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Influence of HDL Cholesterol on Preclinical Carotid Atherosclerosis in Familial Hypercholesterolemia

Mireia Junyent, Montserrat Cofán, Isabel Núñez, Rosa Gilabert, Daniel Zambón, Emilio Ros

Objective—The effect of risk factors on carotid atherosclerosis in heterozygous familial hypercholesterolemia (FH) is unclear. We evaluated carotid intima-media thickness (IMT) by sonography in relation to classical and emergent risk factors in a large FH cohort.

Methods and Results—Risk factors and carotid IMT were assessed in 196 asymptomatic subjects aged ≥ 25 years fulfilling strict diagnostic criteria for clinical FH who were either undertreated or treatment-naive. Conventional risk factors, but not lipoprotein(a), homocysteine, or apolipoprotein E (apoE) genotypes were univariately related to IMT. Age-adjusted and gender-adjusted IMT increased with increasing low-density lipoprotein (LDL) cholesterol and decreased with increasing high-density lipoprotein (HDL) cholesterol. Compared with a total cholesterol/HDL ratio > 5.0 , a ratio ≤ 5.0 was associated with a lower adjusted IMT, with a mean difference of -0.09 mm (95% confidence interval, -0.13 to -0.04). By multivariate analysis, age, HDL cholesterol (negatively), physical exercise, family history of early-onset coronary heart disease, LDL cholesterol, and leukocyte count, in this order, were independent associations of IMT ($r^2=0.429$, $P<0.001$).

Conclusions—Traditional risk factors account for a sizeable proportion of variation in carotid IMT in FH. Because the HDL cholesterol level and the total cholesterol/HDL ratio are strong predictors of preclinical carotid atherosclerosis, HDL cholesterol-raising strategies should have an important therapeutic role in FH. (*Arterioscler Thromb Vasc Biol.* 2006;26:1107-1113.)

Key Words: atherosclerosis ■ carotid intima-media thickness ■ cardiovascular risk factors ■ cholesterol ■ familial hypercholesterolemia ■ lipoprotein

Familial hypercholesterolemia (FH) is a common inherited disorder of lipid metabolism that is usually caused by defects in the low-density lipoprotein (LDL) receptor (LDLR) gene. FH is characterized by lifelong elevation of LDL cholesterol levels, tendon xanthomas, and early-onset coronary heart disease (CHD).¹ Despite its strong genetic background, FH shows a great variability in phenotypic expression in terms of the lipid profile, frequency of xanthomas, and onset and severity of CHD.¹⁻³ Specific LDLR mutations with a differential effect on residual receptor function affect both the lipid phenotype and CHD risk.^{2,3} However, phenotypic variation also occurs in families or populations sharing the same LDLR defect,⁴⁻⁶ suggesting the influence of additional genetic and/or environmental factors. Conventional cardiovascular risk factors are associated with an increased risk for CHD in FH subjects.³ Thus, a recent report from a large retrospective cohort of 2400 individuals with FH showed male gender, smoking, hypertension, diabetes, low high-density lipoprotein (HDL) cholesterol, and elevated lipoprotein(a) to be independent risk factors for cardiovascular disease, although they explained only 18.7% of the variation in its occurrence.⁷

Carotid intima-media thickness (IMT) is a recognized intermediate marker for cardiovascular risk that has also been

examined in FH. Previous studies showed that FH subjects have higher IMT than both age-matched and sex-matched normolipidemic^{8,9} and hypercholesterolemic^{10,11} controls. In FH, carotid IMT has been found to be associated with family history of early-onset CHD,¹⁰ presence of CHD,^{12,13} gender,⁹ lipoprotein levels,^{9,10,12} the type of LDLR defect,^{11,14} and mutations in other genes.¹⁵ However, most of these studies included selected Lipid Clinic patients receiving hypolipidemic treatment, which may variably influence IMT in FH.¹⁶ This might explain why age-adjusted carotid IMT was not associated with CHD in a large cohort of the Utah MEDPED program,¹⁷ in which a larger proportion of patients with than of those without CHD were using lipid-lowering drugs. Therefore, we evaluated with carotid sonography at the time of referral to our Lipid Clinic a large sample of well-phenotyped asymptomatic subjects with clinical FH who were either undertreated or treatment-naive.

Methods

Subjects

From March 1998 to April 2005, we assessed 196 consecutive asymptomatic adults (aged ≥ 25 years) with a clinical diagnosis of FH. They were referred by primary care physicians for diagnosis of

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severe hypercholesterolemia or because of alleged refractoriness to treatment. Within 2 to 6 weeks of the first visit, subjects underwent sonographic assessment of preclinical carotid atherosclerosis according to a protocol approved by the local review board and provided informed consent. For the diagnosis of FH, we used Dutch Lipid Clinic Network criteria,¹⁸ which are based on LDL cholesterol levels above the age-specific and gender-specific 95th percentiles of a Spanish reference population,¹⁹ vertical transmission of hypercholesterolemia, early-onset CHD in the index case and/or first-degree relatives, and presence of tendon xanthomas. Each of these variables is scored, and an overall score is constructed to indicate the diagnostic probability of FH (possible 3 to 5, probable 6 to 7, and certain ≥ 8). Individuals with a FH score ≥ 6 were considered for study.

Subjects with clinical FH are recruited into the Spanish FH Register²⁰ and submitted to DNA testing for identification of *LDLR* mutations and the apoB R3500G mutation following a standard protocol.^{20,21} Molecular screening is still in process and to date 42 different *LDLR* mutations have been identified in 72 of 110 subjects evaluated. Three additional subjects carried the R3500Q mutation in the APOB gene. No *LDLR* or *APOB* defects were found in 35 subjects.

Clinical and Laboratory Characteristics

All subjects were assessed for family history of early CHD (before 55 years in men and 60 years in women), clinical history, medication use, demographic characteristics, physical activity, standard cardiovascular risk factors, and presence of tendon xanthomas. We evaluated leisure-time physical activity by questioning about the average daily time spent walking and the average weekly time spent in athletic and sporting events. Subjects were considered physically active when walking ≥ 1 hour daily and/or participating in sporting activities ≥ 3 hour per week. Body mass index (BMI) was calculated as weight in kilograms divided by the square of height in meters. Blood pressure was measured with a random-zero mercury sphygmomanometer. We used the mean of 2 measurements of systolic and diastolic blood pressure while subjects were sitting after resting for 5 and 10 minutes. To obtain a baseline lipid profile, fasting blood was drawn after at least 4 weeks without hypolipidemic drug treatment. Cholesterol and triglycerides were determined by standard enzymatic methods. We calculated the cholesterol-years score, an estimation of lifetime exposure to hypercholesterolemia, as the sum of average on-treatment and off-treatment cholesterol levels for the lifetime of each subject.²² HDL cholesterol was measured by a precipitation method. LDL cholesterol was estimated with the Friedewald equation. Apolipoproteins (apo) AI and B and lipoprotein(a) were determined by using immunoturbidimetry (Unimate 3; Roche, Basel, Switzerland). Leukocytes were counted with standard automated cell counters. Plasma total homocysteine was measured by a chemiluminescent assay on an ADVIA Centaur automated system (Bayer Advia 1650, Tarrytown, NY). Apolipoprotein (apoE) genotyping was performed by using the method of Wenham et al.²³

Carotid Ultrasonography

B-mode ultrasound imaging of the right and left carotid arteries was performed using SSA 140 A Powervision and Applio instruments (Toshiba, Nasu, Japan) equipped with 7- to 10-MHz broadband linear array transducers. Patients were examined in the supine position with the head turned 45° contralateral to the side of scanning. Before obtaining images for IMT measurements, B-mode and color Doppler sonographic examinations were done in longitudinal and transverse planes to identify vascular stenoses. A standardized imaging protocol was used for the IMT measurements. With the carotid dilatation and flow dividers as anatomic landmarks, the sonographer obtained high-resolution images of the common carotid (1 cm proximal to the bifurcation), the bifurcation (between dilatation and flow divider), and the internal carotid (1 cm distal to the flow divider). A single lateral angle of insonation, optimizing the image for the arterial far wall, was used. The primary variable was mean common carotid IMT (mean CC-IMT), defined as the average of 4 to 8 distances between the far wall lumen-intima and media-

adventitia ultrasound interfaces taken bilaterally. Secondary variables were maximum CC-IMT, defined as the maximum distance in any of the specified segments of right and left common carotids, and maximum carotid IMT, defined as the maximum distance in any of the three available carotid segments of each side. When plaques were present, maximum carotid IMT equaled the highest plaque height. Two experienced sonographers (I.N. and R.G.) performed all examinations and IMTs were measured online. Interobserver variability was examined in 15 subjects. The CVs of paired readings of mean CC-IMT, maximum CC-IMT, and maximum carotid IMT were 5.3%, 6.8%, and 8.2%, respectively.

Statistical Analyses

Data are presented as means \pm SD for continuous variables (medians and interquartile ranges for variables without a normal distribution) and as frequencies or percentages for categorical variables. Differences in mean values were assessed by using analysis of variance and *t* tests or the Mann Whitney *U* test, as appropriate. Categorical variables were compared by using the chi-square test. Pearson correlation coefficients were constructed to test for relationships between continuous variables. Because predictably CIMT was strongly related to age and showed gender differences, age-adjusted and gender-adjusted CIMT values were used when examining associations with other variables. We categorized subjects by tertiles of lipid variables and used ANOVA statistics to calculate tests for trend for CIMT. We used stepwise multiple regression analyses to test the influence of various cofactors on CIMT. Two-sided $P < 0.05$ was considered statistically significant. SPSS software (version 11.0) was used.

Results

Clinical Features and Lipid Profiles

Table 1 shows the clinical characteristics of the study population; 110 (56%) subjects were treatment-naïve and 86 (44%) subjects had been treated previously with lipid-lowering drugs (66 statins, 7 fibrates, 3 resins, and 10 combined resins and statins) for a median period of 24 months (range, 6 to 252). Those treated with statins had received an average daily dose with potency equivalent to 24 mg simvastatin (range, 10 to 40 mg). Such small doses of statins (for FH) indicated ineffective cholesterol-lowering treatment rather than refractoriness to it. Thirteen of 18 subjects with a diagnosis of hypertension were treated with antihypertensive drugs. Hypertension (blood pressure $\geq 140/90$ mm Hg) was newly diagnosed in 5 additional subjects. Mild diabetes (glycosylated hemoglobin $\leq 6.5\%$) was present in 3 subjects, none of whom received hypoglycemic agents. Dutch FH criteria scores were similar in 75 subjects with an identified molecular defect (12.3 ± 4.9), 35 subjects without *LDLR* or *APOB* mutations (11.3 ± 3.6), and 86 subjects still pending DNA diagnosis (11.7 ± 3.9). Women predominated in this cohort, were older than men, and had a higher cholesterol-years score, but smoked less. The HDL cholesterol, apoAI, and lipoprotein(a) concentrations were higher and the cholesterol/HDL ratio was lower in women than men.

The overall distribution of apoE genotypes was: E2/E3 (E2) 6.1%, E3/E3 (E3) 74.0%, E4/E3 18.4%, E4/E4 0.5%, and E2/E4 1.0%, with no sex differences. To test associations with other covariates, E4/E3 and E4/E4 genotypes were grouped together (E4), whereas E2/E4 genotypes were excluded.

Associations of Carotid IMT

Individuals with a family history of early-onset CHD had nonsignificantly higher adjusted IMT values than those with-

TABLE 1. Clinical Characteristics, Biochemical and Lipid Profiles, and Carotid IMT in 196 Asymptomatic Subjects With Familial Hypercholesterolemia

Variable	Men (n=89)	Women (n=107)	Total (n=196)
Age, years (range)†	42 (25–70)	51 (25–76)	47 (25–76)
Previous hypolipidemic drug treatment, n (%)	40 (45)	46 (43)	86 (44)
Family history of CHD, n (%)	48 (54)	64 (60)	112 (57)
Ever smoked, n (%)†	32 (36)	20 (19)	52 (27)
Body mass index, kg/m ²	25.8±3.1	25.6±4.4	25.7±3.8
Physical exercise, n (%)	39 (44)	40 (37)	79 (40)
Tendon xanthomas, n (%)	29 (33)	36 (34)	65 (33)
Dutch Clinic Network criteria score	12.1±4.8	11.9±4.6	12.0±4.7
Hypertension, n (%)	5 (5.6)	18 (16.8)	23 (11.7)
Systolic blood pressure	126±13	127±18	126±16
Diastolic blood pressure	78±8	77±11	77±10
Diabetes mellitus, n (%)	0	3 (2.8)	3 (1.5)
Glucose, mg/dL	94±10	91±12	92±12
Total cholesterol, mg/dL	355±70	372±73	364±72
Cholesterol years score, years×mg/dL†	14 408±4758	17 900±6282	16 278±5878
LDL cholesterol, mg/dL	277±68	283±72	281±70
HDL cholesterol, mg/dL†	51±11	62±14	57±14
Total cholesterol/HDL ratio*	7.3±2.2	6.4±2.4	6.8±2.4
Triglycerides, mg/dL	118 (102–162)	109 (77–143)	114 (87–154)
Apolipoprotein B, g/L	1.94±0.45	1.88±0.51	1.90±0.48
Apolipoprotein AI, g/L†	1.40±0.24	1.61±0.29	1.51±0.29
Lipoprotein(a), g/L*	0.25 (0.10–0.48)	0.35 (0.10–0.65)	0.31 (0.10–0.55)
Leukocyte count, cells/nL	6.6±1.8	6.2±1.6	6.4±1.7
Homocysteine, μmol/L	9.6±3.1	8.4±3.1	8.9±3.1
Mean common carotid IMT, mm	0.63±0.16	0.66±0.17	0.64±0.17
Maximum common carotid IMT, mm	0.77±0.23	0.80±0.25	0.79±0.24
Maximum carotid IMT, mm	1.36±0.89	1.33±0.74	1.34±0.81

Values are mean±SD except for triglycerides and lipoprotein(a) (medians and interquartile ranges).

CHD indicates coronary heart disease.

* $P<0.05$, † $P<0.001$ between genders by unpaired t test.

out (data not shown). Adjusted mean CC-IMT was 0.66 ± 0.18 mm in subjects who had received hypolipidemic drugs and 0.64 ± 0.10 mm in those who were treatment-naive ($P>0.1$). Paradoxically, physically active subjects showed greater adjusted mean CC-IMT than sedentary ones (0.67 ± 0.17 mm versus 0.63 ± 0.11 mm, respectively; $P=0.019$). IMT was similar in subjects with and without genetic defects identified and in those still pending molecular diagnosis (data not shown).

Table 2 shows that the 3 IMT measures correlated strongly with age. As expected, the cholesterol-years score was also associated with IMT. Unadjusted IMT values were similar across genders, but women were older (Table 1). When using age-adjusted IMT, values were nonsignificantly higher in men for both mean and maximum CC-IMT ($P<0.14$) and significantly higher for maximum carotid IMT ($P<0.001$). Consequently, age-adjusted and gender-adjusted IMT was used to assess relationships with other covariates. Smokers tended to have all IMT measures higher than nonsmokers ($P<0.1$). The presence or absence of xanthoma did not influence IMT.

As shown in Table 2, most unadjusted IMT values correlated directly and significantly with conventional lipid and non-lipid risk factors. For some variables, the direct correlations were attenuated or no longer significant after IMT was corrected for age and gender. However, using adjusted IMT strengthened the correlations of HDL cholesterol, total cholesterol/HDL ratios, and leukocyte counts. Lipoprotein(a), homocysteine, or apoE genotypes were unrelated to IMT.

Because cholesterol fractions and systolic blood pressure showed the strongest correlations with adjusted IMT values (Table 2), we further explored these associations by plotting IMT measures against tertiles of these covariates. Age-adjusted and gender-adjusted mean and maximum CC-IMT, but not maximum carotid IMT, increased with increasing LDL cholesterol levels (Figure 1), whereas all measures of adjusted IMT decreased with increasing HDL cholesterol (Figure 2). Adjusted IMT values also increased with increasing systolic blood pressure ($P<0.02$ for all).

We categorized FH subjects by total cholesterol/HDL ratios above or below 5.0, a risk function commonly used in clinical practice for improved CHD prediction.²⁴ Compared

TABLE 2. Correlation Coefficients Between Cardiovascular Risk Factors and IMT (Unadjusted and Adjusted for Age and Gender)

	Mean Common Carotid IMT		Maximum Common Carotid IMT		Maximum Carotid IMT	
	Unadjusted	Adjusted	Unadjusted	Adjusted	Unadjusted	Adjusted
Age	0.544‡		0.471‡		0.484‡	
Body mass index	0.232†	0.092	0.206*	0.079	0.185†	0.034
Systolic blood pressure	0.390‡	0.208†	0.370‡	0.209†	0.355‡	0.170*
Diastolic blood pressure	0.254‡	0.083	0.241*	0.089	0.305‡	0.141†
Glucose	0.307‡	0.156*	0.208†	0.061	0.023	0.013
Total cholesterol	0.274‡	0.199†	0.283‡	0.219†	0.137	0.052
Cholesterol-years score	0.471‡	0.111	0.417‡	0.108	0.342‡	0.037
LDL cholesterol	0.278‡	0.224†	0.298‡	0.250‡	0.142*	0.068
HDL cholesterol	-0.117	-0.268‡	-0.101	-0.214†	-0.067	-0.138
Total cholesterol/HDL ratio	0.241*	0.309‡	0.252‡	0.301‡	0.135	0.149*
Ln triglycerides	0.259‡	0.156*	0.217†	0.116	0.145*	0.002
Apolipoprotein B	0.273‡	0.212†	0.256‡	0.196†	0.227†	0.151*
Apolipoprotein AI	-0.059	-0.116	-0.048	-0.095	-0.124	-0.030
Ln lipoprotein(a)	0.042	0.030	0.028	0.046	0.057	0.022
Leukocyte count	0.190*	0.217†	0.137	0.146*	0.037	0.019
Homocysteine	0.055	0.018	0.011	-0.057	0.123	0.078

*P<0.05; †P<0.01; ‡P<0.001.

with a total cholesterol/HDL ratio >5.0, a ratio ≤5.0 was associated with lower mean and maximum adjusted CC-IMT (Figure 3). The between-group differences for mean CC-IMT and maximum CC-IMT were -0.09 mm (95% confidence interval [CI], -0.13 to -0.04) and -0.10 mm (CI, -0.18 to -0.03), respectively.

Predictors of IMT by Multivariate Analyses

After adjustment for the categorical variables listed in Table 1 and the risk factors listed in Table 2, independent associations of mean CC-IMT by stepwise multiple regression analysis were age, HDL cholesterol, physical exercise, family history of early-onset CHD, LDL cholesterol, and the leukocyte count, in this order (Table 3). Entering the cholesterol/HDL ratio into the regression analysis replaced HDL and LDL cholesterol in the model and showed a higher association than either cholesterol fractions (B=0.0165, P<0.001), but added no more overall information (adjusted r²=0.416). When maximum CC-IMT was the dependent variable, the same factors entered the equation except for familial CHD

and leukocytes. Finally, age, gender, and systolic blood pressure were the sole independent associations of maximum carotid IMT.

Discussion

Carotid IMT is an accepted surrogate measure of CHD risk. No prospective studies of CHD incidence in relation to carotid atherosclerosis have been performed in FH. However, carotid IMT has been related to the presence of CHD in 2 cross-sectional studies of FH patients.^{12,13} The present study showed that age, family history of early-onset CHD, physical exercise, LDL and HDL cholesterol, and the leukocyte count were independent associations of mean CC-IMT, a measure of early carotid atherosclerosis, together explaining ≈43% of its variability in asymptomatic subjects with clinical FH. The robustness of the associations of lipid fractions with IMT increased in the order of total cholesterol/HDL ratio>HDL cholesterol (negative)>LDL cholesterol. Advanced carotid atherosclerosis, as assessed by maximum carotid IMT, was associated with age, gender, and systolic blood pressure.

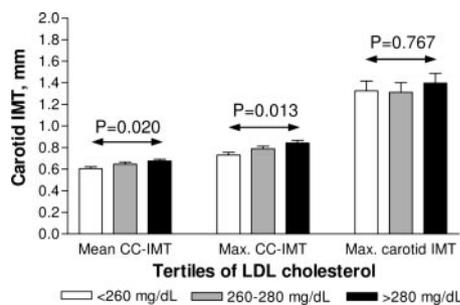


Figure 1. Age-adjusted and gender-adjusted carotid IMT by tertiles of LDL cholesterol. Error bars represent SEM.

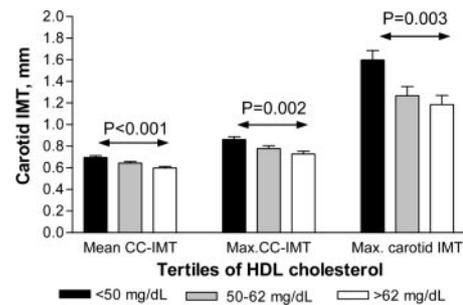


Figure 2. Age-adjusted and gender-adjusted carotid IMT by tertiles of HDL cholesterol. Error bars represent SEM.

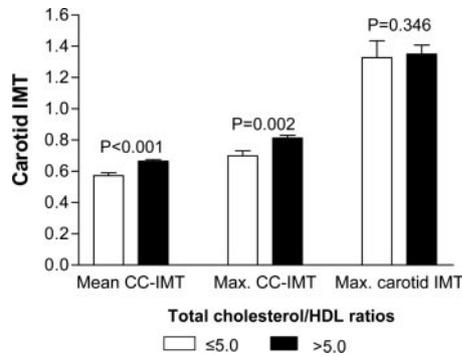


Figure 3. Age-adjusted and gender-adjusted carotid IMT according to total cholesterol/HDL ratios. Error bars represent SEM.

This study has the strengths of a cross-sectional design in a large series of FH subjects defined by strict clinical criteria and not receiving effective cholesterol-lowering treatment that could have influenced IMT. A molecular diagnosis was available in only a subset of subjects, but this had no effect on the clinical phenotype. In this sense, this cohort is representative of asymptomatic persons with FH as they present to a Lipid Clinic, but not of those still undiagnosed within the general population.

The strong association between age and IMT is not surprising because age is a critical predictor of both CHD risk^{1,3,4,17,25} and carotid atherosclerosis^{8,10,12,13} in FH as well as in non-FH populations. Gender and smoking were not independent risk factors for early carotid atherosclerosis in our study. The fact that only asymptomatic subjects were studied probably excluded many men with increased IMT, because men with FH characteristically develop CHD earlier than women.^{1,3} Nevertheless, female gender was a protective factor for advanced carotid atherosclerosis, as assessed by maximum carotid IMT. There were few smokers in this cohort,

thus reducing statistical power. Smoking has been associated with increased CHD risk to a varying degree in FH.^{3,7,17}

As shown by other authors,¹⁰ family history of early-onset CHD was an independent association of IMT in our FH cohort. These findings support the heritability of ultrasound-determined carotid phenotypes.²⁶ Physical exercise was also independently associated with IMT. Given the recognized benefits of physical activity for cardiovascular risk, this observation seems counterintuitive and could be ascribed to unrecognized confounding factors. However, other workers reported similar findings in non-FH subjects and tentatively explained them as a consequence of intermittent exposure to increased systemic arterial pressures during exercise, resulting in periodic elevations in carotid wall tensile stress.^{27,28}

An important finding was that HDL cholesterol had a stronger inverse association with IMT than LDL cholesterol a direct one (Figures 1 and 2). The model in Table 3 predicts that mean CC-IMT is ≈ 0.03 mm thinner for each 10-mg/dL increase in HDL cholesterol and ≈ 0.01 mm thicker for each 10-mg/dL increase in LDL cholesterol. When evaluated in relation to carotid IMT in smaller series of FH subjects, HDL cholesterol was either unrelated⁸ or inversely related at univariate, but not multivariate analyses.^{9,10,12} However, elevated HDL cholesterol was negatively associated with CHD risk in cross-sectional studies of large FH cohorts^{4,7,25} and in a recent case-control study in molecularly defined patients.²⁹ Recently, conclusive evidence of the protective effect of raised HDL cholesterol levels against carotid atherosclerosis progression has been provided.^{30,31} These findings are in line with the known antiatherogenic properties of HDL particles, which not only promote reverse cholesterol transport but also have anti-inflammatory properties and the ability to protect LDL against oxidation.³²

In this FH cohort, HDL cholesterol levels were not reduced, because they were comparable with those of a Spanish

TABLE 3. Independent Determinants of IMT by Stepwise Multiple Regression Analysis

Dependent Variable	Independent Variables	B	SE (B)	β	P	Adjusted R ²
Mean common carotid IMT	Constant	0.2333	0.089		0.010	0.429
	Age, years	0.0078	0.001	0.576	<0.001	
	HDL cholesterol, mg/dL	-0.0028	0.001	-0.229	0.002	
	Physical activity	0.0557	0.023	0.167	0.017	
	Family history of CHD	0.0566	0.023	0.171	0.014	
	LDL cholesterol, mg/dL	0.0009	0.001	0.173	0.014	
	Leukocytes, cells/nL	0.0177	0.001	0.152	0.029	
Maximum common carotid IMT	Constant	0.3071	0.116		0.009	0.319
	Age, years	0.0102	0.002	0.507	0.001	
	LDL cholesterol, mg/dL	0.0013	0.001	0.194	0.012	
	HDL cholesterol, mg/dL	-0.0041	0.001	-0.212	0.007	
	Physical activity	0.0937	0.037	0.188	0.014	
Maximum carotid IMT	Constant	-0.7081	0.509		0.167	0.281
	Age, years	0.0322	0.006	0.487	0.001	
	Gender (female)	-0.3086	0.127	-0.190	0.016	
	Systolic blood pressure, mm Hg	0.0072	0.004	0.160	0.046	

Variables entered into the models were: age, gender, previous cholesterol-lowering treatment, family history of early-onset CHD, smoking habit, physical activity, BMI, tendon xanthomas, cholesterol-years score, systolic and diastolic blood pressure, glucose, Ln triglycerides, LDL cholesterol, HDL cholesterol, apolipoprotein B, apolipoprotein A1, Ln lipoprotein(a), apoE4 genotype, leukocyte count, and homocysteine.

reference population¹⁹ and also with those of Spanish community individuals who had lipid analyses performed in our laboratory and lived in the same geographic area.³³ Except for the effect of specific *LDLR* defects,^{2,3} in genetically heterogeneous FH subjects like those herein described HDL cholesterol levels are probably modulated by genetic and environmental factors similar to those influencing HDL cholesterol in the general population. A plasma factor that plays an important role in determining the HDL cholesterol level is the activity of cholesteryl ester transfer protein (CETP). Low CETP activity was associated with raised HDL cholesterol levels and reduced carotid IMT progression after 2 years of statin therapy in a Dutch FH cohort.³⁴ This suggests that pharmacological CETP inhibition could impart an especial benefit in FH.

Interestingly, the leukocyte count, a nonspecific measure of inflammation, was an independent association of IMT in our study. This variable has been described in few studies in relation to the presence of CHD in FH, and found both to be¹⁷ and not to be²⁵ an independent risk factor. The emerging risk factors lipoprotein(a) and homocysteine were not associated with IMT. The data on these novel risk factors and either IMT^{2,4,9,10} or CHD risk^{2,7,17,25} are FH is conflicting. Definitive evidence for their role in the accelerated atherosclerosis of FH should come from intervention studies relating outcomes to lowering of their serum concentrations. Thus far, there is evidence that moderately reducing lipoprotein(a) with statins does not relate to changes in carotid IMT.³⁵

In conclusion, classical risk factors account for a sizeable part of the variation in preclinical carotid atherosclerosis in FH subjects. HDL cholesterol is a strong factor protecting from carotid IMT enlargement in FH and, similar to the situation in the general population,²³ a total cholesterol/HDL ratio <5 signals subjects with reduced cardiovascular risk, as assessed by IMT. Although not detracting from the pivotal importance of elevated LDL cholesterol levels in the accelerated atherosclerosis of FH, the strong protective role of high HDL cholesterol in these patients has clinical implications and points to the need for improving strategies aimed at raising HDL cholesterol levels.³²

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