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Improvement of Coronary Artery Endothelial Dysfunction With Lipid-Lowering Therapy: Heterogeneity of Segmental Response and Correlation With Plasma-Oxidized Low Density Lipoprotein

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OBJECTIVES This study assessed coronary artery endothelial function in patients with hypercholesterolemia before and after lipid lowering, using quantitative angiography to examine the acetylcholine (Ach) response along the entire analyzable vessel.

BACKGROUND Lipid lowering reverses endothelial dysfunction, but whether improvement occurs only in some segments and not others has not been established. Statistical correlation of improvement with specific lipid moieties remains undefined.

METHODS Quantitative angiography was performed after Ach (10^{-6}, 10^{-5}, 10^{-4}M) in 29 patients with coronary atherosclerosis before and 18 ± 5.2 months after lipid-lowering treatment (statins, bile sequestrant resins). Standard lipid moieties and markers of oxidized low density lipoprotein (LDL) (immunoglobulin G and M autoantibody titers to malondialdehyde-LDL, E06 epitope) were measured serially.

RESULTS Pre-treatment of the vessel diameters at control and with 10^{-6}M, 10^{-5}M and 10^{-4}M Ach were 2.108 ± 0.085, 2.086 ± 0.087, 2.069 ± 0.084 and 1.963 ± 0.097 mm (M ± SE), respectively, and increased at follow-up to 2.139 ± 0.094, 2.119 ± 0.086, 2.127 ± 0.084 and 2.080 ± 0.085 mm ($p < 0.0001$). Improvement in the most constricted and modest declination in the more dilated segments were observed. Change in the E06 and Apolipoprotein A-1 titers correlated with improved vasomotion ($p = 0.027$ and 0.005, respectively). The pre- and post-treatment levels of the E06 epitope, as well as the post-treatment IgM autoantibody titer to MDA-low density lipoprotein, also correlated ($p < 0.028$, < 0.001 and $p < 0.004$, respectively).

CONCLUSIONS Drug treatment reverses endothelial dysfunction, but the effect is heterogeneous. Most coronary segments show enhancement, while others show declination of dilation, underscoring the importance of assessing the entire analyzable artery. Improvement in vasomotion correlates most significantly with markers of plasma-oxidized low-density lipoprotein. (J Am Coll Cardiol 2001;37:766–74) © 2001 by the American College of Cardiology

After lipid lowering, a reversal of coronary artery endothelial dysfunction in patients with hypercholesterolemia has been reported (1–5), suggesting that amelioration of constrictive vasomotion may help reduce coronary events. These studies focused on limited segments of the coronary artery and did not account for the heterogeneity of response to acetylcholine (Ach), such that normal vasodilatation and paradoxical vasoconstriction may occur in different segments of the same artery (6,7). Furthermore, it has not been defined whether improvement in coronary reactivity is predicted by absolute pre- or postlipid-lowering plasma levels of lipid moieties or the magnitude of their change during drug treatment.

In this study we examined the changes with lipid lowering in coronary vasomotor response to Ach using serial quantitative angiography with comprehensive analysis of a number of segments of the same artery. A correlation was then made between the change in vasomotor response and pre- and post-treatment serum lipid moieties.

METHODS

Patient selection, demographics and characteristics. Patients with stable angina, a mildly positive exercise treadmill test and hypercholesterolemia (serum total cholesterol >200 mg/dl) were enrolled in a protocol approved by the Investigational Review Board of the University of California, San Diego, School of Medicine and the Veterans Affairs Medical Center, San Diego. Individuals with a recent myocardial infarction, unstable or variant angina, congestive heart failure or severe peripheral vascular disease were excluded. All patients had mild atherosclerotic luminal irregularities (<50% obstructive) of the artery studied.

Lipid-lowering regimen. After baseline lipid moieties and coronary arteriography had been performed, enrollees were

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Abbreviations and Acronyms

Ach	= acetylcholine
Apo	= apolipoprotein
eNOS	= endothelial nitric oxide synthase
HDL	= high density lipoprotein
HLM	= hierarchical linear model
IgG	= immunoglobulin G
IgM	= immunoglobulin M
LDL	= low density lipoprotein
LR_{fx}	= lipid reduction effect
MDA	= malondialdehyde
NO	= nitric oxide
ox-LDL	= oxidized low density lipoprotein

instructed in the Step I diet of the National Cholesterol Education Program and prescribed a graduated dose of an HMG coenzyme A inhibitor (lovastatin) aimed at reducing their total and low density lipoprotein (LDL) cholesterol to below 200 mg/dl and 100 mg/dl, respectively. Seven of 29 patients were given a bile-acid sequestrant subsequently. Follow-up angiography was performed once the patient's total and LDL cholesterol had each reached a plateau (three successive determinations) on lovastatin of 40 mg/day or greater. If a patient smoked cigarettes, he/she was encouraged to stop although cessation was not required for a patient to remain in the study.

Lipoprotein quantification. Blood samples were collected monthly. Lipid measurements were as described in the Lipid Research Clinic Manual of Laboratory Operations (8). The inter-assay coefficient of variation was 0.8% for total cholesterol, 1.7% for total triglycerides and 2.4% for high density lipoproteins (HDL) cholesterol. Apolipoprotein (Apo)-A1, Apo-B as well as lipoprotein (a) were determined on a Roche-COBAS BIO instrument using appropriate INCSTAR kits and standards.

Immunoglobulin G and M (IgG, IgM) titers of autoantibodies to malondialdehyde-LDL (MDA-LDL) in human plasma were determined using modifications of a previous assay (9). Malondialdehyde-LDL was produced by sequential ultracentrifugation of healthy human donor plasma in the presence of high concentrations of antioxidants (10). The degree of modification of the lysine residues of Apo-B was determined by the trinitrobenzenesulfonic acid assay (11,12) and was consistently above 75%. Data were expressed in relative luminescence units (RLU) per 100 ms. Samples were run in a single assay; the intra-assay coefficient of variation varies from 8% to 10%.

The murine monoclonal antibody E06 recognizes an oxidized phospholipid epitope that is on LDL as it undergoes oxidation. Thus, it is a sensitive index of early stages of oxidized LDL (ox-LDL) (13,14). The E06 epitope concentration in Apo-B 100 containing particles was measured by a chemiluminescence enzyme immunoassay (14). This assay was based on two monoclonal antibodies, an antihuman Apo B-100 antibody, MB47, and a biotin-labeled antioxidized phospholipid antibody, E06. The amount of Apo-B-100 containing particles bound to the MB47 coated plates was measured by using another biotin-labeled antihuman Apo-B-100 monoclonal antibody, MB24, to normalize the bound LDL of each sample. These two anti-Apo-B-100 antibodies bind to distinct Apo-B-100 epitopes. Data were given for absolute levels of E06 bound per well (RLU/100 ms) or as a ratio of E06/MB24. Because each MB24 antibody binds once to each LDL, the E06/MB24 ratio yields an estimate of E06 epitopes/LDL. All samples were run in a single chemiluminescence enzyme immunoassay; the intra-assay coefficient of variation was 6%.

Angiographic protocol. All vasodilator therapy was discontinued 48 h before the angiographic procedure (6). After diagnostic angiography, an end-hole 3F-infusion catheter was placed into the proximal segment of the coronary artery to be studied. Atrial pacing at 80 beats/min was initiated from the right atrium. Biplane coronary angiography was performed at control and during serial, 3-min infusions of Ach at 10^{-6}M, 10^{-5}M and 10^{-4}M; assuming a coronary flow of 80 ml/min, this would yield intracoronary Ach concentrations of 10^{-8}M, 10^{-7}M and 10^{-6}M, respectively. For each injection, 9 ml of Omnipaque 350 (Winthrop-Breon Laboratories, New York) at 6 ml/s were injected using a Medrad Mark IV injector (Medrad Inc., Pittsburgh, Pennsylvania).

Quantitative coronary angiography. In any given patient, all images were acquired at identical X-ray gantry, table height and source-to-image intensifier distances. Projections chosen displayed the maximum length of the artery and minimized foreshortening and vessel overlap. The guiding catheter was imaged unopacified, and the catheter tip was later measured using electronic calipers to calibrate the magnification for each injection. End-diastolic cine frames were digitized at a resolution of 512 × 512 pixels, 8 bits, at end-diastole using a 35 mm SONY (Tokyo, Japan) SME 3500 Cine Video System interfaced to an Imaging Technology, Inc. (Bedford, Massachusetts) 150 Series video digitizer and a Silicon Graphics, Inc. (Mountainview, California) 4D/380/VGX computer. Vessel edges were determined by an automatic edge detection routine (15) with potential for manual override. The vessel centerline and diameters perpendicular to this centerline were calculated and interpolated as previously described (6). The arteriogram was partitioned into successive 3 mm segments, and the diameters in each segment were then averaged, Figure 1. Our routines ensured the geometric identity, or very near correspondence, of segments that were compared as part of the vasomotion analysis.

Statistical analysis. AVERAGE OF SEGMENT DATA PER PATIENT. Data were analyzed as a two-level, repeated measures analysis of variance (SuperANOVA 1.11, Abacus Concepts, Inc., Berkeley, California) with the first level comparing responses at each dose of Ach and the second level comparing results at baseline to those after cholesterol lowering. A statistical contrast was constructed before data analysis in which the single comparison was the combined

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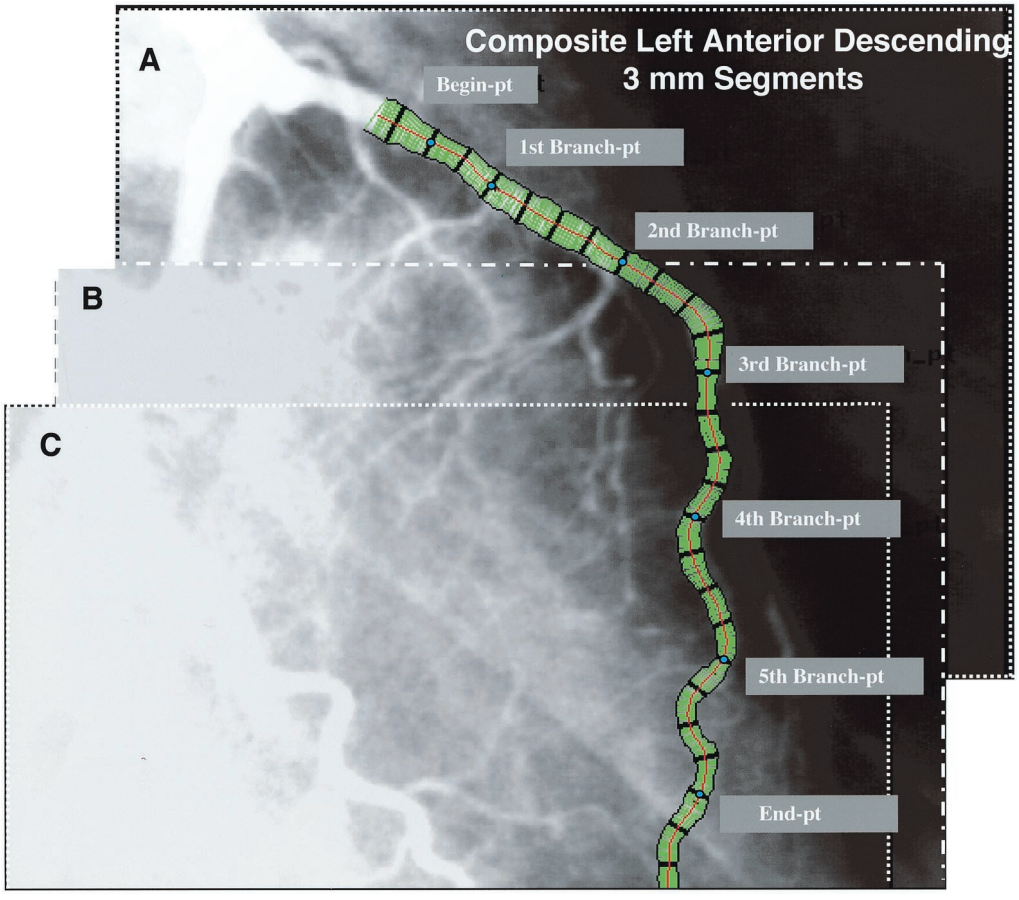

Figure 1. Composite image of a left anterior descending coronary artery. Algorithm-derived edges, the calculated vessel centerline, vessel segments and sequential diameters are displayed. Branch points are used for interpolation of diameters (6). pt = point.

change in the vessel diameter values between baseline and follow-up studies (cell weights ± 0.25 for control, Ach 10^{-6}M, 10^{-5}M and 10^{-4}M for absolute vessel diameter analysis; cell weights ± 0.33 for Ach 10^{-6}M, 10^{-5}M and 10^{-4}M for absolute change [Δ] and percentage change [%Δ] diameter analysis). Because a single comparison was constructed a priori in each contrast analysis, a p value <0.05 was considered significant.

Segment data. Analysis of segment data, using a standard repeated measures analysis of variance, is confounded by an unequal number of segments nested within each patient and the potential for intra-class correlation of these segments in any given patient. Some authors have approached this type of problem by use of a general linear model of intra-class correlation (16). This approach was not suitable here where repeated measures of coronary vasomotion, before and after lipid lowering, were made in each patient. Thus, we applied a hierarchical linear model (HLM) that adjusts variances for patient differences, segment differences and, finally, repeated measures (17). For hierarchical analysis, we used HLM v. 4.01 (Bryk, Raudenbush & Congdon, Scientific Software International, Chicago, Illinois).

Correlation with lipid moieties. The HLM indicated that 58% of the high-level variance in this study was between patients and 42% was between coronary artery segments within patients. Both variance components were statistically significant. Because significant variance was at the segmental level, the effects of lipid moieties on change in coronary vasomotion were developed in level-3 HLM models using nine measured lipid moieties (total cholesterol, LDL, HDL, lipoprotein (a), Apo-A-1, Apo-B, IgM autoantibody, IgG autoantibody and the E06 epitope). Three separate models were used to analyze the influence of the absolute pre-, the absolute post-, and the change (Δ) in lipid moieties. All possible covariates were included so as to obtain a value for μ_0, the estimated residual level-3 error variance. Additional analyses were then run, each one successively excluding one of the set of covariates. The proportion of variance accounted for by a particular covariate was taken as:

$$\text{Proportion of variance} = \frac{\mu_o \text{ for the reduced model} - \mu_o \text{ for the inclusive model}}{\mu_o \text{ for the reduced model}}$$

Only covariates that accounted for at least 5% of the residual error variance were accepted back into the model until the excluded covariates in aggregate accounted for no more than 10% of the residual variance.

Table 1. Effects of Lipid-Lowering Treatment on Lipid Moieties

Lipid Moiety	Prelipid Lowering	Postlipid Lowering	Significance
Total cholesterol	240.0 ± 28.7 (mg/dl)	178.7 ± 24.2 (mg/dl)	$p < 0.0001$
LDL	161.9 ± 27.3 (mg/dl)	103.1 ± 22.2 (mg/dl)	$p < 0.0001$
HDL	49.0 ± 9.4 (mg/dl)	52.4 ± 11.3 (mg/dl)	$p < 0.03$
Lp(a)	29.2 ± 24.6 (mg/dl)	30.6 ± 25.6 (mg/dl)	$p = 0.37$
Apo-A-1	149.2 ± 21.4 (mg/dl)	145.9 ± 19.5 (mg/dl)	$p = 0.28$
Apo-B	114.9 ± 14.8 (mg/dl)	89.8 ± 13.3 (mg/dl)	$p < 0.0001$
IgM autoantibodies to MDA-LDL	155,426 ± 52,524 (RLU/100 ms)	154,040 ± 48,686 (RLU/100 ms)	$p = 0.85$
IgG autoantibodies to MDA-LDL	31,841 ± 17,649 (RLU/100 ms)	36,784 ± 18,181 (RLU/100 ms)	$p = 0.20$
Absolute E06 epitope assay	4,005 ± 2,829 (RLU/100 ms)	6,275 ± 8,187 (RLU/100 ms)	$p = 0.08$
E06/MB24	0.027 ± 0.019 (RLU/100 ms)	0.044 ± 0.058 (RLU/100 ms)	$p = 0.07$

Apo-A-1 = apolipoprotein A-1; Apo-B = apolipoprotein B; E06 = E06 autoantibody to epitope of oxidized LDL; HDL = high density lipoprotein; IgG = Immunoglobin G autoantibody to oxidized LDL; IgM = Immunoglobin M autoantibody to oxidized LDL; LDL = low density lipoprotein; Lp(a) = lipoprotein (a); MDA = malondialdehyde; T Chol = total cholesterol.

RESULTS

Patient attributes and lipid moieties. Forty patients were initially enrolled in the study; seven patients refused follow-up angiography, and in three patients Ach was not given at follow-up, because of progression of coronary artery disease. One patient in whom one dose of Ach was infused was also excluded from final analysis.

The final cohort consisted of 27 men and 2 women, with a mean age of 59.5 ± 8.3 years (M ± SD, range 44 to 73 years). Seven patients were current cigarette smokers, and 17 were former smokers; five had never smoked. Fifteen patients had hypertension, and two had diabetes mellitus. Seven patients were on antioxidant vitamins (Vitamin E, C or both) throughout the protocol. The left anterior descending artery was studied in 24 patients, and the left circumflex artery in five patients. The lipid moiety values, before and after lipid lowering, are given in Table 1.

Quantitative coronary dimensions. AVERAGE SEGMENT RESPONSES PER PATIENT. The mean vessel diameters during the series of infusions were significantly greater at follow-up than they were at baseline ($n = 29$, $p < 0.0001$, Fig. 2, panel A, Table 2). The response to Ach was calculated as the change in vessel diameter (Δ diam) from control infusion for each segment (negative numbers indicate constriction), then averaged for each patient (Fig. 2, panel B, Table 2). The response to Ach was also calculated as the percent change in vessel diameter from control infusion for each segment (negative numbers indicate constriction), then averaged for each patient (Fig. 2, panel C, Table 2). Vasoconstriction induced by the Ach infusions was significantly decreased after lipid lowering ($n = 29$, $p < 0.05$ vs. before lipid lowering).

Hierarchical linear model. In the hierarchical model, the lipid reduction effect (LR_{fx}) is taken as the arithmetical difference between the baseline and follow-up vasomotion response (Δ diam) to each dose of Ach. The effect of lipid lowering is then assessed in terms of the linear relationship between LR_{fx} and the drug dosage of Ach.

In a two-level analysis of covariance, patients were the grouping variable, the mean LR_{fx} for each patient was the dependent variable and dosage of Ach the covariate. The intercept, which is the mean LR_{fx} adjusted across subjects for Ach 10^{-5}M, was not significantly different from zero (coefficient = 0.0364; $t[28] = 0.98$; $p > 0.3$). The common slope estimate, however, was significantly greater than zero (coefficient = 0.0438; $t[28] = 2.05$; $p < 0.05$), indicating a significant dosage effect and, therefore, a significant impact of lipid reduction on arterial vasomotion. The variance in the intercept, which indicates the variability among patients in their responses to treatment, was also significant ($m_{0j} = 0.0317$; $c^2[28] = 128.8$; $p < 0.001$). These findings confirm that, after hypolipidemic therapy, coronary dimensions, on average, manifest less vasoconstriction in response to progressive doses of Ach.

In a three-level analysis (analysis of covariance), both patient-level and segment-level effects are incorporated into a single model: level 1 is the assay level, with dosage of Ach as a covariate; level 2 is the segment level, and level 3 is the patient level. The dependent variable is LR_{fx}, and the dose-response slope is constrained to a homogeneous, fixed estimate by setting the random effects in the slope equations (i.e., r_{1jk} and u_{10k}) to zero. Although the slope coefficient was smaller than that estimated from the patient level, it was much more reliable (coefficient = 0.0287; $t = 3.90$; $p < 0.001$). The variance in the intercept, which indicates the variability among segments and among patients in their responses to treatment, was also significant (for segments, variance = 0.0270; c^2 [553] = 827.8; $p < 0.001$; for patients variance = 0.0361; c^2 [28] = 434.1; $p < 0.001$). Thus, the incorporation of segmental, as well as average patient, information into the statistical model uncovered more fully the significant effect of lipid lowering on coronary vasomotion.

Heterogeneity of change in vasomotor response. A heterogeneous pattern of response (improvement in endothelial function in at least one segment and declination in endo-

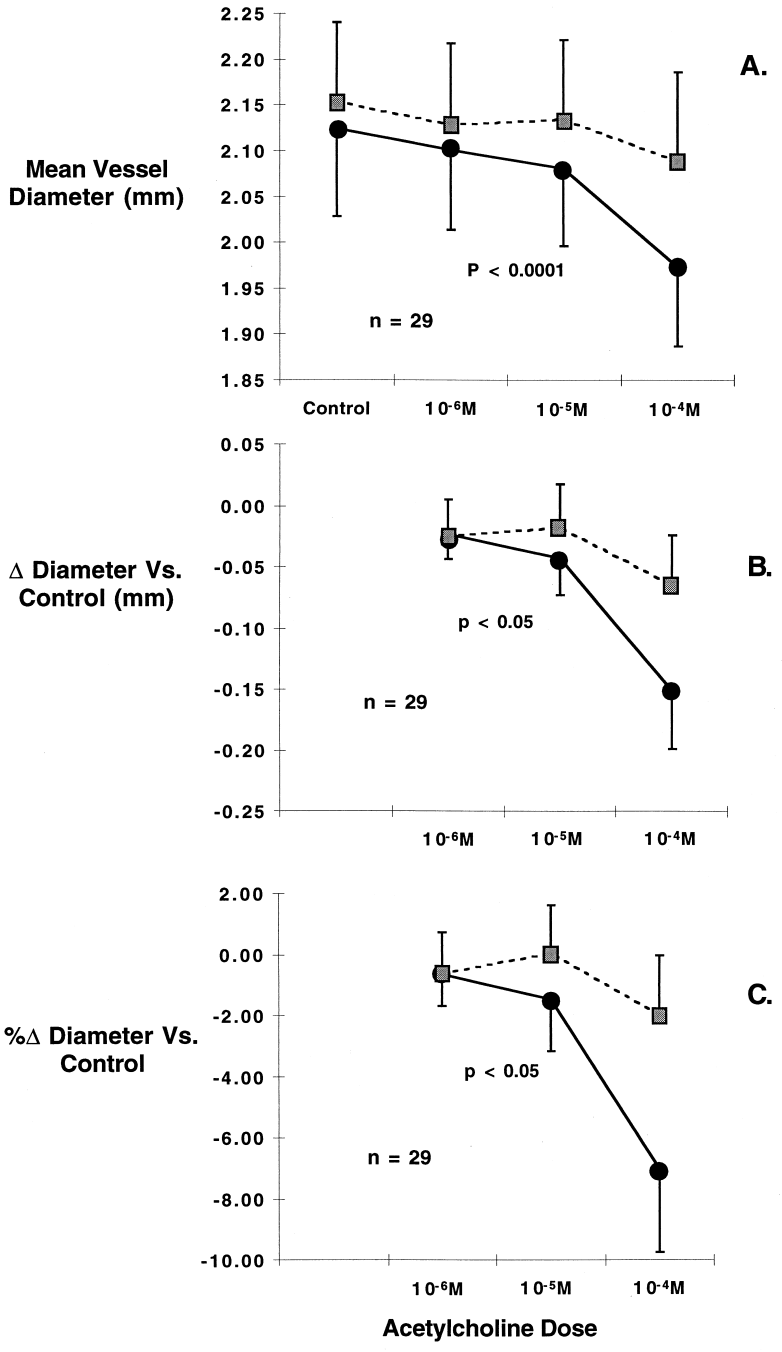

Figure 2. Changes in vessel dimension and vasomotor response to successive Ach infusions are shown as the average of per patient means (n = 29). **(A)** Plot of mean vessel diameter in mm at control and after Ach infused at 10^{-6} to 10^{-4}M. Baseline values (M ± SE) are shown as **closed circles/solid line**; values from follow-up study after cholesterol lowering are shown as **open squares/dashed line**. **(B)** Plot of mean change (in mm) in vessel diameter from control upon Ach infusion at graded doses (M ± SE). **(C)** Plot of mean percent change in vessel diameter from control upon Ach infusion at graded doses (M ± SE). Ach = acetylcholine. **Solid circle** = baseline; **Shaded square** = follow-up.

thelial function in at least one segment) was noted in 25 of the 29 patients at the maximum dose of Ach. Moreover, 20 of the 29 patients had vessel segments that both improved and worsened by 10 or more percentage points (Fig. 3). Also, we ranked the segments from worst to best for each patient (as previously mentioned) and adjusted for the disparate number of segments per patient by normalization on a scale from 0 (most constricted) to 100 (most dilated), then analyzed by decile (Fig. 4). At baseline, the most constricted decile demonstrated a mean change of −18% from control, and the most dilated decile demonstrated a change of +6%. At follow-up, with baseline decile rankings maintained, the range had narrowed, and the slope had flattened due to improved function in the most constricted segment deciles and some declination of function in the segment deciles, which dilated at baseline. These changes in

Table 2. Assessment of Coronary Artery Acetylcholine Responses, Pre- and Postlipid Lowering

Drug State	Prelipid Lowering	Postlipid Lowering
	Absolute Diameters (mm)	
Control	2.108 ± 0.085	2.139 ± 0.094
Acetylcholine 10^{-6}M	2.086 ± 0.087	2.119 ± 0.086
Acetylcholine 10^{-5}M	2.069 ± 0.084	2.127 ± 0.084
Acetylcholine 10^{-4}M	1.963 ± 0.097	2.080 ± 0.085
	Change in Diameter (Δ mm)	
ΔAcetylcholine 10^{-6}M	−0.022 ± 0.021	−0.020 ± 0.029
ΔAcetylcholine 10^{-5}M	−0.038 ± 0.030	−0.012 ± 0.036
ΔAcetylcholine 10^{-4}M	−0.144 ± 0.047	−0.059 ± 0.041
	Percentage Change in Diameter (%)	
%ΔAcetylcholine 10^{-6}M	−0.58 ± 1.06	−0.44 ± 1.32
%ΔAcetylcholine 10^{-5}M	−1.24 ± 1.70	0.31 ± 1.53
%ΔAcetylcholine 10^{-4}M	−6.74 ± 2.73	−1.67 ± 1.94

− = vasoconstriction.

response to Ach after cholesterol lowering did not predominate in any given region of the artery.

Correlation of improvement in vasomotion with lipid moieties. The lipid-lowering E06 autoantibody (as a difference and also as an absolute before or after lipid-lowering variable) showed in aggregate the most powerful influence on improvement in vasomotion. The most potent variable was the postlipid-lowering E06 autoantibody level, which accounted for 22.1% of the variance in vasomotion ($p < 0.007$). The absolute postlipid-lowering IgM autoantibody to oxidized LDL and the change with Apo-A-1 also correlated significantly with the change in coronary vasomotion.

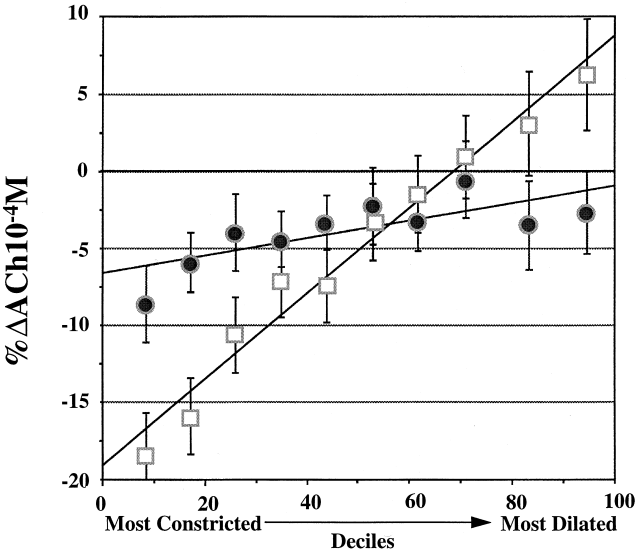

Figure 4. Plot of percentage change in segment diameter versus control **(vertical axis)** ranked by decile from the most constricted to the most dilated **(horizontal axis)**. Data for infusion of Ach 10^{-4}M presented. Note that the most constricted segments improved after lipid lowering while those that dilated showed less dilation or mild constriction. See text for discussion. Ach = acetylcholine. **Open square** = prelipid-lowering; **closed circle** = postlipid-lowering.

By simple linear regression, no statistically significant correlation ($p < 0.05$) was found between the pre–lipid lowering Ach response and any one of the pre-treatment

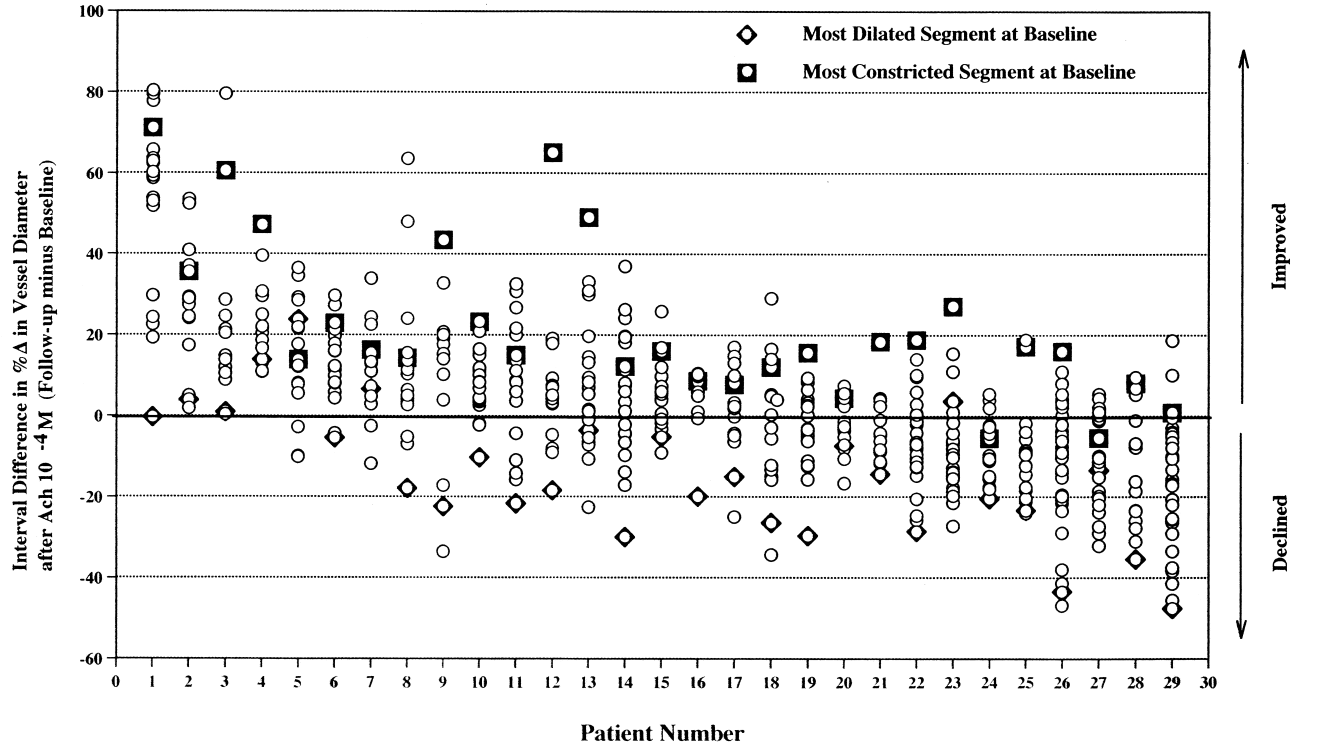

Figure 3. Heterogeneity of vasomotor response after cholesterol lowering demonstrated as the interval change of response of all segments to Ach 10^{-4}M grouped by patient number. See text for further discussion. Ach = acetylcholine.

lipid moieties. No significant correlation was found between coronary vasomotion improvement and the nominal variables of smoking, hypertension, diabetes mellitus or use of antioxidant vitamins.

DISCUSSION

We report here the results of a comprehensive coronary artery segmental analysis in patients with hypercholesterolemia treated with cholesterol-lowering agents. While the significant improvement in endothelial function corroborates previous studies (1–5), assessment of the entire analyzable artery suggests that the phenomenon may be more complex than indicated by investigations that focused on selected coronary segments.

Our quantitative angiographic methodology differs from previous lipid-lowering studies in these respects: 1) a number of contiguous segments are analyzed in the same patient; 2) an interpolation routine ensures correspondence of segments analyzed before and after Ach and before and after lipid lowering; (6) and 3) our statistical analysis incorporates a calculation of data variance at both the patient and segmental levels (17). This latter approach uses the statistical power inherent in analyzing several segments in each patient while accounting for nesting of segments within patients, the repeated measures for analysis of coronary vasomotion and the use of several doses of Ach in testing endothelial integrity.

Heterogeneity of vasomotor response to cholesterol-lowering. The response to cholesterol lowering was dependent upon the initial response of the segment to Ach, with a pattern of improved vasomotion in the most constricted segments from the baseline study and a declination of the most dilated segments. This pattern was incremental throughout the artery when baseline responses to Ach were ranked by decile (Fig. 4). The finding of most marked improvement in the most constricted of selected segments of the control study was also described in the work of Anderson et al. (3) and Treasure et al. (2). This pattern also parallels the results of the MARS trial (18), a study of changes in angiographically defined coronary stenoses after cholesterol lowering. Here there was a small degree of regression of stenosis in those patients in the test group (on lovastatin) compared with the placebo group. Subset analysis revealed that lesions >50% stenotic on the baseline study showed regression (improvement), while lesions <50% at baseline demonstrated progression of stenosis severity (worsening) at follow-up.

Sources of heterogeneity. STATISTICAL. The observed pattern may be due to a regression toward the mean, where extreme outlying points (both the most Ach constricted and dilated segments, arbitrarily chosen for quantification from a normal distribution) fall closer to the central tendency (the average) when resampled and quantified at follow-up. The segmental vasomotor responses also may be constrained by limits of maximal vasoconstriction (i.e., 100%) and vasodilation. Thus, the variability inherent in quantitative analysis may result in less marked mean change at follow-up in segments that exhibit extreme changes upon initial assessment, as there can be more underestimation than overestimation of the subsequent response due to these anatomic constraints.

Pathophysiologic. The heterogeneous response to lipid lowering also may be due to pathophysiologic mechanisms. An obvious source is the segmental distribution of atherosclerosis. Another source could be a nonuniform decrease by lipid lowering in the oxidative milieu of the endothelium and subendothelium. This uneven effect may lead to a variable decrease in sensitivity of the muscarinic receptors of the vascular smooth muscle cells to the effect of Ach. This change could have the most marked effect on the most abnormal vessel segments, resulting in reversal of vasoconstriction, and less effect on the segments that were more normal at the outset and, thus, were not affected predominantly by direct muscarinic stimulation.

Variability in segmental response to lipid lowering may reside in other factors that control vasomotor tone, including shear stress, catecholamines, bradykinin, endothelin-1 and platelet-derived products (19). Isolation of these factors in a clinical investigation is difficult and was not attempted in this protocol. Nevertheless, assuming that coronary blood flow remains constant, one can deduce that basal vasodilation would serve to reduce vessel curvature and diminish shear forces along the wall and in surrounding segments (20,21). This might explain, in part, the reduced dilation with lipid lowering in those segments that initially manifested a more normal response to Ach challenge.

Improvement of the vasomotor response to lipid lowering. The current evidence is compelling that lipid lowering is associated with a net improvement in coronary artery dimensions and the vasomotor response to a drug that stimulates the endothelial-derived relaxation factor, nitric oxide (NO). Several explanations for this improvement appear tenable: 1) reduced potentiation of factors that inactivate endothelial-derived relaxation factor, such as oxidized LDL or its by-products (22–25), 2) an effect of antioxidants (9) or 3) an up-regulation of endothelial nitric oxide synthase (eNOS) as a direct effect of drug treatment (26).

Ox-LDL. Markers of ox-LDL correlated significantly in this study with the response to Ach. Other investigations support an important role for ox-LDL in endothelial dysfunction. Oxidized LDL adversely affects NO production and activity in vascular ring preparations (27,28) in endothelial cells (22,23) and in platelets (24). Anderson et al. measured the susceptibility of LDL to oxidation using the lag phase of conjugated diene formation induced by Cu^{++} and found that the vasodilator response to Ach related significantly to the susceptibility of LDL to oxidation (25). A reported significant ($p = 0.023$) correlation between oxidized LDL levels and dipyridamole-induced coronary flow reserve, as measured by positron emission

tomography, supports this conclusion (29). Tamai et al. (30) reported improved Ach-induced vasodilatation in the forearm with acute LDL apheresis. In these subjects there was a significant inverse correlation between plasma LDL (as well as oxidized LDL) and the maximal concentration in the venous effluent of the forearm of NO_x (a rapidly oxidized, in vivo product of NO) measured before and after Ach administration. Other reports suggest a relationship between autoantibodies of ox-LDL and progression of atherosclerosis (9) as well as risk of myocardial infarction (31).

In the E06 assay utilized here, the murine monoclonal antibody recognizes an oxidized phospholipid epitope on ox-LDL. The number of E06 epitopes per LDL particle was related significantly to the severity of endothelial dysfunction and in the post-therapy study was the single most powerful independent risk factor, accounting for fully 22% of the variance. Because this is a direct assay for an epitope of early stages of ox-LDL, it provides further strong evidence that oxidation of LDL is an important variable influencing abnormal coronary vasomotion in patients with atherosclerosis.

Antioxidants. There is also evidence that ascorbic acid, a vitamin antioxidant, reverses endothelial dysfunction in patients with coronary heart disease (32) and probucol, a potent antioxidant as well as lipid-lowering agent, improves endothelial dysfunction over and above that produced by lipid lowering itself (3). Other investigations, however, have shown no improvement in endothelial dysfunction by either a panel of antioxidant vitamins or superoxide (33). We found no significant correlation between improvement in response to Ach and patient use of antioxidants.

Up-regulation of eNOS. Lauf et al. (26) reported that inhibition of endothelial HMG CoA reductase by simvistatin and lovastatin directly upregulates eNOS, predominantly by post-transcriptional mechanisms. This would suggest that lipid lowering might not be integral to the salutary effect of statins. It may also help explain either the absence or the lack of a strong statistical correlation between changes in serum levels of either total cholesterol or LDL and the improvement in vasomotor response to Ach.

Study limitations. This study examined the vasomotor response of a single main artery in the total coronary arterial tree. A different conclusion might be drawn if all branches were examined in the territory of the artery into which Ach was infused or if all coronary arteries were examined. However, the accuracy of quantitative angiography is suboptimal when very small vessels are measured (34). In our own phantom studies, the accuracy and precision of measurement declines significantly when conduits are 0.6 mm or less in diameter (35). Likewise, optimal angiographic projection for all subbranches is technically challenging, even when biplane views are used. Infusion of Ach in all coronary arteries has not been performed due to the need for separate left and right coronary infusions.

Conclusions. We conclude that, in patients with hypercholesterolemia, lipid-lowering therapy improves endothelial function and causes a small, but measurable, dilation of basal coronary artery dimensions. Nevertheless, the response to Ach is complex, with a heterogeneous pattern of increased diameter in some segments and reduction in others. This pattern underscores the importance of inclusion of the entire analyzable artery when studying coronary vasomotion. Investigations that focus on responses from isolated segments of an artery, especially serial studies that include only segments that demonstrate a visually detectable response to Ach at baseline, would be vulnerable to erroneous or exaggerated conclusions. Despite the variability in segmental vasomotion to Ach challenge, the aggregate coronary artery response correlated highly with markers of plasma ox-LDL, reinforcing its pathogenetic role in the endothelial dysfunction associated with atherosclerosis.

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REFERENCES

1. Leung W, Lau C, Wong C. Beneficial effect of cholesterol-lowering therapy on coronary and endothelium-dependent relaxation in hypercholesterolemic patients. Lancet 1993;34:11496–500.
2. Treasure CB, Klein J, Weintraub W, et al. Beneficial effects of cholesterol-lowering therapy on the coronary endothelium in patients with coronary artery disease. N Engl J Med 1995;332:481–7.
3. Anderson T, Meredith I, Yeung A, Frei B, Selwyn A, Ganz P. The effect of cholesterol-lowering and antioxidant therapy on endothelium-dependent coronary vasomotion. N Engl J Med 1995;332:488–93.
4. Egashira K, Hirooka Y, Kai H, et al. Reduction in serum cholesterol with pravastatin improves endothelium-dependent coronary vasomotion in patients with hypercholesterolemia. Circulation 1994;89:2519–24.
5. Seiler C, Suter TM, Hess OM. Exercise-induced vasomotion of angiographically normal and stenotic coronary arteries improves after cholesterol-lowering drug therapy with bezafibrate. J Am Coll Cardiol 1995;26:1615–22.
6. Penny W, Rockman H, Bhargava M, et al. Heterogeneity of vasomotor response to acetylcholine along the human coronary artery. J Am Coll Cardiol 1995;25:1046–55.
7. El-Tamimi H, Mansour M, Wargovich TJ, et al. Constrictor and dilator responses to intracoronary acetylcholine in adjacent segments of the same coronary artery in patients with coronary artery disease: endothelial function revisited. Circulation 1994;89:45–51.
8. Lipid Research Clinics Program: Manual of Laboratory Operations, Volume 1, Edition 2, Revised 1982: Lipid and Lipoprotein Analysis. U.S. Department of Health, Education and Welfare publication No. (NIH) 76-628. Washington, DC: U.S. Government Printing Office.
9. Salonen JT, Ylä-Herttuala S, Yamamoto R, et al. Autoantibody against oxidized LDL and progression of carotid atherosclerosis. Lancet 1992;339:883–7.
10. Palinski WS, Ylä-Herttuala S, Rosenfeld ME, et al. Antisera and monoclonal antibodies specific for epitopes generated during the oxidative modification of low-density lipoprotein. Arteriosclerosis 1990;10:325–35.
11. Habeeb AFSA. Chemical evaluation of conformational differences in

native and chemically modified proteins by trinitrobenzenesulfonic acid. Biochim BioPhys Acta 1966;15:440–54.
12. Steinbrecher UP, Witztum JL, Parthasarathy S, Steinberg D. Decrease in reactive amino groups during oxidation or endothelial modification of LDL: correlation with changes in receptor-mediated catabolism. Arteriosclerosis 1987;7:135–43.
13. Palinski W, Hörkkö S, Miller E, et al. Cloning of monoclonal autoantibodies to epitopes of oxidized lipoproteins from Apo-E-deficient mice: demonstration of epitopes of oxidized LDL in human plasma. J Clin Invest 1996;98:800–14.
14. Hörkkö S, Miller E, Dudl E, et al. Antiphospholipid antibodies are directed against epitopes of oxidized phospholipids: recognition of cardiolipin by monoclonal antibodies to epitopes of oxidized low-density lipoprotein. J Clin Invest 1996;98:815–25.
15. Dijkstra EW. A note on two problems in connection with graphs. Numer Math 1959;269–72.
16. Gibson CM, Sandor T, Stone PH, Pasternak RC, Rosner B, Sacks FM. Quantitative angiographic and statistical methods to assess serial changes in coronary luminal diameters and implications for atherosclerosis regression trials. Am J Cardiol 1992;69:1286–90.
17. Bryk A, Raudenbush S, Congdon R. Hierarchical Linear Models: Applications and Data Analysis Methods. Newbury Park (CA): Sage Publications, 1992.
18. Blankenhorn DH, Azen SP, Kramsch DM, et al. Coronary angiographic changes with lovastatin therapy. The Monitored Atherosclerosis Regression Study (MARS). Ann Int Med 1193;119:969–76.
19. Drexler H. Endothelial dysfunction: clinical implications. Prog Cardiovasc Dis 1997;39:287–324.
20. Kanai AJ, Strauss HC, Truskey GA, Crews AL, Grunfeld S, Malinski T. Shear stress induces ATP-independent transient nitric oxide release from vascular endothelial cells, measured directly with a porphyrinic microsensor. Circ Res 1995;77:284–93.
21. Niebauer J, Cooke JP. Cardiovascular effects of exercise: role of endothelial shear stress. J Am Coll Cardiol 1996;28:1652–60.
22. Chin JH, Azhar S, Hoffman BB. Inactivation of endothelial derived relaxing factor by oxidized lipoproteins. J Clin Invest 1992;89:10–8.
23. Liao JK, Shin WS, Lee WY, Clark SL. Oxidized low-density lipoprotein decreases the expression of endothelial nitric oxide synthase. J Biol Chem 1995;270:319–24.
24. Chen LY, Mehta P, Mehta JL. Oxidized LDL decreases L-arginine uptake and nitric oxide synthase protein expression in human platelets: relevance of the effect of oxidized LDL on platelet function. Circulation 1996;93:1740–6.
25. Anderson TJ, Meredith IT, Charbonneau F, et al. Endothelium-dependent coronary vasomotion relates to the susceptibility of LDL to oxidation in humans. Circulation 1996;93:1647–50.
26. Laufs U, La Fata V, Plutzky J, Liao JK. Upregulation of endothelial nitric oxide synthase by HMG CoA reductase inhibitors. Circulation 1998;97:1129–35.
27. Simon BC, Cunningham LD, Cohen RA. Oxidized low-density lipoproteins cause contraction and inhibit endothelium-dependent relaxation in the pig coronary artery. J Clin Invest 1990;86:75–9.
28. Kugiyama K, Kerns SA, Morrisett JD, Robert R, Henry PD. Impairment of endothelium-dependent relaxation by lysolecithin in modified low-density lipoproteins. Nature 1990;344:160–2.
29. Raitakari OT, Pitkänen O-P, Lehtimäki T, et al. In vivo low-density lipoprotein oxidation related to coronary reactivity in young men. J Am Coll Cardiol 1997;30:97–102.
30. Tamai O, Matsuoka H, Itabe H, Wada Y, Kohno K, Imaizumi T. Single LDL apheresis improves endothelium-dependent vasodilatation in hypercholesterolemic humans. Circulation 1997;95:76–82.
31. Puurunen M, Manttari M, Manninen V, et al. Antibody against oxidized low-density lipoprotein predicting myocardial infarction. Arch Intern Med 1994;154:2605–9.
32. Levine GN, Frei B, Koulouris SN, Gerhard MD, Keaney JF, Vita JA. Ascorbic acid reverses endothelial vasomotor dysfunction in patients with coronary heart disease. Circulation 1996;93:1107–13.
33. Gilligan DM, Sack MN, Guetta V, et al. Effect of antioxidant vitamins on low-density lipoprotein oxidation and impaired endothelium-dependent vasodilation in patients with hypercholesterolemia. J Am Coll Cardiol 1994;24:1611–7.
34. Reiber JHC, Van Der Zwet PMJ, Von Land CD, et al. Advances in quantitative coronary arteriography. In: Reiber JHC, Serruys PW. Quantitative Coronary Arteriography: Equipment and Technical Considerations. Dordrecht/Boston/London: Kluwer, 1993.
35. Peterson KL. Angiography: role and limitation. In: Sigwart U, Bertrand M, Serruys PW, editors. Handbook of Cardiovascular Interventions. New York: Churchill Livingstone, 1996.