Interrelationships Between Postprandial Lipoprotein B:CIII Particle Changes and High-Density Lipoprotein Subpopulation Profiles in Mixed Hyperlipoproteinemia

Youssef Saïdi, Didier Sich, Anne Camproux, Monique Egloff, Marie-Christine Federspiel, Valérie Gautier, Alain Raisonnier, Gérard Turpin, and Isabel Beucler

We studied the relationships postprandially between triglyceride-rich lipoprotein (TRL) and high-density lipoprotein (HDL) in 11 mixed hyperlipoproteinemia (MHL) and 11 hypercholesterolemia (HCL) patients. The high and prolonged postprandial triglyceridemia response observed in MHL but not HCL patients was essentially dependent on very-low-density lipoprotein (VLDL) changes. This abnormal response was related to decreased lipoprotein lipase (LPL) activity (−48.7%, $P < .01$) in MHL compared with HCL subjects. Cholesteryl ester transfer protein (CETP) activity was postprandially enhanced only in MHL patients, and this elevation persisted in the late period (+19% at 12 hours, $P < .05$), sustaining the delayed enrichment of VLDL with cholesteryl ester (CE). The late postprandial period in MHL patients was also characterized by high levels of apolipoprotein B (apoB)-containing lipoproteins with apoCIII ([LpB:CIII] +36% at 12 hours, $P < .01$) and decreased levels of apoCIII contained in HDL ([LpCIII-HDL] −34% at 12 hours, $P < .01$), reflecting probably a defective return of apoCIII from TRL toward HDL. In MHL compared with HCL patients, decreased HDL2 levels were related to both HDL2b and HDL2a subpopulations (−57% and −49%, respectively, $P < .01$ for both) and decreased apoA-I levels (−53%, $P < .01$) were equally linked to decreased HDL2 with apoA-I only (LpA-I) and HDL2 with both apoA-I and apoA-II ([LpA-I:A-II] −55% and −52%, respectively, $P < .01$ for both). The significant inverse correlations between the postprandial magnitude of LpB:CIII and HDL2-LpA-I and HDL2b levels in MHL patients underline the close TRL-HDL interrelationships. Our findings indicate that TRL and HDL abnormalities evidenced at fasting were postprandially amplified, tightly interrelated, and persistent during the late fed period in mixed hyperlipidemia. Thus, these fasting abnormalities are likely postprandially originated and may constitute proatherogenic lipoprotein disorders additional to the HCL in MHL patients.

HYPERCHOLESTEROLEMIA (HCL) with elevated low-density lipoprotein cholesterol (LDL-C) levels is associated with a high risk of coronary heart disease (CHD).[1] Patients with mixed hyperlipoproteinemia (MHL), ie, a combination of HCL and hypertriglyceridemia, appear to be exposed to additional risk factors, given their hypertriglyceridemia[2] and decreased high-density lipoprotein cholesterol (HDL-C) levels.[3] The relation between fasting hypertriglyceridemia and CHD is still under discussion,[4-6] although several publications[7-9] have reported that the metabolism of triglyceride-rich lipoprotein (TRL), chylomicrons, very–low-density lipoprotein (VLDL), and their remnants is a determinant of fasting HDL-C levels. HDL particles are heterogeneous, considering their density (HDL2 and HDL3),[10] size (HDL2a, 2b, 3a, 3b, and 3c),[11] and apolipoprotein (apo) content (with apoA-I only [LpA-I] or with apoA-I and apoA-II [LpA-I:A-II]).[12] Among these HDL particles, several are considered antiatherogenic markers.[13,14] The TRL-HDL exchanges concern apolipoproteins and neutral lipids and involve lipoprotein-modifying enzyme activities: lipoprotein lipase (LPL), hepatic triglyceride lipase (HTGL), and cholesteryl ester (CE) transfer protein (CETP).[15,16] To better investigate apolipoprotein exchanges, the distribution of apoE and apoCIII within TRL, ie, apoB-containing lipoproteins with apoE or apoCIII (LpB:E or LpB:CIII), and within HDL, ie, LpE-HDL or LpCIII-HDL, could be studied as previously proposed.[17-19]

To date, there are no data on the postprandial changes and interrelationships between LpB:E, LpB:CIII and LpCIII-HDL, LpE-HDL in MHL patients. To focus on the hypertriglyceridemia, these patients were compared with HCL patients. The criteria of selection were proposed to consider both groups with the same level of HCL but differing in fasting triglyceridemia. In addition, to define fasting normal values of the parameters analyzed, we recruited 11 healthy normolipidemic subjects.

SUBJECTS AND METHODS

Subjects

Eleven subjects each for the hyperlipidemic and normolipidemic groups were recruited from the Endocrinology-Metabolism Department at La Pitié Hospital in Paris.

Since there is currently no genetic marker allowing clinical identification of MHL, recruitment of the patients was performed according to fasting plasma LDL-C levels of at least 160 mg/dL and triglyceride (TG) levels of at least 200 mg/dL, both parameters over the 90th percentile for a sex- and age-matched population.[20] Selected HCL patients had fasting plasma LDL-C levels within the same range (≥160 mg/dL) but fasting TG levels less than 120 mg/dL, considered in the normal range. In both groups, lipoprotein(a) (Lp(a)) levels were less than 20 mg/dL. A complete routine medical examination was performed including measurement of weight, height, and diastolic and systolic blood pressure. None of the patients consumed alcohol regularly, and all subjects reported no acute use of alcohol within the previous days. One HCL patient was a smoker for 2 years, but stopped at least 6 months before the study. One HCL patient discontinued lipid-lowering therapy 2 months before the study. All remaining subjects were not taking any medication known to affect lipid metabolism. None had evidence of diabetes mellitus (repeated fasting blood glucose <110 mg/dL) or hepatic, gastrointestinal, thyroidal, and renal disease as ascertained by medical history, physical examination, and routine laboratory analysis.

From the Laboratoire de Biochimie des Lipides et Lipoprotéines, Service de Biomathématiques, and Service d'Endocrinologie-Métabolisme, Hôpital de La Pitié, Paris, France.

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Address reprint requests to Youssef Saïdi, PhD, Laboratoire de Biochimie des Lipides et Lipoprotéines, Pavillon Benjamin Delessert, Hôpital de La Pitié, 83, Bd de l'hôpital, 75651, Paris cedex 13, France.

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At the time of the study, no patient in either group was hypertensive or had abnormal electrocardiogram or exercise electrocardiogram profiles. Ultrasonography of extracranial carotid arteries was performed on a duplex system (Ultramark IV; Squibb Medical System, Reuil Malmaison, France). The right and left common and internal carotid arteries (including bifurcations) were explored using a 7.5-MHz scanning frequency in the B mode and a 3.75-MHz frequency in a pulsed Doppler mode. Four MHL patients presented with premature CHD: three had plaques and one had stenosis (a lesion obstructing >30% of the carotid lumen). The other patients had normal carotid arteries or mere intimal wall thickening (a distance >1 mm between the lumen intima and the media adventicia interface).[21] The aim of the study was explained to each subject, and informed consent was obtained. The protocol was approved by the local ethics committee.

Study Design and Fat-Feeding Protocol

Patients were hospitalized on the day before the study. Between 6:30 and 7:00 PM, they received a dinner of 500 kcal/m^2 body surface area prepared in the Dietetic Department of the hospital, containing 45% of calories as carbohydrate, 19% as protein, and 36% as fat, with 215 mg cholesterol. After fasting overnight, at 7:00 AM the next morning, patients ingested a fat-rich meal as previously used by others.[7,9,22] This contained 729 kcal/m^2 body surface area, with 83% fat (polyunsaturated to saturated fatty acid ratio, 0.06), 14% carbohydrate, and 3% protein, and the cholesterol content was 240 mg/m^2 body surface area. The meal was consumed within 15 minutes, and patients were allowed to be ambulatory throughout the protocol period and to drink water only. The meal was well tolerated by all subjects, and none complained of gastrointestinal problems or steatorrhea.

Blood Sampling

Fasting and 2-, 4-, 6-, 9-, and 12-hour postprandial blood samples were collected via a small forearm indwelling catheter into sterile tubes (Vacutainer, Orsay, France). Samples were immediately centrifuged at 3,000 × *g* for 15 minutes at 4°C, and the plasma was added to proteolytic inhibitors (Sigma, Saint-Quentin Fallavier, France). Samples were kept at +4°C and used for analytical studies within 24 hours. For CETP activity, fasting and postprandial 4-, 6-, 9-, and 12-hour samples were stored at −80°C until assay. For lipase activities, subjects received an intravenous injection of heparin 100 U/kg body weight at least 1 week after a 12-hour fast. Postheparin blood was drawn 10 minutes later into ice-cooled heparin-lithium–containing glass tubes and centrifuged at +4°C, and the plasma was stored at −80°C until assay.

Analytical Assays

Total cholesterol (TC), free cholesterol (FC), TG, and phospholipid (PL) levels in plasma and lipoprotein fractions were determined using commercial enzymatic kits (Biomérieux, Marcy-L'Etoile, and Boehringer, Meylan, France). CE levels were calculated as the CE mass, (TC − FC) × 1.67, and thus represent the sum of esterified cholesterol and fatty acid moieties. HDL-C levels were measured after selective precipitation of apoB-containing lipoproteins with a phosphotungstic acid/$MgCl_2$ reagent (Boehringer). LDL-C was calculated according to the method of Friedewald et al[23] when plasma TG levels were less than 400 mg/dL. Lipid levels were measured on an autoanalyzer (Kone, Evry, France), and apolipoprotein levels were determined by immunoephelometry (BNA; Behring, Reuil Malmaison, France: apoA-I, apoB, apoE, and Lp(a) with Behring kits, and apoA-II with ImmunoFrance antibodies). Intrassay and interassay variation coefficients were less than 2.7% and less than 3.4% for lipids and less than 3.2% and less than 4.1% for apolipoproteins.

Lipoprotein Isolation

Chylomicrons were first isolated at d < 0.99 g/mL, and the remaining lipoprotein fractions were separated on 3-mL aliquots of d > 0.99 g/mL infranatants. For this, a discontinuous density gradient with different NaCl-KBr densities was used as previously described[24]: 2 mL d = 1.25 g/mL, 3 mL d > 0.99 g/mL infranatant adjusted at d = 1.21 g/mL with solid KBr, 2 mL d = 1.063 g/mL, 2.5 mL d = 1.019 g/mL, and 2.5 mL d = 1.006 g/mL. Ultracentrifugation was performed in an SW41 swinging-bucket rotor at 197,568 × *g* for 48 hours at 10°C (L8-55 Ultracentrifuge; Beckman, Gagny, France). Recovered lipoprotein fractions were dialyzed overnight against phosphate-buffered saline containing 1 mmol/L EDTA (pH 7.3). The protein amount was determined by the method of Lowry et al.[25]

Apolipoprotein Phenotyping

ApoE phenotyping was performed by isoelectric focusing according to Bouthillier et al,[26] and the isoelectrophoretic mobility of apoA-I, A-II, and A-IV isoforms was analyzed[27] on a Hoeffer apparatus (Hoeffer Scientific Instrument, San Francisco, CA).

Distribution Profile of HDL Subpopulations

The distribution of HDL subpopulations according to diameter was determined on fasting and postprandial samples for total lipoproteins isolated at d < 1.21 g/mL.[11] Briefly, samples containing 4 parts of dialyzed d < 1.21 g/mL fractions (15 μg protein) and 1 part of a solution consisting of 40% sucrose and 0.01% bromophenyl blue were applied to 4% to 30% nondenaturing polyacrylamide gradient gels. Electrophoresis was performed at 4°C: 2 hours at 30V, 10 hours at 100V, and 8 hours at 200V (Touzart & Matignon, Courtaboeub, France). The migrating buffer contained 25 mmol/L Tris and 186 mmol/L glycine, pH 8.3. At the end of electrophoresis, proteins were fixed for 1 hour (10% sulfosalicylic acid), stained for 1.5 hours in 0.04% Coomassie G-250 and 3.5% perchloric acid, and then destained in 5% acetic acid. The wet gels were then scanned using a Preference densitometer at 570 nm (Sebia, Issy-Les-Moulineaux, France). The limits of each HDL subclass on the scanning curves were derived from the relative migration of protein standards of known Stokes diameter (HMW Calibration Kit; Pharmacia, Saint-Quentin-en-Yvelines, France) applied on the same gels. The relative distribution of each HDL subclass was obtained by calculation of the area under the curve (AUC) of each subclass as defined according to its diameter range: HDL2b, 9.71 to 12.9 nm; HDL2a, 8.77 to 9.71; HDL3a, 8.17 to 8.77; HDL3b, 7.76 to 8.17; and HDL3c, 7.21 to 7.76. To estimate the protein level of each HDL subclass, the relative percentage of the AUC for each HDL subclass was multiplied by the total HDL (HDL2 + HDL3) protein content of the sample.

ApoCIII-, ApoE-, and ApoA-I–Containing Lipoprotein Concentrations

Plasma levels of LpB:CIII were indirectly determined by the difference between the plasma level of total lipoproteins containing apoCIII (LpCIII) and lipoproteins containing apoCIII without apoB (LpCIII-HDL). Both LpCIII and LpCIII-HDL levels were directly measured by electroimmunoassay on ready-to-use Hydragel kits (Sebia). A previous precipitation of apoB-containing lipoproteins by a polyclonal anti-apoB antibody was performed to allow measurement of LpCIII-HDL levels. ApoE-containing lipoproteins (LpE, LpE-HDL, and LpB:E) were assayed with ready-to-use specific Hydragel kits according to the same method. LpA-I levels in plasma and in HDL2 and HDL3 subfractions were measured using the same technique (ready-to-use LpA-I Hydragel kits), and LpA-I:A-II levels were calculated by subtracting LpA-I from total apoA-I levels previously determined in plasma and in HDL2 and HDL3 subfractions by nephelometry. Details

of the method are provided in the manufacturer's instructions. For all parameters, intraassay and interassay variation coefficients were less than 5.6% and less than 6.1%, respectively.

CETP Assay

CETP activity was determined on fasting and 4-, 6-, 9-, and 12-hour postprandial samples using a substrate-independent assay measuring the transfer of radiolabeled CE ([^{3}H]-CE) from exogenous labeled HDL3 ([^{3}H]-CE-HDL3) as CE donors toward exogenous unlabeled LDL as CE acceptors. Exogenous LDL and HDL3 were obtained from a pool of normolipidemic plasma and respectively isolated by sequential ultracentrifugation at $1.019 < d < 1.063$ and $1.125 < d < 1.21$ g/mL. Labeled [^{3}H]-CE-HDL3 was prepared according to Albers et al,[28] and CETP activity was determined according to Mann et al.[29] Briefly, plasma (5 μL) was incubated for 3 hours at 37°C with [^{3}H]-CE-HDL3 (25 nmol CE) and unlabeled LDL (500 nmol CE) in Tris buffer saline containing 50 mmol/L Tris, 150 mmol/L NaCl, and 2 mmol/L EDTA, pH 7.4 (final vol, 100 μL). After incubation, 95 μL of the mixture was added to 5.5 mL NaCl-KBr solution of $d = 1.07$ g/mL (final $d = 1.068$ g/mL) in quick-seal tubes (Beckman) and ultracentrifuged at 560, 196 × *g* for 2.5 hours at 10°C (rotor NVT; Optima XL-90; Beckman). Then, the supernatant (1.5 mL) of $d < 1.068$ g/mL and the infranatant of $d > 1.068$ g/mL were collected and transferred into counting vials (Packard liquid scintillation counter, Les Ulis, France). Blank controls were incubated for the same period without plasma, and nonincubated controls were maintained at 4°C. Results are expressed as nanomoles of [^{3}H]-CE transferred to the $d < 1.068$ g/mL fraction per milliliter of plasma per hour. Intraassay and interassay variation coefficients were less than 5% and less than 8%, respectively.

Postheparin Lipase Activities

LPL and HTGL activities were measured in postheparin plasma as previously reported.[30] Briefly, the LPL assay was performed in the presence of antihuman HTGL antibodies. The substrate was an emulsion containing [9,10-^{14}C]oleic acid-labeled trioleoylglycerol (Amersham, Amersham, England), lysophosphatidyl-choline, and unlabeled triacylglycerol (Sigma) in 0.2 mol/L Tris-HCl buffer (pH 8.0). After sonication, bovine serum albumin (BSA) 4% and heat-inactivated plasma (providing apoC-II as LPL activator) were added. For HTGL activity, the same emulsion was used but contained BSA 1% in 0.2 mol/L Tris-HCl buffer (pH 9.0) and 4 mol/L NaCl (to inhibit LPL activity). Incubations were performed at 37°C for 30 minutes. Reactions were stopped by adding a methanol:chloroform:heptane mixture (1.45:1.25:1.00 vol/vol/vol) and borate buffer, pH 10.5. Radioactivity was determined in 1-mL aliquots of the upper extraction phase. LPL and HTGL activities were expressed as micromoles of free fatty acids released per milliliter of postheparin plasma per hour. Intraassay and interassay variation coefficients were less than 7.5% and less than 8.4% for LPL and less than 6.8% and less than 7.9% for HTGL, respectively.

Statistics

Mean differences between groups were analyzed by the nonparametric Mann-Whitney *U* test. Repeated-measures ANOVA was performed. When the overall F statistic was significant ($P < .05$), further comparisons within a given group were evaluated by the nonparametric Wilcoxon matched-pairs test after Bonferroni correction.[31] The magnitude of TG or lipoprotein responses during the entire 12-hour postprandial period was calculated as the total AUC by the trapezoidal rule, taking the zero concentration as the abscissa axis. Area measurements were expressed as milligrams per deciliter per hour. Associations between lipoprotein variables were determined by Pearson correlation coefficients after adjusting for AUC plasma TG. Analyses were performed by the Statistical Analysis System (SAS) program of the Biomathematics Department of the Hospital.

RESULTS

Anthropometric Data and Fasting Levels of Plasma Lipids, Apolipoproteins, and Lipoproteins

The sex ratio, mean age, and body mass index were not different between the three groups (Table 1). The distribution of apoE phenotypes was similar in hyperlipidemic groups: among 11 patients in each group, there were six E3/E3, three E4/E3, one E3/E2, and one E4/E4. Isoelectrophoretic patterns of apoA-I, apoA-II, and apoA-IV were normal. Levels of TC, LDL-C, and apoB were higher in hyperlipidemic versus normolipidemic subjects. TG and apoE were higher and HDL-C and apoA-I were lower in MHL compared with HCL and normolipidemic subjects. LpB:E and LpB:CIII were higher and LpCIII-HDL was lower in MHL versus HCL and normolipidemic subjects. LpE-HDL levels were similar in the three groups.

Postprandial Levels of Plasma Lipids and Apolipoproteins

The peak of plasma TG was greater (512 ± 97 *v* 218 ± 48 mg/dL, $P < .01$) and delayed (6 *v* 4 hours) in MHL versus HCL patients. At all postprandial times, the levels of TC, LDL-C, and apoB were unchanged; however, HDL-C was decreased at 6 hours only in MHL patients (−10% ± 4%, $P < .05$). ApoE increased by 26% at 6 hours in MHL and by 12% at 4 hours in HCL patients ($P < .05$ for both).

Postprandial Neutral Lipid Changes of VLDL

In MHL patients, the postprandial TRL-TG response resulted essentially from the changes of VLDL (93% at 0 hours, 62% at 6 hours, and 77% at 12 hours), which levels remained higher at

Table 1. Anthropometric Data and Fasting Plasma Lipid, Apolipoprotein and Lipoprotein Levels in MHL and HCL Patients and Normolipidemic Subjects

Parameter	MHL Patients	HCL Patients	Normolipidemic Subjects
Sex (F/M)	5/6	6/5	6/5
Age (yr)	49 ± 5	50 ± 4	48 ± 6
BMI (kg/m²)	24.6 ± 0.9	24.9 ± 1.1	23.7 ± 1.4
Lipids (mg/dL)			
TG	229 ± 26***†††	110 ± 21	111 ± 25
TC	297 ± 20††	304 ± 29‡‡	207 ± 27
FC	98 ± 11††	100 ± 12‡‡	68 ± 9
LDL-C	179 ± 17††	184 ± 21‡‡	132 ± 14
HDL-C	41 ± 9*†	55 ± 11	54 ± 8
Apolipoproteins (mg/dL)			
ApoA-I	144 ± 26**††	172 ± 23	169 ± 25
ApoA-II	45 ± 8	50 ± 14	49 ± 12
ApoB	144 ± 13††	151 ± 18‡‡	114 ± 12
ApoE	7.0 ± 2.2**††	4.4 ± 1.6	3.9 ± 1.0
Lipoprotein particles (mg/dL)			
LpB:E	4.5 ± 1.4**††	1.3 ± 0.6	1.5 ± 0.5
LpE-HDL	2.4 ± 0.4	2.4 ± 0.6	2.2 ± 0.7
LpB:CIII	2.6 ± 0.5**††	1.4 ± 0.3	1.1 ± 0.6
LpCIII-HDL	1.6 ± 0.4**††	3.2 ± 1.0	3.5 ± 1.3

NOTE. Values are the mean ± SD.

Abbreviations: F, female; M, male; BMI, body mass index.

*Between MHL and HCL groups, † between MHL and normolipidemic groups, and ‡ between HCL and normolipidemic groups: *, † or ‡$P < .05$; **, †† or ‡‡$P < .01$; *** or †††$P < .005$.

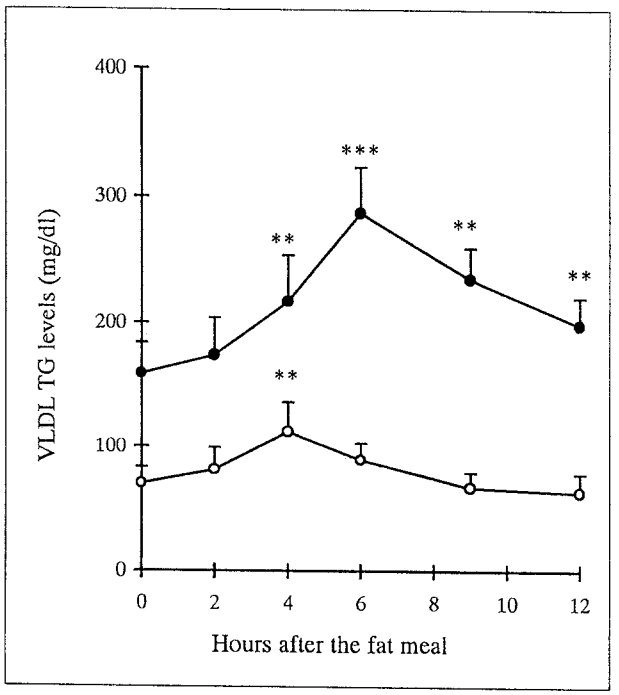

Fig 1. Postprandial VLDL-TG levels in MHL (●) and HCL (○) patients. Significant differences between postprandial and fasting values in each group: **$P < .01$ and ***$P < .005$. Total TG AUC was 2.7-fold higher in MHL *v* HCL patients: 2,661 ± 515 *v* 974 ± 215 mg/dL × h ($P < .005$).

12 hours than at fasting (Fig 1). Thus, we focused the analysis of neutral lipid changes only on this fraction (Table 2). Compared with fasting, the increases of both TG and CE within VLDL at 6 hours were higher in MHL versus HCL patients (81% *v* 60% for TG and 37% *v* 25% for CE, $P < .01$ for both comparisons). At 12 hours, TG and CE remained higher than at fasting in MHL patients (by 25% and 39%, respectively), whereas these neutral lipids returned to basal values in HCL patients. Consequently, VLDLs were first TG-enriched at 6 hours in both groups and CE-enriched at 12 hours only in MHL patients, as indicated by the CE/TG ratio.

HDL Analysis: Neutral Lipids, Subpopulation Profiles, and ApoA-I–Containing Particles

Fasting HDL2-TG and CE levels were about twofold lower in MHL versus HCL patients (Table 2). By contrast, fasting HDL3-TG and CE levels were similar in both groups (data not shown). Postprandially, although HDL2 and HDL3 mass were unchanged in both groups, HDL2 was TG-enriched and CE-depleted at 6 hours in MHL patients only, and these changes persisted partially at 12 hours.

The distribution profile of HDL subpopulations at fasting was characterized by lower levels of both HDL2b and HDL2a and higher levels of both HDL3b and HDL3c in MHL versus HCL and normolipidemic subjects (Table 3). Postprandially, HDL2b decreased at 6 hours (4.4 ± 2.0 *v* 6.9 ± 2.3 mg/dL, $P = .065$) and HDL3c increased at 12 hours (17.9 ± 4.6 *v* 15.3 ± 4.2 mg/dL, $P = .072$) in MHL patients only.

Decreased fasting plasma apoA-I levels in MHL were related to reduced levels of apoA-I, LpA-I, and LpA-I:A-II in HDL2 compared with HCL and normolipidemic controls (Table 3). However, in HDL3, no significant differences were noted between MHL, HCL, and normolipidemic subjects for apoA-I (95 ± 14 *v* 104 ± 21 and 100 ± 17 mg/dL), LpA-I (21 ± 7 *v* 22 ± 8 and 23 ± 7 mg/dL), and LpA-I:A-II (74 ± 16 *v* 82 ± 20 and 79 ± 18 mg/dL).

Postprandially, LpA-I and LpA-I:A-II within HDL2 tended to decrease at 9 hours in MHL patients only, without reaching significance (−7% ± 3% and −6% ± 2%, respectively). The levels of these particles did not vary within HDL3 in any group.

Postprandial TRL-HDL Interrelations: ApoE- and ApoCIII-Containing Particle Analysis

The peak increase of LpB:E levels was higher (29% *v* 20%, $P < .05$) and more delayed (6 *v* 4 hours) in MHL versus HCL patients, and the return to fasting values was also more delayed in the former group (12 *v* 9 hours). In contrast, LpE-HDL levels did not vary in any group (Fig 2A).

The peak increase of LpB:CIII levels was higher (56% *v* 16%, $P < .01$) and more delayed (6 *v* 4 hours) in MHL versus HCL patients, and the elevated levels persisted in the late period (9 to 12 hours) in MHL patients only (Fig 2B). In the same periods, LpCIII-HDL levels were decreased in MHL patients, remaining lower at 12 hours than at fasting, whereas they did not vary in HCL patients. As a consequence, the LpB:E/LpE-HDL and LpB:CIII/LpCIII-HDL ratios, which reflect the distribution of apoE- and apoCIII-containing particles between TRL and HDL, were higher in MHL versus HCL patients (Fig 2A and B). The elevated LpB:CIII/LpCIII-HDL ratio at late postprandial times corresponded to the combined LpB:CIII increase and LpCIII-HDL decrease.

Table 2. Postprandial Neutral Lipid Changes in VLDL and HDL2 in MHL and HCL Patients

Lipid	MHL Patients			HCL Patients		
	0 Hours	TG Peak (6 hours)	12 Hours	0 Hours	TG Peak (4 hours)	12 Hours
VLDL-CE	67 ± 15††	92 ± 17**††	93 ± 22**††	28 ± 7	35 ± 11	28 ± 9
TG	159 ± 21††	287 ± 41**††	198 ± 29*††	70 ± 16	112 ± 22*	63 ± 17
CE/TG	0.42 ± 0.06	0.32 ± 0.05*†	0.47 ± 0.05*	0.40 ± 0.1	0.31 ± 0.07*	0.44 ± 0.08
HDL2-CE	7.1 ± 2.3††	4.5 ± 1.6**††	6.2 ± 1.5††	15 ± 3.8	14.2 ± 3.1	15.2 ± 4.0
TG	4.3 ± 1.6††	6.2 ± 1.7*†	5.0 ± 1.2†	8.6 ± 2.7	9.0 ± 2.1	9.1 ± 1.9
CE/TG	1.65 ± 0.22	0.72 ± 0.17**††	1.24 ± 0.20†	1.74 ± 0.21	1.58 ± 0.16	1.69 ± 0.19

NOTE. Values are the mean ± SD. CE and TG levels are mg/dL.

*Represents a significant difference between postprandial times and the fasting value within a group; † represents the significant difference between the two groups for a given time. * or †$P < .05$ and ** or ††$P < .01$.

Table 3. Fasting Levels of HDL Subpopulations, LpA-I, and LpA-I:A-II in MHL and HCL Patients and Normolipidemic Subjects

Parameter	MHL Patients	HCL Patients	Normolipidemic Subjects
HDL subpopulations (mg/dL)			
HDL2b	6.9 ± 2.3**††	16.0 ± 4.8	18 ± 5.1
HDL2a	14.8 ± 4.0**††	29.1 ± 7.7	27.0 ± 8.2
HDL3a	46.6 ± 9.3	51.2 ± 11.8	50.3 ± 9.8
HDL3b	41.0 ± 6.4*†	33.2 ± 5.1	32.5 ± 7.0
HDL3c	15.3 ± 4.2	12.9 ± 4.4	11.4 ± 3.7
LpA-I and LpA-I:A-II (mg/dL)			
Plasma LpA-I	37 ± 11**††	51 ± 14	54 ± 10
Plasma LpA-I:A-II	107 ± 19	120 ± 32	115 ± 28
HDL2 apoA-I	21 ± 2.8**††	45 ± 4.0	47 ± 5.7
HDL2 LpA-I	10 ± 3.1**††	22 ± 4.4	26 ± 4.0
HDL2 LpA-I:A-II	11 ± 2.7**††	23 ± 3.2	21 ± 3.9

NOTE. Values are the mean ± SD.

Comparisons between groups were made using the Mann-Whitney *U* test. Comparisons between groups are designated: * between MHL and HCL groups and † between MHL and normolipidemic groups. *,†$P < .05$; **, ††$P < .01$.

CETP and Postheparin Lipase Activities

At fasting, CETP activity was not different between hyperlipidemic groups (192 ± 34 *v* 185 ± 29 nmol CE/mL/h, $P = .81$), but was higher than the value in normolipidemic subjects (142 ± 26 nmol CE/mL/h, $P < .01$). Postprandially in MHL patients only, this activity was maximally increased at 6 hours and remained higher at 12 hours than at fasting (Fig 3).

LPL activity was lower (9.7 ± 2 *v* 18.9 ± 5 and *v* 16.2 ± 6 μmol FFA/mL/h, $P < .05$) and HTGL activity was higher (27 ± 7 *v* 18 ± 5 and *v* 21 ± 4 μmol FFA/mL/h, $P < .05$) in MHL versus HCL and normolipidemic subjects, respectively.

Associations Between Lipoprotein Parameters and Lipoprotein-Modifying Enzyme Activities

Levels of HDL2-LpA-I and HDL2b were inversely associated with the postprandial magnitude of LpB:CIII, reaching significance in MHL patients only. Such associations with LpB:E were not significant in either group (Table 4). For the MHL and HCL groups together, CETP activity was positively correlated with the levels of HDL2-TG ($r = .63$, $P < .05$) and VLDL-CE ($r = .64$, $P < .05$) and LPL activity was inversely correlated with the changes in VLDL-TG ($r = -.61$, $P < .05$).

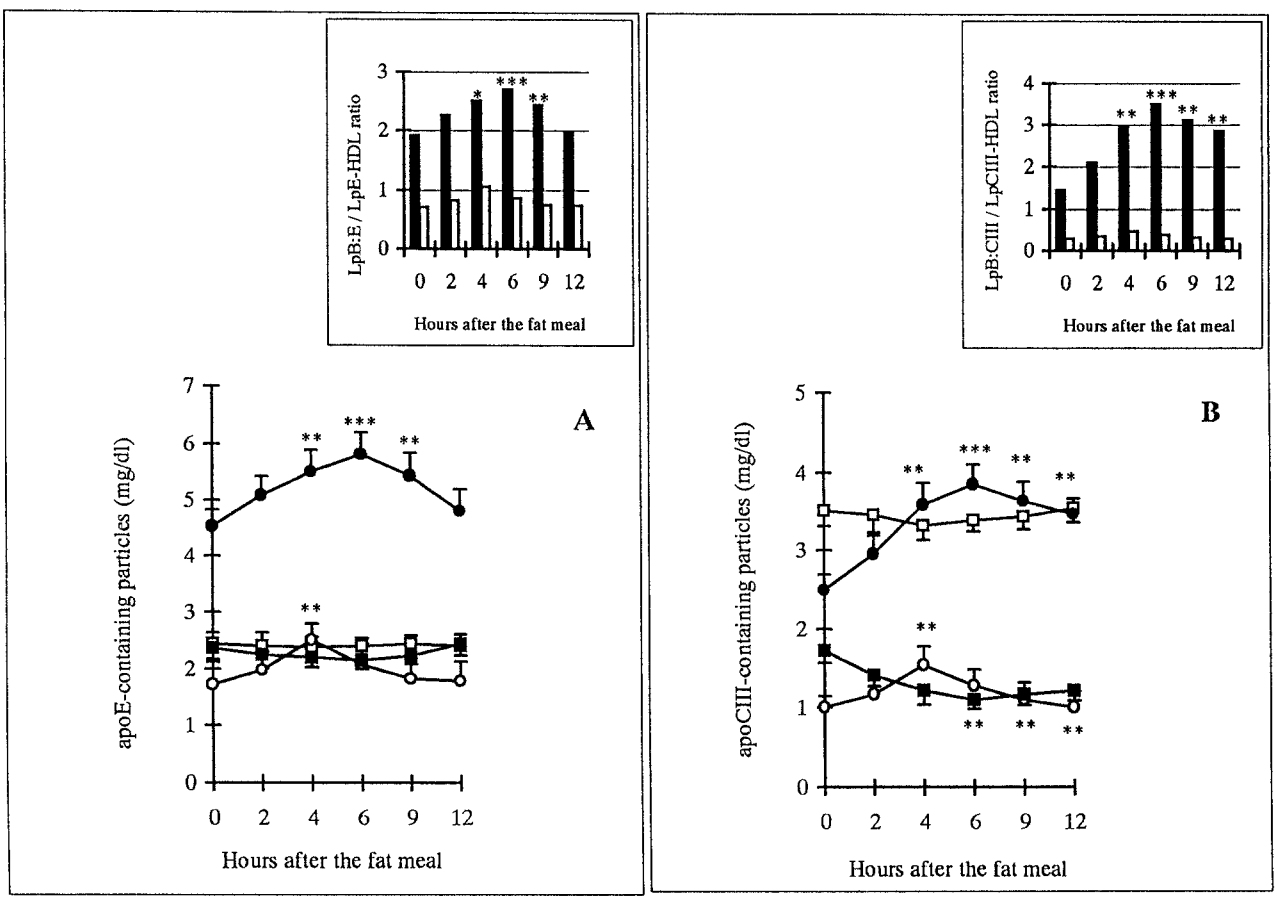

Fig 2. (A) Postprandial changes in LpB:E (●, ○) and LpE-HDL (■, □) in MHL (● and ■) and HCL (○ and □) patients. (B) Postprandial changes in LpB:CIII (●, ○) and LpCIII-HDL (■, □) in MHL (● and ■) and HCL (○ and □) patients. LpB:E/LpE-HDL and LpB:CIII/LpCIII-HDL ratios (enclosed panels) reflect the distribution of apoE- and apoCIII-containing particles between TRL and HDL, respectively, in MHL (■) and HCL (□) patients. Significant differences between postprandial and fasting values in each group: **$P < .01$ and ***$P < .005$.

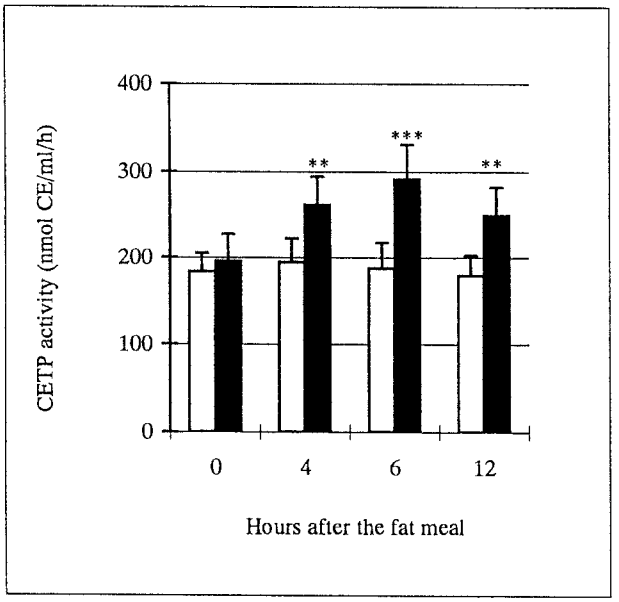

Fig 3. Postprandial CETP activity evolution in MHL (■) and HCL (□) patients. Significant differences between postprandial and fasting values in each group: **$P < .01$ and ***$P < .005$.

The association between LPL activity and LpB:CIII changes reached significance in MHL patients ($r = -.69$, $P < .05$), but not in HCL patients ($r = -.54$, $P = .073$). The activity of HTGL was correlated with HDL3b and HDL3c levels ($r = .61$, $P < .05$ and $r = .51$, $P = .068$, respectively) in MHL patients only.

DISCUSSION

The present study investigated the postprandial changes of TRL and HDL and their interrelationships in MHL compared with HCL. These analyses were focused for the first time on the variations of apoE- and apoCIII-containing TRL and HDL (LpB:E and LpB:CIII in TRL and LpE-HDL and LpCIII-HDL) after an oral fat load. The postprandial changes of neutral lipids in VLDL and HDL2 were studied in relation to lipoprotein-modifying enzyme activities (LPL, HTGL, and CETP).

To study postprandial variations, we used a very–high-fat meal to maximally stress the lipolytic pathways. Such a fatty meal has been used by several investigators who reported that the magnitude of postprandial lipemia varies greatly among individuals but shows a constant response within a given subject.[7,21,32] The elevated and prolonged postprandial triglyceridemia response in MHL patients was relevant to the increase of all TRLs but was mainly associated with VLDL levels, remaining higher at 12 hours than at fasting. This impaired TRL catabolism was likely associated with the significantly reduced LPL activity in these patients. Subsequent to the postprandially increased activity of CETP, known to exchange TG against CE between TRL and HDL,[33] VLDLs were CE-enriched in the late postprandial period, and concomitantly, HDL2 was TG-enriched and CE-depleted, in MHL patients only. Therefore, the combined effects of decreased LPL and enhanced CETP activities in these patients favor the long circulating time for VLDLs and their CE-enrichment associated with the TG-enrichment of HDL2. These latter particles are known to be susceptible to hydrolysis by HTGL and then conversion into small HDL3.[34] In line with this, the elevated HTGL activity and its positive correlations with HDL3b and HDL3c levels in MHL patients are consistent with the role attributed to this enzyme in the generation of increased small-sized HDL3.[34] Thus, the enhanced activities of both CETP and HTGL in MHL patients could explain the abnormal distribution profile of HDL subpopulations, ie, increased levels of small-sized particles (HDL3b and HDL3c) and decreased levels of large-sized particles (HDL2b and HDL2a). Such a profile has been reported to be associated with CHD severity in previous angiographic studies.[13,35] The HDL subclass profile in MHL patients was also characterized by decreased LpA-I and LpA-I:A-II levels within HDL2. The LpA-I decrease likely could be related to the impeded postprandial VLDL catabolism; however, the LpA-I:A-II decrease might be linked to the elevated HTGL activity, considering that LpA-I:A-II particles within HDL2 constitute a substrate avidly hydrolyzed by this enzyme, as reported by Mowri et al.[36,37]

If decreased LpA-I-HDL2 and HDL2b and increased HDL3b and HDL3c levels are known to be associated with impaired postprandial lipemia and thus are considered proatherogenic disturbances,[38,39] there is no available information on the interrelationships between the postprandial changes of LpB:E and LpB:CIII in TRL and LpE-HDL and LpCIII-HDL in mixed hyperlipidemia.

The simultaneous postprandial increases of LpB:E and LpB:CIII in both MHL and HCL patients are likely related to the enhanced hepatic secretion of VLDL associated with decreased LPL activity. At the late postprandial period, the return of LpB:E to basal values indicates that these particles are efficiently cleared, probably by B/E receptors as previously reported.[40] The impaired catabolism of LpB:CIII is the result, at least in part, of an inefficient or weak lipolysis of these particles in MHL patients. This suggestion is sustained by the significant inverse association between postprandial LpB:CIII changes and LPL activity in these patients and by previous data underlining the deleterious effect of increased apoCIII molecules at the surface of VLDL.[41,42] If the decrease of LpCIII-HDL levels in the early postprandial period reflects the transfer of apoCIII from HDL toward newly synthesized TRL, its persistence in the late period is likely associated with the inefficient return of apoCIII toward HDL. Inasmuch as both increased fasting

Table 4. Correlation Coefficients Between HDL2 Parameters (mg/dL) and the Magnitude of LpB:CIII and LpB:E (mg/dL × h) in MHL and HCL Patients

Parameter	HDL2-CE	HDL2-LpA-I	HDL2b
AUC LpB:E			
MHL	−.41	−.35	−.40
HCL	−.32	−.42	−.29
AUC LpB:CIII			
MHL	−.57	−.61*	−.69*
HCL	−.28	−.33	−.37

NOTE. Correlations were evaluated by Pearson correlation coefficients.

*$P < .05$.

LpB:CIII and decreased LpCIII-HDL levels were previously reported to be associated with elevated CHD risk,[43,44] the long postprandial plasma residence of high LpB:CIII levels associated with the depletion of plasma LpCIII-HDL levels could be considered proatherogenic events in mixed hyperlipidemia. In addition, the specific inverse correlations between the postprandial magnitude of LpB:CIII and HDL2 parameters (HDL2-CE, HDL2-LpA-I, and HDL2b) indicate that among VLDLs, the impaired postprandial metabolism of LpB:CIII is tightly associated with low levels of large-sized antiatherogenic HDL2 in MHL patients.

In conclusion, this study found that several fasting TRL and HDL abnormalities were postprandially amplified and closely interrelated in MHL. These postprandial defects were particularly persistent during the late postprandial period, emphasizing the interest in analyzing lipoprotein metabolism disturbances during this period. Among these disorders, the persistence of high levels of LpB:CIII and the reduction of LpCIII-HDL levels represent interesting findings that should be taken into account in the evaluation of cardiovascular risk linked to hypertriglyceridemia.

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