CORE
Metadata, citation and similar papers at core.ac.uk
Provided by AIR Universita degli studi di Milano

Arteriosclerosis, Thrombosis, and Vascular Biology
JOURNAL OF THE AMERICAN HEART ASSOCIATION

Increased Carotid Artery Intima-Media Thickness in Subjects With Primary Hypoalphalipoproteinemia

Damiano Baldassarre, Mauro Amato, Linda Pustina, Elena Tremoli, Cesare R. Sirtori, Laura Calabresi and Guido Franceschini

Arterioscler. Thromb. Vasc. Biol. 2002;22;317-322
DOI: 10.1161/hq0202.104848
Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association. 7272 Greenville Avenue, Dallas, TX 72514
Copyright © 2002 American Heart Association. All rights reserved. Print ISSN: 1079-5642. Online ISSN: 1524-4636

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://atvb.ahajournals.org/cgi/content/full/22/2/317

Subscriptions: Information about subscribing to Arteriosclerosis, Thrombosis, and Vascular Biology is online at
http://atvb.ahajournals.org/subscriptions/

Permissions: Permissions & Rights Desk, Lippincott Williams & Wilkins, a division of Wolters Kluwer Health, 351 West Camden Street, Baltimore, MD 21202-2436. Phone: 410-528-4050. Fax: 410-528-8550. E-mail:
journalpermissions@lww.com

Reprints: Information about reprints can be found online at
http://www.lww.com/reprints

Downloaded from atvb.ahajournals.org by on June 8, 2007

Increased Carotid Artery Intima-Media Thickness in Subjects With Primary Hypoalphalipoproteinemia

Damiano Baldassarre, Mauro Amato, Linda Pustina, Elena Tremoli, Cesare R. Sirtori, Laura Calabresi, Guido Franceschini

Abstract—The plasma concentration of high-density lipoprotein cholesterol (HDL-C) is inversely correlated with the incidence of atherosclerotic vascular events. In the present study, we evaluated pre-intrusive atherosclerosis in subjects with plasma HDL-C at the extremities of normal distribution. Fifty-five subjects with primary hypoalphalipoproteinemia (HypoALP) or hyperalphalipoproteinemia (HyperALP) were compared with fifty-five control subjects with average HDL-C levels, matched for sex, age, and plasma cholesterol. The average and maximal intima-media thicknesses (Avg-IMT and Max-IMT) of 48 carotid segments for each subject were approximately 40% greater in HypoALP than in control subjects (0.94±0.06 versus 0.69±0.04 mm, $P=0.004$, and 1.86±0.16 versus 1.35±0.10 mm, $P=0.025$, respectively). The IMT values in HyperALP subjects (Avg-IMT, 0.71±0.04 and Max-IMT, 1.38±0.14 mm) were the same as in controls. In a large cohort of hyperlipidemic subjects (n=559), significantly greater Avg-IMT and Max-IMT were found in subjects belonging to the first HDL-C quintile (<42 mg/dL) than in all the others. The measurement of carotid IMT in cases with HypoALP and HyperALP, and in a large series of hyperlipidemic patients, thus indicates that a low HDL-C is associated with significant pre-intrusive atherosclerosis, whereas a HDL-C level above average values does not lead to a further reduction of arterial wall thickening. **(*Arterioscler Thromb Vasc Biol*. 2002;22:317-322.)**

Key Words: Intima-media thickness ■ high-density lipoproteins ■ hypoalphalipoproteinemia ■ hyperalphalipoproteinemia ■ preintrusive atherosclerosis

A number of epidemiological studies have consistently demonstrated that a low plasma concentration of high-density lipoprotein cholesterol (HDL-C) is an independent risk factor for atherosclerotic vascular disease.[1] Clinical sequelae are generally preceded by silent changes in the arterial wall, characterized by enhanced lipid deposition and infiltration of blood cells into the subendothelial space.[2] HDL are believed to retard the formation of atherosclerotic lesions in the arterial wall by removing excess cholesterol from cells[3] and by preventing cell adhesion and transmigration.[4] These mechanisms may imply an increased thickening of the arterial wall in individuals with low plasma HDL levels.[5]

High-resolution B-mode ultrasonography is a noninvasive technique that allows pre-intrusive atherosclerotic changes in the walls of the carotid and femoral arteries to be seen and provides reliable and reproducible measurements of the thickness of the arterial intima-media complex, the intima-media thickness (IMT).[6] Cross-sectional and population studies indicate an association between carotid IMT, cardiovascular risk factors,[7,8] and the prevalence of cardiovascular disease.[9] More importantly, in prospective studies,[10,11] carotid IMT was able to predict clinical coronary artery disease (CAD).

Hypoalphalipoproteinemia (HypoALP) defines a group of dyslipidemias characterized by an HDL-C level below the 10th percentile for the age- and sex-matched general population.[12] HypoALP can be a marker of disorders in triglyceride and glucose metabolism, can be caused by environmental factors, or can directly result from mutations in a number of different genes, like those coding for the ABCA1 transporter, the apolipoproteins A-I and A-II, the lecithin:cholesterol acyltransferase (LCAT), or lipoprotein lipase (LPL) enzymes.[3] It is noteworthy that not all of these conditions are associated with an increased risk of premature CAD.[13] Hyperalphalipoproteinemia (HyperALP) is instead characterized by plasma HDL-C levels above the 90th percentile for the age- and sex-matched general population.[14] HyperALP can be acquired by exposure to certain environmental factors, but it is generally inherited as an autosomal dominant trait,[14] caused by mutations in either the cholesteryl ester transfer protein (CETP) or hepatic lipase genes.[3] This condition was early associated with low CAD risk and longevity,[15] but this association has been recently questioned.[16] The main purpose in the present study was to assess the extent of pre-intrusive atherosclerosis in individuals selected because of a primary HypoALP or HyperALP.

Methods

Subjects

Fifty-five consecutive subjects with primary HypoALP (defined by a plasma HDL-C level below the 10th percentile for the general

Received August 2, 2001; revision accepted November 27, 2001.

From the Center E. Grossi Paoletti, Department of Pharmacological Sciences, University of Milan, Italy.

Correspondence to Prof. Guido Franceschini, Center E. Grossi Paoletti, Department of Pharmacological Sciences, Via Balzaretti 9, 20133 Milan, Italy. E-mail Guido.Franceschini@unimi.it

© 2002 American Heart Association, Inc.

Arterioscler Thromb Vasc Biol. **is available at http://www.atvbaha.org**

Downloaded from atvb.ahajournals.org by on June 8, 2007

population[17]) attending the E. Grossi Paoletti Lipid Clinic (Milan, Italy) were recruited for the study. Individuals were excluded if they fulfilled one of the following criteria: obesity (body mass index [BMI] ≥30), diabetes mellitus, liver disease, kidney disease, thyroid dysfunction, or history of alcohol abuse and/or heavy smoking. Age- and sex-matched subjects with HyperALP (defined by a plasma HDL-C above the 90th percentile for the general population) were selected from the Lipid Clinic database, which includes 251 HyperALP subjects. Of these, 53 subjects were excluded because of exclusion criteria. Among the remaining 198 subjects, the 55 subjects who matched for sex and the closest age (in years) to an HypoALP subject were recruited for the study. A third group of subjects (controls), matched for age, sex, and total and LDL cholesterol, with a plasma HDL-C level between the 45th and the 55th percentiles for the general population, was randomly selected from the Lipid Clinic database. No subject was taking medications known to affect plasma lipid/lipoprotein levels.

A cohort of 559 consecutive hyperlipidemic subjects attending the Laboratory for Non-Invasive Diagnosis of the Lipid Clinic also participated in the study. Hyperlipidemia was defined by a low-density lipoprotein cholesterol (LDL-C)>130 mg/dL and/or triglycerides >200 mg/dL.

After an overnight fast, blood was collected into tubes containing Na_2-EDTA (1 mg/mL), and plasma was prepared by low-speed centrifugation. The measurement of plasma lipids was performed by standard enzymatic techniques; HDL-C was determined after precipitation of apoB-containing lipoproteins,[18] and LDL-C was calculated by using the Friedewald's formula.[19] Plasma Lp(a) levels were measured by using ELISA.[20]

Ultrasonography

Ultrasound scanning and reading of carotid arteries were performed by a single expert sonographer, using an 8-MHz transducer with an axial and lateral resolution of ≈0.385 and ≈0.500 mm, respectively.[6,21] The sonographer was blinded to the subject's identity. A standard protocol, scanning the near and far walls of the right and left common, internal and external carotid arteries, and bifurcations in three different projections (anterior, lateral, and posterior), was performed.[21] Eight segments of the right and left carotid arteries in each projection were examined, and the 48 IMT measurements were averaged to calculate the average IMT (Avg-IMT) for each subject. Less than 4% of all IMT measurements was missed because of anatomical reasons; the missing value was replaced in the calculation of Avg-IMT with the average of the remaining measurements for that segment. The highest IMT value among the 48 segments was defined as the maximum IMT (Max-IMT). As age has been shown to be the strongest predictor of IMT,[7,22] age-adjusted IMT values were used in some analyses.

Statistical Analysis

The number of subjects needed to detect a difference in Avg-IMT between the three groups of 0.20 mm with an SD of 0.30 mm, a power of 80%, and $\alpha=0.05$ is 45 per group. Therefore, the study is adequately powered to disprove multiple null hypotheses.

Results are reported as mean±SEM, if not otherwise stated. Logarithmic transformation was performed on individual values of skewed variables. Group differences in continuous and categorical variables were determined by using ANCOVA and a χ^2 test of significance, respectively. Pearson correlation coefficients were computed to assess the association between parameters. Multiple stepwise regression analysis was performed with Avg-IMT or Max-IMT as the dependent variable, and by entering the independent variable with the highest partial correlation coefficient at each step, until no variable remained with an F value of 4 or more. Group differences or correlations with $P<0.05$ were considered statistically significant.

Results

The characteristics of the recruited subjects are shown in Table 1. A trend toward lower BMI and fasting glucose concentrations with increasing average HDL-C levels was found, possibly indicative of the presence of some insulin-resistant subjects in the HypoALP group; none of them, however, was diabetic. Plasma triglycerides were significantly higher in HypoALP than in HyperALP and control subjects. There was no difference in plasma Lp(a), blood pressure, or prevalence of smokers in the three examined groups (Table 1).

TABLE 1. Characteristics of Subjects With HypoALP and HyperALP and of Controls

	HypoALP	Controls	HyperALP
No.	55	55	55
Sex, F/M	20/35	20/35	20/35
Age, y	52.8±1.6	52.9±1.5	52.9±1.6
BMI, kg/m^2	25.4±0.5	24.3±0.4	23.3±0.4†
Total cholesterol, mg/dL	267.8±8.6	263.1±7.3	276.8±8.4
LDL-cholesterol, mg/dL	178.2±8.40	185.7±7.5	183.7±8.4
HDL-cholesterol, mg/dL	30.9±0.8	49.9±0.6‡	70.1±1.2‡
Triglycerides, mg/dL	229 (57–4630)	127 (60–434)†	99 (38–250)†
Lp(a), mg/dL	18 (1–135)	11 (1–101)	25 (1–193)
Glucose, mg/dL	100.8±2.5	95.3±2.6	92.9±1.4*
Systolic BP, mm Hg	135.4±2.4	130.2±2.3	133.5±2.1
Diastolic BP, mm Hg	84.3±1.4	83.0±1.4	83.5±1.5
Hypertension	21 (38.2%)	18 (32.7%)	14 (25.5%)
Smokers	39 (70.1%)	29 (52.7%)	29 (52.7%)

Data are expressed as mean±SEM, except for triglycerides and Lp(a) [median (range)] and hypertension and smokers [n (%)]. BP indicates blood pressure.

*$P<0.05$ vs HypoALP; †$P<0.01$ vs HypoALP.

‡Difference due to selection of study groups.

Mean carotid IMT values in the three groups are shown in Table 2. The intimal thickening of common carotid, bifurcation, and internal carotid was significantly greater in the HypoALP than in subjects with average plasma HDL-C levels. The carotid Avg-IMT and Max-IMT were approximately 40% greater in the HypoALP group than in control subjects. In contrast, the average thickness of the three carotid segments, as well as the Avg-IMT and Max-IMT values, were remarkably similar in HyperALP and control subjects (Table 2). The IMT differences between HypoALP and the other two groups remained significant after data adjustment for BMI, triglycerides, and glucose (not shown). No significant differences were found in Avg-IMT and Max-IMT between the 34 hypertriglyceridemic (triglycerides>200 mg/dL) and the 21 normotriglyceridemic HypoALP subjects (Avg-IMT, 0.91±0.31 versus 0.98±0.10 mm, respectively, $P=0.56$; Max-IMT, 1.79±0.15 versus 2.17±0.28 mm, $P=0.20$).

TABLE 2. Carotid IMT in Subjects With HypoALP and HyperALP and in Controls (mm)

	HypoALP	Controls	HyperALP
Common carotid IMT	0.87±0.06	0.65±0.03‡	0.68±0.03†
Carotid bifurcation IMT	1.12±0.08	0.89±0.05*	0.88±0.06†
Internal carotid IMT	0.87±0.10	0.62±0.04*	0.67±0.04
Avg-IMT	0.94±0.06	0.69±0.03†	0.71±0.04†
Max-IMT	1.86±0.16	1.35±0.10*	1.38±0.14*

Data are expressed as mean±SEM.

*$P<0.05$; †$P<0.01$; and ‡$P<0.001$ vs HypoALP.

Downloaded from atvbahajournals.org by on June 8, 2007

TABLE 3. Characteristics of Hyperlipidemic Subjects

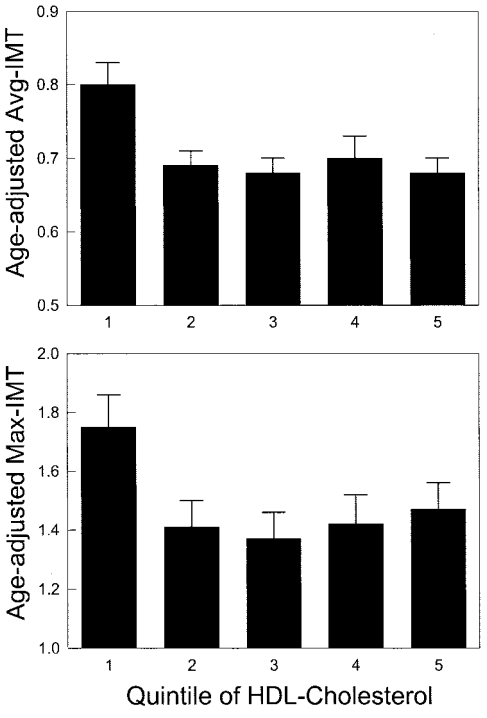
	Hyperlipidemic Subjects		
	Total	Male	Female
No.	559	245	314
Sex, F/M	314/245	0/245	314/0
Age, y	53.6±0.5	51.1±0.8	55.6±0.7‡
BMI, kg/m^2	23.6±0.1	24.2±0.2	23.2±0.2‡
Total cholesterol, mg/dL	265.9±2.4	255.6±3.9	273.7±3.1‡
LDL-cholesterol, mg/dL	186.3±2.4	178.4±3.8	192.4±3.1†
HDL-cholesterol, mg/dL	52.4±0.6	46.7±0.8	56.9±0.7‡
Triglycerides, mg/dL	126 (32–850)	138 (34–850)	115 (32–703)‡
Lp(a), mg/dL	22.1 (0.5–171)	19.0 (0.5–150)	23.7 (0.5–171)
Glucose, mg/dL	94.3±1.0	96.3±1.7	92.9±1.2
Systolic BP, mm Hg	133.4±0.8	133.4±1.2	133.3±1.0
Diastolic BP, mm Hg	82.1±0.4	82.2±0.7	82.0±0.6
Hypertension	152 (27.2%)	54.0 (22.0%)	98.0 (31.2%)
Smokers	250 (44.7%)	170.0 (69.4%)	80.0 (25.5%)†
Common Carotid IMT, mm	0.73±0.015	0.77±0.03	0.70±0.01*
Carotid bifurcation IMT, mm	0.94±0.022	1.01±0.04	0.90±0.02†
Internal carotid IMT, mm	0.72±0.017	0.78±0.03	0.68±0.02†
Avg-IMT, mm	0.74±0.014	0.79±0.03	0.71±0.01†
Max-IMT, mm	1.55±0.042	1.68±0.08	1.45±0.05*

Data are expressed as mean±SEM, except for triglycerides and Lp(a) [median (range)] and hypertension and smokers [n (%)].

*$P<0.05$, †$P<0.01$, and ‡$P<0.001$ vs male subjects.

The measurement of carotid IMT in cases with HypoALP and HyperALP thus indicates that a low HDL-C level is associated with significant pre-intrusive atherosclerosis, whereas a plasma HDL-C level above average values does not lead to further protection from arterial thickening. To further prove this concept, we measured the carotid IMT in a large cohort of hyperlipidemic subjects with widely distributed plasma HDL-C levels. The characteristics of these individuals are reported in Table 3. As expected, women had higher average HDL-C values and lower IMT values at all the analyzed carotid segments, as well as lower Avg-IMT and Max-IMT than the men had (Table 3). The entire cohort of hyperlipidemic subjects was then stratified into quintiles of HDL-C; significantly greater age-adjusted Avg-IMT and Max-IMT values were found in subjects belonging to the first HDL-C quintile (<41 mg/dL) than in all the others (Figure 1). No differences in carotid IMT values were instead detected among subjects belonging to the 2nd to 5th quintiles. When IMT measurements in men and women were considered separately, men displayed higher IMT than did women over the entire HDL-C range (Figure I, please see http://www.atvb.ahajournals.org). Both in men and women, the highest Avg-IMT and Max-IMT values were found in those belonging to the first HDL-C quintile; the impact of a low HDL-C on IMT was greater in men than in women (Figure I).

A significant inverse linear correlation between carotid IMT and plasma HDL-C levels was found in the whole cohort of hyperlipidemic subjects ($r=-0.11$, $P=0.08$, and $P=0.012$, $P=0.003$ for Avg-IMT and Max-IMT, respectively). A number of other parameters correlated significantly with Avg-IMT and Max-IMT in univariate analysis. Therefore, a stepwise multiple regression analysis was performed with Avg-IMT and Max-IMT as the dependent variable, and these various parameters as independent variables. In both cases, age and HDL-C were the first and second strongest predictors of arterial wall thickening, with blood pressure, plasma glucose, and LDL-C making minor additional contributions to these associations (Table 4).



Age-adjusted average (top) and maximal (bottom) carotid IMT in a cohort of 559 hyperlipidemic subjects stratified into quintiles of HDL-Cholesterol. Results are expressed as mean±SEM. The HDL-Cholesterol values in the five quintiles were: <41 mg/dL, 41 to 47 mg/dL, 48 to 54 mg/dL, 55 to 64 mg/dL, and >64 mg/dL.

In view of the known interaction of plasma HDL-C and LDL-C levels in determining individual CAD risk, the association of HDL-C with Avg-IMT and Max-IMT was further investigated by correlation analysis after stratification of the hyperlipidemic subjects into tertiles of LDL-C (Table 5). A significant inverse correlation between plasma HDL-C and Avg-IMT, or Max-IMT was found in each LDL-C tertile; the slope and the significance of the correlations increased from the lower to the higher LDL-C tertiles (Table 5), suggesting an interaction between low HDL-C and high LDL-C in determining individual carotid arterial thickening. When the data from the whole cohort were analyzed together, no significant correlation was found between the individual HDL-C/LDL-C ratio and either Avg-IMT ($r=-0.034$; $P=0.472$), or Max-IMT ($r=0.038$; $P=0.416$).

Discussion

Various population and prospective studies have shown associations between pre-intrusive atherosclerosis, as assessed by measuring carotid arterial wall thickening by noninvasive ultrasonography, and cardiovascular risk factors,

Downloaded from atvb.ahajournals.org by on June 8, 2007

TABLE 4. Stepwise Multiple Regression Analysis of Avg-IMT and Max-IMT to Risk Factors in Hyperlipidemic Subjects

Dependent Variable	Independent Variable	β	SE(β)	F	*P*
Avg-IMT	Age	0.01076	0.00123	76.98	<0.0001
	HDL-cholesterol	−0.00451	0.00121	14.57	0.0002
	Systolic BP	0.00400	0.00105	13.98	0.0002
	LDL-cholesterol	0.00075	0.00024	9.29	0.0024
	Diastolic BP	−0.00483	0.00178	7.33	0.0070
	Glucose	0.00219	0.00099	4.87	0.0278
Max-IMT	Age	0.02703	0.00333	66.04	<0.0001
	HDL-cholesterol	−0.01064	0.00334	10.15	0.0015
	Systolic BP	0.00672	0.00223	9.06	0.0027
	Glucose	0.00670	0.00274	5.99	0.0147

Variables not entered in the Avg-IMT model: total cholesterol, triglycerides, Lp(a), and BMI. Variables not entered in the Max-IMT model: total cholesterol, LDL-cholesterol, triglycerides, Lp(a), diastolic BP, and BMI.

such as increased age, history of coronary heart disease, increased blood pressure, history of smoking, insulin resistance, and dyslipidemia.[5,7–9,22–27] In some[7,8,22–24,27] but not all these studies,[25] an inverse correlation between arterial thickening and HDL-C was found. To the best of our knowledge, this is the first study examining carotid arterial thickening in individuals at the two extremities of the HDL-C distribution, ie, subjects with primary HypoALP or HyperALP, who are generally considered at high and low CAD risk, respectively. The results indicate that: (1) The low plasma HDL-C in subjects with HypoALP is associated with a significant thickening of the carotid arterial wall; in contrast, the high HDL-C in HyperALP subjects does not reduce arterial thickness below that of individuals with average HDL-C. (2) In a large cohort of hyperlipidemic subjects, a curvilinear relationship exists between plasma HDL-C and carotid thickening, with the greatest influence of HDL-C on IMT being from low to average values. (3) HDL-C is a strong independent predictor of carotid wall thickening in hyperlipidemic subjects, and its influence on IMT increases with increasing plasma LDL-C levels. These results may have important clinical implications, because a carotid IMT of the magnitude found in the examined subjects with low HDL-C is associated with future stroke, CAD events, and death.[10,11]

Primary HypoALP includes a variety of HDL deficiency states with heterogeneous genetic, biochemical, and clinical features. Many HypoALP subjects carry mutations in different genes, including those coding for the ABCA1 transporter, apolipoproteins A-I and A-II, LCAT, or LPL.[3] Whereas homozygous and heterozygous carriers of ABCA1 or LPL mutations are generally at high CAD risk, no such clear association between low HDL and CAD risk is found in carriers of apolipoprotein or LCAT mutations.[13] The same uncertainty exists for individuals at the high extremity of the HDL-C distribution; whereas a high HDL-C level is generally considered a protective factor against CAD development,[1,15] some individuals, in whom the high HDL-C reflects the accumulation of dysfunctional HDL,[28] are indeed exposed to a high CAD risk.[16] In view of the present difficulties in defining the CAD risk in individuals with low or high HDL-C levels, we used IMT as a surrogate marker for the presence of CAD.[29]

A major finding in the present study is the remarkable difference in carotid IMT among the three groups of investigated subjects. HypoALP individuals have markedly increased IMT, whereas HyperALP and control subjects have remarkably similar IMT. A larger heterogeneity in arterial thickening, as depicted by the greater SEM values for either single-segment or average IMT measurements, was observed among HypoALP subjects than in subjects with average or high HDL-C values (Table 2). A systematic genetic characterization of HypoALP individuals recruited for the present study is currently under way to investigate the impact of mutations in specific genes on arterial conditions in the

TABLE 5. Correlation of Age-Adjusted Avg-IMT and Max-IMT to HDL-Cholesterol in Hyperlipidemic Subjects Stratified According to Plasma LDL-Cholesterol Levels

	1st LDL-C Tertile (<162 mg/dL)	2nd LDL-C Tertile (162–196 mg/dL)	3rd LDL-C Tertile (>196 mg/dL)
Age-adjusted Avg-IMT			
equation	y=−0.0032x+0.9064	y=−0.0033x+0.8761	y=−0.0057x+1.0646
r	0.17	0.20	0.29
p	0.01	0.002	0.00001
Age-adjusted Max-IMT			
equation	y=−0.0087x+2.0457	y=−0.0115x+2.0708	y=−0.0163x+2.485
r	0.11	0.18	0.28
p	0.09	0.005	0.00001

Downloaded from atvb.ahajournals.org by on June 8, 2007

affected individuals. It is noteworthy that the carriers of the apolipoprotein A-I_{Milano} mutant, who are at low CAD risk despite plasma HDL-C levels lower than those of the present HypoALP subjects, have IMT values remarkably similar to those of present controls, and therefore lower than HypoALP subjects.[30]

Low HDL levels are often found in the presence of other risk factors, such as high triglycerides, elevated concentrations of atherogenic triglyceride-rich lipoprotein remnants, some degree of insulin resistance, and obesity. All these alterations have been associated with increased IMT.[8,23,26] The HypoALP subjects examined in the present study had higher average triglyceride, glucose, and BMI values than control and HyperALP subjects. The increased thickening of carotid arteries in HypoALP subjects compared with controls remained significant after adjustment for BMI, plasma triglyceride, and glucose levels. Moreover, in the large series of hyperlipidemic subjects, a low HDL-C was the second strongest predictor (after age) of increased IMT, whereas no association was found between IMT and plasma triglycerides. All together, these data indicate that a low HDL-C per se is a predictor of pre-intrusive atherosclerosis, independent of plasma triglycerides.

In the hyperlipidemic subjects, both low HDL-C and high LDL-C were independent predictors of carotid thickening, ie, consistent with the independent effect of these two lipoproteins on CAD risk.[1] More interesting, the influence of low HDL-C on both Avg-IMT and Max-IMT increased with increasing plasma LDL-C levels. Previous reports have indeed shown that HDL-C correlates negatively with carotid atherosclerosis in patients with severe hypercholesterolemia,[24] but not in those with moderate elevations of LDL-cholesterol.[25] It could be argued that a low HDL-C increases its pro-atherogenic potential in the presence of a high global risk, as exemplified here by a moderate elevation of LDL-C, and that the present results may not be generalized to healthy populations. However, a low HDL-C was found to be an independent predictor of increased IMT in different populations,[7,22] including those at very low CAD risk.[27]

HDLs are able to remove cholesterol from a variety of cells, including those present in the arterial wall.[31] Cell cholesterol removal is the first step of reverse cholesterol transport, the process by which HDLs transport excess cholesterol from peripheral tissues, including the arterial wall, to the liver for excretion.[3] Studies in humans and in transgenic mice expressing human HDL apolipoproteins have shown that the individual cholesterol efflux capacity is inversely correlated with plasma HDL level.[31] Therefore, a low plasma HDL concentration would result in defective cholesterol removal from the arterial wall and faster plaque growth that would be seen by using ultrasound as a prevalence of echolucent plaques,[32] and a greater carotid IMT, as shown in the present study. HDL can also downregulate the cytokine-induced expression of cell adhesion molecules (CAMs) in cultured endothelial cells.[4] CAMs mediate the adhesion and transmigration of leukocytes, and thus play a critical role in early atherogenesis. Indeed, the level of circulating CAMs is positively associated with the extent of ultrasound-assessed preclinical atherosclerosis.[33] Therefore, a systemic inflammatory state in subjects with low HDL might also contribute to the increased carotid IMT observed in the present study.

HyperALP has been generally associated with longevity and increased protection against the development of atherosclerotic vascular disease.[15] This association has been recently questioned by the observation that an increased plasma HDL-C level in subjects carrying mutations in the CETP gene is an independent risk factor for CAD.[16] In the present study, HyperALP subjects displayed Avg-IMT and Max-IMT values identical to those of age- and sex-matched subjects with average plasma HDL-C levels. Consistent with this finding, hyperlipidemic subjects in the highest HDL-C quintile showed the same degree of carotid intimal thickening of subjects with average plasma HDL-C values. Some of the HyperALP or high-HDL subjects may carry mutations in the CETP genes, leading to defective cholesterol removal from peripheral cells,[28] and increased intimal thickening. More likely, an average plasma HDL-C concentration is sufficient to prevent excessive arterial lipid deposition and thickening.

The present findings may have important clinical implications for current strategies aimed at preventing cardiovascular disease through a raising of plasma HDL-C levels.[34] The observation of a curvilinear relationship between HDL-C and arterial thickening, if confirmed in prospective studies, suggests that there is no need to increase plasma HDL-C above average values and that attention should be focused on individuals with low HDL-C levels and high global risk who are likely to have the greatest benefit from an HDL-elevating therapy.

Acknowledgements

This work was supported in part by grants from the Italian Ministry of University and Scientific Research (Cofin 1998 and Cofin 2000) to G.F.

References

1. Gordon T, Castelli WP, Hjortland MC, Kannel WB, Dawber TR. High density lipoprotein as a protective factor against coronary heart disease: The Framingham Study. *Am J Med*. 1977;62:707–714.
2. Ross R. Atherosclerosis: an inflammatory disease. *N Engl J Med*. 1999; 340:115–126.
3. von Eckardstein A, Nofer JR, Assmann G. High density lipoproteins and arteriosclerosis: role of cholesterol efflux and reverse cholesterol transport. *Arterioscler Thromb Vasc Biol*. 2001;21:13–27.
4. Cockerill GW, Rye KA, Gamble JR, Vadas MA, Barter PJ. High-density lipoproteins inhibit cytokine-induced expression of endothelial cell adhesion molecules. *Arterioscler Thromb Vasc Biol*. 1995;15:1987–1994.
5. Wilt TJ, Rubins HB, Robins SJ, Riley WA, Collins D, Elam M, Rutan G, Anderson JW. Carotid atherosclerosis in men with low levels of HDL cholesterol. *Stroke*. 1997;28:1919–1925.
6. Pignoli P, Tremoli E, Poli A, Oreste P, Paoletti R. Intimal plus medial thickness of the arterial wall: a direct measurement with ultrasound imaging. *Circulation*. 1986;74:1399–1406.
7. Crouse JR, Toole JF, McKinney WM, Dignan MB, Howard G, Kahl FR, McMahan MR, Harpold GH. Risk factors for extracranial carotid artery atherosclerosis. *Stroke*. 1987;18:990–996.
8. Howard G, Manolio TA, Burke GL, Wolfson SK, O'Leary DH. Does the association of risk factors and atherosclerosis change with age? An analysis of the combined ARIC and CHS cohorts: the Atherosclerosis Risk in Communities (ARIC) and Cardiovascular Health Study (CHS) investigators. *Stroke*. 1997;28:1693–1701.
9. Burke GL, Evans GW, Riley WA, Sharrett AR, Howard G, Barnes RW, Rosamond W, Crow RS, Rautaharju PM, Heiss G. Arterial wall thickness is associated with prevalent cardiovascular disease in middle-aged adults: the Atherosclerosis Risk in Communities (ARIC) Study. *Stroke*. 1995; 26:386–391.
10. O'Leary DH, Polak JF, Kronmal RA, Manolio TA, Burke GL, Wolfson SK Jr. Carotid artery intima and media thickness as a risk factor for

Downloaded from atvb.ahajournals.org by on June 8, 2007

myocardial infarction and stroke in older adults: Cardiovascular Health Study Collaborative Research Group. *N Engl J Med*. 1999;340:14–22.
11. Hodis HN, Mack WJ, LaBree L, Selzer RH, Liu CR, Liu CH, Azen SP. The role of carotid arterial intima-media thickness in predicting clinical coronary events. *Ann Intern Med*. 1998;128:262–269.
12. Genest JJ, Bard JM, Fruchart JC, Ordovas JM, Schaefer EJ. Familial hypoalphalipoproteinemia in premature coronary artery disease. *Arterioscler Thromb*. 1993;13:1728–1737.
13. Calabresi L, Franceschini G. High density lipoprotein and coronary heart disease: insights from mutations leading to low high density lipoprotein. *Curr Opin Lipidol*. 1997;8:219–224.
14. Siervogel RM, Morrison JA, Kelly K, Mellies M, Gartside, Glueck CJ. Familial hyper-alpha-lipoproteinemia in 26 kindreds. *Clin Genet*. 1980; 17:13–25.
15. Glueck CJ, Gartside P, Fallat RW, Sielski J, Steiner PM. Longevity syndromes: familial hypobeta and familial hyperalpha lipoproteinemia. *J Lab Clin Med*. 1976;88:941–957.
16. Agerholm-Larsen B, Nordestgaard BG, Steffensen R, Jensen G, Tybjaerg-Hansen A. Elevated HDL cholesterol is a risk factor for ischemic heart disease in white women when caused by a common mutation in the cholesteryl ester transfer protein gene. *Circulation*. 2000;101: 1907–1912.
17. Menotti A, Seccareccia F, Lanti M. Mean levels and distributions of some cardiovascular risk factors in Italy in the 1970's and the 1980's. *G Ital Cardiol*. 1995;25:1539–1572.
18. Warnick GR, Benderson J, Albers JJ. Dextran sulfate precipitation procedure for quantitation of high density lipoproteins. *Clin Chem*. 1982;28: 1379–1388.
19. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of preparative ultracentrifuge. *Clin Chem*. 1972;18:499–503.
20. Werba JP, Safa O, Michelagnoli S, Sirtori CR, Franceschini G. Plasma triglycerides and lipoprotein (a): inverse relationship in a hyperlipidemic Italian population. *Atherosclerosis*. 1993;101:203–211.
21. Baldassarre D, Werba JP, Tremoli E, Poli A, Pazzucconi, Sirtori CR. Common carotid intima-media thickness measurement: a method to improve accuracy and precision. *Stroke*. 1994;25:1588–1592.
22. Stensland-Bugge E, Bonaa KH, Joakimsen O. Age and sex differences in the relationship between inherited and lifestyle risk factors and subclinical carotid atherosclerosis: the Tromso study. *Atherosclerosis*. 2001; 154:437–448.
23. Bokemark L, Wikstrand J, Attvall S, Hulthe J, Wedel H, Fagerberg B. Insulin resistance and intima-media thickness in the carotid and femoral arteries of clinically healthy 58-year-old men: the Atherosclerosis and Insulin Resistance Study (AIR). *J Intern Med*. 2001;249:59–67.
24. Poli A, Tremoli E, Colombo A, Sirtori M, Pignoli P, Paoletti R. Ultrasonographic measurement of the common carotid artery wall thickness in hypercholesterolemic patients: a new model for the quantitation and follow-up of preclinical atherosclerosis in living human subjects. *Atherosclerosis*. 1988;70:253–261.
25. Gariepy J, Simon A, Massonneau M, Linhart A, Levenson J. Wall thickening of carotid and femoral arteries in male subjects with isolated hypercholesterolemia: PCVMETRA Group Prevention Cardio-Vasculaire en Medecine du Travail. *Atherosclerosis*. 1995;113:141–151.
26. Sidhu PS, Naoumova RP, Maher VM, MacSweeney JE, Neuwirth CK, Hollyer JS, Thompson GR. The extracranial carotid artery in familial hypercholesterolaemia: relationship of intimal-medial thickness and plaque morphology with plasma lipids and coronary heart disease. *J Cardiovasc Risk*. 1996;3:61–67.
27. Ferrieres J, Elias A, Ruidavets JB, Cantet C, Bongard V, Fauvel J, Boccalon H. Carotid intima-media thickness and coronary heart disease risk factors in a low-risk population. *J Hypertens*. 1999;17:743–748.
28. Ohta T, Nakamura R, Takata K, Saito Y, Yamashita S, Horiuchi S, Matsuda I. Structural and functional differences of subspecies of apoA-I-containing lipoprotein in patients with plasma cholesteryl ester transfer protein deficiency. *J Lipid Res*. 1995;36:696–704.
29. Hodis HN, Mack WJ, Barth J. Carotid intima-media thickness as a surrogate end point for coronary artery disease. *Circulation*. 1996;94: 2311–2312.
30. Sirtori CR, Calabresi L, Franceschini G, Baldassarre D, Amato M, Johansson J, Salvetti M, Monteduro C, Zulli R, Muiesan ML, et al. Cardiovascular status of carriers of the apolipoprotein A-I_{Milano} mutant: The Limone sul Garda Study. *Circulation*. 2001;103:1949–1954.
31. Rothblat GH, Llera-Moya M, Atger V, Kellner-Weibel G, Williams DL, Phillips MC. Cell cholesterol efflux: integration of old and new observations provides new insights. *J Lipid Res*. 1999;40:781–796.
32. Mathiesen EB, Bonaa KH, Joakimsen O. Low levels of high-density lipoprotein cholesterol are associated with echolucent carotid artery plaques: the tromso study. *Stroke*. 2001;32:1960–1965.
33. Rohde LE, Lee RT, Rivero J, Jamacochian M, Arroyo LH, Briggs W, Rifai N, Libby P, Creager MA, Ridker PM. Circulating cell adhesion molecules are correlated with ultrasound-based assessment of carotid atherosclerosis. *Arterioscler Thromb Vasc Biol*. 1998;18:1765–1770.
34. Boden WE, Pearson TA. Raising low levels of high-density lipoprotein cholesterol is an important target of therapy. *Am J Cardiol*. 2000;85: 645–650.

Downloaded from atvb.ahajournals.org by on June 8, 2007