Improvement in Coronary Flow Reserve Determined by Positron Emission Tomography After 6 Months of Cholesterol-Lowering Therapy in Patients With Early Stages of Coronary Atherosclerosis

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Background—Early stages of coronary atherosclerosis are characterized by a mainly functional impairment of coronary vasodilator capacity under the impact of such risk factors as hypercholesterolemia. The goal of this study was to determine whether 6-month cholesterol-lowering therapy improves coronary flow reserve in patients with angina, reduced flow reserve despite minimally diseased coronary vessels or even normal angiogram, and mild to moderately elevated LDL levels on average.

Methods and Results—We noninvasively investigated 23 consecutive patients (18 men, 5 women; mean age, 56 ± 7.6 years) with a mean LDL level of 165 ± 34 mg/dL at baseline by PET for myocardial blood flow measurement with [13N]ammonia at rest and under dipyridamole stress (0.56 mg/kg) before and after lipid-lowering therapy with simvastatin for 6 months. Between baseline and the 6-month follow-up, total cholesterol concentration fell from 241 ± 44 to 168 ± 34 mg/dL, and the LDL level decreased from 165 ± 34 to 95 ± 26 mg/dL (P < 0.001). Overall, coronary flow reserve increased from 2.2 ± 0.6 to 2.64 ± 0.6 (P < 0.01). Maximal coronary flow increased significantly from 182 ± 36 to 238 ± 58 mL/min×100 g (P < 0.001) at follow-up. Minimum coronary resistance declined significantly from 0.51 ± 0.12 to 0.40 ± 0.14 mm Hg × mL⁻¹ × min×100 g (P < 0.001). Concomitantly, a regression of anginal symptoms was observed in most patients.

Conclusions—Our results suggest that cholesterol-lowering therapy with simvastatin may improve overall coronary vasodilator capacity assessed noninvasively by PET in patients with mild to moderate hypercholesterolemia. Consequently, intensive lipid-lowering therapy is considered a vasoprotective treatment for selected patients in very early stages of coronary atherosclerosis with the potential of preventing further disease progression. (Circulation. 1999;99:2871-2875.)

Key Words: hypercholesterolemia ■ coronary flow reserve ■ tomography ■ atherosclerosis ■ drugs

Hypercholesterolemia represents a major risk factor of atherosclerosis.1,2 In particular, elevated concentrations of LDL are associated with a reduction in coronary flow reserve (CFR)3–5 that is considered a functional precursor of early atherosclerosis. Invasive investigations showed a reduction in pathological vasoconstriction under intracoronary acetylcholine testing after lipid-lowering therapy for 6 to 12 months. This effect was observed preferentially in patients with angiographically documented coronary heart disease (CHD).6–8 Noninvasive studies demonstrating the potential improvement in coronary flow capacity after intensive LDL cholesterol–lowering therapy by semiquantitative PET have been reported in only a few patients with coronary artery disease and high pretreatment LDL levels.9 The objective of our investigation was to examine the reversibility of an LDL cholesterol–associated reduction in coronary vasodilator capacity in patients with angina and reduced CFR despite normal or slightly abnormal angiograms (minimal disease). This constellation of findings may be regarded as a very early, mainly functional stage of coronary atherosclerosis. We report on 23 consecutive patients with only mild to moderate LDL hypercholesterolemia on average whom we studied with regard to their dipyridamole-inducible vasodilator capacity using noninvasive, dynamic, and quantitative PET before and after 6 months of treatment with a lipid-lowering agent (simvastatin).

Methods

Patient Selection and Study Design

Our study included 23 consecutive patients (18 men, 5 women) with a mean age of 56 ± 7.6 years (range, 40 to 75 years) who underwent...
 coronary angiography because of suspected functionally important CHD. Sixteen patients complained of effort angina with a degree of severity of I to II according to the Canadian Cardiac Society classification.10 Seven patients reported atypical angina pectoris. One third of the patients had an abnormal stress test (bicycle exercise, scintigraphy, or stress echocardiography), suggesting coronary artery disease in conjunction with anginal symptoms. All patients were characterized by a reduced coronary vascular reserve assessed with PET compared with a reference group at an equivalent age with normal flow reserve.11 Most patients enrolled in the study had hypercholesterolemia at baseline that had not yet been treated with medication. The primary lipid inclusion criterion was a baseline LDL level of >150 mg/dL. In total, 10 patients had an angiogram that was found to be normal. Thirteen angiograms showed local alterations with wall irregularities and/or reductions in epicardiac vessel caliber ≥30%, called “minimal disease.” Further clinical and demographic data are presented in the Table. After coronary angiography and exclusion of significant CHD, CFR was determined by means of PET. All patients were treated with an HMG-CoA reductase inhibitor (20 mg/d simvastatin) and received adjuvant dietetic counseling concerning a low-fat, cholesterol-reduced diet. Total cholesterol intake was reduced to <300 mg/d and total fat intake to ~25% (<30% limit). Saturated fatty acid content was aimed to be at least ≤30% of total fat intake. Laboratory test values were checked under outpatient conditions after 4 weeks to detect potential side effects of simvastatin medication and to check the lipid values. In addition, cardiac symptoms were carefully recorded with a standardized questionnaire. After being comprehensively informed, all patients consented to the study protocol, which had been approved by the ethics committee of the Medical School of the University of Bochum. Exclusion criteria were pretreatment lipid-lowering drugs, unstable angina, uncontrolled hypertension, smoking, diabetes mellitus, dilated cardiomyopathy, significant echocardiographic left ventricular hypertrophy, and severe concomitant internal diseases. Hemodynamic values of left ventricular function were within normal limits according to invasive and noninvasive criteria. Because some patients were pretreated with various antian- ginal and antihypertensive drugs, study patients were allowed to continue their medication without change during the follow-up period. However, all vasoactive medications were discontinued ≥12 to 24 hours before the patients underwent PET. In detail, 7 patients received β-blocking agents, 2 were given calcium antagonists, and 1 received ACE inhibitor and nitrate. Three patients were on long-term low-dose aspirin medication.

Measurement of Myocardial Blood Flow by PET

Coronary vasodilation capacity was determined PET after any potentially vasoactive concomitant medication was discontinued for ≥12 to 24 hours and without uptake of methyl xanthine or caffeine before the investigation. The technique of measurement, time sequence, and evaluation of the method for quantitative determination of myocardial perfusion in milliliters per minute and tissue unit have been validated and described in detail in both animal experiments and humans.12 Dynamic PET with a Siemens/ECAT-951/R scanner was carried out after administration of 370 to 555 MBq of [15N]ammonia as a slow bolus over 30 seconds under resting conditions and after coronary vasodilation with dipyridamole. Directly after transmission acquisition, a dynamic emission scan was performed with a sequence of 19 frames over a total acquisition period of 10 minutes according to the following frame durations: twelve 10-second frames followed by five 30-second, two 120-second, and one 300-second frame. Image reconstruction and analysis included correction of tissue attenuation for emission data and reorientation of transversal slices in short-axis slices. Blood pressure was measured automatically and oscillometrically (Boso Oscillomat) during data acquisition at 2-minute intervals. ECG and heart rate were registered in parallel. Myocardial perfusion was calculated and analyzed analogously to the method of Hutchins et al.12 For determination of regional myocardial perfusion, the respective arterial and myocardial time-activity curves over the ventricular cavity and over 8 myocardial segments were determined on a region-of-interest basis by 2 septal, anterior, lateral, and inferior segments from representative short-axis sections. A small region of interest was assigned to the right ventricular blood pool to obtain an appropriate input function. Afterward, the regional and mean blood flows were calculated by use of a 3-compartment tracer kinetic model that was described in detail previously.12,13 Because a metabolic correction of flow values for [15N] metabolites seems negligible according to previous investigations,11 this determination of metabolites was not performed, although minor error cannot be excluded. Written results of coronary flow variables were documented by blinded nuclear medical investigators.

Determination of Coronary Flow Capacity With Dipyridamole

The dipyridamole test7,8 was applied for pharmacological recruitment of the maximum inducible vasodilation capacity in the standard dosage of 0.56 mg/kg body weight over a 4-minute intravenous infusion. The dipyridamole approach cannot be regarded as entirely independent of the endothelium in view of its adenosine-mediated mechanism.9,15–19 The following parameters of coronary vasodilation capacity were determined: maximum dipyridamole-inducible coronary blood flow (MCF), minimum coronary vascular resistance (MCR) calculated approximately from the ratio of mean arterial perfusion pressure to dipyridamole flow, and instantaneous CFR calculated as the ratio of dipyridamole to basal flow. Mean aortic perfusion pressure was calculated according to the standard formula: minimum diastolic arterial pressure plus one third of blood pressure amplitude. With regard to noninvasive calculation of MCR, aortic perfusion pressure was not subtracted from diastolic left ventricular opposing pressure because the quantitative influence of the myocardial or extravascular component of resistance can largely be neglected when left ventricular function and end-diastolic pressures are within normal limits.20

Coronary Angiography

High-resolution coronary angiograms were performed with the Judkins technique and the percutaneous femoral approach on a Siemens instrument (digital image processing HICOR 3.0; Polydoros IS-C) with an integrated unit for measuring vessel widths quantitatively. Coronary angiograms were judged with regard to smooth appearance, luminal wall irregularities, and epicardial local or diffuse caliber reduction and stenosis, respectively, by 2 experienced investigators. Coronary arteries were classified as normal if there was neither a discrete stenosis nor wall irregularities.
Laboratory Measurements
Serum lipids and lipoproteins were measured from fasting venous blood at baseline and at the end of the study period (6 months). In detail, total cholesterol was measured by the cholesterol esterase–cholesterol oxidase method (Beckman Instruments); HDL was determined by prior precipitating of VLDL and LDL particles with phosphotungstic acid and magnesium ions (Boehringer Mannheim). For LDL determination, LDL particles were precipitated by polyvinylsulfate (Boehringer Mannheim). LDL was then calculated as the difference between total cholesterol and cholesterol measured in the supernatant. Plasma fibrinogen concentration was determined according to the functional method of Clauss.21

Statistical Analysis
For statistical analysis with the StatView 4.57 software package, mean values and SDs were calculated for all variables. Student’s paired t tests were used for comparison of lipid and coronary parameters before and after study intervention. Statistical significance was assumed when the null hypothesis could be rejected at \( P = 0.05 \).

Results
Serum Lipids and Basic Hemodynamic Variables
Results represent mean values at baseline and after the 6-month (mean±SD, 6.1±0.6-month) follow-up. Total cholesterol concentration decreased from 241±44 mg/dL at baseline to 168±34 mg/dL after treatment with diet and an HMG-CoA reductase inhibitor. LDL cholesterol concentration fell significantly from 165±34 to 95±26 mg/dL (\( P < 0.001 \)). The ratio of LDL to HDL decreased from 4.2±1.3 at baseline to 2.1±0.6 after the lipid-lowering time frame (\( P < 0.001 \)). The HDL fraction showed a slight increase from 43±16 to 48±12 mg/dL (\( P < 0.01 \)). There was no significant change in plasma fibrinogen (292±55 versus 299±55 mg/dL) during the study period. The main determinants of basic hemodynamics, such as mean arterial blood pressure and heart rate at the time of PET data acquisition under dipyridamole and resting myocardial blood flow, did not show significant differences between the first and second PET studies 6 months apart. In detail, mean arterial blood pressure was 89±13 mm Hg at baseline and 87±13 mm Hg (\( P = \text{NS} \)) after lipid-lowering therapy. Heart rate was 82±13 versus 87±15 bpm (\( P = \text{NS} \)). Basal myocardial blood flow was 87±20 mL/min×100 g at baseline and 92±19 mL/min×100 g (\( P = \text{NS} \)) at follow-up.

Changes in Coronary Hemodynamics
Overall, CFR increased from 2.2±0.6 to 2.64±0.6 (\( P < 0.01 \)) during the study period. MCF increased significantly from 182±36 mL/min×100 g at baseline to 238±58 mL/min×100 g (\( P < 0.001 \)) at follow-up. MCR decreased significantly from 0.51±0.12 to 0.40±0.14 mm Hg · mL\(^{-1}\) · min×100 g (\( P < 0.001 \)) at the 6-month follow-up. The main determinants of coronary vasodilator capacity are summarized in the Figure for the total number of patients before and after lipid-lowering therapy.

Changes in Clinical Characteristics
Sixty-five percent of the total number of patients (15 of 23) were free of cardiac symptoms after lipid-lowering therapy, and no patient complained of typical effort angina. Eight patients still reported atypical angina pectoris. The HMG-CoA reductase inhibitor medication was tolerated well as a whole. Only 1 patient developed transient myalgia without a significant increase in creatinine kinase.

Discussion
The primary objective of our investigation was to establish whether the noninvasively determined coronary vasodilator capacity can be improved by intensive medication with a lipid-lowering agent administered for a limited time in patients with angina pectoris and only mild to moderate LDL hypercholesterolemia on average in whom restriction of CFR has been demonstrated despite only minimally diseased coronary vessels or even normal angiographic appearance. Our findings indicate a significant improvement in overall coronary vasodilation capacity in most patients after 6 months of intensive LDL lowering in the very early stages of coronary atherosclerosis. Quantitatively, an increase in dipyridamole-inducible enhancement of coronary blood flow and an increase in the ratio of instantaneous dipyridamole flow to basal flow ratio, called coronary reserve, could be shown to be associated with a significant decrease in MCR. Clinically, the increase in coronary vasodilation capacity was accompanied by a noteworthy improvement in symptoms, with a decrease in effort angina and angina-like symptoms.

Angina Pectoris With Normal or Near-Normal Coronary Angiograms
The syndrome of angina despite a normal coronary angiogram, often called syndrome X, includes several subgroups of patients with different pathophysiological mechanisms leading to anginal pain.22–24 However, increasing evidence3–5
suggests that patients with angina pectoris and a still-normal epicardial angiogram or only minimally affected coronary vessels but restricted CFR who undergo angiography because of suspected CHD may already have a mainly functional early stage in the natural development of coronary sclerosis on the basis of risk factors.\textsuperscript{2.3} Furthermore, it cannot be ruled out that local or diffuse intimal lesions of epicardiovascular regions would already have been detected with intravascular ultrasound in these patients, although endothelial dysfunction may precede the ultrasonographic detection of wall lesions.\textsuperscript{25} With reference to some of our patients with normal angiograms, in patients without angiographic evidence of coronary artery disease, a correlation between the number or coronary risk factors and loss of endothelium-dependent vasodilation has been reported.\textsuperscript{26} In this regard, Seiler and coworkers\textsuperscript{27} demonstrated that hypercholesterolemia causes a reduction in exercise-induced vasodilation in patients with normal coronary angiograms. In addition, transient myocardial perfusion abnormalities on exercise testing suggesting myocardial ischemia have been observed in patients with angina, coronary endothelial dysfunction of resistance vessels, and normal or near-normal coronary arteriograms.\textsuperscript{28}

Impact of Cholesterol-Lowering Therapy on Coronary Vasodilator Function in Previous Trials

Egashira et al\textsuperscript{6} demonstrated an improvement in coronary endothelial vasomotor function in both epicardial and resistance vessels after 6 months of treatment with pravastatin in patients with marked hypercholesterolemia (mean LDL cholesterol, 195±25 mg/dL) and CHD. Leung et al\textsuperscript{6} described an improvement in epicardial coronary vasomotor responses to intracoronary acetylcholine in men with hypercholesterolemia (mean LDL, 219±35 mg/dL) and angiographically normal coronary arteries after 6 months of cholesterol-reducing diet and cholestyramine. In a randomized, double-blind, placebo-controlled trial, Treasure and coworkers\textsuperscript{8} showed that lipid-lowering therapy (diet and lovastatin) had no effect on coronary endothelial function in the short term (12 days) but improved coronary vasoemotion in the longer term (5.5 months) in patients with only mild hypercholesterolemia (mean LDL, 148±7 mg/dL) and symptomatic CHD. Anderson and colleagues\textsuperscript{7} demonstrated in a randomized study that a combined regimen of lipid-lowering diet and antioxidant therapy consisting of lovastatin and probucol significantly improved endothelium-dependent vasodilator responses to acetylcholine in patients with coronary atherosclerosis and a baseline LDL of 145±37 mg/dL on average after 1 year of therapy. Finally, Gould et al\textsuperscript{9} using PET to evaluate myocardial perfusion for the first time, suggested that lipid-lowering therapy may have beneficial effects on the integrative dipyridamole-induced coronary flow capacity in patients with CHD and marked hypercholesterolemia (mean LDL level, 213±79 mg/dL) after 3 months of therapy (diet combined with lovastatin and cholestyramine).

Possible Pathophysiological Mechanisms

An improvement in CFR by regression of structural vascular changes appears very improbable owing to the small time span in our patient population with still-normal or only slightly altered coronary angiograms. Several components may contribute to impaired vasodilation under conditions of relative LDL hypercholesterolemia. In this regard, an increased production of superoxide anions by oxidized LDL seems to be a major mechanism.\textsuperscript{30,31} In addition, reduced expression of endothelial nitric oxide (NO) synthase by oxidized LDL may also be involved.\textsuperscript{32} Moreover, oxidized LDL potentiates the effect of vasoconstrictor hormones.\textsuperscript{33} In humans, hypercholesterolemia leads to endothelial dysfunction of both the epicardial vessels and the coronary resistance vessels in the microcirculation.\textsuperscript{2.3,6} This disturbance of basal and metabolic regulation of vascular tone may occur a long time before angiographically visible stenoses and can be corrected, at least partially, in the resistance vessels by administration of l-arginine.\textsuperscript{34} The precise mechanism of the moderate improvement in integrative coronary vasodilation capacity after effective lipid-lowering that we documented with PET has not been clarified. However, at present it may be assumed that the major pathophysiological mechanism is probably an increase in vasoactive NO bioavailability. In this regard, in a recent randomized, placebo-controlled, double-blind study, an increased bioavailability of NO after lipid-lowering therapy was reported for the first time in patients with hypercholesterolemia (LDL ≥160 mg/dL) after 6-month treatment with fluvastatin.\textsuperscript{35} In addition, newer findings suggest that the HMG-CoA reductase inhibitor simvastatin might have beneficial effects on atherosclerosis beyond that attributed to the lowering of LDL cholesterol by direct upregulation of endothelial NO synthase activity.\textsuperscript{36}

The dipyridamole-recruitable CFR represents an integrative marker of both smooth muscle relaxation of resistance vessels and, at least in part, endothelial function resulting from NO-mediated flow-dependent vasodilation.\textsuperscript{15–19} It thus approximately reflects the entire coronary vasodilation capacity.\textsuperscript{9,15} Detection of abnormal CFR in patients with risk factors and still-absent or only minimal changes in coronary angiogram may be considered a manifestation of a functional disorder of vascular tonus regulation in the early stage of coronary sclerosis.\textsuperscript{4} In our patient group, improvement in coronary vasodilation capacity after intensive lipid lowering might have resulted from an increase in flow-dependent vasodilation in small resistance arteries of microcirculation and possibly in epicardial vessels after initial dipyridamole-induced flow enhancement. Although endothelial function was not specifically tested in our noninvasive study, improvement in endothelial function may have contributed to an increase in flow-mediated endothelial-dependent dilation in response to the initial increase in flow caused by the direct action of dipyridamole,\textsuperscript{37} similar to improvements in myocardial perfusion during dipyridamole-PET after cholesterol lowering in patients with CHD studied by Gould and coworkers.\textsuperscript{9}

Study Limitations

The absence of a nontreated placebo group must be mentioned. However, with current knowledge about the atherogenic and vasoconstrictive effects of LDL hypercholesterolemia, inclusion of a sufficiently large placebo group of patients even at the beginning of coronary atherosclerosis hardly seems justified when the radiopharmaceutical loading caused by repeated nuclear radiation during several PET studies in a relatively short time frame also is taken into
account. Consequently, such a placebo-controlled study would not have been accepted by the ethics committee. Instead, each patient served as his or her own control.

Conclusions
The results of our study indicate that cholesterol-lowering therapy with principally HMG-CoA reductase inhibition for an average of 6 months improves the integrative dipyridamole-recruitable CFR in most patients with mild to moderate hypercholesterolemia at very early, predominantly functional stages of coronary atherosclerosis. Consequently, intensive lipid-lowering therapy may contribute to functional reversal of impaired CFR, leading to a potential reduction in the risk of myocardial ischemia, with concomitant regression of anginal symptoms and possibly prevention of further disease progression.

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