Evidence that hepatic lipase deficiency in humans is not associated with proatherogenic changes in HDL composition and metabolism

Isabelle L. Ruel,*,† Patrick Couture,† Jeffrey S. Cohn,§ Andre Bensadoun, Michel Marcil,†† and Benoit Lamarche[1],*,†**

Institute on Nutraceuticals and Functional Foods,* Laval University, Québec City, Québec, Canada; Lipid Research Center,† Laval University Hospital Center Research Center, Québec City, Québec, Canada; Hyperlipidemia and Atherosclerosis Research Group,§ Clinical Research Institute of Montréal, Montréal, Québec, Canada; Division of Nutritional Sciences,** Cornell University, Ithaca, NY; and Cardiovascular Genetics Laboratory,†† Royal Victoria Hospital, McGill University Health Center, Montréal, Québec, Canada

Abstract The aim of the present study was to characterize the composition and metabolism of HDL in subjects with complete hepatic lipase (HL) deficiency. Analyses were carried out in three complete and three partial HL-deficient subjects as well as in eight normotriglyceridemic (NTG) and two hypertriglyceridemic controls. Complete HL deficiency was associated with hypertriglyceridemia and with a 3.5-fold increase in HDL-triglyceride (TG) levels. The in vivo kinetics of apolipoprotein A-I (apoA-I) and apoA-II (d < 1.25 g/l) were studied in the fasted state using a primed-constant infusion of L-(5,5,5-D_3)leucine for 12 h. Complete HL deficiency was associated with a reduced fractional catabolic rate of apoA-I in the HL-deficient female proband (−47%) and in her two brothers (−21%) compared with gender- and TG-matched controls. Total plasma and HDL from complete HL-deficient patients were able to mediate normal cholesterol efflux from human skin fibroblasts labeled with [^{3}H]cholesterol. Complete HL deficiency was also associated with normal levels of preβ-migrating apoA-I-containing HDL separated by two-dimensional gel electrophoresis and with an accumulation of large HDL particles compared with NTG controls. These results suggest that HL activity is important for adequate HDL metabolism, although its presence may not be necessary for normal HDL-mediated reverse cholesterol transport.—Ruel, I. L., P. Couture, J. S. Cohn, A. Bensadoun, M. Marcil, and B. Lamarche. **Evidence that hepatic lipase deficiency in humans is not associated with proatherogenic changes in HDL composition and metabolism. *J. Lipid Res.* 2004.** 45: **1528–1537.**

Supplementary key words kinetics • high density lipoprotein • cholesterol efflux

Manuscript received 4 March 2004 and in revised form 12 May 2004.

Published, JLR Papers in Press, June 1, 2004.
DOI 10.1194/jlr.M400090-JLR200

The inverse relationship between plasma levels of HDL and the risk of coronary artery disease (CAD) has been demonstrated in numerous epidemiological studies (1–3). The cardioprotective effect of HDL has been attributed to, among other factors, its role in reverse cholesterol transport (RCT), in which cholesterol is mobilized from the periphery for the delivery and catabolism at the liver (4). The sequential events of the RCT process consist of the efflux of cellular cholesterol into lipid-free apolipoprotein A-I (apoA-I), esterification of cholesterol within HDL by LCAT, exchange of cholesteryl esters for triglycerides (TGs) to apoB-containing lipoproteins by the cholesteryl ester transfer protein (CETP), and removal of these lipoproteins by the liver. The HDLs are also concurrently remodeled through the action of hepatic lipase (HL), after which they may be taken up mainly by the liver (5). The efficacy of this dynamic process as a whole, as well as its individual steps (such as the rate of cholesterol flux through HDL subspecies and the activity of enzymes, transfer proteins, and receptors), thus defines the susceptibility of an individual to atherosclerosis.

In the HDL remodeling step of the RCT pathway, HL modulates the phospholipid and TG content of HDL particles and therefore contributes significantly to determining their lipid composition, density, size, and metabolic fate (6). The importance of TG enrichment of HDL as an

Abbreviations: apoA-I, apolipoprotein A-I; CAD, coronary artery disease; CETP, cholesteryl ester transfer protein; EL, endothelial lipase; FCR, fractional catabolic rate; HL, hepatic lipase; HTG, hypertriglyceridemic; NTG, normotriglyceridemic; 1D-PAGGE, one-dimensional polyacrylamide gradient gel electrophoresis; QHLD, Québec-based hepatic lipase deficiency; RCT, reverse cholesterol transport; TG, triglyceride.

[1] To whom correspondence should be addressed.
e-mail: benoit.lamarche@inaf.ulaval.ca

important process regulating its catabolism is now well recognized (7). In the presence of active intravascular HL, TG enrichment of HDL significantly enhances the fractional catabolic rate (FCR) of HDL apoA-I, the major protein moiety of HDL (7). This enhanced clearance of HDL apoA-I from the circulation is one of the mechanisms thought to underlie the low HDL apoA-I and HDL cholesterol levels in hypertriglyceridemic (HTG) subjects (8). It has also been shown that TG-enriched HDL particles have diminished interaction with cell surface receptors (9–11) and reduced capacity to facilitate sterol balance (12), thereby potentially altering several steps in RCT (13–16).

It has previously been shown that HL deficiency in humans is associated with hypercholesterolemia and hypertriglyceridemia (17–20). One of the unique features found in subjects with HL deficiency is a marked TG enrichment of HDL particles, and despite having increased HDL_2-cholesterol levels, patients with HL deficiency have often been shown to have premature atherosclerosis (17–19, 21, 22). Thus, it can be hypothesized that the absence of HL activity in vivo may significantly compromise the capacity of HDL to play its role in the RCT process. However, the extent to which potentially disturbed HDL metabolism may contribute to increased risk of CAD in HL-deficient patients is unknown.

To better understand the pathophysiological properties of HDL in HL deficiency, we characterized the in vivo kinetics of plasma apoA-I and apoA-II in three patients with complete HL deficiency, three patients with partial HL deficiency, nonaffected family members of the Québec-based hepatic lipase deficiency (QHLD) kindred (23), as well as control subjects with normal and increased TG levels [normotriglyceridemic (NTG) and HTG control subjects]. The capacity of total plasma and HDL particles to efflux cellular cholesterol from normal skin fibroblasts, and the characterization of apoA-I-containing HDL subspecies by nondenaturing two-dimensional electrophoresis, were also examined.

METHODS

Subjects

HL-deficient patients from the QHLD kindred have been described in detail elsewhere (23). Only middle-aged subjects from the second generation of the QHLD kindred have been selected to participate in the kinetic studies and have been included in the present study. Briefly, the proband (subject 1-1) and her two brothers (subjects 1-5 and 1-6) were arbitrarily referred to as patients with complete HL deficiency because they had extremely low to undetectable HL activities with normal LPL activities. All three subjects were compound heterozygotes for the A174T and T383M mutations in the HL gene but were apoE3 homozygotes. We also characterized three partial HL-deficient patients from the QHLD kindred (subjects 1-2, 1-8, and 1-9; **Table 1**). They had low but detectable HL activity compared with unaffected individuals. The female control subjects were family members of the QHLD kindred (subjects 1-3, 1-4, and 1-7), whereas the male control subjects were recruited from the Lipid Clinic at the Clinical Research Institute of Montreal and from the Lipid Research Center in Quebéc. Control subjects were matched with complete HL-deficient patients for gender, age, waist circumference, and body mass index. None of the subjects were using hypolipidemic medications or were smokers at the time of evaluation. At the time of experimentation, one compound heterozygous male (subject 1-5) was being treated for primary hypothyroidism with 100 μg/day levothyroxine; his plasma thyroid-stimulating hormone and free thyroxine levels remained within the normal limits during the experiment. One control female was taking oral contraceptives (Diane®-35; 2 mg of cyproterone acetate and 0.035 mg of ethinylestradiol). All participants gave their written informed consent to participate in the study, which received approval from local ethics committees.

TABLE 1. Characteristics of partial and complete HL-deficient patients and control subjects

Subjects	HL Mutations	Age[a]	Body Mass Index[a]	Waist Girth[a]	Plasma Cholesterol[a]	LDL-Cholesterol[a]	Plasma TG[a]	HL Mass	HL Activity	LPL Activity
		years	*kg/m²*	*cm*	*mmol/l*	*mmol/l*	*mmol/l*	*ng/ml*	*μmol/ml/h*	*μmol/ml/h*
Males										
Complete HLdef										
1-5	A174T/T383M	37	30.5	107	7.7	3.7	4.53	20	0	2.57
1-6	A174T/T383M	35	32.3	103	7.7	1.9	9.82	14	0.87	3.81
Mean ± SD	—	36 ± 1	31.4 ± 1.3	105 ± 3	7.7 ± 0.0	2.8 ± 1.3	7.17 ± 3.74	17 ± 4	0.44 ± 0.61	3.2 ± 0.9
Partial HLdef										
1-2	T383M	42	25.7	96	5.6	3.8	1.36	24	4.75	2.56
1-9	A174T	37	26.1	89	7.1	5.4	1.11	24	5.76	4.27
Mean ± SD	—	39 ± 3	25.9 ± 0.3	92 ± 5	6.3 ± 1.1	4.6 ± 1.2	1.23 ± 0.18	24 ± 0	5.2 ± 0.7	3.4 ± 1.2
HTG controls (N = 2)										
Mean ± SD	—	39 ± 1	25.9 ± 0.3	—	6.1 ± 0.0	—	4.50 ± 1.93	—	—	—
NTG controls (N = 5)										
Mean ± SD	—	39 ± 4	29.0 ± 4.9	99 ± 13	5.2 ± 0.9	3.3 ± 0.8	1.58 ± 0.76	416 ± 53	20.5 ± 4.6	5.1 ± 2.1
Females										
Complete HLdef										
1-1	A174T/T383M	42	25.7	78	6.3	3.6	2.02	11	0.24	2.82
Partial HLdef										
1-8	A174T	41	26.7	79	6.1	3.8	1.85	15	4.88	2.14
NTG controls (N = 3)										
Mean ± SD	—	39 ± 1	26.7 ± 3.3	85 ± 9	5.0 ± 1.1	3.3 ± 0.9	1.12 ± 0.36	122 ± 170	9.0 ± 4.3	3.1 ± 1.2

HL, hepatic lipase; HLdef, HL deficiency; HTG, hypertriglyceridemic; NTG, normotriglyceridemic; TG, triglyceride.
[a] Data previously published (23).

Lipid and lipoprotein analyses

Fasted blood samples were drawn in tubes containing 0.15% EDTA and plasma was isolated by centrifugation (1,500 *g* at 4°C, 15 min). VLDL, intermediate density lipoprotein/LDL, and total HDL were separated using sequential ultracentrifugation and precipitation methods, whereas HDL_2 and HDL_3 subfractions were isolated using dextran sulfate and magnesium chloride as described previously (24). Lipid content of plasma and lipoprotein subfractions was determined by enzymatic methods with an AutoAnalyzer RA-1000 (Technicon Instruments) according to standardized procedures that have been described previously (25). Plasma apoA-I and apoA-II levels were measured by nephelometry (Dade Behring). The A174T and T383M mutations in the HL gene were detected as described previously (23). LPL and HL activities were measured in subjects at the end of the kinetic protocol, in the fasted state, 10 min after an intravenous injection of heparin (60 IU/kg body weight) (23). Lipase activities were expressed as micromoles of free fatty acids released per milliliter of plasma per hour. Plasma postheparin HL protein concentration was determined by ELISA (26). One-dimensional nondenaturing 4–30% polyacrylamide gradient gel electrophoresis (1D-PAGGE) was used to determine the HDL particle size (27).

Stable isotope infusion protocol

After a 12 h overnight fast, all subjects received a primed-constant infusion of L-[5,5,5-D_3]leucine, as previously described (28), to evaluate the kinetics of plasma apoA-I and apoA-II. Subjects first received a bolus injection of 10 μmol/kg body weight L-[5,5,5-D_3]leucine followed by a constant intravenous infusion of 10 μmol/kg/h over a 12 h period in the fasting state. Subjects remained in the fasting state throughout the kinetic protocol. Blood samples (20 ml) were collected at baseline and at 15, 30, and 45 min and 1, 1.5, 2, 2.5, 3, 4, 5, 6, 7, 8, 10, and 12 h.

Isolation of apoA-I and apoA-II and isotopic enrichment determination

Total lipoproteins were isolated from plasma by ultracentrifugation ($d < 1.25$ g/ml, 50,000 rpm, 48 h). Infranatant was dialyzed overnight in a NaCl-Tris-EDTA buffer, incubated with cysteamine for 4 h at 37°C, and delipidated using acetone-ethanol and diethylic ether. Plasma apoA-I and apoA-II were isolated by preparative isoelectric focusing on 7.5% polyacrylamide-urea (8 M) gels (4–7 pH gradient) as previously described (29). ApoA-I and apoA-II bands were excised from gels, hydrolyzed in 6 N HCl at 100°C for 24 h, and dried (29). The hydrolyzed amino acids were isolated by Dowex AG-50W-X8 100 to 200 mesh cation-exchange chromatography and derivatized with acetylchloride-acidified 1-propanol and heptafluorobutyric anhydride. The isotopic enrichment was determined by selected ion monitoring of derivatized samples at mass-to-charge ratios of 349 and 352 and with the use of negative chemical ionization and methane as the reagent gas. Tracer/tracee ratios (percentage) were derived from isotopic ratios for each sample using the equation proposed by Cobelli, Toffolo, and Foster (30). Plasma amino acids were also separated by cation-exchange chromatography, derivatized, and analyzed for the determination of plasma leucine isotopic enrichment.

Modeling

Tracer-to-tracee ratios of plasma apoA-I and apoA-II were fitted to a monoexponential function using SAAM II computer software (SAAM Institute). We assumed a constant enrichment of the precursor pool and used the total plasma leucine enrichment as the forcing function to drive the appearance of tracer into apoA-I or apoA-II, but the use of the VLDL apoB-100 enrichment plateau yielded identical results (data not shown). The plasma apoA-I or apoA-II production rate (milligrams per kilogram of body weight per day) was calculated as [FCR (pools/day) × apoA-I or apoA-II concentration (grams per liter) × plasma volume (liters)]/body weight (kilograms) (29). ApoA-I and apoA-II pool sizes were determined by multiplying the measured apolipoprotein concentration by plasma volume (4.5% of body weight).

Cholesterol efflux studies

Cultures of normal human skin fibroblasts were established from 3.0 mm punch biopsies of the forearm of healthy individuals as described previously (31). We seeded 5×10^4 cells on 12-well plates. The efflux protocol for efflux studies has been previously described (31). Briefly, cells were labeled with [^{3}H]cholesterol (0.2 μCi/ml; New England Nuclear-Dupont) until confluence and then loaded with nonlipoprotein cholesterol (20 μg/ml) for 24 h. Efflux studies were carried out in the presence of 50 μg of apoA-I from the HDL fraction ($d = 1.063$–1.21 g/ml) dialyzed in PBS per milliliter of culture medium (31) or with total fasting plasma (20% of total volume of experiment) isolated in tubes containing streptokinase (10 U/ml). Efflux was determined for specified periods of time (3, 6, 12, and 24 h), and an efflux period of 8 h was chosen for end point experiments because preliminary data in several subjects indicated that the efflux kinetic curve was linear up to 12 h. Efflux medium was collected and aliquots were taken for radioactivity counting. Cell layers were treated with 0.1 N NaOH before the determination of radioactivity and protein concentrations. Each experiment was performed in triplicate. Efflux was determined as the percentage of total cholesterol in the medium (^{3}H in medium divided by ^{3}H in medium plus ^{3}H in cell lysate). The coefficient of variation for the analysis was 4.6%.

Two-dimensional nondenaturing gradient gel electrophoresis

Lipoproteins in fasted plasma were separated by two-dimensional nondenaturing gradient gel electrophoresis using a modification of a procedure described previously (32). Briefly, plasma samples (15 μl) with 5 μl of Tris buffer were separated in the first dimension by 0.75% agarose gel (100 V, 8 h, 4°C) and in the second dimension by nondenaturing 3–24% PAGGE (1 h prerun at 70 V, 12 h at 30 V, and 8 h at 80 V, 4°C). A high-molecular-mass protein standard (71–170 Å; Amersham Biosciences) was run as a standard in the second dimension on each gel. The gel was then transferred to a nitrocellulose membrane (30 V, 20 h, 4°C) and immunoblotted with rabbit anti-human apoA-I antibody (1:1,000; Calbiochem). ApoA-I was visualized by incubating the membrane with horseradish peroxidase-conjugated anti-rabbit antibody (1:10,000; Amersham Biosciences), followed by development with enhanced chemiluminescence reagents (Perkin-Elmer).

Statistical analyses

The Spearman rank test was used for univariate correlation analyses, performed with the SAS software package (SAS Institute).

RESULTS

Plasma lipid and lipoprotein data

Characteristics of complete and partial HL-deficient subjects, which have been previously published (23), as well as those of control subjects who participated in the kinetic study are shown in Table 1. The three subjects re-

ferred to as complete HL-deficient subjects (subjects 1-1, 1-5, and 1-6) had very low postheparin HL mass and lipolytic activities compared with control subjects. Their postheparin plasma LPL activities were within the normal range. Data for male and female patients are presented separately, because complete HL deficiency in males (unlike the female proband) was associated with abdominal obesity and hypertriglyceridemia. In addition to abdominal obesity, the two males with complete HL deficiency had abnormal lipoprotein-lipid profiles compared with NTG control subjects. They had marked increases in plasma TG compared with NTG controls (+354%) and cholesterol levels (+48%). Because the two complete HL-deficient patients were HTG, we examined two healthy individuals with similarly increased plasma TG levels. These HTG control subjects were characterized by reduced HDL cholesterol (−46%) compared with the NTG control subjects. Unlike her two brothers with complete HL deficiency, the female proband had a more favorable lipoprotein-lipid profile associated with only minor increase of plasma cholesterol and TG concentrations. Heterozygous subjects for the A174T or T383M mutation in the HL gene had reduced but detectable postheparin HL mass and catalytic activity, which was associated with a lipoprotein-lipid profile relatively similar to that of the control subjects in both males and females. The relation between plasma postheparin HL protein concentration and activity was studied in all subjects and is shown in **Fig. 1**. There was a significant positive correlation between plasma HL mass and HL activity ($r = 0.89$, $P < 0.0001$), with carriers of the A174T and T383M combination presenting the lowest HL mass and activity.

Table 2 presents the composition of total HDL in subjects from the QHLD kindred and in control subjects. The two complete HL-deficient males had normal HDL cholesterol levels (1.18 ± 0.32 vs. 1.25 ± 0.30 mmol/l in NTG controls), despite their increased body mass index. The major abnormality of HDL particles in those patients was a marked 4-fold enrichment in TG compared with NTG control subjects. As shown in **Table 3**, the marked TG enrichment of HDL particles in the two complete HL-deficient males was mostly attributable to a 10-fold increase in the TG content of HDL_2 particles and a more subtle 2-fold increase in HDL_3-TG levels compared with NTG controls. The phospholipid content of total HDL in complete HL deficiency did not differ from that of the NTG controls (1.37 ± 0.08 vs. 1.12 ± 0.17 mmol/l). The female proband was characterized by increased HDL cholesterol levels (2.28 vs. 1.42 ± 0.14 mmol/l) and had a 3-fold increase in HDL-TG levels compared with her sex-matched controls. Like her brothers, she was characterized by increased HDL_2-cholesterol, TG, and phospholipid levels, with no change in the lipid composition of HDL_3. One-dimensional nondenaturing 4–30% PAGGE confirmed that the three patients with severe HL deficiency had larger HDL particles compared with NTG controls (106.7 ± 2.3 vs. 83.2 ± 0.7 Å). The composition of total HDL as well as HDL_2/HDL_3 subfractions in partial HL-deficient subjects was not significantly different from that in control subjects.

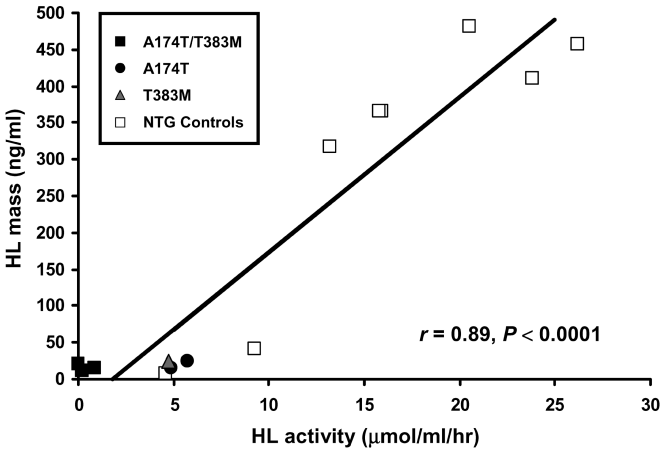

Fig. 1. Correlation between plasma postheparin hepatic lipase (HL) mass and HL activity. Postheparin plasma was obtained in the fasted state 10 min after an intravenous injection of heparin (60 IU/kg body weight). r = Spearman's rho.

Plasma apoA-I and apoA-II kinetics

Analyses of deuterated plasma amino acids for all subjects indicated that plasma leucine enrichments remained constant throughout the course of the infusion (data not shown). **Table 4** presents the plasma apoA-I and apoA-II pool sizes, FCRs, and production rates in various subjects. No difference in the kinetic parameters of apoA-I was observed in the two males with complete HL deficiency compared with sex-matched NTG control subjects. The two HTG control subjects were characterized by reduced plasma apoA-I pool size (3,587 ± 198 vs. 5,677 ± 371 mg) as well as a 30% increase in apoA-I catabolism compared with the NTG control subjects. Complete HL deficiency in males was associated with a 21% lower apoA-I FCR and an increased apoA-I pool size (6,428 ± 761 vs. 3,587 ± 198 mg) compared with those of HTG control subjects. Plasma apoA-II pool size was also increased in complete HL-deficient males compared with NTG controls (2,047 ± 61 vs. 1,472 ± 213 mg), mostly because of a nonsignificant increase in the production of apoA-II (2.90 ± 0.44 vs. 2.37 ± 0.64 mg/kg/day). Complete HL deficiency in the female proband was associated with a 60% increase in apoA-I pool size compared with her sex-matched controls, mostly attributable to a reduction in the FCR of apoA-I (−47%), with no apparent change in production rate. In addition, we observed a 35% reduction in FCR of plasma apoA-II in the complete HL-deficient female compared with her sex-matched control subject. Partial HL deficiency in males and females was also associated with reductions in the FCR and production rate of apoA-I (−27% and −21%, respectively) and apoA-II (−25% and −20%, respectively), but these values were not significantly different from those obtained in NTG controls.

Cholesterol efflux

The capacity of total plasma and HDL particles (d = 1.06–1.21 g/ml) to efflux cellular cholesterol was assessed

TABLE 2. Characteristics of HDL in partial and complete HL-deficient patients and control subjects

Subjects	HDL Cholesterol[a]	HDL TG	HDL Phospholipids	Plasma apoA-I	Plasma apoA-II	HDL-TG/ HDLapoA-I	HDL Size
	mmol/l			*g/l*			*Å*
Males							
Complete HLdef							
1-5	1.41	0.69	1.43	1.50	0.45	0.49	106.6
1-6	0.96	1.01	1.31	1.44	0.49	0.74	109.1
Mean ± SD	1.18 ± 0.32	0.85 ± 0.23	1.37 ± 0.08	1.47 ± 0.04	0.47 ± 0.03	0.62 ± 0.18	107.8 ± 1.7
Partial HLdef							
1-2	1.18	0.28	1.21	1.30	0.34	0.24	82.9
1-9	1.35	0.22	1.21	1.42	0.43	0.18	83.5
Mean ± SD	1.26 ± 0.12	0.25 ± 0.04	1.21 ± 0.00	1.36 ± 0.08	0.38 ± 0.06	0.21 ± 0.04	83.2 ± 0.4
HTG controls (N = 2)							
Mean ± SD	0.68 ± 0.13	0.15 ± 0.03	—	1.06 ± 0.06	—	—	—
NTG controls (N = 5)							
Mean ± SD	1.25 ± 0.30	0.21 ± 0.02	1.12 ± 0.17	1.43 ± 0.24	0.36 ± 0.05	0.16 ± 0.03	83.1 ± 0.9
Females							
Complete HLdef							
1-1	2.28	0.64	1.88	2.47	0.43	0.27	104.4
Partial HLdef							
1-8	1.51	0.34	1.46	1.73	0.39	0.23	83.8
NTG controls (N = 3)							
Mean ± SD	1.42 ± 0.14	0.23 ± 0.01	1.20 ± 0.16	1.43 ± 0.16	0.35 ± 0.05	0.16 ± 0.02	83.4 ± 0.1

apoA-I, apolipoprotein A-I.
[a] Data previously published (23).

using normal human skin fibroblasts labeled with [^{3}H]cholesterol. Mean efflux values for NTG controls in each experiment were arbitrarily set at 100%. Efflux for patients was then expressed as a percentage relative to controls. In both complete and partial HL-deficient male patients, total plasma and HDL-mediated cholesterol efflux was normal, with values ranging from 62% to 130% of cholesterol efflux in controls (**Fig. 2**). The ability of plasma and HDL particles to efflux cholesterol in complete and partial HL-deficient females was also similar to that of sex-matched control subjects (data not shown).

Two-dimensional nondenaturing gradient gel electrophoresis

ApoA-I-containing HDL subpopulations of patients and controls were compared after qualitative two-dimensional gel electrophoresis of frozen plasma samples. **Figure 3** shows the distribution of apoA-I-containing HDL subpopulations for one male and one female patient with partial HL deficiency, one male and one female patient with complete HL deficiency, and two NTG control subjects of each gender. A clear increase in large HDL (α_1) and a concomitant decrease in smaller HDL (α_3) was evident

TABLE 3. Characteristics of HDL_2 and HDL_3 subfractions in partial and complete HL-deficient patients and control subjects

Subjects	HDL_2 Cholesterol	HDL_2 TG	HDL_2 Phospholipids	HDL_3 Cholesterol	HDL_3 TG	HDL_3 Phospholipids
			mmol/l			
Males						
Complete HLdef						
1-5	0.83	0.42	0.68	0.58	0.27	0.75
1-6	0.59	0.59	0.61	0.37	0.42	0.70
Mean ± SD	0.71 ± 0.17	0.50 ± 0.12	0.64 ± 0.05	0.47 ± 0.15	0.34 ± 0.11	0.72 ± 0.03
Partial HLdef						
1-2	0.52	0.10	0.43	0.66	0.18	0.78
1-9	0.58	0.06	0.41	0.77	0.16	0.80
Mean ± SD	0.55 ± 0.04	0.08 ± 0.03	0.42 ± 0.01	0.71 ± 0.08	0.17 ± 0.01	0.79 ± 0.01
NTG controls (N = 5)						
Mean ± SD	0.45 ± 0.15	0.05 ± 0.02	0.28 ± 0.07	0.80 ± 0.19	0.16 ± 0.03	0.83 ± 0.16
Females						
Complete HLdef						
1-1	1.61	0.41	1.06	0.67	0.23	0.82
Partial HLdef						
1-8	0.71	0.14	0.55	0.80	0.20	0.91
NTG controls (N = 3)						
Mean ± SD	0.58 ± 0.09	0.05 ± 0.03	0.39 ± 0.01	0.84 ± 0.15	0.18 ± 0.03	0.81 ± 0.15

HDL subfractions were not isolated in HTG controls.

TABLE 4. Kinetics of plasma apoA-I and apoA-II in partial and complete HL-deficient patients and control subjects

Subjects	Plasma apoA-I			Plasma apoA-II		
	Pool Size	Fractional Catabolic Rate	Production Rate	Pool Size	Fractional Catabolic Rate	Production Rate
	mg	*pools/day*	*mg/kg/day*	*mg*	*pools/day*	*mg/kg/day*
Males						
Complete HLdef						
1-5	6,966	0.155	10.46	2090	0.128	2.59
1-6	5,890	0.205	13.31	2004	0.146	3.21
Mean ± SD	6,428 ± 761	0.180 ± 0.035	11.88 ± 2.01	2047 ± 61	0.137 ± 0.013	2.90 ± 0.44
Partial HLdef						
1-2	5,031	0.133	7.75	1316	0.117	1.80
1-9	4,997	0.149	9.54	1513	0.090	1.74
Mean ± SD	5,014 ± 24	0.141 ± 0.011	8.64 ± 1.27	1414 ± 139	0.103 ± 0.019	1.77 ± 0.04
HTG controls (N = 2)						
Mean ± SD	3,587 ± 198	0.227 ± 0.083	11.00 ± 4.62	—	—	—
NTG controls (N = 5)						
Mean ± SD	5,677 ± 371	0.175 ± 0.046	10.89 ± 1.19	1472 ± 213	0.144 ± 0.035	2.37 ± 0.64
Females						
Complete HLdef						
1-1	7,325	0.126	14.01	1275	0.110	2.13
Partial HLdef						
1-8	5,130	0.153	11.88	1157	0.137	2.40
NTG controls (N = 3)						
Mean ± SD	4,565 ± 619	0.236 ± 0.083	14.88 ± 3.94	1125 ± 289	0.169 ± 0.033	2.61 ± 0.39

in complete HL-deficient patients compared with sex-matched NTG control subjects, whereas the distribution of these subpopulations was unaltered in partial HL-deficient patients. Levels of preβ-migrating HDL-apoA-I were normal in both partial and complete HL-deficient patients.

DISCUSSION

The present study has documented the effect of complete and partial HL deficiency on HDL composition and metabolism. Patients with complete HL deficiency were found to have large HDL particles that were markedly enriched in TG compared with control subjects. Results of kinetic analyses showed that they had an accumulation of plasma apoA-I mostly attributable to reduced apoA-I catabolism compared with TG-matched controls. ApoA-II concentrations were also increased in complete HL deficiency. We found that α- and preα-migrating HDL subfractions separated by two-dimensional gel electrophoresis were shifted toward larger particles in complete HL deficiency relative to control subjects, with apparently normal levels of apoA-I-containing preβ-HDL. No difference in cholesterol efflux was observed for the HDL of patients with complete HL deficiency and control subjects. Finally, partial HL-deficient patients were found to have HDL profiles, compositions, and kinetics that were relatively similar to those of control subjects.

HL deficiency in humans is one of the rarest genetic abnormalities of lipoprotein metabolism. One of the most consistent findings in the few patients who have been investigated is a marked TG enrichment of HDL particles (17, 19, 21). Complete HL-deficient subjects from the QHLD kindred displayed a similar phenotype. The TG content of HDL has been shown to have a major impact on the metabolism of HDL particles. In healthy humans, TG-enriched HDL particles are associated with an enhanced catabolism of apoA-I (7). One of the mechanisms underlying the increased FCR of TG-rich HDL apoA-I in vivo could be an enhanced lipolytic modification of TG-enriched HDL by HL (8). Recent studies in rabbits have further demonstrated that TG enrichment of HDL, as generally observed in HTG states and patients with HL deficiency, resulted in an enhanced clearance of HDL in the presence but not in the absence of HL activity (33). The present study is the first to examine the impact of an absence of HL mass or activity on HDL clearance in humans, and our results are consistent with these concepts because we found that in the absence of active HL, TG enrichment of HDL particles was not associated with an increased catabolism of HDL particles. In fact, the three complete HL-deficient patients from the QHLD kindred were characterized by slower HDL clearance compared with sex- and TG-matched controls. The TG enrichment of HDL and the presence of an active HL represent only two elements regulating HDL catabolism in HTG states. Other mechanisms involving, for example, cellular receptors (34) and other intravascular lipolytic enzymes such as endothelial lipase (EL) (35) may also play important roles in modulating the metabolic fate of HDL. Although the reduced apoA-I FCR was evident in the female proband with complete HL deficiency, the absence of HL on apoA-I FCR in the brothers of the proband was less apparent. It must be kept in mind that these two HL-deficient patients were abdominally obese and had marked hypertriglyceridemia. It is possible that other factors associated with these states may have attenuated the impact of HL deficiency in these patients. In contrast, the normal HDL composition and metabolism in subjects with partial HL

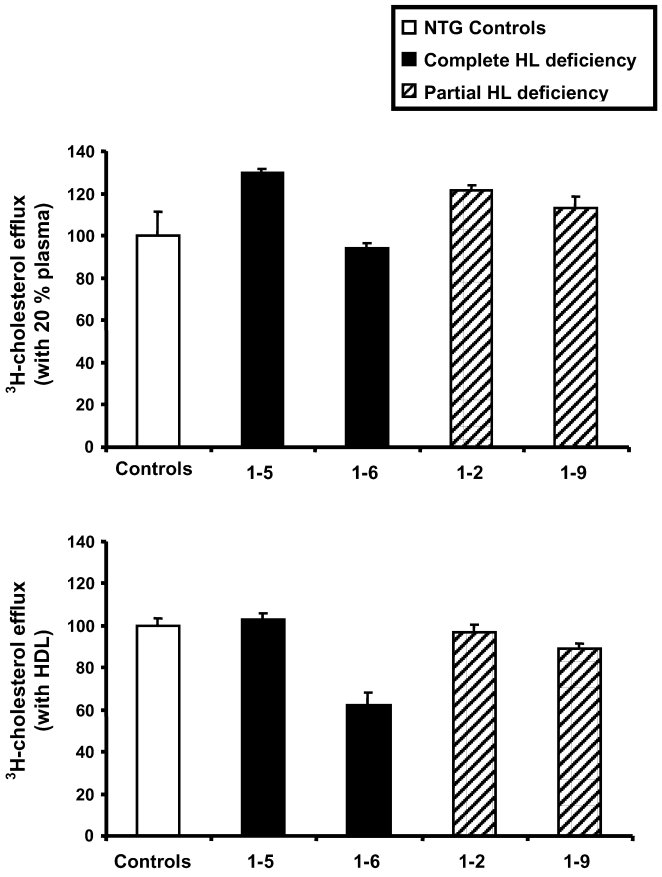

Fig. 2. Bar graph showing the total percentage of [^{3}H]cholesterol in medium after an incubation of total fasting plasma (20% of total volume of experiment; top panel) or 50 μg of apolipoprotein A-I (apoA-I) from HDL per milliliter of culture medium (bottom panel) with [^{3}H]cholesterol-labeled normal skin fibroblasts during 8 h. The [^{3}H]cholesterol efflux for each subject, which was derived from experiments performed in triplicate, is relative to the mean percentage of [^{3}H]cholesterol efflux of the control subjects (N = 5) fixed at 100%. Subjects are designated 1-5, 1-6, etc. Error bars correspond to the SD.

deficiency suggest that partial HL activity ensures an adequate processing of HDL particles.

Individuals with complete HL deficiency, despite having increased HDL cholesterol levels and large HDL particles, two characteristics that are generally known to protect against the development of atherosclerosis (36, 37), have also been reported to be at increased risk for premature CAD compared with subjects with normal HL activity (17–20). To assess the extent to which the disturbed HDL composition and metabolism may have deleterious implications for the RCT in complete HL deficiency, we first examined the in vitro capacity of HDL to remove cholesterol from cells, which represents the first step in the RCT. We used human skin fibroblasts that exhibited normal ABCA1 transporter. There is no clinical evidence suggesting a genetic variation in the ABCA1 gene among subjects of the present study or that ABCA1 function per se may be perturbed as a result of the mutations in the HL gene, other than the fact that HDLs have gross composition abnormalities. Thus, using total plasma or isolated HDL particles, we found no difference in the capacity of HDL or plasma from complete HL-deficient individuals to efflux cholesterol relative to the NTG control subjects, suggesting that complete HL deficiency does not compromise the lipidation of lipid-poor apoA-I during the cholesterol efflux process in vitro. The preferential acceptors of peripheral cellular cholesterol in plasma are preβ-HDL particles (38, 39). The efflux of cellular cholesterol through the ABCA1 transporter into lipid-free apoA-I promotes the formation of preβ_1-HDL particles. It must be stressed that only the early steps of the RCT pathway were investigated in this experiment and that it is still possible that large HDLs found in complete HL-deficient patients may be less effective in delivering cholesterol to the liver. However, because the early steps of the RCT pathway, such as the formation of preβ_1-HDL particles, are believed to be rate-limiting steps (40), we believe that increased circulating levels of preβ_1-HDL particles are good surrogates of an enhanced RCT. The study of how various HDL subclasses contribute to promoting cholesterol efflux remains a topic of interest that will have to be investigated in future studies.

Previous studies have shown that lipid-free apoA-I, which may become preβ_1-HDL particles, are generated by the remodeling of TG-enriched HDL after a sequence of events involving the exchange of cholesteryl esters for TG through the action of CETP, transfer of phospholipids through the action of phospholipid transfer protein, and hydrolysis of TG and phospholipid by HL (41, 42). To examine the impact of the absence of HL activity on the formation of preβ-HDL particles and consequently on RCT in complete HL deficiency, we qualitatively characterized the various apoA-I-containing HDL subfractions using two-dimensional gel electrophoresis. The apparently normal levels of preβ-HDL indicated that the absence of HL does not limit the processing of lipid-free apoA-I. Finally, spherical α_3-HDL as well as larger α_2- and α_1-HDL particles were also observed in complete HL-deficient patients, thus indicating, as one would expect, that esterification of cholesterol was also normal in the absence of HL activity. Based on these results, we suggest that, despite the reduced HDL catabolism and pronounced TG enrichment of HDL particles, the absence of lipolytically active HL has no or very little impact on the early steps of the overall RCT in patients with HL deficiency.

HL-mediated lipolysis accounts for a significant amount of the effect of HL on HDL metabolism. However, HL may also serve as a ligand that mediates the interactions of lipoproteins with cell surface proteoglycans and receptors, facilitating the uptake of lipoprotein (43). It has been shown in knockout mice expressing catalytically inactive HL that even in the absence of lipolysis, HL was still associated with reduced circulating plasma levels of HDL (44). In the present study, HL activity correlated strongly with HL mass concentrations. Carriers of the A174T and T383M mutations in the HL gene, characterized by very low to undetectable postheparin HL activity, presented with very low amounts of HL protein compared with

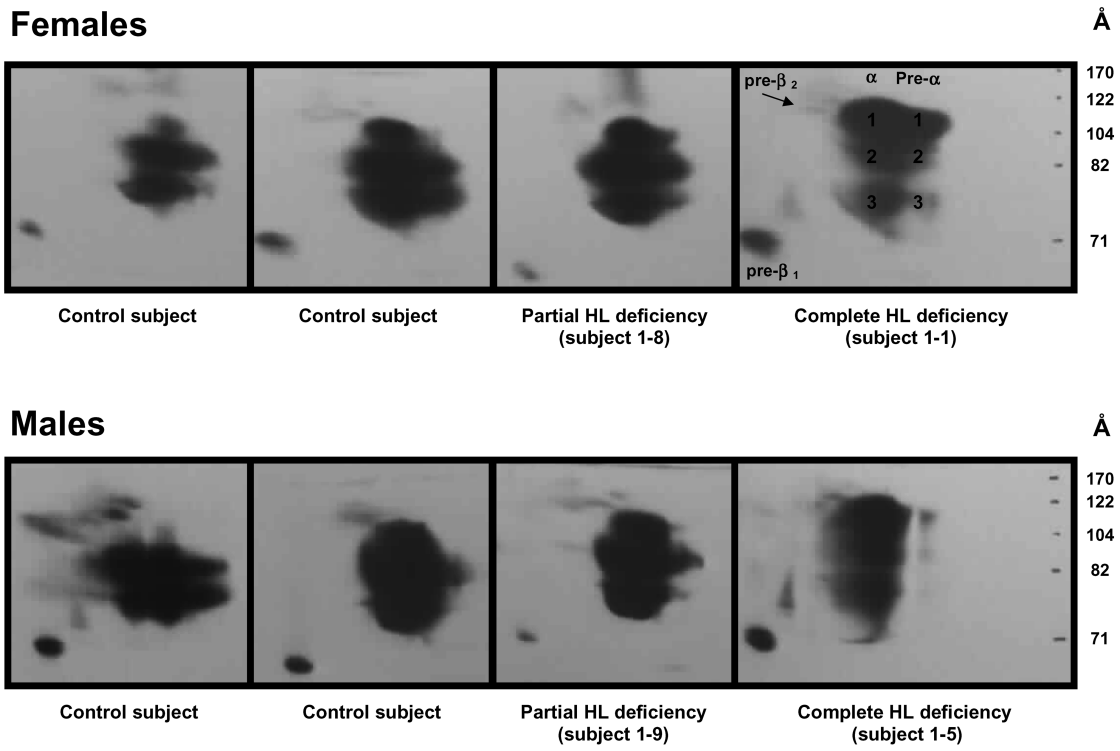

Fig. 3. Two-dimensional gel electrophoresis of apoA-I-containing lipoproteins from plasma of control and HL-deficient subjects. The first dimension consists of agarose gel electrophoresis of plasma lipoproteins, and the second dimension involves a nondenaturing polyacrylamide (3–24%) gradient gel. Lipoproteins containing apoA-I were detected after electrotransfer to nitrocellulose membranes with a polyclonal anti-human apoA-I antibody followed by horseradish peroxidase-conjugated anti-rabbit antibody and enhanced chemiluminescence treatment. For practical purposes, preβ_1 and preβ_2 subpopulations, as well as α and preα particles, were identified in the female proband. The sizes of the molecular standards are shown at right in angstroms.

control subjects. Heterozygous carriers of the A174T or T383M mutation were also characterized by low HL mass. In vitro studies demonstrated that the T383M mutation in the HL gene results in an 80% reduction of HL secretion compared with the wild-type protein (45), and the present results suggest that the A174T mutation may also impair the enzyme secretion. We therefore conclude that the HDL phenotype attributed to HL deficiency in the present study could not have been modulated by the presence of significant amounts of catalytically inactive HL. Recent studies have suggested that EL, a recently discovered member of the lipase family, may play a central role in HDL metabolism (35). Patients with HL deficiency had normal HDL phospholipid levels compared with controls. EL acts predominantly as a phospholipase (46), and we have no reason to expect that EL activity was disturbed in our patients. The extent to which EL may contribute to the normalization of the RCT in complete HL deficiency remains to be determined.

Recently, it was reported that patients with CAD are characterized by lower levels of large α_1- and preα_{1-3}-mobility apoA-I-containing HDL subfractions and higher α_3-HDL concentrations compared with control individuals (47). In the present study, complete HL-deficient patients from the QHLD kindred were qualitatively characterized as having large α_1-HDL and fewer α_3-HDL particles by two-dimensional gel electrophoresis. These findings were further supported by quantitative analyses of HDL particle size by 1D-PAGGE, which demonstrated that patients with complete HL deficiency, even in the presence of marked hypertriglyceridemia in males, were characterized by a significantly increased HDL particle size compared with NTG controls. As previously indicated (23), there is a history of premature CAD in the QHLD kindred, but the three patients with complete HL deficiency were aged 44, 38, and 36 years at the time of this study, were perfectly healthy, and had no signs of premature CAD. It is recognized that complete HL deficiency has been associated with profound disturbances in apoB-containing lipoprotein metabolism, with marked accumulations of small, partially catabolized TG-rich lipoprotein remnants (48). Because the accumulation of these atherogenic remnant particles may be associated with an accelerated development of premature atherosclerosis (49), we suggest that the altered apoB-containing lipoprotein metabolism, rather than apparent disturbances in HDL metabolism, is more likely to be responsible for the presence of premature atherosclerosis previously associated with human HL deficiency. Moreover, there are properties and functions of HDL other than RCT that are well documented in vitro and that are known to be potentially antiatherogenic. For example, HDLs have anti-inflammatory (50), antioxidant (51), and antithrombotic (52) functions. The extent to which these properties were altered in complete HL-defi-

cient patients from the QHLD kindred remains to be established.

In summary, we have shown that complete HL deficiency in the QHLD kindred was associated with HDL particles of abnormal composition, which have apparently reduced plasma catabolism but which are still effective in promoting the removal of cholesterol from cells. We observed that partial HL activity was sufficient to ensure a normal metabolism of HDL. Our data indicate that lack of HL mass or activity in humans does not affect the ability of HDL to mediate the early steps of the RCT process, thereby potentially counteracting the impact that accumulating levels of remnants may have on CAD risk in these patients.

I.L.R. is the recipient of a scholarship from the Heart and Stroke Foundation of Canada/Canadian Institutes of Health Research (CIHR) Health Research Partnership Fund. P.C. is the recipient of a fellowship from the Fonds de la Recherche en Santé du Québec. J.S.C. is the recipient of a CIHR/Research and Development Investigator Salary Award (DSC-64146) and a CIHR operating grant (MOP-14684). B.L. is the recipient of the Canada Research Chair in Nutrition, Functional Foods, and Cardiovascular Health. This research was supported by the CIHR (MOP-37950).

REFERENCES

1. Miller, G. J., and N. E. Miller. 1977. Plasma high density lipoprotein concentration and development of ischaemic heart disease. *Lancet.* **1:** 16–18.
2. Durrington, P. N. 1993. How HDL protects against atheroma. *Lancet.* **342:** 1315–1316.
3. Gordon, D. J., J. L. Probstfield, R. J. Garrison, J. D. Neaton, W. P. Castelli, J. Knoke, D. R. Jacobs, Jr., S. Bangdiwala, and H. A. Tyroler. 1989. High-density lipoprotein cholesterol and cardiovascular disease. Four prospective American studies. *Circulation.* **79:** 8–15.
4. Glomset, J. A. 1968. The plasma lecithins:cholesterol acyltransferase reaction. *J. Lipid Res.* **9:** 155–167.
5. Fielding, C. J., and P. E. Fielding. 1995. Molecular physiology of reverse cholesterol transport. *J. Lipid Res.* **36:** 211–228.
6. Kuusi, T., P. Saarinen, and E. A. Nikkila. 1980. Evidence for the role of hepatic endothelial lipase in the metabolism of plasma high density lipoprotein2 in man. *Atherosclerosis.* **36:** 589–593.
7. Lamarche, B., K. D. Uffelman, A. Carpentier, J. S. Cohn, G. Steiner, P. H. Barrett, and G. F. Lewis. 1999. Triglyceride enrichment of HDL enhances in vivo metabolic clearance of HDL apo A-I in healthy men. *J. Clin. Invest.* **103:** 1191–1199.
8. Lamarche, B., S. Rashid, and G. F. Lewis. 1999. HDL metabolism in hypertriglyceridemic states: an overview. *Clin. Chim. Acta.* **286:** 145–161.
9. Chen, G. C., W. Liu, P. Duchateau, J. Allaart, R. L. Hamilton, C. M. Mendel, K. Lau, D. A. Hardman, P. H. Frost, and M. J. Malloy. 1994. Conformational differences in human apolipoprotein B-100 among subspecies of low density lipoproteins (LDL). Association of altered proteolytic accessibility with decreased receptor binding of LDL subspecies from hypertriglyceridemic subjects. *J. Biol. Chem.* **269:** 29121–29128.
10. McKeone, B. J., J. R. Patsch, and H. J. Pownall. 1993. Plasma triglycerides determine low density lipoprotein composition, physical properties, and cell-specific binding in cultured cells. *J. Clin. Invest.* **91:** 1926–1933.
11. Toyota, Y., T. Yamamura, Y. Miyake, and A. Yamamoto. 1999. Low density lipoprotein (LDL) binding affinity for the LDL receptor in hyperlipoproteinemia. *Atherosclerosis.* **147:** 77–86.
12. Fielding, P. E., C. J. Fielding, R. J. Havel, J. P. Kane, and P. Tun. 1983. Cholesterol net transport, esterification, and transfer in human hyperlipidemic plasma. *J. Clin. Invest.* **71:** 449–460.
13. Morton, R. E., and D. B. Zilversmit. 1983. Inter-relationship of lipids transferred by the lipid-transfer protein isolated from human lipoprotein-deficient plasma. *J. Biol. Chem.* **258:** 11751–11757.
14. Ahnadi, C. E., T. Masmoudi, F. Berthezene, and G. Ponsin. 1993. Decreased ability of high density lipoproteins to transfer cholesterol esters in non-insulin-dependent diabetes mellitus. *Eur. J. Clin. Invest.* **23:** 459–465.
15. Lagrost, L. 1994. Regulation of cholesteryl ester transfer protein (CETP) activity: review of in vitro and in vivo studies. *Biochim. Biophys. Acta.* **1215:** 209–236.
16. Skeggs, J. W., and R. E. Morton. 2002. LDL and HDL enriched in triglyceride promote abnormal cholesterol transport. *J. Lipid Res.* **43:** 1264–1274.
17. Hegele, R. A., J. A. Little, C. Vezina, G. F. Maguire, L. Tu, T. S. Wolever, D. J. Jenkins, and P. W. Connelly. 1993. Hepatic lipase deficiency. Clinical, biochemical, and molecular genetic characteristics. *Arterioscler. Thromb.* **13:** 720–728.
18. Breckenridge, W. C., J. A. Little, P. Alaupovic, C. S. Wang, A. Kuksis, G. Kakis, F. Lindgren, and G. Gardiner. 1982. Lipoprotein abnormalities associated with a familial deficiency of hepatic lipase. *Atherosclerosis.* **45:** 161–179.
19. Connelly, P. W., G. F. Maguire, M. Lee, and J. A. Little. 1990. Plasma lipoproteins in familial hepatic lipase deficiency. *Arteriosclerosis.* **10:** 40–48.
20. Connelly, P. W., S. Ranganathan, G. F. Maguire, M. Lee, J. J. Myher, B. A. Kottke, A. Kuksis, and J. A. Little. 1988. The beta very low density lipoprotein present in hepatic lipase deficiency competitively inhibits low density lipoprotein binding to fibroblasts and stimulates fibroblast acyl-CoA:cholesterol acyltransferase. *J. Biol. Chem.* **263:** 14184–14188.
21. Carlson, L. A., L. Holmquist, and P. Nilsson-Ehle. 1986. Deficiency of hepatic lipase activity in post-heparin plasma in familial hyper-alpha-triglyceridemia. *Acta Med. Scand.* **219:** 435–447.
22. Little, J. A., and P. W. Connelly. 1986. Familial hepatic lipase deficiency. *Adv. Exp. Med. Biol.* **201:** 253–260.
23. Ruel, I. L., P. Couture, C. Gagne, Y. Deshaies, J. Simard, R. A. Hegele, and B. Lamarche. 2003. Characterization of a novel mutation causing hepatic lipase deficiency among French-Canadians. *J. Lipid Res.* **44:** 1508–1514.
24. Warnick, G. R., J. Benderson, and J. J. Albers. 1982. Dextran sulfate-Mg^{2+} precipitation procedure for quantitation of high-density-lipoprotein cholesterol. *Clin. Chem.* **28:** 1379–1388.
25. Couillard, C., J. P. Despres, B. Lamarche, J. Bergeron, J. Gagnon, A. S. Leon, D. C. Rao, J. S. Skinner, J. H. Wilmore, and C. Bouchard. 2001. Effects of endurance exercise training on plasma HDL cholesterol levels depend on levels of triglycerides: evidence from men of the Health, Risk Factors, Exercise Training and Genetics (HERITAGE) Family Study. *Arterioscler. Thromb. Vasc. Biol.* **21:** 1226–1232.
26. Bensadoun, A. 1996. Sandwich immunoassay for measurement of human hepatic lipase. *Methods Enzymol.* **263:** 333–338.
27. Pérusse, M., A. Pascot, J. P. Després, C. Couillard, and B. Lamarche. 2001. A new method for HDL particle sizing by polyacrylamide gradient gel electrophoresis using whole plasma. *J. Lipid Res.* **42:** 1331–1334.
28. Cohn, J. S., D. A. Wagner, S. D. Cohn, J. S. Millar, and E. J. Schaefer. 1990. Measurement of very low density and low density lipoprotein apolipoprotein (Apo) B-100 and high density lipoprotein Apo A-I production in human subjects using deuterated leucine. Effect of fasting and feeding. *J. Clin. Invest.* **85:** 804–811.
29. Batal, R., M. Tremblay, L. Krimbou, O. Mamer, J. Davignon, J. J. Genest, and J. S. Cohn. 1998. Familial HDL deficiency characterized by hypercatabolism of mature apoA-I but not proapoA-I. *Arterioscler. Thromb. Vasc. Biol.* **18:** 655–664.
30. Cobelli, C., G. Toffolo, and D. M. Foster. 1992. Tracer-to-tracee ratio for analysis of stable isotope tracer data: link with radioactive kinetic formalism. *Am. J. Physiol.* **262:** E968–E975.
31. Marcil, M., L. Yu, L. Krimbou, B. Boucher, J. F. Oram, J. S. Cohn, and J. Genest, Jr. 1999. Cellular cholesterol transport and efflux in fibroblasts are abnormal in subjects with familial HDL deficiency. *Arterioscler. Thromb. Vasc. Biol.* **19:** 159–169.
32. Krimbou, L., M. Tremblay, J. Davignon, and J. S. Cohn. 1997. Characterization of human plasma apolipoprotein E-containing lipoproteins in the high density lipoprotein size range: focus on

pre-beta1-LpE, pre-beta2-LpE, and alpha-LpE. *J. Lipid Res.* **38:** 35–48.
33. Rashid, S., D. K. Trinh, K. D. Uffelman, J. S. Cohn, D. J. Rader, and G. F. Lewis. 2003. Expression of human hepatic lipase in the rabbit model preferentially enhances the clearance of triglyceride-enriched versus native high-density lipoprotein apolipoprotein A-I. *Circulation.* **107:** 3066–3072.
34. Fidge, N. H. 1999. High density lipoprotein receptors, binding proteins, and ligands. *J. Lipid Res.* **40:** 187–201.
35. Jaye, M., K. J. Lynch, J. Krawiec, D. Marchadier, C. Maugeais, K. Doan, V. South, D. Amin, M. Perrone, and D. J. Rader. 1999. A novel endothelial-derived lipase that modulates HDL metabolism. *Nat. Genet.* **21:** 424–428.
36. Gordon, D. J., and B. M. Rifkind. 1989. High-density lipoprotein—the clinical implications of recent studies. *N. Engl. J. Med.* **321:** 1311–1316.
37. Miller, N. E. 1987. Associations of high density lipoprotein subclasses and apolipoproteins with ischemic heart disease and coronary atherosclerosis. *Am. Heart J.* **113:** 589–597.
38. Castro, G. R., and C. J. Fielding. 1988. Early incorporation of cell-derived cholesterol into pre-beta-migrating high-density lipoprotein. *Biochemistry.* **27:** 25–29.
39. Kawano, M., T. Miida, C. J. Fielding, and P. E. Fielding. 1993. Quantitation of pre beta-HDL-dependent and nonspecific components of the total efflux of cellular cholesterol and phospholipid. *Biochemistry.* **32:** 5025–5028.
40. Sviridov, D., and P. Nestel. 2002. Dynamics of reverse cholesterol transport: protection against atherosclerosis. *Atherosclerosis.* **161:** 245–254.
41. Liang, H. Q., K. A. Rye, and P. J. Barter. 1994. Dissociation of lipid-free apolipoprotein A-I from high density lipoproteins. *J. Lipid Res.* **35:** 1187–1199.
42. Liang, H. Q., K. A. Rye, and P. J. Barter. 1995. Cycling of apolipoprotein A-I between lipid-associated and lipid-free pools. *Biochim. Biophys. Acta.* **1257:** 31–37.
43. Dichek, H. L., W. Brecht, J. Fan, Z. S. Ji, S. P. McCormick, H. Akeefe, L. Conzo, D. A. Sanan, K. H. Weisgraber, S. G. Young, J. M. Taylor, and R. W. Mahley. 1998. Overexpression of hepatic lipase in transgenic mice decreases apolipoprotein B-containing and high density lipoproteins. Evidence that hepatic lipase acts as a ligand for lipoprotein uptake. *J. Biol. Chem.* **273:** 1896–1903.
44. Dugi, K. A., M. J. Amar, C. C. Haudenschild, R. D. Shamburek, A. Bensadoun, R. F. Hoyt, Jr., J. Fruchart-Najib, Z. Madj, H. B. Brewer, Jr., and S. Santamarina-Fojo. 2000. In vivo evidence for both lipolytic and nonlipolytic function of hepatic lipase in the metabolism of HDL. *Arterioscler. Thromb. Vasc. Biol.* **20:** 793–800.
45. Durstenfeld, A., O. Ben-Zeev, K. Reue, G. Stahnke, and M. H. Doolittle. 1994. Molecular characterization of human hepatic lipase deficiency. In vitro expression of two naturally occurring mutations. *Arterioscler. Thromb.* **14:** 381–385.
46. McCoy, M. G., G. S. Sun, D. Marchadier, C. Maugeais, J. M. Glick, and D. J. Rader. 2002. Characterization of the lipolytic activity of endothelial lipase. *J. Lipid Res.* **43:** 921–929.
47. Asztalos, B. F., P. S. Roheim, R. L. Milani, M. Lefevre, J. R. McNamara, K. V. Horvath, and E. J. Schaefer. 2000. Distribution of ApoA-I-containing HDL subpopulations in patients with coronary heart disease. *Arterioscler. Thromb. Vasc. Biol.* **20:** 2670–2676.
48. Demant, T., L. A. Carlson, L. Holmquist, F. Karpe, P. Nilsson-Ehle, C. J. Packard, and J. Shepherd. 1988. Lipoprotein metabolism in hepatic lipase deficiency: studies on the turnover of apolipoprotein B and on the effect of hepatic lipase on high density lipoprotein. *J. Lipid Res.* **29:** 1603–1611.
49. Karpe, F., G. Steiner, K. Uffelman, T. Olivecrona, and A. Hamsten. 1994. Postprandial lipoproteins and progression of coronary atherosclerosis. *Atherosclerosis.* **106:** 83–97.
50. Cockerill, G. W., K. A. Rye, J. R. Gamble, M. A. Vadas, and P. J. Barter. 1995. High-density lipoproteins inhibit cytokine-induced expression of endothelial cell adhesion molecules. *Arterioscler. Thromb. Vasc. Biol.* **15:** 1987–1994.
51. Klimov, A. N., V. S. Gurevich, A. A. Nikiforova, L. V. Shatilina, A. A. Kuzmin, S. L. Plavinsky, and N. P. Teryukova. 1993. Antioxidative activity of high density lipoproteins in vivo. *Atherosclerosis.* **100:** 13–18.
52. Nofer, J. R., M. Walter, B. Kehrel, S. Wierwille, M. Tepel, U. Seedorf, and G. Assmann. 1998. HDL3-mediated inhibition of thrombin-induced platelet aggregation and fibrinogen binding occurs via decreased production of phosphoinositide-derived second messengers 1,2-diacylglycerol and inositol 1,4,5-tris-phosphate. *Arterioscler. Thromb. Vasc. Biol.* **18:** 861–869.