served as a control group to the baseline examination of the patients with hypercholesterolemia.

Study design. The study was approved by the ethics committee of the University of Erlangen-Nürnberg, and performed according to the "good clinical practice" guidelines. Written informed consent was obtained from all patients before study entry. After evaluation of inclusion and exclusion criteria, possible concomitant use of lipid-lowering drugs was discontinued in a first washout period (day −56 to day −42). During this time period, all patients who had not been on a stable diet for hyperlipidemia received dietary instructions according to the "American Heart Association Step I diet" from a registered dietitian. The washout period was followed by a placebo run-in period for six weeks (day −42 to day 0). Patients were finally included according to the mean of their LDL cholesterol concentrations measured on days −14 and −7. The following active treatment phase (day 0 to 14) lasted 14 ± 3 days. The primary end point of the study was the assessment of endothelium-dependent vasodilation on day 0 (baseline) and day 14 (after treatment).

Assessment of forearm blood flow. Forearm blood flow (FBF) and responses to different vasoactive drugs were assessed by forearm plethysmography. Studies were performed in the morning at the same time in an undisturbed temperature-controlled environment (22 to 24°C). An intra-arterial catheter (20 G, 8 cm, Vygon, France) was inserted into the brachial artery of the nondominant arm using Seldinger's technique. After cannulation, subjects rested for 60 min before the study was started. Subjects lay supine with their left forearm supported above the level of the right atrium. Forearm vascular responsiveness to vasoactive agents was assessed by strain gauge venous-occlusion plethysmography (EC 5R Plethysmograph, Hokanson, Bellevue, Washington). Drugs were infused intra-arterially at the rate of 1.5 ml/min using syringe pumps. The following substances were administered (each dose was infused intra-arterially for 4 min): 1) acetylcholine (ACh) to assess endothelium-dependent vasodilation at sequential doses of 12 and 48 μg/min; 2) sodium-nitroprusside to test endothelium-independent vascular relaxation (3.2 and 12.8 μg/min); and 3) simultaneous infusion of N(G) monomethyl-L-arginine (L-NMMA) (4 μmol/min) and ACh (12 and 48 μg/min) to test whether any improvement in endothelium-dependent vasodilatation can be blocked by this NO synthase inhibitor. Before each intervention, saline was infused for 15 min to let the FBF return to resting levels. The left hand was excluded from the circulation by the inflation of a wrist cuff to 220 mm Hg during each measurement period. The upper arm cuff was inflated to 40 mm Hg by a rapid cuff inflator (E20, Hokanson, Bellevue, Washington) to occlude venous outflow during each measurement. Output from the strain gauges was displayed on a monitor in real-time. A software program coordinated the acquisition, storage and display of data as well as inflation and deflation of the arm cuffs. Forearm blood flow was obtained from an average of measurements recorded for 9 s of every 15 s during the final 2 min of each infusion period.

Measurement of biochemical parameters. Lipoprotein(a) was determined from serum by an immunonephelometric method (Dade-Behring, Liederbach, Germany). Thrombomodulin was determined from EDTA-plasma by ELISA (Pharmacia, Freiburg, Germany); tissue-type Plasmingen activator (t-PA) and von Willebrand factor antigen were determined from citrate plasma by ELISA (Roche, Imtec, Mannheim, Germany). The inhibitory effect of plasminogen activator inhibitor type 1 (PAI-1) on t-PA was measured by the reduction of cleavage of a chromogenic substrate by t-PA (Dade-Behring, Liederbach, Germany). Serum homocystein was determined by high performance liquid chromatography.

Treatment. After the baseline evaluation, patients were randomly assigned to one of two treatments: cerivastatin 0.4 mg or placebo once daily in the evening. Both substances were identical in appearance. Patients and treating physicians were blinded with regard to the chosen therapy.

Statistical analysis. Differences between treatment groups in clinical characteristics, lipid profiles, other biochemical parameters and changes in FBF were analyzed by unpaired and paired Student *t* test between before (day 0) versus after (day 14) treatment. In addition, analysis of variance (ANOVA) for repeated measurements was applied to test differences in dose response curves between groups and treatment phases. Vascular reactivity data are expressed as blood flow in ml/min/100 ml forearm tissue (x ± SD) and as the percent change (x ± SEM) from the corresponding baseline. Biochemical parameters are expressed in absolute values (x ± SD) and as the percent change (x ± SEM) from the corresponding baseline. Linear correlation analysis (Pearson) was used to test correlations between changes in endothelium-dependent vasodilation and changes in lipid profiles after therapy. Two-sided p values are given throughout the text. A two-sided p value of < 0.05 was considered statistically significant.

RESULTS

Changes in lipid profiles. After two weeks of lipid-lowering therapy with cerivastatin, a decrease was found in total cholesterol levels (286 ± 54 mg/dl before therapy vs. 209 ± 51 mg/dl after therapy, $p < 0.001$), LDL cholesterol (198 ± 48 mg/dl vs. 132 ± 47 mg/dl, $p < 0.001$), triglycerides (174 ± 103 mg/dl vs. 109 ± 47 mg/dl, $p < 0.01$), very low density lipoprotein (46 ± 51 mg/dl vs. 22 ± 10 mg/dl, $p < 0.05$), homocysteine (9.6 ± 2.6 mg/dl vs. 8.7 ± 2.5 mg/dl, $p < 0.01$), apolipoprotein B levels (143 ± 30 mg/dl vs. 106 ± 29 mg/dl, $p < 0.001$) and apolipoprotein E levels (5.1 ± 1.5 vs. 3.4 ± 0.6, $p = 0.001$). High density lipoprotein (HDL) cholesterol increased (51 ± 16 mg/dl vs. 55 ± 15 mg/dl, $p < 0.05$). There were no significant changes in lipid profiles in the placebo

Table 2. Comparison of Changes in Lipid Profiles After the Two Week Treatment Period (>Δ% ± SEM, n = 35) With Cerivastatin or Placebo

Percent Change in	Cerivastatin n = 17	Placebo n = 18	p Value
Total cholesterol (%)	−26.2 ± 3.5	+3.3 ± 3.4	0.001
LDL cholesterol (%)	−33.2 ± 4.1	+1.8 ± 4.0	0.001
HDL cholesterol (%)	+6.1 ± 3.8	+3.2 ± 3.7	NS
LDL/HDL ratio (%)	−36.7 ± 2.9	−1.7 ± 2.8	0.001
VLDL (%)	−32.1 ± 8.0	+15.4 ± 7.7	0.001
Triglycerides (%)	−29.2 ± 7.3	+13.5 ± 7.1	0.001
Apolipoprotein B (%)	−26.3 ± 2.4	+0.6 ± 2.3	0.001
Apolipoprotein E (%)	−31.2 ± 4.5	+7.7 ± 4.4	0.001
Lipoprotein (a) (%)	+18.8 ± 13.0	+13.3 ± 12.6	NS
Homocysteine (%)	−8.4 ± 3.1	−0.8 ± 3.0	NS
Thrombomodulin (%)	+0.4 ± 11.0	+8.5 ± 10.7	NS
t-PA (%)	+0.7 ± 13	+12 ± 13	NS
PAI-1 (%)	+29 ± 22	+18 ± 21	NS
vWF-antigen (%)	−9.6 ± 11.3	−1.4 ± 11.0	NS

HDL = high density lipoprotein; LDL = low density lipoprotein; PAI-1 = plasminogen activator inhibitor type 1; t-PA = tissue-type plasminogen activator; VLDL = very low density lipoprotein; vWF antigen = von Willebrand factor antigen.

group. Lipid profiles improved in the treatment group in comparison with the placebo group after treatment (ANOVA) (Table 2).

FBF responses to ACh. Baseline FBF was 4.12 ± 2.34 ml/min/100 ml in the control subjects and 3.95 ± 2.32 ml/min/100 ml in patients with hypercholesterolemia, respectively (NS). Intra-arterial administration of ACh caused an increase in FBF, but with diminished increases of FBF in the patients with hypercholesterolemia for all doses of ACh (Fig. 1). A significant correlation (r = −0.34, p = 0.01) was found between LDL cholesterol serum levels and the maximum response to ACh before therapy in the combined group of study patients and healthy controls.

Forearm blood flow at baseline was similar in the cerivastatin group and placebo group both before and after treatment (Table 3). Intra-arterial administration of ACh caused an increase in FBF with increasing doses in the placebo and the treatment group, before and after therapy (ANOVA all $p < 0.001$). In the cerivastatin group, however, the ACh-induced increases in FBF were enhanced after two weeks of lipid-lowering therapy compared with pretreatment evaluation and the placebo group (ANOVA, $p < 0.05$, Fig. 2). If FBF values are given in absolute terms for the different doses of ACh, a significant improvement of the vasodilator response to ACh could be demonstrated after therapy in the cerivastatin group for each single dose of ACh ($p < 0.01$ and $p < 0.02$, respectively) (Table 3). In contrast, we found no differences before and after therapy in the placebo group (Table 3).

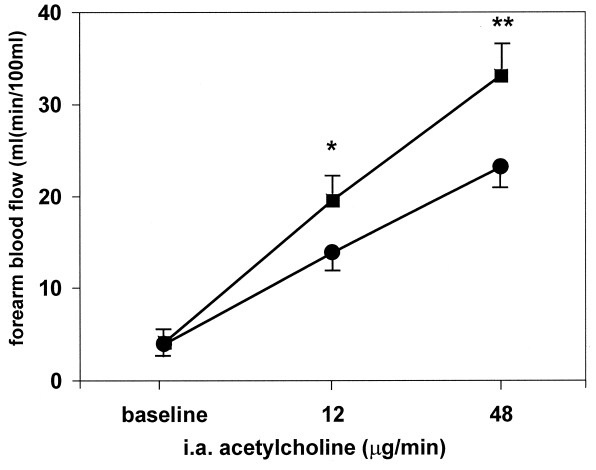

Figure 1. Impaired endothelium-dependent vasodilation in patients with hypercholesterolemia in comparison with controls. Increase in forearm blood flow (ml/min/100 ml) after the infusion of increasing doses of acetylcholine in patients with hypercholesterolemia (n = 35, **solid circle**) and in healthy controls (n = 19, **solid box**) (**$p < 0.01$, *$p < 0.05$).

To analyze the effects in the treatment group versus those in the placebo group, we subtracted the percent change of FBF from baseline in response to ACh after therapy from those before therapy. After two weeks of treatment, cerivastatin improved the vasodilator response compared with the placebo group significantly at dose 48 μg/min (+203 ± 85% vs. −26 ± 71%, p = 0.047) with a trend at dose 12 μg/min (+181 ± 59% vs. +41 ± 68%, p = 0.13).

Finally, in the treatment group we analyzed whether the improvement of endothelium-dependent vasodilation after lipid-lowering therapy was related to the degree of decreases in LDL cholesterol, the LDL/HDL ratio or the apolipoprotein B serum levels. No relations could be found between the percent change of improvement of endothelium-dependent vasodilation and the improvement of lipid profiles after therapy (LDL cholesterol: r = −0.30, p = 0.09; LDL/HDL-ratio: r = 0.27, p = 0.12; apolipoprotein B: r = −0.23, p = 0.19).

FBF responses to nitroprusside. Administration of the endothelium-independent vasodilator sodium-nitroprusside caused dose-dependent increases in FBF in the placebo group before and after therapy as well as in the cerivastatin group before and after therapy (ANOVA $p < 0.001$). No differences in the observed increases after administration of sodium-nitroprusside could be found between the

Table 3. Forearm Blood Flow (ml/min/100 ml) at Baseline and Changes From Baseline for Different Doses of Intra-Arterial ACh and Sodium Nitroprusside in the Cerivastatin and the Placebo Group (x ± SD)

Change (ml/min/100 ml)	Cerivastatin (n = 17)			Placebo (n = 18)		
	Before Therapy	After Therapy	p Value	Before Therapy	After Therapy	p Value
Ach						
Baseline	4.19 ± 2.43	4.38 ± 2.71	NS	3.71 ± 1.29	3.97 ± 1.83	NS
12 μg/min	+11.2 ± 7.88	+22.3 ± 21.3	0.01	+9.00 ± 8.04	+12.9 ± 15.3	NS
48 μg/min	+19.1 ± 12.7	+31.2 ± 26.1	0.02	+19.4 ± 12.7	+20.7 ± 15.8	NS
Nitroprusside						
Baseline	5.35 ± 3.22	5.84 ± 4.31	NS	5.05 ± 1.85	5.74 ± 2.81	NS
3.2 μg/min	+12.6 ± 7.04	+16.2 ± 10.4	NS	+10.5 ± 5.28	+11.5 ± 4.56	NS
12.8 μg/min	+24.3 ± 11.4	+25.6 ± 9.43	NS	+22.7 ± 12.9	+22.0 ± 12.8	NS

Corresponding p values for comparison before and after the treatment period (Student *t* test).
ACh = acetylcholine.

cerivastatin and the placebo group both before and after treatment, respectively (Table 3, Fig. 3).

FBF responses to ACh with simultaneous L-NMMA infusion. Before the simultaneous intra-arterial infusion of L-NMMA (4 μmol/min) and two increasing doses of ACh, baseline FBF before treatment with cerivastatin was 4.62 ± 2.53 ml/min/100 ml, and after treatment it was 5.08 ± 2.79 ml/min/100 ml, respectively (NS). The significant improvement in ACh-induced vasodilation after treatment with cerivastatin (see above) was no longer observed, if L-NMMA was coinfused (ANOVA NS). Forearm blood flow increased from baseline at dose ACh 12 μg/min by 268 ± 53% before versus 318 ± 60% after treatment and at ACh 48 μg/min by 553 ± 85% before versus 505 ± 86% after treatment with cerivastatin (all differences NS) (Fig. 4).

DISCUSSION

Rapid improvement of NO availability. This study documents that lipid-lowering therapy with the HMG-CoA reductase inhibitor, cerivastatin, can rapidly improve endothelial function and the bioavailability of NO after two weeks of therapy.

Treatment with cerivastatin improved endothelium-dependent vasodilation in comparison with pretreatment analysis and placebo as it has been documented for lipid-lowering therapy in previous studies after one to 12 months of therapy in coronary (1,2) and peripheral arteries (3,4,13). However, the issue that improved endothelial function is mediated by improved bioavailability of NO has been demonstrated after only six months of therapy (4). In this study, administration of the NO synthase inhibitor, L-NMMA, blunted the improvement of endothelium-dependent vasodilation after therapy. This finding indicates

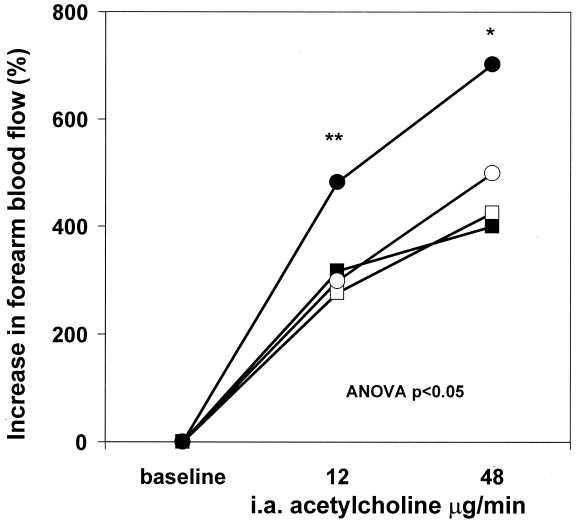

Figure 2. Improved endothelium-dependent vasodilation after two weeks of cerivastatin. Increase in forearm blood flow in percent from baseline (Δ%) after the infusion of acetylcholine with increasing doses in the cerivastatin group (n = 17, **open circle** = before therapy, **solid circle** = after therapy) and the placebo group (n = 18, **open box** = before therapy, **solid box** = after therapy). Comparison of the changes between treatment group and placebo (*$p < 0.05$, **$p < 0.01$; analysis of variance [ANOVA] $p < 0.05$).

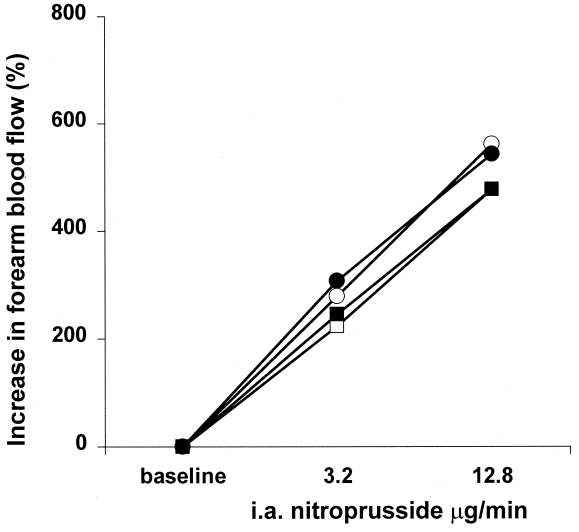

Figure 3. Unaffected endothelium-independent vasodilation after two weeks of cerivastatin. Increase in forearm blood flow in percent from baseline (Δ%) after the infusion of nitroprusside with increasing doses in the cerivastatin group (n = 17, **open circle** = before therapy, **solid circle** = after therapy) and the placebo group (n = 18, **open box** = before therapy, **solid box** = after therapy). Comparison of the changes between the treatment group and placebo (all NS).

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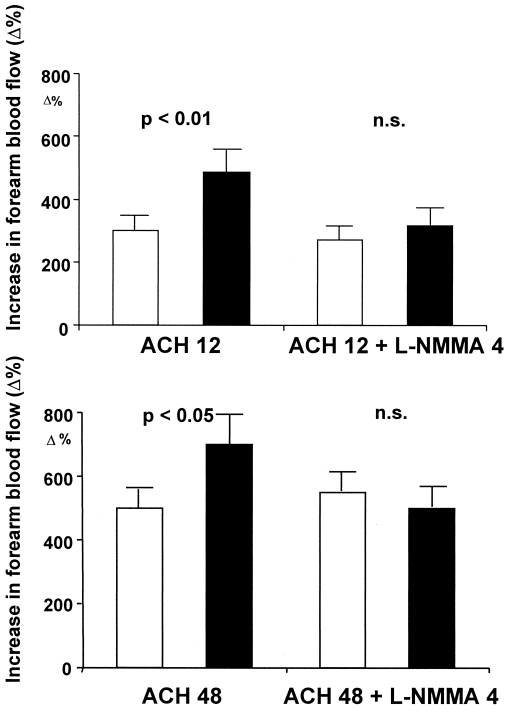

Figure 4. Improved endothelium-dependent vasodilation after cerivastatin therapy can be blocked with L-NMMA. Changes in forearm blood flow in percent from baseline (Δ%) after the infusion of acetylcholine (ACh) 12 μg/min (ACh 12, **upper panel**) and 48 μg/min (ACh 48, **lower panel**) without (**left two columns**) and with (**right two columns**) coinfusion of the nitric oxide synthase inhibitor L-NMMA 4 μmol/min before (**open box**) and after (**solid box**) lipid-lowering therapy with cerivastatin for two weeks. L-NMMA = N(G)-monomethyl-L-arginine.

that a rapidly increased bioavailability of NO mediates improved endothelium-dependent vasodilation found after two weeks. To our knowledge, this is the shortest time period demonstrated for HMG-CoA reductase inhibitors to improve NO bioavailability. For LDL apheresis, improved endothelium-dependent vasodilation has been suggested after a single LDL apheresis (15,16). However, in these studies, endothelial function may have been affected not only by reduction of LDL cholesterol but also by the administration of heparin during apheresis. Heparin has recently been shown to affect the vascular endothelium by activating endothelium NO synthase and the NO-cGMP pathway leading to improved endothelium-dependent vasodilation (18,19).

Thrombostatic parameters. It was previously suggested that lipid-lowering therapy could improve thrombostatic parameters that may serve as endothelial cell markers in patients with hypercholesterolemia (20). In our study, PAI-1, t-PA, von Willebrand factor and thrombomodulin were not elevated in patients with hypercholesterolemia with impaired endothelial function. Thus, not surprisingly, no treatment effect could be detected on these parameters after lipid-lowering therapy.

Potential mechanisms. In patients with hypercholesterolemia, the bioavailability of NO is impaired either through decreased synthesis of NO or, even more importantly, through increased breakdown of NO (21). It has been suggested that hypercholesterolemia stimulates the generation of superoxide radicals by the endothelial NO synthase itself or through stimulation of different oxidases, such as NAD(P)H-dependent oxidases, which are influenced by the renin-angiotensin system (22,23). Superoxide directly inactivates NO and may also increase the subsequent oxidation of LDL particles by the formation of the powerful oxidant, peroxynitrite (21). A reduction in serum cholesterol is associated with the normalization of oxygen-derived free radical production (24). In this study, LDL cholesterol decreased by 33% after two weeks and was accompanied by a decrease in LDL/HDL ratio, triglycerides, very low density lipoprotein and apolipoprotein B and E. Thus, decrease of free radical production and consecutively less degradation of NO could explain the observed improvement in the bioavailability of NO and, thus, in endothelium-dependent vasodilation in our patients.

Although LDL cholesterol and superoxide production in particular are clearly associated with endothelial dysfunction and reduced bioavailability of NO, therapy with statins may improve endothelial function through other mechanisms independent of their lipid-lowering effects. In this context, statins have been demonstrated to activate endothelial NO synthase, independent of their cholesterol-lowering actions (25) and to decrease superoxide formation (26) by decreasing inducible NO synthase induction (27) and decreasing NAD(P)H oxidase activity. The latter effect is probably related to a statin-dependent down-regulation of angiotensin II type 1 receptor expression on smooth muscle cells, which has been demonstrated to be up-regulated in hypercholesterolemia and to stimulate NAD(P)H oxidase-dependent superoxide formation (22,28). Thus, independent of their lipid-lowering actions, statins may improve endothelial function by a further increase of NO synthesis and decrease of oxidative stress. The improvement of endothelium-dependent vasodilation found in our study was not clearly related to the reduction in LDL cholesterol or other lipid fractions. Although our study was clearly not designed to investigate such lipid-independent effects of statin therapy, we consequently cannot exclude that, in addition to the improved lipid profiles, other effects may have contributed to the improvement of endothelium-dependent vasodilation.

Possible clinical implications. Despite the well-recognized efficacy of lipid-lowering therapy in the secondary prevention of coronary syndromes, treatment of hypercholesterolemia is usually delayed during the acute phase of coronary syndromes (17). Concern with the validity of elevated in-hospital cholesterol values and the lack of demonstrated short-term benefits of lipid-lowering therapy may explain this delay in therapy. Lipid-lowering therapy is often started only after hospital discharge by the patient's general practitioner.

In this study, we have shown that lipid profile improvement can be achieved rapidly within two weeks. Cerivastatin effectively reduced total cholesterol, LDL cholesterol, very low density lipoprotein and triglycerides and increased HDL cholesterol after this short period. These improved lipid profiles were accompanied by improved bioavailability of NO, an effect that is highly desirable during the acute phase of coronary syndromes. Thus, our data support the concept that lipid-lowering therapy with statins may play a role, not only for prevention, but for therapy of acute coronary syndromes.

However, in this study only patients with hypercholesterolemia but without overt atherosclerosis or even coronary events, were investigated. The very recently published Coronary Artery Reactivity After Treatment with Simvastatin (CARATS) study in patients with mild-to-moderate coronary artery disease did not reveal any benefits of therapy with simvastatin on endothelial function after six months of therapy (29). However, in this study the average baseline LDL cholesterol was lower (average LDL cholesterol 130 mg/dl) than it was in participants in previous studies that showed improvement in coronary endothelial vasodilator function with statin therapy (1,2). Recently, the REduction of Cholesterol in Ischemia and Function of the Endothelium (RECIFE) trial demonstrated an improved endothelial function in patients with acute coronary syndromes after six weeks of therapy with pravastatin (14). In this trial endothelial function was measured as flow-mediated dilation of the brachial artery by ultrasound, a method that does not specifically assess the NO-cGMP pathway. Our data obtained by intra-arterial administration of vasoactive substances affecting the NO-cGMP pathway provide evidence of rapidly achieved benefits of lipid-lowering therapy. Further trials are required to determine whether this rapid improvement of the bioavailability of NO can be achieved in patients with acute coronary syndromes in the early recovery phase and whether this is associated with a reduction of cardiovascular events and improved outcome.

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