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Angiographically Silent Atherosclerosis Detected by Intravascular Ultrasound in Patients With Familial Hypercholesterolemia and Familial Combined Hyperlipidemia: Correlation With High Density Lipoproteins

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Objectives. **This study sought to evaluate the extent of atherosclerosis in coronary and iliac arteries in patients with heterozygous familial hypercholesterolemia or familial combined hyperlipidemia, using intravascular ultrasound imaging.**

Background. **Intravascular ultrasound imaging provides cross-sectional tomographic views of the vessel wall and allows quantitative assessment of atherosclerosis.**

Methods. **Forty-eight nonsmoking, asymptomatic patients with heterozygous familial hypercholesterolemia or familial combined hyperlipidemia underwent intravascular ultrasound imaging of the left anterior descending coronary, left main coronary and common iliac arteries. Angiography showed only minimal or no narrowing in these vessels. Intravascular ultrasound images obtained during catheter pullback underwent morphometric analysis. Plaque burden was expressed as the mean and maximal intimal index (ratio of plaque area and area within the internal elastic lamina) and as the percent of vessel surface covered by plaque.**

Results. **Intravascular ultrasound detected plaque more frequently than angiography in the left anterior descending (80% vs. 29%, respectively), left main (44% vs. 16%) and iliac arteries (33% vs. 27%). Plaque burden was higher in the left anterior descending (mean intimal index [±SD] 0.25 ± 0.16) than in the left main (0.11 ± 0.16, $p < 0.001$) and iliac arteries (0.02 ± 0.04, $p < 0.001$). Angiography detected lumen narrowing only in coronary arteries with a maximal intimal index ≥0.42 (left anterior descending artery) and ≥0.43 (left main artery). The area within the internal elastic lamina increased with plaque area in the left anterior descending ($r = 0.82$, $p < 0.001$) and left main arteries ($r = 0.53$, $p < 0.001$). By stepwise multiple regression analysis, the strongest predictor for plaque burden in the left anterior descending artery was the level of high density lipoprotein (HDL) cholesterol and total/HDL cholesterol ratio for the left main artery.**

Conclusions. **In patients with heterozygous familial hypercholesterolemia and familial combined hyperlipidemia, extensive coronary plaque is present despite minimal or no angiographic changes. Compensatory vessel enlargement and diffuse involvement with eccentric plaque may account for the lack of angiographic changes. Levels of HDL cholesterol and total/HDL cholesterol ratio are far more powerful predictors of coronary plaque burden than are low density lipoprotein cholesterol levels in these patients with early, asymptomatic disease.**

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Contrast angiography is the standard technique for detection and quantification of atherosclerotic plaque in humans. Most of our contemporary understanding of the relation of various risk factors to plaque burden in the coronary arteries is derived from this technique (1–4). Angiography has also been used in regression trials to monitor the response of plaque to various therapies (5–8). However, angiography is at best an indirect measure of atherosclerotic plaque because it visualizes the silhouette of the vessel lumen. Antemortem (9,10) and postmortem (11–13) studies have shown only limited correlation between angiographic and histologic extent of atherosclerotic plaque.

Intravascular ultrasound is a new technique that provides cross-sectional tomographic views of vessels, allowing reliable measurement of lumen and wall dimensions (14–19).

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In the present study, we used intravascular ultrasound to image coronary and peripheral arterial segments in which only minimal or no atherosclerosis was shown by angiography. The patients had a high likelihood of atherosclerosis because they had heterozygous familial hypercholesterolemia or familial combined hyperlipidemia. Intravascular ultrasound was used to 1) determine the extent of coronary and peripheral atherosclerotic plaque burden; and 2) correlate the extent of atherosclerotic plaque with lipoprotein variables and other risk factors.

Methods

Patients. Forty-eight patients (32 men, 16 women; mean [±SD] age 48 ± 12 years, range 29 to 70) with heterozygous familial hypercholesterolemia or familial combined hyperlipidemia were recruited from the Lipid Clinic of the University of California, San Francisco. The diagnosis of heterozygous familial hypercholesterolemia was based on the presence of tendon xanthomas in 29 patients or a first-degree relative and a mean serum level of low density lipoprotein (LDL) cholesterol and total triglycerides of 284 ± 59 mg/dl and 166 ± 81 mg/dl, respectively. Heterozygous familial combined hyperlipidemia was diagnosed in 19 patients on the basis of mean serum levels of LDL cholesterol and total triglycerides of 213 ± 98 mg/dl and 223 ± 98 mg/dl, respectively, in the absence of tendon xanthomas. A family history could be obtained in 18 of the 19 patients. In each of these kindreds, at least one similarly affected person was identified. Patients with secondary hyperlipidemia and those homozygous for apolipoprotein E-2 were excluded. All patients underwent angiography and intravascular ultrasound imaging of coronary and iliac arteries. A complete medical history, including risk factors and use of medication, was obtained. All patients were asymptomatic at the time of the study and had no prior history of coronary disease. Systolic and diastolic blood pressures were averaged from measurements during three different visits. All patients gave written informed consent to participate in the study, and the University of California, San Francisco, Committee on Human Research approved the study.

Angiography. Coronary angiography was performed through the right femoral artery using 7F catheters. Cineangiograms were filmed at 60 frames/s through a lens with a focal length of 135 mm, with an X-ray field of 15 cm. Multiple pairs of perpendicular views (90°) of the left and right coronary arteries were obtained. For arteriography of the iliac arteries, a 7F pigtail catheter was placed in the infrarenal abdominal aorta. A cineangiogram in the anteroposterior view was obtained by injecting 30 ml of contrast media at 15 ml/s using a pressure injector. Films were viewed at ×5 magnification (Vanguard Instruments) by two experienced angiographers (T.A.P., P.G.Y.). The left main coronary, left anterior descending coronary and common iliac arteries were evaluated for the presence of lumen narrowing and calcium deposits in the vessel wall.

Intravascular ultrasound imaging. Intravascular ultrasound imaging was performed using a commercially available imaging system (Cardiovascular Imaging Systems) with a catheter size of 4.3F and a center frequency of 30 MHz. The theoretic resolution of the transducer was ~80 μm in the axial direction and ~150 μm in the lateral direction. Ultrasound was performed only when angiography showed only mild or no coronary narrowing (<40% lumen diameter reduction) in the left main, left anterior descending and iliac arteries. The decision to perform intravascular ultrasound imaging was based on the estimation of lesion severity during the procedure. An 8F guiding catheter was used to advance the intravascular ultrasound catheter over a 0.014-in. guide wire into the midportion of the left anterior descending artery; the catheter location was controlled by fluoroscopy and angiography. The overall gain, contrast and reject settings of the ultrasound scanner were optimized to obtain well balanced gray scales. Using a motorized, timed pullback device, the intravascular ultrasound catheter was withdrawn at 1 mm/s until the imaging element reached the guiding catheter. For imaging of the iliac artery, the catheter was placed proximal to the aortic bifurcation and pulled back in the same manner. The intravascular ultrasound imaging procedure added 10 to 15 min to the catheterization study. The images during pullback were acquired at 33-ms intervals and stored on 0.5-in. videotape. A standard electrocardiogram (ECG) (lead II) was recorded simultaneously.

The recordings were replayed on a video screen. Using branch points (septal and diagonal branches, circumflex artery) as references, the left main artery and the proximal portion of the left anterior descending artery were identified on the ultrasound recordings. The common iliac artery was identified on the ultrasound recording with reference to the aortic bifurcation and the branch point of the internal iliac artery. Only images obtained ≥5 s after the start of the pullback were included in the analysis because of potential "slack" in initiating catheter movement. The end-diastolic frames were identified by the beginning of the QRS complex on the ECG; only segments with ≥10 end-diastolic images/segment were included. The images were digitized in 8 bits (RasterOps) in a 300 × 300-pixel matrix and stored on computer disk.

Analysis of ultrasound images. The digitized frames were recalled from memory, and morphometric analysis was performed using custom-developed software for geometric computations. Calibration was accomplished by cross-hair markers on the video images. The vessel wall was defined as normal when no three-layered appearance could be detected or when a three-layered appearance was seen on the ultrasound image, but the intima consisted only of a thin line on the ultrasound image with a thickness <200 μm (20). The internal elastic lamina was defined as the inner border of the echolucent zone in wall segments with three-layered appearance or as the border between lumen and vessel wall in segments without three clear layers (Fig. 1). The lumen border and the internal elastic lamina were traced using a cursor-controlled planimeter, and the areas were calculated. The plaque area was

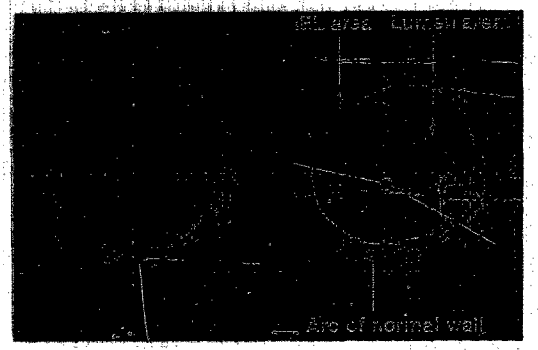


Figure 1. Morphometric measurements of intravascular ultrasound images. Left, Original ultrasound image. Right, Delineation of lumen, plaque and normal vessel wall using cursor-driven computer software. The following values were obtained: lumen area = 15.2 mm^2; plaque area = 9.8 mm^2; intimal index = 0.36; arc of normal wall = 166°; eccentricity index = 3.1. IEL = internal elastic lamina.

determined as the difference between lumen area and area within the internal elastic lamina. When plaque did not involve the entire wall circumference, each end of the nondiseased wall arc was identified, and the angle from the geometric center of the vessel lumen was calculated (12) (Fig. 1). Maximal and mean intimal thicknesses were calculated from 360 equally spaced radial measurements with the geometric lumen center as a reference. Calcium was identified as an abnormally bright ultrasound region with a corresponding shadow in the periphery; each end of the calcified segment was identified, and the angle was calculated. In segments with a calcium arc >60°, the internal elastic lamina was not traced because of potential inaccuracy due to the shadowing. In segments with more than one area of calcium, only those with >90° separation between the deposits were included. A plaque eccentricity index was determined as the ratio between maximal and mean intimal thickness (21). The *intimal index* was calculated as Intimal index = (Plaque area/Total area within the internal elastic lamina including lumen). To determine interobserver and intraobserver variabilities, one ultrasound image was randomly selected from each patient and measured by two independent observers.

The measurements of all individual end-diastolic images from a segment were pooled to derive variables describing the plaque burden of the entire segment comparable to previous pathoanatomic studies (22). Due to the pullback speed of 1 mm/s, the end-diastolic images considered for measurements were spaced ~1 mm, depending on the patient's heart rate. The *mean intimal index* was averaged from the intimal index of all individual cross sections in the segment; this variable is equivalent to the ratio between plaque volume and total vessel volume (e.g., a plaque volume index). The *maximal intimal index* within each vessel segment was identified and considered a variable for the maximal severity of atherosclerosis. On the basis of the relative extent of diseased and nondiseased wall circumference in each individual cross section, the *percent plaque surface area* (wall area covered with plaque) was calculated for the segment (23). Similarly, the *wall area covered with calcified plaque* was calculated from the calcium arc in the individual images.

Table 1. Baseline Characteristics of 48 Study Patients

Men	32 (66)
Age (yr)	48 ± 12
Heterozygous FH	29 (60)
FCH	19 (40)
Apolipoprotein E phenotype	
E-3/E-2	1 (2)
E-3/E-3	31 (65)
E-4/E-3	9 (19)
E-4/E-4	5 (10)
Unknown	2 (4)
Hypertension	1 (2)
Medication	
Beta-blockers	4 (8)
Estrogen	3 (6)
H_2-blockers	3 (6)

Data presented are mean value ± SD or number (%) of patients. FCH = familial combined hyperlipidemia; FH = heterozygous familial hypercholesterolemia.

Lipid measurements. Blood was drawn after a 12-h fast. Serum was separated at room temperature for determination of cholesterol and triglyceride levels. Cholesterol and triglyceride levels were measured for very low, LDL and high density lipoprotein (HDL) cholesterol after separation by ultracentrifugation (24). Analyses were standardized against reference material supplied by the National Center for Disease Control. Agarose gel electrophoresis was performed by the method of Pagnan et al. (25), and the apolipoprotein E phenotype was determined by isoelectric focussing. Apolipoprotein(a) was assessed semiquantitatively as the pre-beta-lipoprotein in the 1.063-g/cm^3 infranatant in a modification of the method of Rhoads et al. (26).

Statistical analysis. For final statistical analysis, each individual patient was the unit of the analysis unless otherwise specified. Results are expressed as mean value ± SD. Univariate regression analysis was used to compare the relation between risk factors [age; gender; total, LDL and HDL cholesterol; triclycerides; total/HDL cholesterol ratio; lipoprotein(a)] and extent of atherosclerotic plaque. Indexes for the extent of plaque were mean and maximal intimal indexes and plaque surface area. In addition, stepwise multiple regression analyses (maximum likelihood ratio) were performed to assess the relative contribution of these risk factors (independent variables) to each of the indexes of plaque burden (dependent variables). Significance was assumed at $p < 0.05$. Intraobserver and interobserver variabilities for the morphometric measurements were determined using Pearson's correlation.

Results

Patients. Baseline characteristics of the 48 patients are shown in Table 1. Risk factors other than hyperlipidemia were

Table 2. Baseline Levels of Serum Lipoproteins

	Familial Hypercholesterolemia (mean ± SD)	Familial Combined Hyperlipidemia (mean ± SD)
Cholesterol		
Total	369 ± 61	302 ± 45
HDL	55 ± 18	56 ± 15
VLDL	26 ± 21	33 ± 22
LDL	284 ± 59	213 ± 34
Triglycerides		
Total	166 ± 81	223 ± 98
HDL	19 ± 8	37 ± 48
VLDL	91 ± 60	146 ± 84
LDL	46 ± 15	46 ± 19

HDL = high density lipoprotein; LDL = low density lipoprotein; VLDL = very low density lipoprotein; other abbreviations as in Table 1.

rare in this cohort. One patient was hypertensive (diastolic blood pressure ≥90 mm Hg), and four others were normotensive with antihypertensive agents. No patient had diabetes mellitus or other reasons for secondary hyperlipidemia. Four patients had stopped smoking ≥8 years before the study, and no patient was a smoker at the time of the study. Levels of lipoprotein(a) were elevated in three patients (10%) with familial hypercholesterolemia and seven patients (37%) with familial combined hyperlipidemia (Table 2).

Angiographic data were obtained for coronary arteries in all 48 patients and for iliac arteries in 45 patients (92%). Lumen narrowing was present in the left anterior descending coronary artery of 17 patients (35%), the left main coronary artery of 4 (8%) and the common iliac artery of 12 (27%) (Fig. 2). Calcium deposits were observed in the left anterior descending artery of nine patients (19%), the left main artery of three patients (6%) and the iliac artery of four (9%) patients.

Intravascular ultrasound imaging. Ultrasound images of the left main coronary artery were available for 36 patients, left anterior descending coronary artery in 35 and common iliac artery in 45. In 10 patients, intravascular ultrasound imaging of the coronary arteries was not performed because of advanced

Figure 2. Incidence of lumen narrowing detected by angiography (ANGIO) and incidence of atherosclerotic plaque detected by intravascular ultrasound (IVUS).

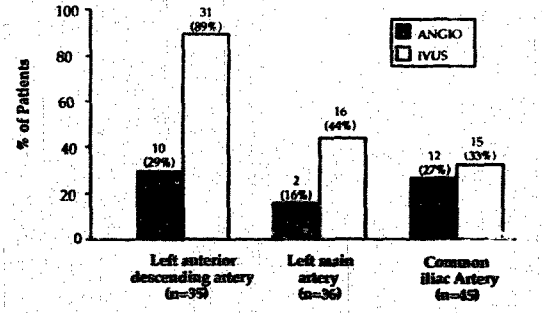


Table 3. Morphometric Measurements of Atherosclerotic Plaque From Intravascular Ultrasound Imaging (mean ± SD)

Variable	LAD (n = 35)	LMCA (n = 36)	Common Iliac Artery (n = 45)
Mean intimal index	0.25 ± 0.16	0.11 ± 0.16	0.02 ± 0.04
		$p < 0.001$	$p < 0.001$
		$p < 0.001$	
Maximal intimal index	0.32 ± 0.19	0.12 ± 0.16	0.04 ± 0.06
		$p < 0.001$	$p < 0.001$
		$p < 0.001$	
Plaque surface area (%)	54 ± 39	33 ± 40	6 ± 18
		$p < 0.05$	$p < 0.001$
		$p < 0.001$	
Calcium plaque surface area (%)	3.9 ± 7.6	2.7 ± 7.7	3.3 ± 4.1
		NS	NS
		NS	

LAD = left anterior descending coronary artery; LMCA = left main coronary artery.

disease (6 patients) or image quality that was insufficient for analysis (4 patients). In one patient, only images of the left main artery were obtained because of an advanced lesion in the proximal left anterior descending artery. The mean number of individual intravascular ultrasound images analyzed for each segment was 11 ± 3 (range 10 to 18) for the left main artery 12 ± 4 (range 10 to 21) for the left anterior descending artery and 44 ± 14 (range 30 to 56) for the common iliac artery. Intraobserver and interobserver correlations between measurements were 0.96 and 0.91 for intimal index, 0.97 and 0.90 for plaque area, 0.87 and 0.91 for the arc of the nondiseased wall segment and 0.97 and 0.98 for the arc of calcium plaque, respectively. Vessel spasm was observed in 4 (10%) of 39 patients undergoing intravascular ultrasound imaging of coronary arteries and was immediately reversible after intracoronary application of nitroglycerin in all patients. No other complications occurred.

For all three vessels, atherosclerotic plaque was seen by intravascular ultrasound imaging in more patients than lumenal narrowing detected by angiography (Fig. 2). Mean and maximal intimal indexes as well as plaque surface area were higher for the left anterior descending than for the left main or common iliac artery (Table 3). The wall area covered with calcified plaque was not significantly different in the left main, left anterior descending and iliac arteries.

All patients with angiographic narrowing of the left anterior descending coronary artery had a maximal intimal index of ≥0.42 by ultrasound imaging; angiographic narrowing of the left main coronary artery was detected only in patients with a maximal intimal index ≥0.43 (Fig. 3). Figure 4 shows the ultrasound image of atherosclerotic plaque in an angiographically normal coronary artery. In the common iliac artery, patients with angiographically detected plaque had a maximal intimal index ≥0.11 (Fig. 3). All coronary or iliac segments

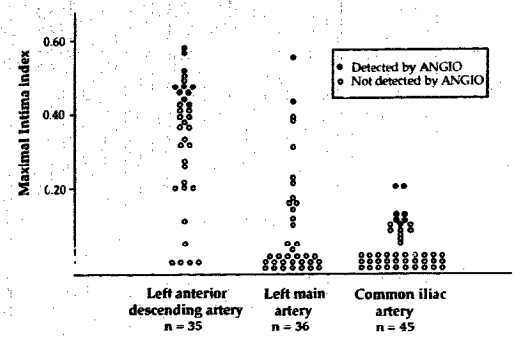


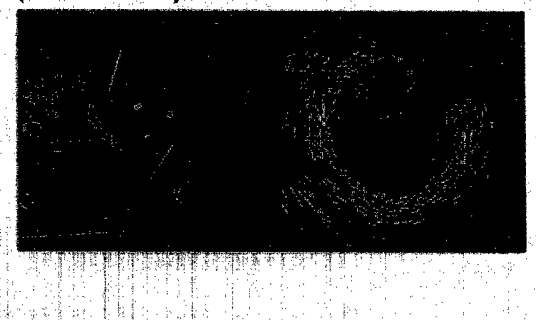
Figure 3. Maximal intimal index assessed by intravascular ultrasound in the left anterior descending coronary, left main coronary and common iliac arteries. ANGIO = angiography.

with a normal appearance on ultrasound imaging also appeared normal on angiography.

For the maximal diseased site in each coronary segment, the correlation between plaque area and the area within the internal elastic lamina as measured on the intravascular ultrasound images is shown in Figure 5. In the left anterior descending coronary artery, the area within the internal elastic lamina increased 1.34 mm^2 for every 1-mm^2 increase in plaque burden, suggesting that the vessel enlarges in response to plaque accumulation ($r = 0.82$, $p < 0.001$). Similar results were obtained for the left main coronary artery (Fig. 5); the area within the internal elastic lamina increased 0.71 mm^2 for every 1-mm^2 increase in plaque burden ($r = 0.53$, $p < 0.001$).

Intravascular ultrasound imaging showed that plaque accumulation was predominantly eccentric in the vessel. The eccentricity index in relation to the intimal index in the left anterior descending coronary artery for all 188 individual cross sections with plaque is shown in Figure 6. Although plaque eccentricity decreased with plaque burden, most cross sections showed eccentric disease (eccentricity index >2). Similar results were obtained for the relation between plaque eccentricity in the left main coronary artery and intimal index ($y = -0.050x + 3.89$, $r = 0.51$, $p < 0.01$).

Figure 4. Left, Normal angiographic appearance of the left anterior descending coronary artery in a patient with coronary atherosclerosis. **White line** indicates the plane of the corresponding intravascular ultrasound image. **Right,** Intravascular ultrasound image shows an eccentric plaque in the proximal left anterior descending artery (intimal index = 0.39).



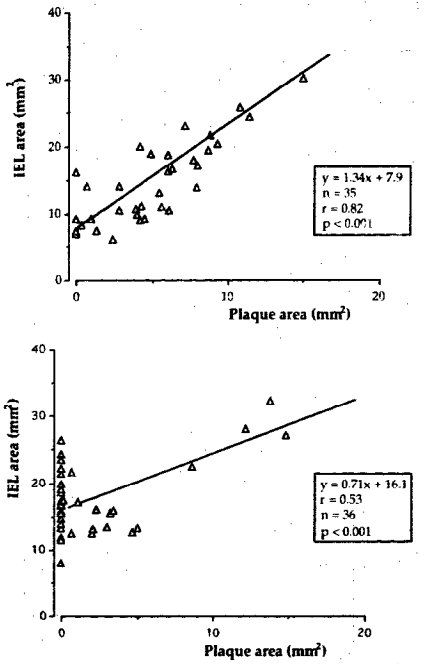


Figure 5. Plots of plaque area and area within the internal elastic lamina (IEL) for the left anterior descending **(top)** and left main coronary arteries **(bottom)**. Area of internal elastic lamina significantly increases with plaque area in both vessels.

Correlation with risk factors. *Univariate regression analysis.* Lipoprotein variables and other risk factors were compared with the extent of atherosclerotic plaque burden as determined

Figure 6. Plot of eccentricity and intimal indexes for the 188 cross-sectional ultrasound images obtained in the left anterior descending coronary artery. Some data points represent more than one data pair. Values for eccentricity index >2 **(dashed line)** indicate eccentric plaque.

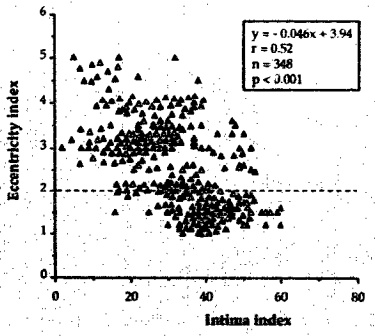


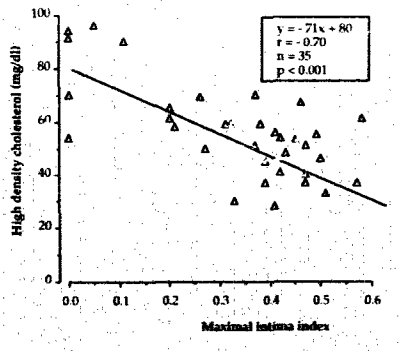
Table 4. Univariate Regression Analysis Between Risk Factors and Extent of Coronary Atherosclerosis Assessed by Intravascular Ultrasound Imaging

Variable	LAD			LMCA	
	Mean Intimal Index	Plaque Surface Area	Maximal Intimal Index	Mean Intimal Index	Plaque Surface Area
Age	−0.21	−0.14	−0.22	0.01	−0.07
Gender	−0.43*	−0.34†	−0.45*	−0.26	−0.31
Diastolic blood pressure	0.25	0.13	0.20	0.07	0.01
Total cholesterol	−0.11	0.01	−0.19	0.27	0.21
LDL cholesterol	−0.04	0.06	−0.11	0.24	0.23
HDL cholesterol	−0.70‡	−0.54*	−0.70‡	−0.28	−0.46*
Total/HDL cholesterol	0.47*	0.38†	0.49*	0.38†	0.56‡
Triglycerides	0.02	0.01	0.07	−0.06	0.02
Lp(a)	−0.10	0.04	0.13	0.05	0.05

*$p < 0.01$. †$p < 0.05$. ‡$p < 0.001$. Lp(a) = lipoprotein(a); other abbreviations as in Tables 2 and 3.

by intravascular ultrasound imaging (mean intimal index, plaque surface area, maximal intimal index). Plaque burden in the iliac arteries was not analyzed because of the small number of diseased vessels in this study. For the left anterior descending coronary artery, gender, HDL cholesterol level and total/HDL cholesterol ratio correlated significantly with mean and maximal intimal index and plaque surface area (Table 4). Figure 7 shows that the maximal intimal index correlates inversely with HDL cholesterol levels. Among the 12 patients with HDL cholesterol ≥60 mg/dl, 8 (66%) had only minimal or no disease (intimal index ≤0.2) compared with 2 patients (9%) with HDL cholesterol <60 mg/dl ($p < 0.001$). In the left main coronary artery, HDL cholesterol levels were significantly related to plaque surface area; the total/HDL cholesterol ratio was significantly related to plaque surface area as well as mean and maximal intimal indexes measured by intravascular ultrasound imaging (Table 4). Extent of atherosclerotic plaque burden as determined by intravascular ultrasound imaging was not significantly different in patients with familial hypercholesterolemia compared with patients with familial combined hyperlipidemia and in patients with normal compared with elevated levels of lipoprotein(a).

Figure 7. Plots of maximal intimal index for the left anterior descending coronary artery and levels of high density lipoprotein (HDL) cholesterol. Levels of HDL cholesterol inversely correlate with maximal intimal index.



Stepwise multiple regression analysis. The relative contribution of various risk factors to severity and extent of coronary atherosclerosis was analyzed. Age; gender; total, LDL and HDL cholesterol; triglycerides; total/HDL cholesterol ratio; and lipoprotein(a) were the independent variables. In the left anterior descending coronary artery, HDL cholesterol level was the only independent variable associated with maximal intimal index ($p < 0.001$), mean intimal index ($p < 0.001$) or plaque surface area ($p < 0.001$). In contrast to the results of univariate analysis, gender and total/HDL cholesterol ratio were not independently related to these markers of plaque burden. For the left main coronary artery, the total/HDL cholesterol ratio was the only independent predictor of maximal intimal index ($p < 0.05$), mean intimal index ($p < 0.001$) and plaque surface area ($p < 0.001$).

Discussion

The present study evaluated direct measurements of plaque burden in coronary and peripheral arterial segments with minimal or no angiographically detectable atherosclerosis. The study patients were asymptomatic but had a high likelihood of atherosclerosis because they had heterozygous familial hypercholesterolemia or familial combined hyperlipidemia. Intravascular ultrasound imaging revealed several important findings in these patients: 1) Extensive atherosclerosis can be present in angiographically normal coronary arteries; 2) compensatory enlargement of the vessels and diffuse involvement with predominately eccentric plaque are potential explanations for this lack of angiographic change; 3) HDL cholesterol levels and total/HDL cholesterol ratio are the strongest predictors of extent of coronary atherosclerotic plaque in this patient group.

Imaging of atherosclerotic plaque. Numerous studies have shown that antemortem (9,10) and postmortem angiography (11–13) provides a weak reflection of the histologic extent of atherosclerotic plaque. The discrepancies between angiographic and anatomic extent of atherosclerosis can be attrib-

uted to different mechanisms: 1) Compensatory mechanisms during plaque development cause vessel enlargement in relation to plaque growth. Lumen narrowing may be delayed until the plaque occupies 30% to 40% of the potential lumen (13,27). These findings may be explained in part by observations that vessels enlarge with increased flow velocity, tending to maintain normal wall shear stress. Localized, outward bulging of eccentric plaque has also been described, suggesting that this pattern of plaque growth may promote vessel expansion. Stiehl et al. (13) showed that vessel enlargement indeed accounts for angiographic underestimation of mild atherosclerotic plaque in vitro. 2) Angiographic detection of lumen narrowing is based on comparison of a "lesion" with a reference segment that is considered normal. Histologic (10) and epicardial echocardiographic (28) studies suggest that coronary arteries often develop diffuse atherosclerotic involvement, leading to angiographic underestimation of atherosclerotic plaque. 3) The media, the principal component of the nondiseased arterial wall, is substantially attenuated in advanced coronary atherosclerosis due to primary atrophy or intravasation of atherosclerotic plaque (29). This may facilitate local expansion of the vessel at sites of plaque accumulation.

Intravascular ultrasound imaging is a new method that provides cross-sectional, tomographic views of the vessel in vivo. This technique provides accurate quantification of lumen and wall dimensions (14–19) and allows assessment of plaque composition (17,30). We measured atherosclerotic plaque burden using morphometric analysis of intravascular ultrasound images. Morphometric techniques are based on the assumption that the amount of atherosclerotic plaque within a segment can be quantified by averaging tomographic measurements when random or equally spaced sections are considered (22). Images were sampled during continuous intravascular ultrasound catheter pullback through a segment and measurements averaged for the segment. This approach has several advantages: 1) The average ratio of plaque and lumen in the individual cross sections in a segment represents the ratio for plaque and lumen integrated in the entire segment. 2) It is not essential to know the precise location of the intravascular ultrasound catheter in the segment; this may be especially helpful when results of serial studies are to be compared. 3) Ratios between lumen and intimal areas were used in the present study. Measurements are therefore independent of the angle between the intravascular ultrasound image plane and the vessel axis. In contrast, absolute lumen and vessel wall areas are overestimated when the intravascular ultrasound catheter position is nonorthogonal (31).

Using this approach, plaque in coronary and iliac arteries was quantified in patients with heterozygous familial hypercholesterolemia or familial combined hyperlipidemia. Despite only minimal or no angiographic changes, an average of 11% (left main coronary artery) and 25% (left anterior descending coronary artery) of the potential lumen cross-sectional area was occupied by plaque. Changes in coronary vessels were detected angiographically only when the plaque occupied more than ~40% of the potential vessel lumen. The area within the internal elastic lamina (plaque plus lumen area) was significantly related to plaque area in both left anterior descending and left main coronary arteries (Fig. 5). In the left anterior descending artery, vessel enlargement was greater than the corresponding increase in plaque area, indicating that vessel expansion effectively "overcompensates" for plaque growth, as shown by previous histologic findings in humans (27) and animals (32). However, compensatory vessel enlargement can only be proved when serial studies in the same subjects show that the vessel enlarges with plaque growth. The occurrence of vessel expansion in the patients studied here is likely to be related to the increased tendency for aneurysmal disease observed in patients with familial hypercholesterolemia. This process is strongly correlated with HDL cholesterol levels (33).

Another explanation for absent or minimal angiographic changes despite advanced atherosclerosis in our patients was plaque distribution (10,12,28). Plaque accumulation was usually eccentric and diffuse, that is, large amounts of vessel surface (34% in the left main coronary artery, 55% in the left anterior descending artery) were covered by plaque.

Intravascular ultrasound seems to be an effective tool when serial changes in plaque burden are to be quantified (e.g., regression trials). Intravascular ultrasound is clearly superior to angiography in detecting early atherosclerotic plaque not resulting in lumen compromise. Angiography might miss changes in early atherosclerotic plaque but possibly also changes in advanced plaque that do not result in parallel changes in lumen dimensions. Furthermore, intravascular ultrasound provides insight into plaque composition, which might also change during therapeutic interventions.

The discrepancy in plaque detection using intravascular ultrasound versus angiography was considerably smaller in the iliac than in the coronary arteries. The differential in threshold for detection by angiography may reflect different lesion morphology and vessel size. Finally, plaque accumulation was greater in the left anterior descending than in the left main artery in our patients. Atherosclerosis was also more extensive in the coronary arteries than the iliac artery, in keeping with clinical experience in patients with familial hypercholesterolemia. This disparity is important when imaging is limited to peripheral vessels (34) and is taken as an indirect marker for coronary disease.

High density lipoprotein cholesterol and atherosclerosis. Pathoanatomic studies in different age groups have shown that serum levels of HDL cholesterol are a powerful predictor for the extent of coronary atherosclerosis as determined by the amount of intimal surface covered with plaque (23,35,36). Prospective observational studies have shown (37–39) that the incidence of clinical coronary events is also independently and inversely related to HDL cholesterol levels. Furthermore, reduction in coronary death and myocardial infarction is significantly related to changes in HDL cholesterol levels during lipid-lowering therapy (37,40). Baseline levels and changes from baseline levels in HDL cholesterol during intense lipid-lowering therapy are independent predictors of angiographic regression or decreased progression of coronary

atherosclerosis (5–8). The relation between HDL cholesterol and angiographic extent of atherosclerosis is controversial. Several studies (1–4) have concluded that HDL cholesterol is a strong predictor of the presence and severity of disease, whereas others did not confirm these findings. However, in those studies coronary atherosclerosis was considered to be absent with no angiographic changes (1,2) or with lumen diameter reduction <50% by angiography (3,4). According to our findings, even vessels with no angiographic changes can have extensive plaque burden. Thus, discrimination based on angiographic criteria may have categorized patients as falsely normal, potentially contributing to the contradictory results of these angiographic trials (1–4).

The present study showed that HDL cholesterol levels (left anterior descending coronary artery) and total/HDL cholesterol ratio (left main coronary artery) are powerful predictors of atherosclerotic plaque burden as assessed by intravascular ultrasound imaging. This relation was found to hold for different markers of plaque burden (e.g., mean and maximal intimal indexes and plaque surface area). These findings suggest that even with very high total or LDL lipoprotein cholesterol levels, high HDL cholesterol levels are associated with a smaller plaque burden. Gender was significantly related to plaque burden only by univariate analysis; after introduction of HDL cholesterol into a stepwise regression model, gender was not significant. This result is in agreement with many observations that suggest that the primary effect of female gender or of exogenous estrogens is mediated by changes in HDL cholesterol levels. The mechanisms by which this effect is exerted remain unclear, and multiple mechanisms may be operative. The role of HDL cholesterol in the pathway for retrieval of cholesterol from peripheral tissues may comprise an important component of the protective effects of these lipoproteins (41–43). The discovery that HDL cholesterol can inhibit the oxidative modification of LDL cholesterol (44–46) may also contribute to the retardation of atherogenesis by reducing the uptake of lipoproteins by scavenger receptors (47). Our finding of the inverse correlation of HDL cholesterol levels with early atherosclerosis suggests that HDL cholesterol may be involved especially with events that initiate atherosclerotic lesions, perhaps playing a role in maintaining the integrity of endothelium or by inhibiting the formation of foam cells.

Study limitations. Our study has some intrinsic limitations: 1) Only a small portion of the epicardial coronary vasculature was studied by intravascular ultrasound, and among these regions only vessel segments with lumen diameter reduction <40% by angiography were examined to provide maximal safety using currently available intravascular ultrasound equipment (48). However, lesions in the left main and left anterior descending coronary arteries have the highest impact on clinical outcome. 2) Intimal thickening may be present even before a three-layered appearance is seen by intravascular ultrasound imaging, and an intimal thickness <200 μm by intravascular ultrasound does not exclude a morphologically abnormal vessel wall (20). Because the lumen border and the internal elastic lamina are not seen by intravascular ultrasound in the absence of a three-layered appearance, the border between the lumen and the wall was analyzed. This absence might have caused underestimation of plaque area. 3) Precise delineation of the internal elastic lamina as the outer border of the plaque is not always possible on intravascular ultrasound images. Instead, the adventitial surface may have been considered the plaque border in some cases, which might have caused overestimation of plaque area.

Conclusions. Extensive atherosclerosis in the left main and proximal left anterior descending coronary arteries often remains angiographically silent. Only coronary plaques involving >40% of the potential cross-sectional area of the vessel were detected by angiography. Compensatory enlargement and diffuse involvement with eccentric plaque may be responsible for the lack of angiographic change. The levels of HDL cholesterol are inversely related, and total/HDL cholesterol ratios directly related, to the extent and severity of coronary plaque as assessed by intravascular ultrasound imaging.

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