Coronary Atherosclerotic Wall Thickening and Vascular Reactivity in Humans

Elevated High-Density Lipoprotein Levels Ameliorate Abnormal Vasoconstriction in Early Atherosclerosis

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Background Abnormal vascular reactivity represents a fundamental disturbance in vascular biology with the development of atherosclerosis. Because endothelial vasodilator function plays a pivotal role in controlling vasomotor tone, we hypothesized that atherosclerotic wall thickening might directly interfere with deficient endothelium-mediated dilation and thereby contribute to the abnormal reactivity of atherosclerotic arteries in vivo.

Methods and Results In 26 patients without focal stenoses in the left anterior descending coronary artery, acetylcholine (0.036 to 3.6 μg/mL) was infused into the artery to evaluate endothelium-mediated vasodilation. Segmental vasomotor responses to acetylcholine were correlated with the local extent of atherosclerotic wall thickening quantitated by intracoronary ultrasound examination. Seventeen of the patients also underwent cold pressor testing to assess the vasoreactivity to sympathetic activation. The response of coronary artery segments to acetylcholine varied from 35% dilation to 52% constriction and demonstrated a segmental pattern, with dilation and constriction observed in different segments of the same artery. The vasomotor response to acetylcholine was closely correlated with the extent of local atherosclerotic wall thickening (r = −.82, P < .0001). Over the entire range of atherosclerotic wall thickening, segments from patients with elevated high-density lipoprotein (HDL) cholesterol serum levels (>75th percentile) demonstrated a significantly blunted constrictor response to acetylcholine (P < .01 at the maximal acetylcholine concentration) compared with segments from patients with HDL cholesterol <75th percentile. The degree of constriction or dilation in response to the acetylcholine infusion correlated with the response to cold pressor testing (r = .75, P < .0001). Again, the cold pressor test–induced constrictor response was significantly (P < .05) blunted in segments from patients with elevated HDL cholesterol serum levels compared with those from patients with HDL cholesterol <75th percentile despite equal degrees of atherosclerotic wall thickening.

Conclusions Coronary vasomotor responses to the endothelium-dependent dilator acetylcholine and to sympathetic stimulation by cold pressor test correlate with local atherosclerotic wall thickening. Thus, the degree of abnormal local vascular reactivity is closely related to the extent of atherosclerotic "plaque load" in human epicardial arteries in vivo. Elevated HDL cholesterol serum levels ameliorate abnormal vasoconstriction at any given extent of atherosclerotic wall thickening, suggesting that HDL cholesterol exerts a beneficial effect on abnormal vascular reactivity, a fundamental functional disturbance associated with coronary atherosclerosis.

Key Words • coronary artery disease • atherosclerosis • ultrasound • endothelium • cholesterol

Abnormal vascular reactivity represents a fundamental disturbance in vascular biology with the development of atherosclerosis. Experimental studies have demonstrated the importance of the vascular endothelium in controlling arterial tone1,2 and its role in mediating abnormal constrictor responses in the presence of atherosclerosis.3-5 We and others have shown in patients studied during cardiac catheterization that the disturbance of vasomotor function by atherosclerosis leads to a paradoxical constriction of coronary arteries in response to cold pressor testing.6,7 Exercise,8 pacing,9 mental stress,10 and intracoronary thrombus formation.11 These studies also demonstrated that the abnormal constrictor responses correlated with independent local evidence of endothelial vasodilator dysfunction, suggesting that endothelial dysfunction may also be important in the pathogenesis of abnormal coronary artery constriction in atherosclerotic human arteries in vivo. In addition, we demonstrated a progressive impairment in endothelial vasoactive function with angiographic evidence of progression of the atherosclerotic disease process.12

However, radiographic visualization of atherosclerosis merely reflects luminal encroachment by the atherosclerotic plaque and therefore is not suitable to use to assess the extent of early stages of atherosclerosis. In contrast, the evaluation of arterial wall architecture and circumferential dimensions by intravascular ultrasound allows for a more precise quantitation of atherosclerotic "plaque load" in vivo in the intact human coronary circulation.13

Thus, the purpose of the present study was to investigate how atherosclerotic plaque load locally interferes with coronary arterial vasoreactivity. Our hypothesis was that dysfunction of the endothelium might be related to the extent of atherosclerosis and thereby contribute to the abnormal vasomotor responses of
atherosclerotic epicardial coronary arteries. In addition, we sought to identify variables that might affect the relation between atherosclerotic plaque load and local epicardial vasoreactivity.

Methods

Patient Population

Twenty-six patients undergoing routine diagnostic cardiac catheterization were prospectively studied. Prerequisite for inclusion in the study was the absence of hemodynamically significant stenoses in the left anterior descending coronary artery (the vessel under study). In addition, the proximal part of the left anterior descending coronary artery had to be at least 3 mm in diameter to accommodate the ultrasound catheter without flow restriction. Patients with unstable angina, recent myocardial infarction, a clinical history suggestive of variant angina, valvular heart disease, clinical evidence of heart failure, and diabetes mellitus were excluded. Written informed consent was obtained from all patients before the study. The study protocol had been approved by the Ethical Committee of the University of Freiburg.

Study Protocol

Vasoactive medications, including calcium channel blockers, angiotensin-converting enzyme inhibitors, and long-acting nitrates, were withheld at least 24 hours before cardiac catheterization. No patient received β-adrenergic blockers within 48 hours before the study. Diagnostic left-side heart catheterization and coronary angiography were performed using a standard percutaneous femoral approach. After completion of the diagnostic catheterization, an additional 5000 U heparin IV was given, and an 8F guiding catheter was introduced into the left main coronary artery. A 2.7F infusion catheter was advanced into the proximal part of the left anterior descending coronary artery via a 0.014-in. guidewire.

Acetylcholine was selectively infused into the left anterior descending coronary artery via the infusion catheter to assess endothelium-dependent vasomotor responses of the left anterior descending coronary artery. Increasing doses of acetylcholine (0.036, 0.36, and 3.6 μg/mL) were infused at a rate of 2 mL/min, lasting 2 minutes for each concentration. The lowest dose of 0.036 μg acetylcholine/mL corresponds to an estimated blood concentration in the coronary bed of 10⁻⁸ mol/L, assuming a blood flow rate of 80 mL/min. Stepwise acetylcholine infusions were terminated either when vessel occlusion occurred or when the largest dose (3.6 μg/mL) was reached. After the maximal dose of acetylcholine, 0.25 mg nitroglycerin was injected into the left main stem via the guiding catheter to assess endothelium-independent vasodilator capacity of the epicardial artery under study. As in previous studies, intra-coronary infusion of acetylcholine and nitroglycerin in the doses used did not significantly affect the systemic hemodynamic parameters heart rate and blood pressure.

In 17 of the patients, before the acetylcholine infusion, a cold pressor test was performed by immersion of the left forearm into ice water for 90 seconds as previously reported. Throughout the study, heart rate and aortic pressure were continuously measured via the guiding catheter. Serial hand injections of nonionic contrast material were performed during the control period, after cold pressor testing, during a second recontrol period, at the end of each acetylcholine infusion, and after the injection of nitroglycerin. After the angiogram after nitroglycerin injection, the guidewire was reintroduced into the left anterior descending coronary artery to perform the intracoronary ultrasound examination.

Intracoronary Ultrasound Examination

The intracoronary imaging system includes a 30-MHz ultrasound transducer enclosed within an acoustic housing on the tip of a 4.3F flexible, rapid-exchange catheter (CVIS). The ultrasound beam is reflected against a mechanically driven rotating mirror creating a 360° cross-sectional image perpendicular to the catheter. Images are acquired at 30 frames per second and recorded on S-VHS videotape for subsequent off-line analysis.

The ultrasound catheter was advanced over the 0.014-in. guidewire into the midportion of the left anterior descending coronary artery. Then, the ultrasonic catheter was slowly retracted under combined intermittent fluoroscopic and continuous ultrasound guidance. Ultrasonic visualization of the take-off of sidebranches was used to identify the precise position of the ultrasound transducer, and the positions were documented by the ultrasound technician to relate ultrasound images to angiographic segments during off-line analysis. Special care was taken to maneuver the ultrasound catheter to a central and coaxial a position in the coronary artery as feasible, primarily by catheter rotation and guidewire movement. Ultrasonic gain settings were individually adjusted for optimal visualization of the lumen-intima interface and the outer boundary of the vessel wall by the highly echo-reflective adventitial layer.

Quantitative Coronary Angiography

The method of quantitative coronary angiography has been described. In brief, with a simultaneous biplane multidirectional isocentric radiograph system (Siemens Bicor), the coronary arteries under study were positioned near the isocenter, biplane cine-angiograms were recorded at a rate of 25 frames per second, and end-diastolic cine frames were video-digitized and stored in the image-analysis system (Mipron I, Kontron Electronics) in a 512x512 matrix with an 8-bit gray scale. With the 12-cm field of view, the resulting pixel density was 7.3 pixels per mm. Automatic contour detection was performed by a previously described and validated method using a geometrical edge-differentiation technique, and the exact radiological magnification factor of the measured segment was calculated to scale the data from pixels to millimeters. The accuracy and precision of this technique as well as the reproducibility of serial measurements under routine clinical conditions have been established in previous studies.

Quantitative measurements were performed in two distinct 5- to 8-mm-long straight segments distal to the tip of the infusion catheter. The length of the segment was chosen to be 5 to 8 mm to minimize measurement errors for serial measurements and to obtain an average diameter value in segments with luminal irregularities. Therefore, the number of segments to be analyzed was limited in general to two segments in the proximal and mid portions of the left anterior descending coronary artery per patient because the size of the arteries did not accommodate the ultrasound catheter without flow restriction in the more distal parts of the vessel. The segments had to be clearly defined in between the take-off of two sidebranches, which were used to identify the corresponding ultrasound images. Tapered or curved segments were excluded from the analysis. The arterial segments used for quantitative angiographic measurements were selected and analyzed by an observer who was unaware of the ultrasound appearance of the coronary artery. Whenever possible, measurements were performed in both views of the biplane images using the take-off of sidebranches as anatomic landmarks for identification of corresponding vessel segments, and the vessel's cross-sectional area was calculated from both views assuming an elliptical shape. Only single-plane analysis was performed for coronary segments demonstrating overlapping with other parts of the coronary tree in one view; in those cases (8 of 26 patients, 32%), vessel cross-sectional area was calculated assuming a circular shape.

Ultrasound Image Analysis

Ultrasound image analysis was performed by an independent observer who was unaware of the angiographic measure-
ments. Based on the protocol obtained during the examination at the time of cardiac catheterization, ultrasound images were selected by reviewing the video recordings and identification of the take-off of the sidebranches defining the vessel segment that was selected for quantitative angiography. Because the angiographic measurements were averaged along a 5- to 8-mm-long segment and intracoronary ultrasound provided a number of cross-sectional images along the entire length of the angiographically analyzed segment, the ultrasound video recordings of the defined vessel segment were carefully reviewed to select images for quantitative analysis. Because the majority of the selected vessel segments were angiographically smooth and because tapered and curved segments were excluded, the ultrasound images did not demonstrate major differences in luminal area or wall thickness along the length of an individual 5- to 8-mm-long segment used for angiographic analysis, although considerable wall thickening frequently could be observed despite the angiographically smooth appearance. Therefore, when no major qualitative differences in arterial wall thickness along the length of the selected segment could be detected during review of the video recordings, only a single ultrasound image was used for quantitative analysis. However, when review of the video recordings revealed variations in wall thickness along the length of the selected segment, two or more ultrasound frames were analyzed, and a mean value was calculated for the derived parameters. Ultrasound images with extensive fibrotic or calcific deposits obscuring details of the subjacent arterial wall were excluded from the analysis. The selected high-quality videotaped ultrasound sequences were digitized into a 512x512x8-bit matrix using an image-processing computer (Kontron) capable of digitization and storage of a series of 62 images, permitting at least two complete cardiac cycles to be digitized for each analyzed sequence. Review of the dynamic imaging sequence was routinely used to facilitate measurements in the frame with optimal delineation of the blood-intima border because a continuous border was not always visible along the entire circumference in an individual frame. The acoustic interface between the lumen and the intimal leading edge was traced manually to obtain the luminal cross-sectional area by planimetry, and the total arterial area was planimetered by tracing the leading edge of the adventitia. Absolute wall area was calculated as total arterial area minus lumen area. Area adjustment for magnification was performed, using a distance scale automatically recorded within each ultrasound image. To normalize for different vessel size, relative wall area was calculated as absolute wall area divided by total arterial area multiplied by 100, thus representing the atherosclerotic plaque load of an individual segment.

Intraobserver and interobserver variability measurements (evaluated by measuring two randomly selected frames from 14 patients by one observer at two separate times and once by two separate observers, respectively) revealed excellent reproducibility with an error of 4.9% (r=.95) for luminal area and 5.8% (r=.94) for total arterial area for intraobserver variability and 3.3% (r=.97) and 4.3% (r=.95), respectively, for interobserver variability. These data correspond exactly to previously published studies assessing reproducibility of quantitative ultrasound image analysis.15,17

Serum Lipid Analysis

Fasting venous blood was obtained before heparinization at the time of cardiac catheterization. Serum lipid levels were determined by established methods.18 High-density lipoprotein (HDL) cholesterol serum levels exceeding the 75th percentile adjusted for age were considered to be elevated.19

Statistical Analysis

All data are given as mean±SD unless otherwise stated. Statistical comparisons were made by ANOVA followed by the Student-Newman-Keuls test. Linear regression analysis was used to compare vasomotor responses to acetylcholine with relative arterial wall area. To assess the effects of HDL serum cholesterol levels >75th percentile on vasomotor responses to acetylcholine, regression models were fit according to Liang and Zeger20 using MACRO GEE (SAS Institute). An exchangeable correlation structure was used to adjust for several segments from one patient. Multivariate analysis using multiple stepwise regression techniques was performed to examine potential interactions among age, sex, total serum cholesterol level, low-density lipoprotein (LDL) and HDL serum cholesterol levels, smoking, and a history of hypertension on acetylcholine-induced vasomotor responses. Statistical significance was assumed if a null hypothesis could be rejected at the .05 probability level.

Results

Patient Characteristics

Twenty-six patients with a mean age of 53±7 years (range, 39 to 69 years), were enrolled in the study. Four patients were women, and 22 were men. Mean total serum cholesterol level was 243±38 mg%. Twelve patients had a history of arterial hypertension, and 8 patients were smokers. The left anterior descending coronary artery (the vessel under study) was angiographically smooth in 16 patients and demonstrated minor luminal irregularities (<30% luminal narrowing) in the remaining 10 patients. Left ventricular ejection fraction and left ventricular end-diastolic pressures were within normal limits in all patients, and no patients had evidence of valvular heart disease.

Responses of Epicardial Arteries to Acetylcholine

A total of 45 segments of the left anterior descending coronary artery from 26 patients were analyzed. From 22 patients, two segments of the left anterior descending coronary artery could be analyzed by angiography and ultrasound, whereas from 3 patients only 1 arterial segment was available for angiographic analysis, and from 1 patient, high-quality ultrasound images suitable for quantitative analysis could be obtained in only 1 segment. Three ultrasound examinations revealed significant calcification obscuring the subjacent arterial wall in the segments to be analyzed and therefore were excluded. The vasomotor response to the maximal dose of acetylcholine varied from 35% dilation to 52% constriction. The vasomotor response to acetylcholine was a local segmental phenomenon because both vaso-dilation and constriction were observed in different segments of the left anterior descending coronary artery from the same patient (Fig 1).

Responses to Acetylcholine and Atherosclerotic Wall Thickening

Intravascular ultrasound revealed a mean absolute wall area of 6.5±2.6 mm² and a mean total arterial area of 16.9±4.5 mm² in the 45 analyzed segments. The extent of atherosclerotic wall thickening, defined as relative wall area, ranged from 18% to 58%.

Fig 2 illustrates that the local response to acetylcholine correlated with the extent of local atherosclerotic wall thickening, suggesting that the degree of deficient endothelium-mediated dilatation is related to the extent of local atherosclerotic plaque load in human epicardial arteries in vivo. Mean relative wall area was 24.6±4.4% in the 11 dilating segments from 8 patients, whereas mean relative wall area was 42.8±8.7% in the 34 constricting segments (P<.001). There was a significant
correlation between the response to acetylcholine and atherosclerotic wall thickening at all three concentrations of acetylcholine ($r = -0.60, P < 0.0001$ at 0.036 $\mu g/mL; r = -0.75, P < 0.0001$ at 0.36 mg/mL; $r = -0.82, P < 0.0001$ at 3.6 $\mu g/mL$).

Multivariate analysis by multiple stepwise regression techniques revealed that relative wall area ($P < 0.0001$), HDL serum cholesterol level $>75$th percentile ($P < 0.0001$), LDL serum cholesterol levels ($P < 0.0001$), and a history of hypertension ($P < 0.05$) were all significant independent predictors of the acetylcholine-induced epicardial artery vasomotor response. In contrast, age, sex, total serum cholesterol levels, and smoking were not independently related to the epicardial vasoreactivity in response to acetylcholine (Table 1).

**TABLE 1. Independent Predictors of Acetylcholine-Induced Epicardial Artery Vasomotor Responses by Multivariate Analysis With Multiple Stepwise Regression Techniques**

<table>
<thead>
<tr>
<th>Variable</th>
<th>$P$</th>
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<tbody>
<tr>
<td>Relative wall area</td>
<td>.0001</td>
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<tr>
<td>HDL serum cholesterol $&gt;75$th percentile</td>
<td>.0001</td>
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<tr>
<td>LDL serum cholesterol level</td>
<td>.0001</td>
</tr>
<tr>
<td>History of hypertension</td>
<td>.012</td>
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<tr>
<td>Age</td>
<td>NS</td>
</tr>
<tr>
<td>Sex</td>
<td>NS</td>
</tr>
<tr>
<td>Total serum cholesterol level</td>
<td>NS</td>
</tr>
<tr>
<td>Smoking</td>
<td>NS</td>
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</tbody>
</table>

HDL indicates high-density lipoprotein; LDL, low-density lipoprotein.
TABLE 2. Patient Characteristics

<table>
<thead>
<tr>
<th></th>
<th>HDL &gt;75th percentile (n=11)</th>
<th>HDL &lt;75th percentile (n=15)</th>
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<tr>
<td>Age, y</td>
<td>52.7±4.0</td>
<td>53.3±9.4</td>
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<tr>
<td>Sex, female/male</td>
<td>3/8</td>
<td>1/14</td>
</tr>
<tr>
<td>Smoking, yes/no</td>
<td>3/8</td>
<td>5/10</td>
</tr>
<tr>
<td>Hypertension, yes/no</td>
<td>5/6</td>
<td>7/8</td>
</tr>
<tr>
<td>Total serum cholesterol, mg%</td>
<td>258.8±48.9</td>
<td>240.4±37.1</td>
</tr>
<tr>
<td>LDL serum cholesterol, mg%</td>
<td>178.4±28.1</td>
<td>167.5±29.6</td>
</tr>
<tr>
<td>HDL serum cholesterol, mg%</td>
<td>89.6±18.5</td>
<td>46.9±10.9*</td>
</tr>
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Ultrasound data

<p>| | |</p>
<table>
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<tbody>
<tr>
<td>Total arterial area, mm²</td>
<td>16.4±3.5</td>
</tr>
<tr>
<td>Absolute wall area, mm²</td>
<td>6.1±2.1</td>
</tr>
<tr>
<td>Relative wall area, %</td>
<td>37.5±11.1</td>
</tr>
</tbody>
</table>

HDL indicates high-density lipoprotein; LDL, low-density lipoprotein. Values are given as mean±SD.

*P<.001.

HDL Cholesterol Levels and Vasoreactivity

Eleven patients had HDL cholesterol levels >75th percentile adjusted for age in the present study group, whereas HDL cholesterol levels ≤75th percentile in 15 patients. Fig. 2 illustrates that the regression lines relating relative wall area to the response to acetylcholine were significantly (P<.001) different for segments from patients with HDL cholesterol levels <75th percentile compared with segments from patients with HDL cholesterol levels >75th percentile. Thus, over the entire range of atherosclerotic wall thickening, the vasoconstrictor responses to acetylcholine were significantly blunted in segments from patients with HDL cholesterol levels >75th percentile compared with segments from patients with HDL cholesterol levels <75th percentile.

The blunted constrictor response in patients with elevated levels of HDL cholesterol was further substantiated when the acetylcholine dose-response relation was evaluated separately for the two patient groups. Table 2 demonstrates that the two groups did not differ with respect to age, sex, smoking habits, history of hypertension, total serum cholesterol levels, or LDL serum cholesterol levels. In addition, intracoronary ultrasound–derived total arterial wall area, absolute wall area, and relative wall area were comparable in the two groups, indicating a similar extent of atherosclerotic plaque load (Table 2). However, the vasoconstrictor response to acetylcholine was considerably blunted in the segments from patients with elevated HDL cholesterol levels, achieving statistical significance at the 0.36 and 3.6 μg/mL concentrations (Fig 3). The segments from both groups dilated equally in response to nitroglycerin (22.2±20.7% and 23.4±17.5%, respectively), suggesting that there were no significant differences in vascular tone at baseline. There also were no significant differences in baseline angiographic luminal area for the two groups (7.4±3.1 and 7.4±3.9 mm², respectively).

Response to Cold Pressor Testing

The response to cold pressor testing was correlated with the vasomotor response to acetylcholine in all 23 segments available for analysis from the 17 patients who underwent both tests (Fig 4). Importantly, despite similar increases in the rate-pressure product (33.5±8.9% and 34.1±10.3%, respectively), cold pressor test–induced vasoconstriction was significantly greater in patients with HDL cholesterol levels <75th percentile (n=11) compared with the response in patients with HDL cholesterol levels >75th percentile (n=6; Fig 5). At the same time, relative wall thickening did not differ for the two groups (37.0±12.3% and 44.9±8.3%, respectively). Thus, despite equal atherosclerotic plaque load, coronary artery segments from patients with elevated HDL cholesterol levels exhibited a significantly blunted constrictor response not only to acetylcholine but also to cold pressor testing.

Fig 3. Bar graph of effects of acetylcholine on the luminal area of the constricting left anterior artery segments from patients with high-density lipoprotein (HDL) cholesterol serum levels >75th percentile (n=12) and HDL cholesterol serum levels <75th percentile (n=22). The change in luminal area is expressed as the percent change from baseline (±SEM). Acetylcholine induced a dose-dependent constrictor response of −4.8±2.3% at 0.036 μg/mL, −10.2±2.8% at 0.36 μg/mL, and −16.8±2.4% at 3.6 μg/mL in segments from patients with HDL >75th percentile and of −9.0±2.3%, −16.8±2.3%, and −33.3±3.2%, respectively, in segments from patients with HDL <75th percentile. The changes differed significantly among the two groups at the 0.36-μg/mL and at the 3.6-μg/mL acetylcholine concentrations (P<.05 and P<.01, respectively, by ANOVA).

Fig 4. Scatterplot of relation between the percent change in luminal area in response to cold pressor testing (CPT) and the change in response to acetylcholine (3.6 μg/mL) in the same epicardial artery segments. There was a significant association (P<.0001, r=.75) between the two responses.
Discussion

This study demonstrates that the local vasomotor response to acetylcholine, an agent used to assess endothelial vasodilator function, correlates with the extent of atherosclerotic plaque load. In addition, the response to acetylcholine is closely related to the local vasomotor response to sympathetic stimulation by cold pressor testing. These results suggest that the extent of atherosclerotic wall thickening is associated with the degree of deficient endothelium-mediated vasodilation and thereby contributes to the altered coronary vascular reactivity observed in early stages of atherosclerosis. Most important, elevated levels of HDL cholesterol appear to reduce the degree of vasoconstriction in response to both stimuli—acetylcholine and cold pressor test—at any given degree of atherosclerotic plaque load, indicating that HDL cholesterol ameliorates the local deficiency of endothelium-mediated vasodilation in coronary atherosclerosis.

Epicardial Artery Vasoreactivity and Wall Thickness

The results of the present study clearly demonstrate that deficient endothelium-mediated vasodilation is a local segmental phenomenon, because both dilation and constriction in response to acetylcholine were observed in different coronary artery segments from the same patients depending on the extent of atherosclerotic wall thickening. The correlation between the degree of local impairment in endothelium-dependent vasodilation and the extent of the atherosclerotic plaque load suggests that atherosclerotic wall thickening may constitute a physical or functional barrier to the endothelium-derived relaxing factor (EDRF) released on stimulation with acetylcholine. Interestingly, an almost identical relation between the extent of intimal proliferation and the magnitude of impairment in endothelium-dependent relaxation has been recently observed in a rabbit model of atherosclerosis. Once EDRF has been released from the endothelium, it traverses the subintimal space by diffusion to act on the smooth muscle cells in the media. EDRF, believed to be nitric oxide or a related compound, is a highly reactive compound with a very short half-life. In addition, EDRF is rapidly inactivated by superoxide anions and oxidized LDLs, both of which are present within the atherosclerotic vessel wall. Thus, the atherosclerotic plaque load not only might impose a physical barrier onto diffusion of EDRF but also may act as a functional barrier by directly inactivating EDRF. Two recent experimental findings strongly support this notion. First, it has been shown that the release of nitric oxide is not reduced but rather enhanced in diet-induced experimental atherosclerosis. Second, in situ hybridization of atherosclerotic arteries demonstrated normal or even increased expression of nitric oxide synthase mRNA in endothelial cells overlaying an atherosclerotic plaque. Thus, it appears that the deficient endothelium-dependent vasodilation observed in atherosclerosis is a consequence of increased inactivation of EDRF rather than of decreased EDRF production within the endothelial cell layer.

This assumption is also supported by our finding that increasing LDL serum cholesterol levels are independently related to an impaired epicardial vasodilator response to acetylcholine. Elevated LDL serum cholesterol levels have been implicated to play a significant role in the presence of oxidatively modified LDL within the atherosclerotic plaque. Thus, in addition to increased wall thickness due to the atherosclerotic plaque load itself, elevated LDL serum cholesterol levels appear to independently contribute to an enhanced constrictor response to acetylcholine, suggesting that additional functional mechanisms are involved. In contrast, increasing age is associated with increased wall thickness, even in angiographically normal human coronary arteries. Thus, when wall thickness is taken into account, increased age no longer represents an independent predictor of an abnormal vasomotor response to acetylcholine in epicardial arteries.

However, we cannot exclude that the correlation between atherosclerotic wall thickening and local acetylcholine response observed in the present study merely reflects the severity of the disease process affecting the endothelium. In addition, we cannot exclude the possibility that increased vasoconstriction with increasing wall thickness is due to the release of an endothelium-derived constricting factor or even due to increased constrictor activity of smooth muscle cells to the direct effects of acetylcholine. Nevertheless, regardless of the specific mechanisms involved, the results of the present study conclusively establish the usefulness of intracoronary infusion of acetylcholine as a diagnostic test to detect abnormal local vascular reactivity related to the extent of atherosclerotic plaque load in human epicardial arteries in vivo. However, in addition to plaque load, low HDL and elevated LDL serum cholesterol levels as well as a history of hypertension appear to be independent predictors of impaired epicardial vasodilator responses to acetylcholine, suggesting that these risk factors for coronary artery disease exert additional adverse effects on coronary artery endothelial vasodilator function.

Potential Mechanisms of Protective Action of HDL Serum Cholesterol

The association of elevated HDL cholesterol serum levels with a reduced degree of vasoconstriction in
response to both acetylcholine and cold pressor testing at any given extent of atherosclerotic wall thickening is a very intriguing finding. A relation between HDL cholesterol levels and the vasomotor response to acetylcholine has been recently suggested; however, because the extent of atherosclerosis was not assessed, interpretation of these results is limited. Recent in vitro studies have demonstrated that HDL cholesterol not only prevents oxidative modification of LDL in the extravascular microenvironment but also inhibits monocyte transmigration in cocultures of human arterial wall cells. Both monocyte-derived macrophages producing large amounts of superoxide anions and oxidatively modified LDL within the atherosclerotic arterial wall are highly potent inactivators of EDRF. Combining these experimental findings with the results of the present study, it is very intriguing to speculate that elevated levels of HDL cholesterol ameliorate the local deficiency in endothelium-mediated vasodilatation by providing a microenvironment within the atherosclerotic vessel wall that contains less oxidized LDL and superoxide-producing macrophages, resulting in diminished inactivation of EDRF as it traverses the vessel wall. In addition, HDL cholesterol has been shown to stimulate arterial endothelial cell prostacyclin synthesis and to acutely reverse the impairment of endothelium-dependent relaxation induced by oxidatively modified LDL, which also could contribute to the reduced constrictor response of atherosclerotic vessels.

### Clinical Implications

Numerous epidemiological studies have documented an inverse relation between HDL cholesterol levels and the incidence of atherosclerotic coronary artery disease. Patients with low levels of HDL cholesterol exhibit a significantly increased risk of developing atherosclerotic coronary events. An increase in HDL cholesterol levels was identified as the most important predictor of a favorable outcome with respect to a reduction in myocardial infarction rates after lipid-lowering therapy. The association of elevated HDL cholesterol levels with protection against coronary artery disease has generally been attributed to indicating the efficiency of reverse cholesterol transport involved in removing cholesterol from the atheroma. Importantly, recent lipid-modifying studies demonstrated a considerable reduction in the number of coronary events despite only minimal changes in coronary luminal diameters achieved by elevating HDL cholesterol levels, suggesting that functional mechanisms are also involved. The results of the present study identify such a functional mechanism, namely, amelioration of abnormal vascular reactivity, that might contribute to the beneficial effects of elevated HDL cholesterol levels on the incidence of atherosclerotic coronary events.

The correlation of vasomotor responses to cold pressor testing with those to the endothelium-dependent dilator acetylcholine extends previous published observations by our group and by Ganz and coworkers, suggesting that in atherosclerosis unopposed constriction caused by a local failure of endothelium-mediated dilation causes the coronary arteries to respond abnormally to sympathetic stimulation. Importantly, elevated HDL cholesterol levels also reduced the vasoconstrictor responses to cold pressor testing at any given degree of atherosclerotic plaque load, indicating that the beneficial effect of HDL cholesterol on coronary vasoreactivity is not a phenomenon specifically related to the mechanisms of action of acetylcholine but rather a generalized protective phenomenon.

In summary, results of the present study demonstrate that the degree of abnormal local vascular reactivity as a fundamental functional disturbance in vascular biology of atherosclerosis is closely related to the extent of atherosclerotic plaque load in human epicardial arteries in vivo. Elevated plasma levels of HDL cholesterol ameliorate abnormal vasoconstriction at any given degree of atherosclerosis. This protective effect of high HDL cholesterol levels on the functional disturbance associated with coronary atherosclerosis might contribute to the beneficial effects of HDL cholesterol in preventing atherosclerotic coronary events.

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