Hyperalphalipoproteinemia: Characterization of a Cardioprotective Profile Associating Increased High-Density Lipoprotein$_2$ Levels and Decreased Hepatic Lipase Activity

Didier Sich, Youssef Saïdi, Philippe Giral, Laurent Lagrost, Monique Egloff, Claude Auer, Valérie Gautier, Gérard Turpin, and Isabel Beucler

The aim of the present study was to investigate the high-density lipoprotein (HDL) structural characteristics and metabolism in hyperalphalipoproteinemic (HALP) patients (HDL-cholesterol [HDL-C], 92 ± 14 mg/dL) with combined elevated low-density lipoprotein-cholesterol (LDL-C) levels (LDL-C, 181 ± 33 mg/dL). Patients were subjected to a complete cardiovascular examination, including ultrasonographic investigation of carotid arteries. Two HALP profiles were identified according to the HDL_2/HDL_3 ratio. HALP profile A was characterized in 28 patients by increased HDL_2/HDL_3 ratio, HDL_{2b}, and lipoprotein (Lp)A-I levels compared with normolipidemic subjects, and HALP profile B, including the 12 remaining patients, was characterized by a HDL_2/HDL_3 ratio within the normal range and by the increase of all HDL subclasses ($HDL_{2b,2a,3a,3b,3c}$), LpA-I, and LpA-I:A-II levels. With regard to the exploration of carotid arteries, in HALP profile A, 20 patients were free from lesions and eight had only intimal wall thickening. In HALP profile B, only one patient was free from lesions, four had intimal wall thickening, and seven displayed plaques, but none had stenosis. Taking into account the number of patients with plaques within each group, HALP profile A was associated with a low prevalence of atherosclerotic lesions, whereas HALP profile B was less cardioprotective (odds ratio, 77.7 [95% confidence interval, 3.7 to 1,569.7]; $P < .0001$). For both HALP profiles, cholesteryl ester transfer protein (CETP) deficiency was discarded and activities of phospholipid transfer protein (PLTP) and lipoprotein lipase (LPL) were normal. However, hepatic lipase (HL) activity was significantly decreased in HALP profile A, but within the normal range for HALP profile B. In conclusion, an HALP profile A with a low prevalence of atherosclerosis was characterized by an increased HDL_2/HDL_3 ratio, HDL_{2b}, and LpA-I levels associated with decreased HL activity.

NUMEROUS STUDIES have shown that plasma lipoprotein levels are important determinants of atherosclerosis and cardiovascular disease. Increased plasma levels of low-density lipoprotein-cholesterol (LDL-C) and high-density lipoprotein-cholesterol (HDL-C) are associated with, a high incidence of cardiovascular disease[1,2] and with cardioprotection,[3,4] respectively. Both LDL and HDL are known to be heterogeneous particles in their physical and chemical properties and it is thought that the most atherogenic LDL are of small size,[5,6] and that some HDL particles, but not others, are protective against atherosclerosis.[7,8] In this regard, it is of interest to consider the distribution of HDL according to their density (HDL_2 and HDL_3),[9] but also to their size (HDL_{2b}, HDL_{2a}, HDL_{3a}, HDL_{3b}, HDL_{3c} subclasses in order of decreasing diameters),[10] and to their apolipoprotein (apo) content, lipoprotein (Lp)A-I (particles containing apoA-I but not apoA-II) and LpA-I:A-II (particles containing both apoA-I and apoA-II).[11] Thus, according to their distribution within the HDL subclasses, LpA-I can be of large size in HDL_{2b}, of medium size in HDL_{2a}, HDL_{3a}, and HDL_{3b}, and of small size in HDL_{3c}.[12] Most of the LpA-I are large, whereas LpA-I:A-II are mostly distributed within HDL_{2a}, HDL_{3a}, and HDL_{3b}.[12,13] The levels of large LpA-I correlate inversely and significantly with the incidence of coronary artery disease (CAD) and are considered to be the HDL particles that are the most protective against atherosclerosis.[14] The distribution and the concentrations of the HDL subfractions are under the control of several plasma factors: lecithin:cholesterol acyltransferase (LCAT), cholesteryl ester transfer protein (CETP), phospholipid transfer protein (PLTP), lipoprotein lipase (LPL), and hepatic lipase (HL). CETP promotes the transfer of neutral lipids between apoB-containing lipoproteins and HDL, inducing the formation of large triglyceride-rich HDL_2.[15,16] PLTP, exchanging phospholipids between HDL, converts HDL_3 into both larger and smaller HDL.[17,18] LPL, during its lipolytic activity on triglyceride-rich lipoproteins (TGRL), generates small HDL that join the general metabolism of HDL.[19] The part played by HL in HDL metabolism is to convert large and triglyceride-rich HDL_2 into small HDL_3.[20]

Hyperalphalipoproteinemia (HALP) characterized by high plasma HDL_2 levels has been related either to CETP deficiency[21,22] or to decreased HL activity,[20] both abnormalities that were associated with a longevity syndrome or with cardiovascular protection. However, a controversial study had reported a premature atherosclerosis in HALP patients with CETP deficiency associated with decreased HL activity.[23] Therefore, it remains difficult at present to unequivocally state if HALP and its related metabolic disorders are always associated with cardioprotection.

This study was undertaken to investigate in HALP subjects, the plasma concentrations of the main HDL sufractions (HDL_2, HDL_3, LpA-I, and LpA-I:A-II), the HDL particle size distribution, and the CETP, PLTP, LPL, and HL activities. HALP patients were selected with a combined hypercholesterolemia characterized by elevated LDL-C levels and were considered at high risk for atherosclerosis. They were submitted to careful investigation of their cardiovascular state, with the purpose to

From the Laboratoire de Biochimie des Lipides and Service d'Endocrinologie-Métabolisme, Hôpital de la Pitié, Paris; and the Laboratoire de Biochimie des Lipoprotéines, INSERM CFJ 93-10, Faculté de Médecine, Dijon, France.

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Address reprint requests to Didier Sich, PhD, Laboratoire de Biochimie des Lipides, Pavillon B, Delessert, Hôpital de la Pitié, 83 Boulevard de l'Hôpital, 75651 Paris Cedex 13, France.

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determine whether HALP could be considered to have a cardioprotective profile.

MATERIALS AND METHODS

Subjects

The patients (N = 40) included in this study had elevated LDL-C (≥160 mg/dL for both genders) and HDL-C (≥80 mg/dL for women or ≥65 mg/dL for men) levels, with these selection limits being higher than the 90th percentiles for their age and gender.[24,25] Triglycerides and Lp(a) levels were within the normal range (<100 and 30 mg/dL, respectively). These biological selection criteria were found at least twice before inclusion in the study. Control subjects (N = 20) were healthy and had normal fasting plasma lipid levels: LDL-C ≤ 130 mg/dL and HDL-C from 40 to 65 mg/dL for both genders. The aim of the study was explained to each subject and informed consent was obtained. Patients who were taking lipid-lowering drugs or corticosteroids were excluded from the selection. Other exclusion criteria were as follows: alcoholism, diabetes mellitus, major endocrine disorders (such as thyroid dysfunction), and liver, renal, or gastrointestinal tract diseases. All patients were questioned on their own and family medical history and whether they smoked. Measurements of weight and height allowed the calculation of body-mass index (BMI). The complete medical examination performed by a physician included the determination of systemic blood pressure at least three times using a sphygmomanometric procedure and a resting ECG. Exercise ECG was performed using a bicycle ergometer in the upright position, starting at 30 W and with an increase of load by 30 W every 3 minutes. The right and left common and internal extracranial carotid arteries (including the bifurcations) were explored by ultrasonography on a duplex system (Ultramark IV; Squibb Medical Systems, USA), using a 7.5-MHz scanning frequency in B-mode and 3.75-Mhz frequency in a pulsed Doppler mode. Ultrasonographic results were divided into four categories: 1, normal arteries; 2, intimal wall thickening, characterized by a distance of greater than 1 mm between the lumen-intima and the media-adventicia interface; 3, plaque, considered as a distinct area that obstructed less than 30% of the carotid lumen; and 4, stenosis, ie, a lesion obstructing more than 30% of the carotid lumen. The reliability of this classification of carotid lesions has been validated elsewhere.[26]

Seven patients had smoked or were still smoking, although occasionally or moderately, as their cigarette consumption was of 15 ± 10 packs per year. All patients had a normal BMI (<25.5 $kg \cdot m^{-2}$) and one hypertensive patient was corrected by medication during the study. The structural interview and the medical examination of patients had clearly established that at the time of the inclusion in the study, all were asymptomatic for CAD and none had a familial origin of hypercholesterolemia. ECGS and exercise ECGS were normal in all patients, allowing us to exclude any silent ischemia. Ultrasonographic assessment of carotid arteries showed that 21 patients were free from lesions, 12 displayed moderate lesions (intimal wall thickening), and seven had arterial plaques, but none displayed stenosis.

Blood Samples

Venous blood samples were collected after a 12-hour overnight fast and were drawn into sterile EDTA-containing tubes (Vacutainer; Becton Dickinson, Le Pont de Clair, France). Plasma was then promptly separated by a 10-minute centrifugation at 3,000× *g* at 10°C. Proteolytic inhibitors (aprotinin 10 μg/mL, D-phenylalanyl-propyl-L-arginine chloromethyl ketone 0.5 μg/mL, benzamidine 30 μg/mL, and of soy bean and lima bean antitrypsin 20 μg/mL [final concentrations]; Sigma, St Quentin Fallavier, France) and a LCAT inhibitor (iodoacetic acid 1.5 mmol/L final concentration) were added to plasma samples that were kept at 4°C for 24 hours for usual determinations and all the analytical investigations. Postheparin plasma samples were obtained 10 minutes after intravenous injection of heparin (100 IU/kg body weight) and collected into ice-cold heparinized tubes. Immediately, samples were centrifuged at 4°C and 3,500× g for 10 minutes. For CETP, PLTP, LPL, and HL activity assays, samples were frozen and kept at −80°C.

Analytical Procedures

Lipids in plasma and in isolated lipoprotein fractions were quantified on a Specific Supra analyser (Kone, Espoo, Finland) by usual enzymatic methods with bioMérieux kits (bioMérieux, Marcy l'Etoile, France) for total cholesterol (TC) and triglycerides (TG), with a Boerhinger Mannheim kit (Mannheim, Germany) for free cholesterol (FC) and with a Wako phospholipid kit (Wako Chemicals, Neuss, Germany) for phospholipids (PL). HDL-C was determined using a phosphotungstic acid/$MgCl_2$ reagent (Boehringer Mannheim) to precipitate the apoB-containing lipoproteins, and cholesterol was measured in the supernatant, as described earlier for plasma. LDL-C was calculated by Friedewald's formula.[27] The cholesteryl esters (CE) mass was calculated as: 1.67× (TC − FC) mass. ApoA-I, A-II, and B, and Lp(a) were measured with nephelemetric assays (Behring BNA, Marburg, Germany), using antibodies provided by Behring for apoA-I and B and Lp(a), and by Immuno AG (Heidelberg, Germany) for apoA-II. Lipids and apolipoproteins were controlled daily with French Committee for Research Coordination on Atherosclerosis and Cholesterol (ARCOL)-specific controls. LpA-I particles were determined in total plasma by electroimmunodiffusion on ready-to-use plates (Hydragel LpA-I kit; Sebia, Issy les Moulineaux, France).[28] LpA-I:A-II levels were calculated, subtracting the LpA-I from the apoA-I concentrations obtained with nephelemetric assays as described earlier.

Isolation of HDL_2 and HDL_3

Gradient density ultracentrifugation enabled isolation of the plasma lipoproteins with a continuous KBr gradient using the general procedure of Chapman et al.[29] Briefly, the density (*d*) of the plasma was first adjusted to 1.21 g/mL by the addition of solid dry KBr. A discontinuous gradient was then constructed in a 12-mL tube (Ultraclear; Beckman, Palo Alto, CA), overlaying 2 mL of a NaCl-KBr solution at $d = 1.25$ g/mL, 3 mL of plasma at $d = 1.21$ g/mL, 2 mL of a NaCl-KBr solution at $d = 1.063$ g/mL, 2.5 mL of a NaCl-KBr solution at $d = 1.109$ g/mL, and 2.5 mL of a NaCl solution at $d = 1.006$ g/mL. Tubes were immediately centrifuged in a SW41-Ti rotor, on a L8-55 ultracentrifuge (Beckman) for 48 hours at 40,000 rpm (197,568× *g*) and 10°C. In isolated LDL, HDL_2, and HDL_3, TC, FC, CE, PL, and TG levels were determined as described earlier for total plasma and the protein concentrations were measured using the method of Lowry et al,[30] with bovine serum albumin as the standard. Lipoprotein mass corresponded to the sum of the mass of FC, CE, PL, TG, and proteins. ApoA-I, LpA-I, and LpA-I:A-II concentrations were determined in HDL_2 and HDL_3 as described earlier for total plasma.

Electrophoretic Separation of LDL and HDL Subclasses

LDL and HDL size distributions were analyzed by nondenaturing polyacrylamide gradient gel (PAGG) electrophoresis.[10,31] Total lipoprotein fractions were isolated at $d < 1.21$ g/mL from 3 mL of plasma (quick-seal tubes) by preparative ultracentrifugation for 3.5 hours at 90,000 rpm (560, 196× *g*) and 10°C in a NVT 90 rotor on a Optima XL 90 ultracentrifuge (Beckman). LDL and HDL subfractions were separated by electrophoresis in 20 to 160 g/L and 40 to 300 g/L PAGG, respectively.[10,31] Migration buffer solution consisted of Tris (hydroxymethyl) aminomethane (Trizma-base; Sigma) 0.025 mol/L and glycine 0.186 mol/L, pH 8.3. Gels were cast and run in a Penguin vertical electrophoresis system (OWL Scientific, Woburn, MA). Electrophoresis was performed at 5°C for 24 hours with rising voltages: for LDL, 2 hours at 30 V, 10 hours at 100 V, and 8 hours at 200 V; and for HDL, 2 hours at 30 V, 12 hours at 80 V, and 10 hours at 200 V. Gels were fixed

and stained with Coomassie brilliant blue G and destained in a acetic acid (5%) solution.

The mean apparent diameters of the isolated lipoprotein subfractions were determined by comparison with calibration curves constructed with proteins of known Stokes diameters (High Molecular Weight Protein calibration kit; Pharmacia, Uppsala, Sweden). For LDL subfractions, the calibration curve was constructed with ferritin (12.2 nm), thyroglobulin (17 nm), and carboxylated latex beads (38 nm) (Duke Scientific, Palo Alto, CA). For HDL subfractions, the calibration curve was constructed with albumin (7.1 nm), lactate dehydrogenase (8.16 nm), ferritin (12.2 nm), and thyroglobulin (17 nm). Two patterns of LDL size distribution were defined according to Austin et al[6]: pattern A with LDL size higher than 25.5 nm, and pattern B with LDL size lower than 25.5 nm. The LDL distribution profiles were monodisperse or polydisperse; in the latter cases, the mean apparent diameter was calculated according the mobility of the major peak. Five HDL subpopulations were identified according to Blanche et al[10] as follows: HDL_{2b} (9.71 to 12.9 nm), HDL_{2a} (8.77 to 9.71 nm), HDL_{3a} (8.17 to 8.77 nm), HDL_{3b} (7.76 to 8.17 nm), and HDL_{3c} (7.21 to 7.76 nm). To calculate the percentage distribution of HDL subclasses, areas under the scan curves were integrated within the size limits, on a Preference densitometer (Sebia) at 570 nm, and by relating them to the total area considered as 100% of HDL. To estimate the plasma concentrations of HDL subclasses, the percentage of each HDL subclass was multiplied by the sum of the protein content of HDL_2 and HDL_3.

CETP Activities

Determination of plasma CETP activity with endogenous lipoproteins. This assay was made according to the method previously described by Lagrost et al,[31] measuring the capacity of the plasma CETP to transfer the radiolabeled CEs ([^{3}H]-CE) from a tracer amount of exogenous [^{3}H]-CE-HDL_3 considered as CE donors, to the endogenous apoB-containing lipoproteins of total plasma (VLDL + LDL) considered as CE acceptors. Under these experimental conditions, CETP activity values reflect the modulation of concentration and composition of endogenous lipoproteins. HDL_3 ($1.125 < d < 1.21$ g/mL) were isolated from a pool of normolipidemic plasma samples by sequential ultracentrifugation, and labeled [^{3}H]-CE-HDL_3 was prepared according to the general procedure developed by Albers et al.[32] Briefly, aliquots of plasma (50 µL) were incubated for 3 hours at 37°C with [^{3}H]-CE-HDL_3 (5 nmol of cholesterol), iodoacetate (150 nmol), in Tris-buffer-saline (TBS) made of Tris 50 mmol/L NaCl 150 mmol/L, and EDTA 2 mmol/L, pH 7.4 (final volume, 100 µL). Nonincubated controls were maintened at 4°C for the same time. The apoB-containing lipoproteins were isolated by preparative ultracentrifugation (in quick-seal tubes for 2.5 hours at 90,000 rpm [560,196× *g*] and 10°C in a NVT 90 rotor on a Optima XL 90 ultracentrifuge [Beckman]) adding 60 µL of the incubation mixture to 5.5 mL of a NaCl-KBr solution at $d = 1.070$ g/mL (final $d = 1.068$ g/mL).[31] The $d < 1.068$ (1.5 mL) and the $d > 1.068$ g/mL lipoprotein fractions were collected and the radioactivity was counted in a Packard Bell (Warrenville, IL) liquid scintillation counter. Each plasma and blank was assayed in duplicate in two experiments. Results were expressed in nanomoles of [^{3}H]-CE transferred from labeled HDL_3 to endogenous VLDL + LDL per milliliter of plasma and per hour (nmol/mL/h). Intraassay and interassay coefficients of variation (CVs) were 4.8% and 6.3%, respectively.

Determination of plasma CETP activity with exogenous lipoproteins. CETP activity with exogenous lipoproteins was determined measuring the transfer of [^{3}H]-CE between exogenous [^{3}H]-CE-HDL_3 as CE donnors and exogenous unlabeled LDL as CE acceptors, according to the method of Mann et al.[33] Under these conditions, this assay minimizes the relative effects of donnor and acceptor lipoproteins and provides a measure of CETP mass. Exogenous LDL ($1.019 < d < 1.063$ g/mL) and HDL_3 ($1.125 < d < 1.21$ g/mL) were isolated from a pool of normolipidemic plasma samples by sequential ultracentrifugations. Briefly, aliquots of plasma (5 µL) were incubated for 3 hours at 37°C with [^{3}H]-CE-HDL_3 (25 nmol of CE) and unlabeled LDL (500 nmol of CE) in TBS (final volume, 100 µL). Simultaneously, blank controls were incubated without plasma and nonincubated controls were maintained at 4°C for the same time. The [^{3}H]-CE-LDL were isolated as described earlier for the former CETP assay. Results were expressed in nanomoles of [^{3}H]-CE transferred from labeled HDL_3 to unlabeled LDL per milliliter of plasma and per hour (nmol/mL/h). Intraassay and interassay CVs were 4.3% and 6.1%, respectively.

Isotopic Assay of Plasma PLTP Activity

PLTP activity was determined measuring the transfer of labeled phosphatidylcholine [^{14}C]-PC from [^{14}C]-PC-liposomes to the plasma HDL fraction as previously described by Lagrost et al[34] and with a general procedure derived from Damen et al.[35] Briefly, plasma (30 µL), [^{14}C]-PC-liposomes (125 nmol of PC), and iodoacetate (120 nmol) were incubated for 30 minutes at 37°C in a final volume of 80 µL. In blank controls, incubation mixtures were maintened at 4°C. After incubation, [^{14}C]-PC-liposomes and apoB-containing lipoproteins were precipitated with 60 µL of a solution containing NaCl (500 mmol/L), $MnCl_2$ (215 mmol/L), and heparin (445 U/mL). After removal of precipitates by low-speed centrifugation, the resulting supernatants containing the [^{14}C]-PC-HDL were counted for radioactivity in a Packard Bell liquid scintillation counter. Each plasma and blank was assayed in duplicate in two experiments. PLTP activity was calculated as the percentage of total radiolabeled phospholipids transferred from [^{14}C]-PC-liposomes to plasma HDL fraction per milliliter and per hour (%/mL/h) after deduction of the radioactivity contained in control samples maintained at 4°C.

HL and LPL Activities

Activities of HL and LPL were independently measured according to the method of Nilsson-Ehle and Ekman.[36] Briefly, HL activity assays were performed by incubation for 30 minutes at 37°C of 5 µL of postheparin plasma with 0.2 mL of an emulsion consisting of labeled [9, 10-^{14}C] trioleylglycerol (specific activity, 65 mCi/mmol; Amersham, Eire, UK), unlabeled trioleylglycerol, and lysophosphatidylcholine, made in a Tris-HCl buffer 0.2 mol/L, pH 9, containing BSA (1%) and NaCl (4 mol/L) to inhibit the LPL activity. LPL activities were assayed using the same emulsion as for HL assay incubated for 30 minutes at 37°C, but with the following differences: BSA (4%) and heat-inactivated human serum 10% (as apoCII source) were added only after emulsion of the substrate and no NaCl was added. Both HL and LPL enzymatic reactions were stopped by adding 3.5 mL methanol/chloroform/heptane mixture (1.41/1.25/1.0, vol/vol/vol) and free fatty acids (FFA) were extracted using a borate buffer consisting of potassium tetraborate (0.1 mmol/L) and carbonate (0.1 mol/L), at pH 10.5.[37] Released FFA were recovered in the upper extraction phase and were counted for radioactivity. Blank controls were constituted using aliquots of plasma collected before the intravenous injection of heparin. In each experiment, two postheparin plasma controls obtained from normolipidemic subjects (one man and one woman) were assayed simultaneously to validate the assay. Each plasma and control was assayed in duplicate in two experiments. Both HL and LPL activity values were expressed in micromoles of FFA released from the radiolabeled emulsion per milliliter of plasma and per hour (µmol/mL/h). Intraassay and interassay CVs for HL were 6.8% and 7.9% and for LPL were 7.5% and 8.4%, respectively.

Statistical Analysis

Comparisons of two groups were made with Student's *t* test for normal continuous variables and by the Mann-Whitney *U* test for nonnormal variables. Comparisons of three groups were made by the Kruskall-Wallis test, followed by two by two comparisons with

Mann-Whitney U test, with a threshold of significance determined by the Bonferroni adjustment. We systematically decided to compare the three groups using nonparametric tests, because the number of subjects in the third group was low. Differences in the frequency of category variables were tested with the χ^2 test. For correlation analysis, the coefficients r and P were calculated using the Spearman rank correlation analysis. A P value less than .05 was considered significant. An odds ratio was calculated to evaluate the prevalence of carotid plaques between the two HALP profiles.

RESULTS

Plasma Levels of Lipids, Apolipoproteins, and Lipoproteins of all Patients and Controls

Mean plasma levels of TC, LDL-C, HDL-C, apoB, and apoA-I were higher in all HALP patients than in control subjects ($P < .001$ for all), as were apoA-II, LpA-I, and LpA-I:A-II levels ($P < .01$ for all) (Table 1).

Concentrations of HDL_2 and HDL_3 Subfractions: Definition of Two HALP Profiles

In the 20 normolipidemic subjects, the concentrations of HDL_2 and HDL_3 were entirely consistent with those previously observed in healthy adults (HDL_2, 92 ± 38; HDL_3, 173 ± 46 mg/dL; HDL_2/HDL_3, 0.53 ± 0.15).[29,38] When examining closely the distribution of HDL_2 and HDL_3 in HALP patients, 28 had isolated increased HDL_2 levels ($P < .001$ v controls) and a HDL_2/HDL_3 ratio ($P < .001$ v controls) (Fig 1). The 12 remaining HALP patients had both elevated HDL_2 and HDL_3 levels ($P < .01$ and $P < .05$ v controls, respectively), resulting in a HDL_2/HDL_3 ratio within the normal range (Fig 1). On the basis of the HDL_2/HDL_3 ratio, two HALP profiles were defined: HALP profile A characterized by a HDL_2/HDL_3 ratio greater than 0.815, which corresponds to the 95th percentile of the normal distribution, and HALP profile B, with a HDL_2/HDL_3 ratio within the normal range (Fig 1). Consequently, in HALP profile A, HDL_2 and HDL_3 concentrations were not significantly different, whereas in HALP profile B, HDL_2 concentrations were significantly lower ($P < .01$) than HDL_3 concentrations, as observed in normolipidemic subjects (Table 1).

Table 1. Plasma Levels of Lipids, Apolipoproteins, and Lipoproteins in HALP Patients and Controls

Parameter	HALP Patients			Controls
	All	Profile A	Profile B	
No.	40	28	12	20
Age (yr)	55 ± 14	54 ± 12	59 ± 5	49 ± 15
Sex ratio (F/M)	30/10	21/7	9/3	13/7
TC (mg/dL)	288 ± 39*	293 ± 42*	286 ± 40*	191 ± 20
LDL-C (mg/dL)	181 ± 23*	184 ± 18*	180 ± 20*	114 ± 24
HDL-C (mg/dL)	92 ± 14*	95 ± 15*	90 ± 8*	58 ± 9
HDL_2 (mg/dL)	202 ± 54*	217 ± 49*	156 ± 51†	92 ± 38
HDL_3 (mg/dL)	202 ± 56*	185 ± 43	244 ± 62‡	173 ± 46
ApoB (mg/dL)	125 ± 24*	126 ± 24*	126 ± 22*	95 ± 14
ApoA-I (mg/dL)	211 ± 25*	211 ± 27*	213 ± 24*	157 ± 21
ApoA-II (mg/dL)	57.6 ± 14†	54.4 ± 14‡§	65.3 ± 12†	43 ± 11
LpA-I (mg/dL)	91 ± 21†	96.4 ± 20†§	77.3 ± 18‡	51 ± 19
LpA-I:A-II (mg/dL)	120 ± 28*†	114 ± 26¶	136 ± 28†	106 ± 19

NOTE. Data concern 40 HALP patients: 28 of profile A, 12 of profile B, and 20 normolipidemic subjects. Chemical analyses were performed as described in the Methods. Values are given as means ± SD.

Abbreviations: F, female; M, male.

*$P < .001$, †$P < .01$, and ‡$P < .05$ compared with control subjects.

§$P < .05$ and ¶$P < .01$, compared with subjects of profile B.

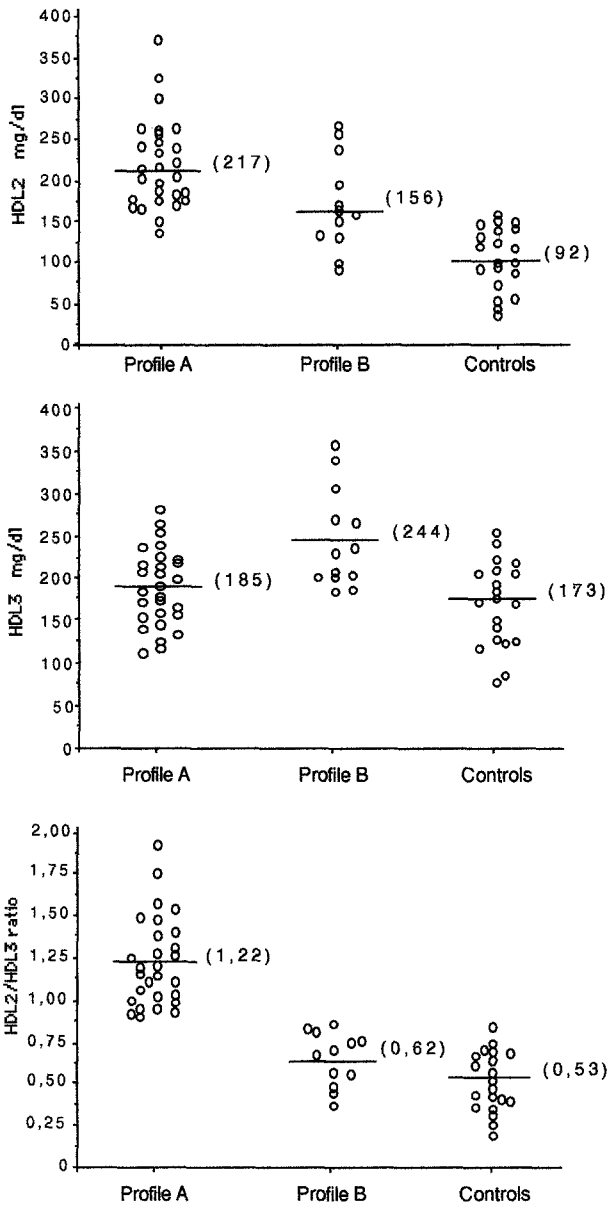

Fig 1. HDL_2 and HDL_3 concentrations and HDL_2/HDL_3 ratios in both groups of HALP patients and controls. Data concern 40 HALP patients: 28 of profile A, 12 of profile B, and 20 normolipidemic subjects. Horizontal lines indicate respective values of arithmetic means expressed in mg/dL in parenthesis.

Anthropometric Characteristics, Plasma Lipid, Apolipoprotein, and Lipoprotein Levels in Patients of Both HALP Profiles

Patients with both HALP profiles A and B had similar mean age (54 ± 12 and 59 ± 5 years, respectively), sex ratio (women/men, 21/7 and 9/3, respectively), and mean BMI (22.9 ± 1.4 and 22.6 ± 2.0 kg · m^{-2}, respectively). Compared with values in control subjects, mean plasma levels of TC, LDL-C, HDL-C, apoB, and apoA-I were higher in both HALP profiles ($P < .001$

for all), as well as those of apoA-II ($P < .05$ for HALP profile A and $P < .01$ for HALP profile B), LpA-I ($P < .01$ for HALP profile A and $P < .05$ for HALP profile B), and LpA-I:A-II ($P < .01$ for only HALP profile B) (Table 1). Plasma levels of TC, LDL-C, HDL-C, apoB, and apoA-I were not significantly different between both HALP profiles, but in HALP profile A compared with HALP profile B, the LpA-I concentrations were increased ($P < .05$) and apoA-II and LpA-I:A-II concentrations were decreased ($P < .05$ and $P < .01$, respectively) (Table 1).

Levels of apoA-I, LpA-I and LpA-I:A-II, in HDL_2 and HDL_3 Subfractions of Both HALP Profiles

ApoA-I concentrations in HDL_2 and HDL_3 were not significantly different in HALP profile A; however, in HALP profile B, they were lower in HDL_2 than in HDL_3 ($P < .01$) (Table 2). LpA-I concentrations in HDL_2 of HALP profile A were higher than (1) in HDL_3 of the same profile ($P < .05$), and (2) in HDL_2 and HDL_3 of HALP profile B ($P < .05$ for both) (Table 2). LpA-I:A-II concentrations were lower in both HDL_2 and HDL_3 of HALP profile A and in HDL_2 of HALP profile B than in HDL_3 of HALP profile B ($P < .05$ for all) (Table 2).

LDL Size and HDL Subclass Levels

The size of LDL in all patients and controls showed a pattern, A,[6] characterized by LDL mean apparent diameters greater than 25,50 nm: HALP profile A, 27.12 ± 0.46 nm with a range from 27.8 to 25.52 nm; HALP profile B, 27.34 ± 0.45 nm with a range from 27.9 to 26.38 nm; and control subjects, 26.96 ± 0.60 nm with a range from 27.8 to 25.87 nm, values that were not statistically different between the two HALP profiles and controls. HDL were divided into five electrophoretic subclasses varying according to their diameters from 12.9 to 7.2 nm and their levels were given in milligrams of protein per deciliter (Table 3). In HALP profile A, only the levels of HDL_{2b} (9.71 to 12.9 nm) were significantly increased compared with controls and with HALP profile B ($P < .001$ for both comparisons). In contrast, in HALP profile B compared with controls, all of the HDL subclass levels were increased, significantly for HDL_{2b}, HDL_{2a}, HDL_{3a}, and HDL_{3b} ($P < .05$ for all), but without significance for HDL_{3c}. However, in HALP profile B, compared with HALP profile A, only HDL_{3a} and HDL_{3b} subclass levels were increased ($P < .05$ for both) (Table 3).

Table 2. Distribution of ApoA-I, LpA-I, and LpA-I:A-II Particles in HDL_2 and HDL_3 of the Two Groups of HALP Patients

Parameter	HALP Profile A		HALP Profile B	
	HDL_2	HDL_3	HDL_2	HDL_3
ApoA-I (mg/dL)	84 ± 17	82 ± 14	75 ± 10*	92 ± 9
LpA-I (mg/dL)	44 ± 10†	31 ± 9	30 ± 2	33 ± 14
LpA-I:A-II (mg/dL)	41 ± 16‡	51 ± 16‡	44 ± 14‡	60 ± 19

NOTE. ApoA-I were determined by nephelemetric assay and LpA-I by electroimmunodiffusion, in HDL_2 and HDL_3 isolated by ultracentrifugation. Levels of LpA-I:A-II were calculated subtracting the LpA-I concentrations from apoA-I levels. Values are means ± SD.

*$P < .01$, compared with HDL_3 of HALP profile B.

†$P < .05$, compared with HDL_2 of HALP profile A and to both HDL_2 and HDL_3 of HALP profile B.

‡$P < .05$, compared with HDL_3 of HALP profile B.

Table 3. HDL Subclass Concentrations in Both Groups of HALP Patients and Controls

HDL Subclass	HALP Patients		Controls
	Profile A	Profile B	
HDL_{2b} (mg/dL)	73.8 ± 17.9*	50.3 ± 14.5†	34.0 ± 8.5
HDL_{2a} (mg/dL)	45.2 ± 10.9	50.9 ± 15.6†	29.8 ± 13.6
HDL_{3a} (mg/dL)	41.0 ± 12.6	63.9 ± 22.4‡	36.2 ± 13.7
HDL_{3b} (mg/dL)	16.2 ± 4.6	25.7 ± 6.9‡	15.3 ± 7.1
HDL_{3c} (mg/dL)	5.9 ± 4.0	6.6 ± 3.4	4.8 ± 3.5

NOTE. The nomenclature used for HDL subclasses is in accordance with Blanche et al[10] and the concentrations are expressed in mg/dL. Values are means ± SD.

*$P < .001$, compared with control group and to HALP profile B.

†$P < .05$, compared with control group.

‡$P < .05$, compared with control group and with HALP profile A.

CETP, PLTP, LPL, and HL Activities

Values of the CETP activity measured with both assays (with endogenous or exogenous lipoproteins) in all patients of both HALP profiles were statistically higher than values observed in control subjects ($P < .001$ for both CETP assays) and they were not significantly different between both HALP profiles (Table 4). Values of PLTP and of LPL activities were not statistically different between both HALP profiles and were similar to those observed in control subjects (Table 4). In contrast, HL activity values were markedly lower in HALP profile A than in HALP profile B and in the control group ($P < .001$ for both comparisons) (Table 4). In addition, HL activities were not statistically different between HALP profile B and controls.

Clinical Results

With regard to the results of the ultrasonographic exploration of carotid arteries (Table 5), among the 28 patients with HALP profile A, 20 were free from lesions and eight had intimal wall thickening. Among 12 patients with HALP profile B, one was free from lesions, four displayed intimal wall thickening, and seven had plaques. The incidence of medium lesions such as

Table 4. Activities of CETP, PLTP, LPL, and HL in Both Groups of HALP Patients and in Controls

Activity	HALP Patients		Controls
	Profile A	Profile B	
CETP assay with endogenous lipoproteins	13.2 ± 3.1*	14.1 ± 2.9*	9.6 ± 3.0
CETP assay with exogenous lipoproteins	158.8 ± 34.5*	163.5 ± 42.2*	90 ± 12.0
PLTP	723 ± 96	771 ± 108	670 ± 110
LPL	17.6 ± 3	18.9 ± 4	16.5 ± 3
HL	12.6 ± 3.9†	20.5 ± 6	19.8 ± 3.7

NOTE. CETP activities in both assays were expressed in nanomoles of [^{3}H]-CE transferred per milliliter of plasma and per hour (nmol/mL/h). PLTP activities were measured as the percentage of [^{14}C]-PC transferred per milliliter of plasma and per hour (%/mL/h). LPL and HL activities are given in micromoles of free fatty acid released per milliliter of postheparin plasma and per hour (μmol/mL/h). Data concern 40 HALP patients: 28 of profile A, 12 of profile B, and 20 normolipidemic subjects.

*$P < .001$, compared with controls.

†$P < .001$, compared with HALP profile B and with controls.

Table 5. Ultrasonographic Findings of the Extracranial Carotid Arteries in Both Groups of HALP Patients

Ultrasonographic Result	HALP Patients	
	Profile A (n = 28)	Profile B (n = 12)
Absence of lesions (n)	20*	1
Intimal wall thickening (n)	8	4
Plaques (n)	0	7†

NOTE. Data concern 40 HALP patients: 28 of profile A and 12 of profile B. Absence or presence of atherosclerotic lesions such as intimal wall thickening and plaques was diagnosed on the basis of ultrasonographic findings of right and left extracranial common and internal carotid arteries.

*$P < .001$ compared with subjects of profile B.

†$P < .001$ compared with subjects of profile A.

intimal wall thickening was not significantly different between the two HALP profiles; however, considering the absence of lesions in the first group and the presence of plaques in the second group, the prevalence of atherosclerotic lesions was significantly lower in HALP profile A than in HALP profile B ($P < .001$, χ^2 test). The absence of plaques and the increase of HDL_2/HDL_3 ratio were closely related, as shown by the odds ratio (77.7; 95% confidence interval, 3.76 to 1,569.7; $P < .0001$). The cut-off value for the HDL_2/HDL_3 ratio, used to calculate the odds ratio, was 0.815 and corresponded to the 95th percentile of the control group distribution.

Correlations

(1) Between HDL subfraction and HDL subclass concentrations. Several positive and significative correlations were established: (a) between levels of HDL_2 subfraction and both HDL_{2b} ($r = +.84$, $P < .001$) and HDL_{2a} ($r = +.46$, $P < .01$) subclasses; and (b) between levels of HDL_3 subfraction and both HDL_{3a} ($r = +.83$, $P < .001$) and HDL_{3b} ($r = +.74$, $P < .001$) subclasses.

(2) Between plasma levels of LpA-I and LpA-I:A-II and HDL subclass concentrations. Several positive and significative correlations were established: (a) between plasma levels of LpA-I and HDL_{2b} subclass ($r = +.48$, $P < .01$); and (b) between plasma levels of LpA-I:A-II and HDL_{2a}, HDL_{3a}, and HDL_{3b} subclasses ($r = +.46$, $r = +.48$, $r = +.42$, $P < .01$ for all, respectively).

(3) Between CETP activities and LDL-C concentrations. CETP activities assayed with both methods correlated positively with the LDL-C levels ($r = +.70$ for the CETP assay with endogenous lipoproteins, and $r = +.55$ for the CETP assay with exogenous lipoproteins; $P < .001$ for both).

(4) Between HL activity values and HDL subfraction, HDL subclass, plasma LpA-I, and LpA-I:A-II levels. HL activity values correlated (a) negatively and significantly with HDL_2 subfraction ($r = -.34$, $P < .05$), HDL_{2b} subclass ($r = -.46$, $P < .01$), and plasma LpA-I concentrations ($r = -.44$, $P < .01$); and (b) positively and significantly with HDL_3 subfraction ($r = +.35$, $P < .05$), HDL_{3a} subclass ($r = +.40$, $P < .01$), and plasma LpA-I:A-II concentrations ($r = +.32$, $P < .05$).

DISCUSSION

The present study was undertaken to investigate the lipoprotein characteristics of hyperalphalipoproteinemia, with the purpose to define to what extent this unusual lipoprotein profile may be considered as protective against premature atherosclerosis in hypercholesterolemic patients. In the selected 40 patients with elevated levels of both HDL-C and LDL-C, two profiles of HALP were identified that differed in the distribution of HDL_2 and HDL_3 subfractions. HALP profile A was characterized by an elevated HDL_2/HDL_3 ratio consequent to the sole increase of the HDL_2 subfraction. The further lipoprotein characteristics of this HALP profile were elevated HDL_{2b} subclass and LpA-I levels, associated with low HL activity values. In contrast, in HALP profile B, the HDL_2/HDL_3 ratio was within the normal range, since both HDL_2 and HDL_3 subfractions were increased. In addition, this HALP profile was characterized by elevated levels of all the HDL subclasses and of both LpA-I and LpA-I:A-II, and was associated with HL activity values that were within the normal range.

To establish in these patients the extent of atherosclerotic lesions, the clinical investigation included noninvasive ultrasonographic exploration of the common and internal carotid arteries; furthermore, the extent of atherosclerosis of these arteries is well correlated with that of coronary arteries.[26,39,40] In the 28 patients with HALP profile A, lesions were either totally absent or moderate, whereas in the 12 patients with HALP profile B, atherosclerotic lesions were much more frequent and severe, although stenosis was not observed. Given that the patients of both HALP profiles had LDL with the same size and lipid composition (data not shown) and similarly increased LDL-C levels, they were considered as being exposed to the same potential risk for atherosclerosis.[41] Therefore, we can assert that patients with the HALP profile A displayed a cardioprotection that was not observed in patients with HALP profile B.

The antiatherogenic properties of HDL have been ascribed, in numerous epidemiological and experimental studies, to HDL_2 and more precisely to the HDL_{2b} subclass[42,43] and to LpA-I.[44,45] This is in part due to large LpA-I distributed within the HDL_{2b}, an interval size[12,13] that, compared with medium and small LpA-I and with LpA-I:A-II,[14] are the most effective particles to promote cholesterol efflux from cultured cells.[46,47] Therefore in HALP profile A, considering the predominant distribution of LpA-I within the HDL_2 density range and the increase of the sole HDL_{2b} subclass, the LpA-I are assumed to be mainly distributed within HDL_{2b} and thus to be of large size. In contrast, in HALP profile B, considering the equal distribution of LpA-I within HDL_2 and HDL_3 density ranges and the increase of all the HDL subclasses, large LpA-I are likely not predominant over medium and small LpA-I. Furthermore, in HALP profile B in comparison to HALP profile A, plasma levels of LpA-I:A-II are not only increased, but also distributed mainly within HDL_3. Therefore, the distribution of LpA-I and LpA-I:A-II within HDL subclasses of both HALP profiles can account for the reduced prevalence of atherosclerosis in HALP profile A.

To address the metabolic origins of the lipoprotein characteristics of both HALP profiles, we investigated the role of the CETP, PLTP, LPL, and HL. LCAT was not further explored, since the CE/TC ratio in the plasma and in the lipoprotein fractions of all patients (data not shown) was similar to that of controls.[29] CETP deficiency has been reported to induce HALP characterized by increased levels of large and CE-enriched-HDL_2,[21,22] and thus our first design was to investigate the

activity of this transfer protein. However, the CETP activities were increased in all patients compared with normolipidemic subjects and were not different between both HALP profiles. Given that the observed elevated CETP activity values could be related to the increased LDL and HDL levels of the HALP patients, another assay was performed in which the CE were transferred not towards endogenous, but towards exogenous lipoproteins. Therefore, the increased plasma CETP activities were confirmed, which shows that both HALP profiles cannot originate from a CETP deficiency and CETP is probably not involved in the difference of the HDL subclass distribution observed between HALP profiles. However, the elevated CETP activities of HALP patients are rather relevant as regards their similarly elevated LDL-C levels, as increased synthesis of CETP has been previously related to hypercholesterolemia[48,49] in response to high levels of circulating LDL.[50,51] To date, it remains difficult to precisely determine if the CETP is a proatherogenic or antiatherogenic factor. In several studies, CETP deficiency has been associated with cardioprotection and longevity syndrome[21,22]; however, recent studies have reported that CETP-deficient subjects presented with CAD.[23,52,53] Thus, neither the origin of HALP profiles nor the difference in the cardioprotection between the patients seems to be related to CETP. PLTP is known to generate HDL_{2b} from HDL_{3a} during the transfer of phospholipids,[54,55] and therefore, an increased PLTP activity could be at the origin of the elevated HDL_{2b} levels in the HALP profile A. However, the plasma PLTP activities were similar in all patients of both HALP profiles and in controls, and therefore PLTP is unlikely to be the origin of the reported HALP. Activities of LPL and HL also are known to influence the HDL_2/HDL_3 ratio in a reciprocal manner. In healthy subjects, LPL is the main actor of TGRL metabolism, and its activity in the fasting state is positively correlated with concentrations of HDL_2.[56] In contrast, HL activity is inversely correlated with HDL_2 levels, since this enzyme hydrolyzes both TGs and PLs of HDL_2, which are thus converted into HDL_3.[19,20,57] However, HL also acts in TGRL metabolism, converting VLDL remnants into LDL. The LPL activities of all HALP patients were similar to those of control subjects; therefore, LPL action appeared to be normal in HALP patients and does not seem to account for their difference in the HDL_2/HDL_3 ratios. In contrast, HL activities were strikely different between HALP profiles: in HALP profile A, HL activities were markedly decreased compared with controls, whereas in HALP profile B, they were within the normal range. This difference was confirmed by the negative correlations established between HL activities and HDL_2, HDL_{2b}, and LpA-I levels, and by the positive correlations between HL activities and HDL_3, HDL_{3a}, and LpA-I:A-II levels. According to Mowri et al,[58] the increase of LpA-I levels within HDL_2, as observed in HALP profile A, may be relevant on the preferential hydrolysis by HL of LpA-I:A-II over LpA-I particles. According to the cases reported by Hegele et al, the decreased HL activity in HALP profile A could result from a heterozygous form of the familial HL deficiency.[59] However, in this large kindred family, only two of 15 simple heterozygotes displayed isolated HALP, whereas all of the others had abnormal TGRL metabolism. Therefore, the familial HL deficiency in its heterozygous form appears to be much more involved in TGRL abnormalities than to be at the origin of HALP.[59] Since no individual or familial history of TGRL abnormalities were observed in our HALP patients, a familial HL deficiency, as described by Hegele et al,[59] seems to be discarded in the HALP profile A. Thus, we can hypothesize that the decreased HL activity might originate from another disorder, such as an allelic variation in the HL gene.[60] However, no metabolic disorder seems to account for the HALP profile B, for which another explanation remains to be found. High levels of HDL have been reported either linked to allelic variations on the A-I/C-III/A-IV cluster[60] or to polymorphism in the 5′-flanking region in the apoA-I gene promoter.[61] We can also assume that high levels of HDL may originate from decreased HDL catabolism. For instance, the recently identified scavenger receptor SR-BI[62] can be an actor to consider, since its potential dysfunction may induce a defective clearance of HDL by hepatocytes or macrophages.

In summary, the present study concerned HALP patients for whom it has been demonstrated that CETP deficiency was not involved. Two HALP profiles were identified, but only HALP profile A is associated with prevention of the onset or progression of atherosclerotic lesions. This HALP profile was characterized by an increase of the HDL_2/HDL_3 ratio associated with increased HDL_{2b} and LpA-I levels and decreased HL activity. Consequently, the cardiovascular protection usually attributed to increased levels of HDL-C has to be carefully considered.

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