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Cholesteryl ester transfer activity in liver disease and cholestasis, and its relation with fatty acid composition of lipoprotein lipids

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Abstract

Liver disease is accompanied by major qualitative and quantitative disturbances in plasma lipoprotein metabolism, the extent and intensity of which depend on the degree of parenchymal damage, cholestasis, or both. The main objective of this study was to determine the cholesteryl ester transfer CETP activity and its association with the lipoprotein neutral lipid composition in patients with either liver cirrhosis or cholestasis, as compared to normal controls. Lipoproteins were isolated by ultracentrifugation, lipids and apolipoproteins were measured by conventional methods, and the fatty acid composition was established by gas chromotography; CETP activity in lipoprotein-deficient plasma was measured by determining the transfer of [^{3}H]cholesteryl esters from HDL to VLDL. Lipoprotein lipase and hepatic lipase activities were measured in post-heparin plasma by radiochemical methods. In patients with liver cirrhosis, low levels of VLDL, HDL, apo B, and Lp(a) were observed, as well as a change in the composition of HDL particles, with increases in the relative proportion of tri-

Abbreviations: ALAT, alanine aminotransferase; ALP, alkaline phosphatase; ASAT, aspartate aminotransferase; CETP, cholesteryl ester transfer protein; GGT, γ-glutamyl transferase; HDL, high density lipoproteins (d = 1.063–1.21 g/ml); HL, hepatic triglyceride lipase; LCAT, lecithin:cholesterol acyltransferase; LDL, low density lipoproteins (d = 1.006–1.063 g/ml); Lp(a), lipoprotein(a); LPL, lipoprotein lipase; VLDL, very low density lipoproteins (d 1.006 g/ml).

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glyceride and free cholesterol. Respectively, the last two changes could be attributed in part to the low hepatic lipase activity observed in this study, and to the low lecithin:cholesterol acyltransferase activity previously observed by others. In patients with cholestasis, a moderate hyperlipidemia due to the elevation of LDL was found. In contrast, HDL and apo A-I levels were very low reflecting a low number of HDL particles, which also had altered compositions with increases in the triglyceride and free cholesterol contents relative to apo A-I and esterified cholesterol, respectively. As regards the fatty acid composition of lipoprotein lipids, the two groups of patients showed, in general, a lower proportion of linoleic acid and a compensating higher proportion of oleic acid as compared to the controls, changes that were observed in both cholesteryl esters and triglycerides. In contrast, the proportions of oleic and palmitoleic acids in phospholipids were increased, whereas that of stearic acid was decreased in patients as compared to controls. In patients with liver cirrhosis, as well as in controls, no changes were observed in the fatty acid compositions of cholesteryl ester, triglycerides, or phospholipids among the different lipoproteins, which probably reflects the equilibration reached by the action of CETP. In patients with cholestasis, no differences were observed in fatty acid composition among the lipoprotein phospholipids but, interestingly, cholesteryl esters from VLDL had a significantly lower linoleic acid content than those from HDL, whereas triglycerides from VLDL had significantly higher oleic acid and lower linoleic acid contents than those from HDL. This distinct fatty acid composition of the neutral lipids between lipoproteins was associated with a significant decrease (25%) in the cholesteryl ester transfer activity in patients with cholestasis. We suggest that fat malabsorption due to the biliary defect may induce a decrease in cholesteryl ester transfer protein synthesis or secretion, which in turn would slow the equilibration of the neutral lipids among plasma lipoproteins.

Keywords: Cholesteryl ester transfer protein; Lipoproteins; Fatty acid composition; Liver disease; Cholestasis

1. Introduction

Cholesteryl ester transfer protein (CETP) mediates the transfer of cholesteryl esters from HDL to apo B-containing lipoproteins in exchange for triglycerides [1–3]. In this manner, the cholesteryl esters formed in the HDL particles by the action of lecithin:cholesterol acyltransferase (LCAT) are mainly channelled to VLDL and LDL, and are finally removed by tissues, constituting the so-called 'reverse cholesterol transport', in which the major target organ is the liver. Besides this, the liver plays a crucial role in other aspects of lipoprotein metabolism, including the synthesis of VLDL, nascent HDL and also important enzymes such as hepatic triglyceride lipase (HL) and LCAT [4]. Therefore, it is not surprising that hepatic disease is accompanied by major disturbances in plasma lipoprotein metabolism. Whether the disease affects parenchymal tissue or results in cholestasis, the lipoprotein alterations are quite different. Thus, in patients with acute hepatitis or cholestasis both triglyceride and cholesterol plasma levels are usually elevated, whereas plasma levels of cholesterol and phospholipids are normal

or reduced in patients with hepatocellular damage [5,6]. A variety of intermediate situations can be found depending on the stage and the co-existence of parenchymal affection and cholestasis [7–11].

There is very limited information on CETP in liver disease. It was originally observed that CETP activity was reduced in rabbits with D-(+)-galactosamine-induced hepatitis [12] and the same occurs during the most active part of hepatocellular damage in human patients with viral hepatitis [13]. However, in patients with chronic hepatitis, or either compensated or decompensated liver cirrhosis, or hepatocellular carcinoma, plasma CETP levels are similar to those in control subjects [13]. Finally, CETP has recently been observed to be increased in patients with primary biliary cirrhosis who had hyperalphalipoproteinemia, but it was normal in those with average HDL levels [14].

CETP mRNA has been detected in several sites including the liver, intestine, adipose tissue and macrophages [15–18], and the secretion of active CETP by perfused rabbit liver [19,20], cultured HepG2 cells [21], and the CaCo-2 intestinal cell model [22] has been demonstrated directly. In the latter cells, CETP secretion is twice as high as in HepG2 cells, and regulation of this process differs greatly between these two cell types, since fatty acids stimulate CETP synthesis in CaCo-2, but they do not in HepG2 [21,22]. This makes CETP activity in patients with hepatic jaundice unpredictable because, besides the possible damage to the liver parenchyma, fat intolerance and malabsorption usually coexist in these patients due to their biliary defect [23].

For the above reasons, in order to know whether hepatic disease affects CETP, we studied plasma CETP activity in patients with liver disease or cholestasis, and in a control group, and determined the fatty acid composition of esterified lipids in the different lipoproteins to ascertain whether the eventual alteration of CETP in these pathological states resulted in an inhomogeneous distribution of cholesteryl ester species among lipoproteins.

2. Patients and methods

We studied 25 patients with liver disease or cholestasis, aged 28–78 years. One group comprised 14 subjects (12 male, 2 female) affected with liver cirrhosis, which was alcoholic cirrhosis in 9, two had primary biliary cirrhosis stage IV, one had viral hepatitis type B, and the origin was unknown in the last two. At the beginning of the study, the patients had clinical symptoms of liver cirrhosis of more than 1 year of evolution. A second group consisted of 11 patients (7 males, 4 females), 2 of whom had sclerosing cholangitis, and 9 had extrahepatic cholestasis due either to choledocholithiasis (7) or to pancreatic cancer (2). Except for some patients with decompensated cirrhosis,

who received furosemide and were recommended a low-salt diet, none of the other patients had received any medication known to influence lipoprotein metabolism. In all cases, diagnosis was based on clinical history, X-ray examination, histological examination of liver biopsy, and biochemical and immunological analyses. The control group consisted of 20 healthy normolipidemic volunteers, 9 female, 11 male, aged 24–65, all from the Hospital staff/personnel. Informed consent was obtained from each patient and normal control.

Blood samples were drawn after 12 h of fasting. Two samples were collected from each subject, one in basal conditions and a second 10 min after the i.v. injection of heparin (50 IU/kg of body weight). Basal blood was processed for routine biochemical analyses of serum and for lipid analyses and lipoprotein isolation. Postheparin plasma was frozen at −70°C until used for lipoprotein lipase and hepatic lipase activity determination.

Lipoproteins were isolated from plasma by sequential ultracentrifugation [24]. The lipoprotein-containing supernatants (VLDL, $d < 1.006$ g/ml; LDL, $d = 1.006–1.063$, and HDL, $d = 1.063–1.21$) were used for lipid analyses and for fatty acid composition. The CETP-containing fraction (infranatant of $d = 1.21$ g/ml) was exhaustively dialyzed against 0.15 mol/l NaCl, 1 mmol/l Na_2-EDTA, brought to the initial plasma volume with this medium and stored at −70°C until processing.

Total cholesterol, free cholesterol, triglyceride and phosphatidylcholine were measured enzymatically in a Technicon RA-1000 autoanalyzer (Technicon Ltd., Dublin, Ireland). Apolipoproteins A-I and B were determined by immunonephelometry (Array System, Beckman Instruments, Palo Alto, CA) using the respective reagents and WHO standards. Lipoprotein(a) was quantified in frozen samples by a sandwich-type enzyme-linked immunoassay (ELISA; TintElizeLp(a), BioPool AB, Umea, Sweden).

Hepatic triglyceride lipase activity in post-heparin plasma was determined using a [^{3}H]triolein-based substrate stabilized in gum arabic according to Huttunen et al. [25]. Lipoprotein lipase (LPL) activity was measured in post-heparin plasma using VLDL prelabelled with [^{3}H]triolein as substrate, as described elsewhere by us [26]. LPL activity was calculated as the difference between total [^{3}H]VLDL hydrolysis and that observed in the presence of 1 mol/l NaCl, and expressed in pkat of triglyceride hydrolyzed/ml plasma by considering the specific radioactivity of [^{3}H]VLDL and the amount of triglyceride present in the sample.

CETP activity of lipoprotein-free plasma was determined as the activity which transferred cholesteryl esters from HDL to VLDL as previously described by us [26,27]. An apo E-free HDL fraction prelabelled with [^{3}H]cholesteryl oleate was used as substrate [28]. After incubation in the presence of the sample and a fixed amount of exogenous VLDL, the newly-

formed [^{3}H]VLDL were isolated by selective precipitation with dextran sulfate/$MgCl_2$ and the radioactivity counted [26,27]. CETP activity was expressed as nmol cholesteryl ester transferred to VLDL per ml of plasma.

Fatty acid profile analysis was performed by gas-liquid chromatography. Briefly, lipoprotein lipids were extracted with chloroform-methanol (2:1, v/v) [29] and then phospholipids, triglycerides and cholesteryl esters were separated by thin layer chromatography using hexane/ethyl ether/acetic acid (80:20:1, v/v/v). After saponification, the fatty acids were methylated [30] and analyzed with a Perkin Elmer 3920 B gas liquid chromatograph (Perkin Elmer Corp., Norwalk, Connecticut, USA), equipped with a 2 m × 2 mm glass column filled with 5% diethyleneglycol succinate on gas chrom Q 100–120 mesh. Quantitation was based on the relative responses of the fatty acid methyl ester in the sample as compared with the response of pure standards (Sigma Chemical Co, St. Louis, MO, USA).

Results were expressed as mean ± S.E.M. Statistical comparisons were performed by ANOVA and Neuman-Keuls multiple range analysis, and by the Kruskal-Wallis one way ANOVA on ranks, followed by the Student-Neuman-Keuls pairwise multiple comparisons method when indicated. In the case of Lp(a), statistical comparisons were done by the Kruskal-Wallis one way ANOVA on ranks and the Dunn methods. Calculations were done using Statgraphics (Statistical Graphics Corporation STSC Inc., Tockville, MD) and SigmaStat Statistical Analysis System (Jandel Scientific GmbH, Erkrath, Germany).

3. Results

Laboratory data of patients with either liver cirrhosis or cholestasis, and controls are shown in Table 1. As expected, serum bilirubin, γ-glutamyl

Table 1
Serum markers of hepatic function serum markers in liver cirrhosis, cholestasis and controls

	Liver cirrhosis	Cholestasis	Controls
Bilirubin*	3.3 ± 0.7^{a}	11 ± 3^{b}	0.8 ± 0.1^{c}
GGT**	153 ± 33^{a}	391 ± 118^{b}	27 ± 6^{c}
ASAT**	123 ± 32^{a}	107 ± 26^{a}	19 ± 1^{b}
ALAT**	55 ± 6^{a}	183 ± 72^{b}	22 ± 3^{c}
ALP**	439 ± 60^{a}	1102 ± 195^{b}	155 ± 9^{c}

Values are *mg/dl plasma, **IU/l, means ± S.E.M.
Statistical comparisons between groups by ANOVA and Neuman-Keuls multiple range analysis. Different superscript letters indicate that statistically significant differences ($P < 0.05$) exist between the respective groups.

Table 2
Plasma lipids, lipoproteins and apolipoproteins in liver cirrhosis, cholestasis and controls

	Liver cirrhosis	Cholestasis	Controls
Total triglyceride	81 ± 7[a]	231 ± 45[b]	137 ± 15[c]
Total cholesterol	155 ± 13[a]	292 ± 46[b]	203 ± 10[c]
VLDL-triglyceride	20 ± 4[a]	95 ± 6[b]	92 ± 13[b]
VLDL-cholesterol	7 ± 2[a]	18 ± 8[a,b]	16 ± 3[b]
LDL-cholesterol	111 ± 10[a]	217 ± 50[b]	137 ± 10[a]
HDL-cholesterol	36 ± 5[a]	22 ± 2[b]	47 ± 3[a]
apo B	52 ± 4[a]	107 ± 12[b]	66 ± 4[c]
apo A-I	79 ± 14[a,b]	55 ± 11[b]	114 ± 10[a]
Lp(a)	2.9[a] (1.8–8.8)	4.5[a,b] (2.0–22.3)	19.2[b] (10.1–35.1)

Values are mg/dl plasma, means ± S.E.M, except for Lp(a) where median (interquartile range) values are given. Statistical comparisons between groups by ANOVA and Neuman-Keuls multiple range analysis, in the case of Lp(a) the Kruskal-Wallis one way ANOVA on ranks and Dunn's method for multiple comparisons were used. Different superscript letters indicate that statistically significant differences ($P < 0.05$) exist between the respective groups.

transpeptidase (GGT), aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT), and alkaline phosphatase (ALP) were moderately elevated in patients with liver cirrhosis. With the exception of ASAT, these parameters were much more elevated in patients with cholestasis, especially total bilirubin and ALP.

Table 3
Lipid and apolipoprotein mass ratios in ultracentrifugally isolated HDL in liver cirrhosis, cholestasis and controls

	Liver cirrhosis	Cholestasis	Controls
A-I/esterified cholesterol	3.3 ± 0.3[a]	4.6 ± 0.8[a]	3.5 ± 0.4[a]
A-I/free cholesterol	6 ± 1[a]	6 ± 1[a]	12 ± 1[b]
A-I/triglyceride	13 ± 2[a]	13 ± 2[a]	19 ± 1[b]
A-I/phospholipid	2.0 ± 0.1[a]	2.3 ± 0.3[a,b]	2.6 ± 0.2[b]
Esterified cholesterol/free cholesterol	1.6 ± 0.3[a]	1.3 ± 0.3[a]	3.7 ± 0.2[b]

Values are mg/mg, means ± S.E.M. Statistical comparisons between groups by ANOVA and Neuman-Keuls multiple range analysis: different superscript letters denote statistically significant differences ($P < 0.05$) between the respective groups.

Plasma lipid and apolipoprotein concentrations are shown in Table 2. Patients with liver cirrhosis had moderately, but significantly, diminished total triglyceride values as compared to controls, which mainly corresponded to a decrease in the VLDL fraction. Total cholesterol was also diminished in patients with liver cirrhosis, due to decreases of the cholesterol content in every lipoprotein fraction, especially in both VLDL and HDL, which were significantly lower than in controls. In correlation with the changes observed in plasma lipid concentrations, plasma levels of both apo B and Lp(a) were significantly lower and those of apo A-I were only slightly lower in patients with liver cirrhosis than in controls (Table 2). Most of the patients with cholestasis had moderate hyperlipidemia, which affected both total triglyceride and cholesterol. Despite that the LDL fraction from these patients potentially contained some LpX, the elevation of both the LDL-cholesterol and the apo B concentration probably indicates the increased number of LDL particles in these subjects. On the contrary, HDL-cholesterol and apo A-I were very low in patients with cholestasis, indicating the lower number of HDL particles in these patients than in either controls or cirrhotic patients. Lp(a) plasma concentration in these patients was moderately, but not significantly, different from that in normal controls (Table 2).

To study the composition of ultracentrifugally isolated HDL, several lipid mass ratios were calculated (Table 3). With the exception of the apo A-I/esterified cholesterol, all the other mass ratios shown in Table 3 were lower in patients with liver cirrhosis than in controls. These data indicate the profound alteration of HDL composition in these patients, especially the enrichment in triglyceride and phospholipids in proportion to apo A-I, and the enrichment in free cholesterol in relation to esterified cholesterol. The HDL

Table 4

Lipolytic activities in post-heparin plasma and cholesteryl ester transfer activity in liver cirrhosis, cholestasis and controls

	Liver cirrhosis	Cholestasis	Controls
Lipoprotein lipase*	518 ± 73[a]	392 ± 53[a]	403 ± 35[a]
Hepatic lipase*	422 ± 133[a]	267 ± 66[a]	941 ± 71[b]
Cholesteryl ester transfer activity**	194 ± 14[a,b]	156 ± 12[b]	208 ± 10[a]

Values are *pkat/ml post-heparin plasma, **nmol cholesteryl ester transferred/8 h/ml plasma.

Means ± S.E.M. Statistical comparisons between groups by ANOVA and Neuman-Keuls multiple range analysis: different superscript letters denote statistically significant differences ($P < 0.05$) between the respective groups.

mass ratios in patients with cholestasis were very similar to those in patients with liver cirrhosis, showing the enrichment in both free cholesterol and triglycerides when compared to the HDL from controls (Table 3).

To elucidate the underlying mechanisms of these changes in lipoprotein

Table 5
Fatty acid composition of cholesteryl esters from VLDL, LDL and HDL in liver cirrhosis, cholestasis and controls

	Liver cirrhosis	Cholestasis	Controls
C14:0			
VLDL	1.19 ± 0.23	2.06 ± 1.11	0.86 ± 0.21
LDL	0.83 ± 0.13	0.72 ± 0.21	0.74 ± 0.14
HDL	0.86 ± 0.26	0.68 ± 0.15	0.63 ± 0.12
C16:0			
VLDL	13.0 ± 1.2	14.1 ± 0.1	13.1 ± 1.5
LDL	14.2 ± 1.0	13.2 ± 0.2	12.1 ± 0.6
HDL	14.4 ± 1.2	12.2 ± 1.1	13.2 ± 1.6
C16:1			
VLDL	4.01 ± 0.88	4.70 ± 1.14	3.74 ± 0.77
LDL	4.27 ± 0.75	4.13 ± 1.10	2.65 ± 0.32
HDL	4.46 ± 0.99	3.23 ± 0.35	2.74 ± 0.34
C18:0			
VLDL	2.54 ± 0.81	2.50 ± 0.62	2.70 ± 0.44
LDL	2.31 ± 0.76	1.40 ± 0.23	1.34 ± 0.42
HDL	3.37 ± 0.81	3.22 ± 1.33	1.35 ± 0.39
C18:1			
VLDL	22.5 ± 2.8	29.5 ± 1.9^{+}	23.0 ± 1.7
LDL	27.1 ± 2.2^{a}	26.9 ± 1.3^{a}	18.8 ± 1.0^{b}
HDL	26.6 ± 2.1^{a,b}	28.3 ± 1.5^{a}	21.1 ± 2.3^{b}
C18:2			
VLDL	51.1 ± 6.4	40.5 ± 1.1*	51.3 ± 9.2
LDL	46.4 ± 3.2^{a}	46.5 ± 2.3^{a*}	58.0 ± 1.6^{b}
HDL	44.4 ± 3.8^{a,b}	46.9 ± 2.0^{a}	54.4 ± 2.0^{b}
C20:4			
VLDL	5.75 ± 1.39	6.47 ± 0.60	5.25 ± 0.64
LDL	4.80 ± 0.57^{a}	7.56 ± 0.30^{b}	6.39 ± 0.59^{a,b}
HDL	5.49 ± 0.70	6.86 ± 0.42	6.69 ± 0.68

Values are mol % of total fatty acids, means ± S.E.M. Statistical comparisons between groups by ANOVA and Neuman-Keuls multiple range analysis: different superscript letters denote statistically significant differences ($P < 0.05$) between the respective groups. Statistical comparisons between lipoprotein fractions from the same patient group were analyzed by the Kruskal-Wallis one way ANOVA on ranks and the Student-Neuman-Keuls pairwise multiple comparisons method: *significantly different ($P < 0.05$) than HDL; +significantly different ($P < 0.05$) than LDL. Only annotations indicating significant differences are shown.

plasma levels and HDL composition, we measured CETP activities in lipoprotein-deficient plasma, as well as LPL and HL in post-heparin plasma (Table 4). In patients with chronic liver disease, whether liver cirrhosis or cholestasis, LPL activity was not statistically different, whereas HL was

Table 6
Fatty acid composition of triglycerides from VLDL, LDL and HDL in liver cirrhosis, cholestasis and controls

	Liver cirrhosis	Cholestasis	Controls
C14:0			
VLDL	1.73 ± 0.31^{a}	0.89 ± 0.16^{b}	$1.39 \pm 0.14^{a,b}$
LDL	1.05 ± 0.12	1.09 ± 0.18	1.19 ± 0.20
HDL	2.16 ± 0.42^{a}	$1.74 \pm 0.40^{a,b}$	0.90 ± 0.14^{b}
C16:0			
VLDL	24.6 ± 0.7	23.9 ± 1.0	24.1 ± 0.9
LDL	24.0 ± 0.7	22.1 ± 1.03	23.9 ± 1.0
HDL	23.6 ± 0.8	20.8 ± 0.8	24.0 ± 1.2
C16:1			
VLDL	4.05 ± 0.79	3.95 ± 0.53	4.20 ± 0.32
LDL	4.40 ± 0.39	3.59 ± 0.46	4.05 ± 0.34
HDL	5.37 ± 0.60^{a}	$4.09 \pm 0.49^{a,b}$	3.59 ± 0.29^{b}
C18:0			
VLDL	6.16 ± 1.35^{a}	3.86 ± 0.25^{b}	3.19 ± 0.25^{b}
LDL	5.31 ± 0.26	4.00 ± 0.18	4.57 ± 0.76
HDL	7.68 ± 1.10^{a}	5.38 ± 0.44^{b}	4.63 ± 0.51^{b}
C18:1			
VLDL	$40.9 \pm 4.8^{a,b}$	$49.4 \pm 1.9^{a*}$	41.3 ± 1.5^{b}
LDL	$49.2 \pm 1.8^{a,b}$	$50.9 \pm 1.6^{a*}$	44.0 ± 2.0^{b}
HDL	44.7 ± 2.3	43.5 ± 2.3	43.8 ± 2.1
C18:2			
VLDL	$20.5 \pm 5.0^{a,b}$	$16.3 \pm 2.0^{a*}$	24.9 ± 2.0^{b}
LDL	14.3 ± 1.8^{a}	$16.5 \pm 1.5^{a,b*}$	21.5 ± 2.1^{b}
HDL	14.2 ± 1.5^{a}	23.3 ± 3.6^{b}	22.0 ± 2.4^{b}
C20:4			
VLDL	2.04 ± 0.18^{a}	1.54 ± 0.27^{a}	0.81 ± 0.13^{b}
LDL	1.63 ± 0.32^{a}	1.94 ± 0.16^{a}	0.92 ± 0.16^{b}
HDL	2.18 ± 0.50	2.72 ± 0.62	1.15 ± 0.30

Values are mol % of total fatty acids, means ± S.E.M. Statistical comparisons between groups by ANOVA and Neuman-Keuls multiple range analysis: different superscript letters denote statistically significant differences ($P < 0.05$) between the respective groups. Statistical comparisons between lipoprotein fractions from the same patient group were analyzed by the Kruskal-Wallis one way ANOVA on ranks and the Student-Neuman-Keuls pairwise multiple comparisons method: *significantly different ($P < 0.05$) than HDL. Only annotations indicating significant differences are shown.

Table 7
Fatty acid composition of phospholipids from VLDL, LDL and HDL in liver cirrhosis, cholestasis and controls

	Liver cirrhosis	Cholestasis	Controls
C14:0			
VLDL	0.42 ± 0.03	0.37 ± 0.10	0.47 ± 0.10
LDL	0.69 ± 0.12	0.50 ± 0.08	0.61 ± 0.10
HDL	0.66 ± 0.16[a]	0.88 ± 0.19[a]	0.22 ± 0.04[b]
C16:0			
VLDL	26.9 ± 2.2	29.0 ± 0.2	30.7 ± 1.0
LDL	31.0 ± 0.6	32.1 ± 2.2	33.9 ± 0.9
HDL	27.9 ± 1.3	29.9 ± 1.3	31.2 ± 0.9
C16:1			
VLDL	1.54 ± 0.23	1.46 ± 0.39	1.16 ± 0.25
LDL	1.67 ± 0.27[a]	1.62 ± 0.27[a]	0.74 ± 0.13[b]
HDL	1.87 ± 0.31[a]	1.32 ± 0.25[a]	0.63 ± 0.10[b]
C18:0			
VLDL	17.8 ± 0.5	19.3 ± 0.6	15.9 ± 0.4
LDL	14.2 ± 1.0[a,b]	12.6 ± 1.1[a]	16.5 ± 0.6[b]
HDL	13.6 ± 1.4[a]	15.6 ± 1.2[a,b]	17.5 ± 0.6[b]
C18:1			
VLDL	14.4 ± 1.6	16.1 ± 1.9	13.0 ± 0.6
LDL	19.3 ± 2.2[a]	18.0 ± 1.6[a]	12.5 ± 0.7[b]
HDL	19.0 ± 1.9[a]	16.0 ± 1.2[a]	12.1 ± 0.7[b]
C18:2			
VLDL	26.5 ± 3.9	20.1 ± 1.0	24.2 ± 0.8
LDL	22.7 ± 1.5	21.6 ± 1.3	23.5 ± 0.6
HDL	25.7 ± 2.4	20.4 ± 0.9	23.6 ± 1.0
C20:3			
VLDL	2.16 ± 0.66	3.57 ± 1.69	2.94 ± 0.34
LDL	2.84 ± 0.74	3.00 ± 0.54	2.80 ± 0.39
HDL	2.26 ± 0.53	3.84 ± 0.67	3.17 ± 0.38
C20:4			
VLDL	10.3 ± 2.5	11.2 ± 1.0	11.4 ± 0.8
LDL	7.5 ± 0.8	10.2 ± 0.8	9.4 ± 0.8
HDL	9.3 ± 1.0	12.1 ± 0.9	12.6 ± 1.1

Values are mol % of total fatty acids, means ± S.E.M. Statistical comparisons between groups by ANOVA and Neuman-Keuls multiple range analysis: different superscript letters denote statistically significant differences ($P < 0.05$) between the respective groups. Statistical comparisons between lipoprotein fractions from the same patient group were analyzed by the Kruskal-Wallis one way ANOVA on ranks and the Student-Neuman-Keuls pairwise multiple comparisons method. Only annotations indicating significant differences are shown.

substantially reduced as compared to normal controls. CETP activity in patients with liver cirrhosis was similar to that found in controls; however, it was significantly decreased in patients with cholestasis, showing values 25% lower than in the controls (Table 4).

The interexchange mediated by CETP results in the homogenization of neutral lipids between lipoproteins. To ascertain whether this process was altered in cholestatic patients, who had a decreased CETP activity, the fatty acid composition of cholesteryl esters, triglycerides, and phospholipids of VLDL, LDL and HDL were determined separately (Tables 5, 6 and 7, respectively). Only the quantitatively important fatty acids are shown in the tables. In the two groups of patients studied, the proportion of linoleic acid in cholesteryl esters from LDL and HDL was lower than in normal controls and, conversely, the proportion of oleic acid was increased (Table 5). In VLDL, however, no difference in the proportion of any fatty acid in cholesteryl esters between patients and controls was observed (Table 5). The fatty acid composition of triglycerides is shown in Table 6. In patients with cholestasis, the proportion of linoleic acid was decreased and those of oleic and arachidonic acids were increased, these changes being statistically significant in both VLDL and LDL but not in HDL. In patients with liver cirrhosis, the percentage of linoleic acid in triglycerides was decreased in every lipoprotein, and this change was compensated by slight increases in stearic, arachidonic and palmitoleic acid (Table 6). Finally, the fatty acid composition of phospholipids revealed a higher proportion of oleic acid and palmitoleic acid in patients than in controls, which was partially compensated by a slight decrease in the percentage of stearic acid (Table 7). These differences were statistically significant in phospholipids from LDL and HDL but not from VLDL (Table 7).

The fatty acid composition of each lipid class was compared between the different lipoproteins. Differences were only found in patients with cholestasis. The proportion of linoleic acid in VLDL-cholesteryl esters in these patients was significantly lower than in LDL and HDL (Table 5), whereas their VLDL-triglycerides were proportionally richer in oleic acid but poorer in linoleic acid than the corresponding HDL-triglycerides (Table 6). Fatty acid composition of phospholipids was similar in every lipoprotein within each study group, indicating the equilibrium of this lipid class among plasma lipoproteins (Table 7).

4. Discussion

We studied several aspects of lipoprotein metabolism in two groups of patients with chronic liver disease or cholestasis. Besides confirming the profound alterations in lipoprotein plasma levels and composition, this study

demonstrates that patients with cholestasis have decreased CETP activity and, in correlation with this, the cholesteryl ester and triglyceride species are not homogeneously distributed among the plasma lipoproteins, as indicated by their distinct fatty acid composition. To our knowledge, this is the first time the association between acquired deficiency of CETP activity and an inhomogeneous distribution of lipid species among lipoproteins has been documented in humans.

The patients with liver cirrhosis had major alterations in liver function and lipoprotein metabolism. They showed low levels of total triglycerides, cholesterol, apo B and apo A-I. In relation to lipoprotein fractions, VLDL and HDL were decreased, but their LDL levels were unaffected. LPL activity was normal in these patients, reflecting the extrahepatic origin of this enzyme activity in post-heparin plasma. Normal LPL, together with the adequate apo C-II and apo C-III in VLDL from these patients [31], should guarantee the rapid catabolism of plasma VLDL-triglycerides and eventually would lead to VLDL with low triglyceride/cholesterol mass ratio (Table 2). Lp(a) plasma concentration was intensely decreased in patients with liver cirrhosis, which is in agreement with recent results by others [32]. In contrast with Lp(a), LDL-cholesterol levels were unaffected in these patients, reflecting the distinct metabolism of these lipoproteins.

Composition studies in isolated HDL confirmed the small number of these particles in liver cirrhosis patients and revealed their proportional enrichment in free cholesterol, which is probably related to the low LCAT activity that is also characteristic of these patients [13,32,34]. Besides this change in free cholesterol content, HDL from patients with liver cirrhosis were proportionally enriched in both triglycerides and phospholipids, which is probably the result of the low HL activity found in the present study (Table 4) and others [10,11]. As regards the fatty acid composition of lipoprotein lipids, the proportion of linoleic acid in both cholesteryl esters and triglycerides was remarkably lower in lipoproteins from cirrhotic patients than in those from controls; this finding agrees with previous observations by others in total plasma [35]. This alteration in the fatty acid profile affected all lipoproteins to a similar extent, since no significant differences were detected when the compositions of the cholesteryl esters, triglycerides or phospholipids in the different lipoproteins were compared. In other words, in patients with liver cirrhosis, as in normal controls, lipid species were equilibrated among the different lipoprotein fractions.

This equilibration in human plasma is reached in part by the exchange action of CETP [36,37]. CETP activity in patients with liver cirrhosis was not significantly different from that in controls, a result that is in agreement with recent observations by Tahara et al. in patients with various chronic liver diseases and degrees of hepatocellular damage [13]. In contrast, CETP mass

and activity were found to be increased in some patients with primary biliary cirrhosis, but only in those with hyperalphalipoproteinemia [14]. Our study group included two patients with state IV primary biliary cirrhosis who had low to normal HDL levels; the fact that CETP was not affected in patients with normal values of HDL, as observed in the present study and by Hiraoka et al. [14], is striking and its mechanism remains unknown. The liver of several species has been found to secrete or synthesize CETP [15–17,38], however, only the non-parenchymal cells appear to be an important source of CETP as demonstrated in the primate liver [39]. Moreover, many other cell types have been reported to contain mRNA CETP or secrete CETP, including adipose tissue [17,18], macrophages [40], and the CaCo-2 model of human enterocytes [22]. Therefore, liver parenchymal disease should not necessarily lead to a change in plasma CETP activity, and whether synthesis from these cells is compromised or not, non-parenchymal liver cells or other tissues still might compensate total CETP production.

As expected, the lipid profiles of cholestatic patients were notably different from those of patients with cirrhosis or the normal controls. Cholestatic patients had moderate hyperlipidemia that mainly affected LDL. On the contrary, HDL were highly decreased, as indicated by both the HDL-cholesterol and the apo A-I plasma levels. The HDL composition was also impaired in these patients, showing increases in the proportional contents of both triglycerides and free-cholesterol. These changes may be attributed, respectively, to the low HL activity shown in this study (Table 4) and others [10,11], and to the diminished LCAT activity observed by others in this pathology [33,34]. In general, the present results show the profound alterations in lipid and lipoprotein metabolism which are characteristic of this disease [7–11].

Interestingly, patients with cholestasis had significantly lower CETP activity than controls, a change that may have important consequences in the lipoprotein metabolism. Tahara et al. found a decrease of plasma CETP activity at the moment of the most active hepatocellular damage in acute hepatitis [13]; no patient with this pathology was included in our study group, but several metabolic alterations in cholestatic patients are similar to those found in acute hepatitis, including the cholestasis itself. The mechanism for the decrease of CETP activity in these patients is not obvious because of the multiple origin of CETP [1–3]. The presence of a CETP-inhibitor [41,42] specifically in samples from cholestatic patients is ruled out because the recovery of CETP activity from control samples after the addition of lipoprotein-deficient plasma from patients was satisfactory (>88%, data not shown). Since fat malabsorption is usually present in cholestatic patients [23,43], and since fatty acid absorption regulates CETP secretion in CaCo-2 intestinal cells [22], it could be suggested that fat malabsorption is responsible for the decrease of CETP activity in cholestatic pa-

tients. It is well-known that fat-rich diets increase CETP activity in both experimental animals [18,38,44,45] and in humans [46–48]. Whether there is a direct participation of the small intestine in CETP production or whether the reduced chylomicron transport from the intestine to liver that is present in cholestasis [23] affects CETP synthesis by the liver deserves further investigation.

Besides diet, many other factors modulate CETP activity. CETP is increased in several primary and secondary hyperlipidemias [26,27,48–50], the highest levels being observed in lipoprotein lipase deficient patients with hyperchylomicronemia [26,46]. An increase in CETP is associated with an enrichment of triglycerides in HDL [26,27,51] and with a lowering of HDL-cholesterol and apo A-I concentrations [26,52,53], events that are causally related, as recently demonstrated in transgenic mice expressing human CETP [54,55]. On the contrary, decreases in CETP activity have been observed in hypothyroidism [56] and after alcohol consumption [57], and these changes may contribute to the elevations in HDL-cholesterol generally observed in these conditions. The present paper describes a pathological state, cholestasis, with decreased CETP activity associated with moderate hyperlipidemia and low levels of HDL; it offers an interesting model for studying both the regulation and action of CETP on lipoprotein metabolism.

A remarkable, coexisting alteration in cholestasis was the inhomogeneous distribution of cholesteryl ester and triglyceride species among the lipoprotein fractions. Centering our interest on cholesteryl linoleate because it is the major cholesteryl ester species in human plasma and is considered to be formed in HDL by LCAT action [35,58], we observed that it was less abundant in VLDL than in the other lipoproteins in these patients (Table 5). On the other hand, the percentage of oleic acid in triglycerides was significantly higher and that of linoleic acid lower in VLDL or LDL than in HDL (Table 6). In contrast, the fatty acid profile in phospholipids was very similar in every lipoprotein fraction (Table 7). These results demonstrate that CETP-exchangeable lipid species were not homogeneously distributed among lipoproteins in our patients with cholestasis, and this contrasted with the equilibrium observed in patients with liver cirrhosis or in normal subjects (present work; [58,59]). Therefore, this inhomogeneous distribution in cholestasis may be attributed to the CETP reduction, which would imply a slower transfer of triglyceride species from VLDL to HDL and cholesteryl esters from HDL to VLDL. In keeping with this is the observation that triglyceride species in LDL were similar to those in VLDL (Table 6), which reflects the metabolic origin of LDL from VLDL. Surprisingly, LDL cholesteryl esters were different from those in VLDL and similar to those in HDL (Table 5). This finding needs verification in other conditions with CETP deficiency, and could be attributed to a unidirectional transfer of cholesteryl esters from HDL to LDL as recently described in animals lacking CETP [60].

A major finding of this study is the inhomogeneous distribution of both plasma cholesteryl ester and triglyceride species associated with decreased CETP activity in cholestasis. Whether this relationship is causal needs to be confirmed directly, however, a similar lack of cholesteryl ester equilibration was also found by others in homozygous human CETP deficiency [58,61] and in animal species devoid of CETP [37]. Therefore, existing evidence allows us to suggest that the different proportion of cholesteryl linoleate between HDL and VLDL in cholestasis is due to decreased CETP. Studies are needed to elucidate how CETP activity is reversed in patients recovering from extrahepatic cholestasis and how this might affect lipid distribution and lipoprotein metabolism.

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