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Cholesteryl Ester Transfer Activity

Localization and Role in Distribution of Cholesteryl Ester among Lipoproteins in Man

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Summary

The cholesteryl ester exchange/transfer protein is involved in the transport of cholesteryl ester from high density lipoproteins (HDL) to very low density lipoproteins (VLDL) and low density lipoproteins (LDL). Localization of cholesteryl ester transfer activity (CETA) in plasma was studied by measuring CETA in various delipidated fractions from a single step density ultracentrifugation gradient of plasma. CETA was measured in an in vitro system by calculating the exchange of cholesteryl ester in a standard mixture of [^{3}H]CE-HDL and LDL. The method used for the delipidation of plasmas and fractions to be tested was critical. Optimal results were obtained by delipidation with diisopropylether-butanol (60 : 40, v/v) at 0°C.

The bulk of CETA was detected in HDL_3 ($1.125 < d < 1.210$ g/ml) when the lipoproteins were separated by single-step density gradient ultracentrifugation and in the 'lipoprotein-free' fraction ($d > 1.250$ g/ml) when the lipoproteins were separated by flotation ultracentrifugation including two washes. To determine whether CETA plays a role in the distribution of cholesteryl ester among the various lipoproteins, it was measured in whole plasma from normal and hyperlipidemic subjects. Plasma was delipidated before the assay in order to prevent bias due to variation of cholesterol content. CETA was higher in delipidated plasma of hyperlipidemic

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subjects (117.3 ± 36.5 nmol CE/ml/h) than in delipidated plasma of normolipidemic controls (68.7 ± 17.6 nmol CE/ml/h) ($P < 0.005$). A positive correlation ($r = 0.80$, $P < 0.005$) was found between CETA and (VLDL + LDL) cholesterol levels. A negative correlation ($r = 0.57$, $P < 0.05$) existed between CETA and HDL cholesterol. This correlation was found both in the group as a whole and within the normal and the hyperlipidemic groups separately. The activity of the cholesteryl ester transfer appears to be a regulatory factor in the distribution of cholesteryl ester over the various lipoproteins.

Key words: *Cholesteryl ester transfer activity – High density lipoprotein – Human plasma – Low density lipoprotein – Very low density lipoprotein*

Introduction

The rediscovery of the inverse relationship between high density lipoproteins (HDL) [1,2] and cardiovascular disease in epidemiological studies has renewed interest in the metabolism of HDL. Cholesteryl esters in human plasma are formed predominantly in plasma HDL in a reaction catalyzed by the enzyme lecithin:cholesterol acyl transferase (LCAT) [3]. Cholesteryl esters are then rapidly transferred and/or exchanged between lipoproteins in a process facilitated by a protein or protein complex [4–9].

Plasma proteins which facilitate the transfer/exchange of cholesteryl esters have been isolated and partially characterized from human plasma d > 1.21 g/ml infranatant [10,11,13] and from HDL [9,12]. Whether different proteins or protein complexes are involved in these processes is not yet clear.

Precise knowledge concerning the localization of these protein(s) in circulating plasma is still lacking. In vivo studies in rabbits and pigs suggest that an active cholesteryl ester transfer protein (CETP) is necessary to achieve the in vivo transfer of esterified cholesterol from HDL to other lipoproteins [14]. We first studied cholesteryl ester transfer activity (CETA) in various lipoproteins and in HDL subfractions from normal plasma separated by one-step density gradient ultracentrifugation. In order to gain further knowledge of a possible role of CETA in the distribution of cholesteryl ester among various lipoproteins in human plasma, we have studied CETA in delipidated plasma of normolipidemic and hyperlipidemic subjects and its relation of plasma lipoprotein cholesterol levels.

Material and Methods

Subjects

Twenty normolipidemic men, mean age 28.7 ± 6.2 years (range 22–43 years) and 10 normolipidemic women, mean age 28.0 ± 9.7 years (range 20–47 years) volunteered for this study. Blood was also collected from 12 hyperlipidemic patients, 7 women, mean age 48.3 ± 9.4 years (range 38–61) and 5 men, mean age 51.3 ± 15.4 years (range 30–64) who visited the outpatient lipid clinic.

Preparation of lipoprotein fractions

Blood from normolipidemic female donors, unless otherwise stated, was collected into tubes containing EDTA (1 mg/ml) as anticoagulant. Plasma was immediately separated from erythrocytes. VLDL, LDL, HDL and the lipoprotein-free fraction were subsequently separated by one-step density gradient ultracentrifugation [15]. The various fractions were pipetted off and dialyzed against 0.9% NaCl, 1 mM EDTA, and 0.01% NaN_3. Labelled VLDL, LDL, and HDL were prepared by incubating 20 ml of freshly prepared plasma with a filter paper disc impregnated with 100 μCi [7(n)-^{3}H]cholesterol (spec. act. 8 Ci/mmol, The Radiochemical Centre, Amersham) for 22 h at 37°C. After incubation, the plasma was washed to remove excess radioactive cholesterol with an amount of packed red blood cells equal to 2 vol. of plasma, for 1.5 h. This washing procedure was repeated twice. Labelled VLDL, LDL, and HDL were isolated and dialyzed as described above. Analysis of the preparations indicated that respectively 65–75%, 70–80%, and 80–90% of the label present in each fraction was associated with cholesteryl ester. For the localization of the cholesteryl ester exchange activity plasma was separated by a modification of the density gradient ultracentrifugation [15]. Samples of plasma were adjusted to d = 1.25 with KBr and 3.5-ml aliquots were pipetted into 14 mm × 95 mm polyallomer tubes. The plasma sample was carefully overlayered with 2.0 ml of KBr/NaCl, 1 mM EDTA solution of d = 1.21, 2.0 ml of d = 1.125, 2.0 ml of d = 1.063 and 2.3 ml of d = 1.019. The samples were centrifuged for 29 h at 12°C at 40 000 rpm in an SW 40 Ti rotor in a Beckman L8-80 ultracentrifuge. Fractions of 0.5 ml were carefully aspirated, dialyzed, and delipidated before assaying the cholesteryl ester exchange activity.

Delipidation

Plasma, lipoproteins, and fractions from the gradient ultracentrifugation were delipidated, using diisopropylether/butanol (60 : 40, v/v) at 0°C [16].

Incubation procedure

Cholesteryl ester exchange activity was measured between HDL and LDL. A typical assay mixture consisted of HDL (200 nmoles cholesteryl ester, spec. act. 0.1 mCi/mmol), and LDL (500 nmoles cholesteryl ester) in a volume of 1.0 ml, containing the following components: 0.02 M Tris/HCl, pH 7.4, 0.001 M EDTA, 0.9% NaCl, 0.01% NaN_3, and 0.002 M parachloromercuriphenylsulfonate (PCMPS) to inhibit lecithin:cholesterol acyltransferase [17]. In order to correct for cholesteryl ester exchange activity bound to substrate HDL, which accounted for 25–30% of the CETA in each assay, incubations were conducted with and without the addition of lipoprotein-free plasma (6–9 mg protein), delipidated plasma (4–6 mg protein), or delipidated fractions from gradient ultracentrifugation. Incubations were carried out at 37°C for 16 h. Under these conditions there was linearity of the cholesteryl ester exchange with added protein and with time. The exchange was terminated by placing the tubes on ice. LDL was precipitated by heparin/$MnCl_2$ [18], the supernatant was removed and the precipitate washed twice with 1.0 ml 0.9% NaCl, 1 mM EDTA and precipitated again with heparin/$MnCl_2$. The precipitate was finally

redissolved in 1 ml NaCl/KBr solution, d = 1.063 g/ml and the radioactivity measured. Control incubations were carried out at 0°C and radioactivity in the LDL precipitate was subtracted. Lipids were extracted [19] and subjected to thin layer chromatography on silicagel H using hexane/diethylether/acetic acid (70 : 30 : 1, v/v/v) as development solvent to separate free and esterified cholesterol. Both were scraped off the plates and assayed for radioactivity. The exchange of unesterified cholesterol was slightly dependent on the temperature and independent of the addition of samples containing CETA. In all experiments, TLC was carried out for incubation at 0°C and 37°C in the presence and absence of a CETA-containing sample.

Radioactivity was determined by liquid scintillation counting using instagel as scintillator in a Packard Tricarb Liquid scintillation counter equipped with external standardization. The exchange of cholesteryl ester between HDL and LDL was calculated [20].

In order to test whether VLDL- or LDL-bound CETA was present, VLDL (100 nmoles cholesteryl ester) and LDL (400 nmoles cholesteryl ester) containing labelled cholesteryl ester, were incubated and exchange of radioactive labelled cholesteryl ester was estimated. VLDL and LDL were separated by adding an equal amount of plasma to the incubation medium, followed by centrifugation at 100 000 rpm (160 000 × g) for 2.5 h in a Beckman airdriven ultracentrifuge.

Other methods

Concentrations of cholesterol and cholesteryl ester were determined by an enzymatic method using a commercial kit (Boehringer, Mannheim, F.R.G.). Protein concentrations were measured by the dye-binding assay [21] with a protein assay kit (BioRad Laboratories, NY).

The density of the fractions after centrifugation was measured with an Abbe refractometer against a standard curve of known NaCl/KBr concentrations, except for the bottom fractions which were measured against plasma to which NaCl/KBr was added.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was carried out [22], using 12.5% acrylamide and 0.1% SDS.

Groups of data were compared using Student's t-test.

Correlations were calculated according to the method of least squares [23].

Results

Localization of CETA

To measure CETA, incubation mixtures were used in which HDL, containing [^{3}H]cholesteryl ester, and LDL were present. Cholesteryl ester exchange was linear with time for up to at least 36 h. During this incubation no alteration in the cholesterol or cholesteryl ester mass of HDL and LDL could be measured. This suggests an exchange reaction, rather than a transfer reaction. Cholesteryl ester exchanged between HDL and LDL in the presence and absence of the plasma fraction with a density > 1.21 g/ml. Addition of this fraction increased cholesteryl

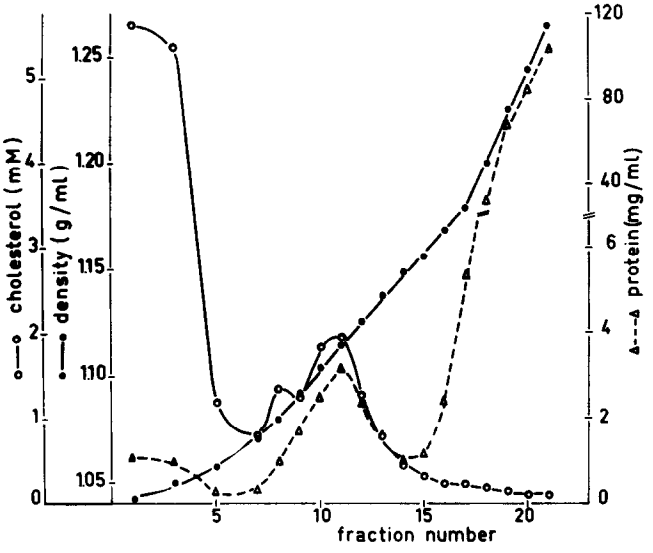

Fig. 1. Density gradient ultracentrifugation of plasma. Plasma (3.5 ml) was centrifuged over the modified gradient (see Methods) and fractions of 0.5 ml were taken and analyzed for cholesterol and protein concentrations.

ester transfer. In incubations in which VLDL and LDL were used mutually as acceptor and donor of radioactive-labelled cholesteryl esters, no exchange could be measured when the plasma fraction with a density > 1.21 g/ml was not present. This means that CETA is not located in VLDL or LDL. The mean specific activities ($\pm$SD) of cholesteryl ester exchange in HDL and in the lipoprotein-free plasma (d > 1.21) from normal donors were 4.52 ± 1.19 and 0.33 ± 0.17 nmoles CE/mg protein/h (n = 7).

To further localize CETA in HDL, plasma was centrifuged by the modified density gradient ultracentrifugation (see Methods). After separation each fraction of the density gradient ultracentrifugation was assayed for protein and cholesterol. CETA was tested in the fraction in the region of the gradient corresponding to HDL and lipoprotein-free plasma. The cholesterol and protein profiles of this gradient are shown in Fig. 1. SDS-PAGE showed no detectable levels of apo-B in fractions with density > 1.08 g/ml, which means that LDL is well separated from HDL. Albumin appeared in fractions with densities > 1.17 g/ml.

Before the estimation of CETA, the various fractions obtained from gradient ultracentrifugation, lipoproteins and plasmas were delipidated, in order to exclude bias due to variations in the cholesterol content of the samples. We evaluated various extraction procedures. After extraction with either chloroform/methanol (2 : 1, v/v) or ethanol/diethylether (2 : 1, v/v), only a few percent of the original CETA could be recovered in the residual protein. Delipidations according to Cham and Knowles [16] gave optimal results. As can be seen in Table 1, using this procedure, the composition of the diisopropylether/butanol mixture and the temperature were critical. The procedure used throughout this study, i.e. delipidation with diisopro-

TABLE 1

SPECIFIC ACTIVITY OF CHOLESTERYL ESTER TRANSFER IN PLASMA, FRACTION d > 1.21, AND HDL AFTER VARIOUS DELIPIDATION PROCEDURES

Delipidation has been carried out with diisopropylether/butanol of 75:25, v/v (procedure I) or 60:40 v/v (procedure II) [12]. Incubations were performed at 37°C in the presence of 197 nmol HDL-CE and 504 nmol LDL-CE for 16 h. Control incubations were carried out at 0°C. Specific activity is expressed as nmol cholesteryl ester/mg protein/h. Means ± SD of duplicate incubations are presented.

Fraction	Non-delipidated	Procedure I		Procedure II	
		0°C	20°C	0°C	20°C
Plasma		0.30 ± 0.01	0.04 ± 0.01	0.60 ± 0.04	0.10 ± 0.03
Fraction d > 1.21	0.46 ± 0.02	0.30 ± 0.03	n.d.	0.44 ± 0.03	0.05 ± 0.02
HDL	3.73 ± 0.01	n.d.	n.d.	13.20 ± 1.60	n.d.

n.d.: not detectable.

pylether/butanol (60:40, v/v) at 0°C (procedure II) gave more than 85% recovery of CETA both for lipoprotein-free plasma and for HDL. The recovery of whole plasma protein and lipoprotein-free plasma protein exceeded 85%. The recovery of HDL protein was between 30 and 50%. As a consequence, the specific CETA was increased after delipidation in HDL, whereas it remained unaltered in lipoprotein-free plasma For whole plasma, no figures can be given since undelipidated plasma cannot be used. Added lipoproteins would influence the assay. The specific activity

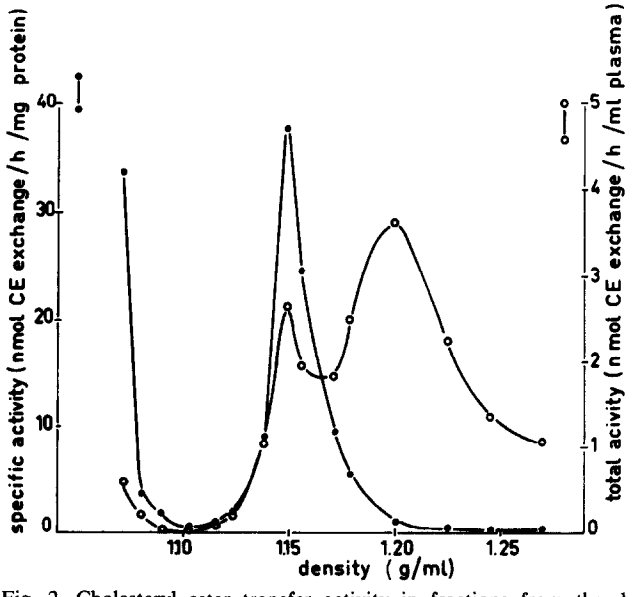

Fig. 2. Cholesteryl ester transfer activity in fractions from the density gradient ultracentrifugation. Fractions of densities > 1.06 g/ml were dialyzed, delipidated and assayed for cholesteryl ester exchange activity. Incubations contained 191 nmol HDL cholesteryl ester and 504 nmol LDL cholesterol ester and were carried out at 37°C for 16 h. Control incubations were carried out at 0°C.

TABLE 2

CHOLESTERYL ESTER TRANSFER ACTIVITY IN PLASMAS OF NORMOLIPIDEMIC CONTROLS AND HYPERLIPIDEMIC PATIENTS

Delipidated plasmas were incubated for 16 h at 37°C in a medium containing 200 nmoles HDL-CE and 500 nmoles LDL-CE. Control incubations were performed at 0°C. Results are means ± SD.

Normolipidemic controls	Total TG (mM)	Total chol (mM)	HDL chol (mM)	CETA (nmol CE/ml plasma/h)
Male (n = 23)	0.86 ± 0.27	5.11 ± 1.04	1.42 ± 0.21	69.7 ± 20.5
Female (n = 10)	0.70 ± 0.24	4.85 ± 0.46	1.62 ± 0.36	65.1 ± 11.3
Total (n = 33)				68.7 ± 17.6
Hyperlipidemic patients	**Total TG (mM)**	**Total chol. (mM)**	**HDL chol. (mM)**	**CETA (nmol CE/ml plasma/h)**
Male (n = 5)	8.94 ± 6.22	14.46 ± 2.89	0.60 ± 0.07	114.1 ± 42.8 [a]
Female (n = 7)	4.41 ± 7.75	12.37 ± 2.15	1.02 ± 0.36	119.5 ± 34.7 [b]
Total (n = 12)				117.3 ± 36.5 [b]

[a] $P < 0.005$ between hyperlipidemic patients and normolipidemic controls.
[b] $P < 0.01$ between hyperlipidemic patients and normolipidemic controls.

and the total activity of cholesteryl ester transfer in the various delipidated fractions of the density gradient ultracentrifugation are shown in Fig. 2. No CETA was detectable in the region of HDL_2. The rate of cholesteryl ester transfer increase suddenly at density 1.13 g/ml and was thereafter found in HDL_3 and lipoprotein-free plasma. From this figure it can be seen that the bulk of the cholesteryl ester transfer activity is found in HDL_3 ($1.125 < d < 1.210$ g/ml) and that the peak of the specific activity lies between $d = 1.140$ and 1.175 g/ml.

From the three normal plasma samples fractionated, one plasma showed a high specific CETA at $d = 1.07$ g/ml. Whether this activity is due to a specific fraction in HDL or LDL is not known. The activity at $d = 1.07$ g/ml represented only 3% of the total CETA in this sample.

CETA in normal and hyperlipidemic subjects

Whole plasma of 12 hyperlipidemic patients and 30 normolipidemic controls was assayed for CETA after delipidation with diisopropylether/butanol (60 : 40, v/v) at 0°C. The assay system contained fixed amounts of one batch of HDL and LDL. The results are presented in Table 2. CETA was higher ($P < 0.005$) in hyperlipidemic patients than in normolipidemic subjects. The coefficients of correlation between concentration of lipoprotein lipids and total cholesteryl ester transfer activity in delipidated plasma are given in Table 3. There was a positve correlation between CETA and total cholesterol as well as (VLDL + LDL) cholesterol in the group as a whole and within both the normal and the hyperlipidemic groups. An inverse correlation existed in the entire group between CETA and HDL cholesterol, whether expressed in absolute terms or as a fraction of total or (VLDL + LDL) cholesterol. The highest coefficient of correlation was found between CETA and (VLDL + LDL) cholesterol (Fig. 3).

TABLE 3

COEFFICIENTS OF CORRELATION BETWEEN CHOLESTERYL ESTER EXCHANGE ACTIVITY IN DELIPIDATED PLASMA AND CONCENTRATION OF LIPOPROTEINS

	Normolipidemic (n = 30)	Hyperlipidemic (n = 12)	Combined (n = 42)
Total chol	0.59 [b]	0.50 [a]	0.79 [b]
VLDL chol	–	−0.17	–
LDL chol	–	0.48	–
HDL chol	−0.21	−0.30	−0.57 [b]
HDL/total chol	−0.51 [b]	−0.48	−0.42 [b]
HDL/(VLDL + LDL) chol	−0.46 [b]	−0.58 [a]	−0.71 [b]
(VLDL + LDL) chol	0.62 [b]	0.57 [a]	0.80 [b]
Tot TG	0.15	−0.26	0.30

[a] $P < 0.05$.
[b] $P < 0.005$.

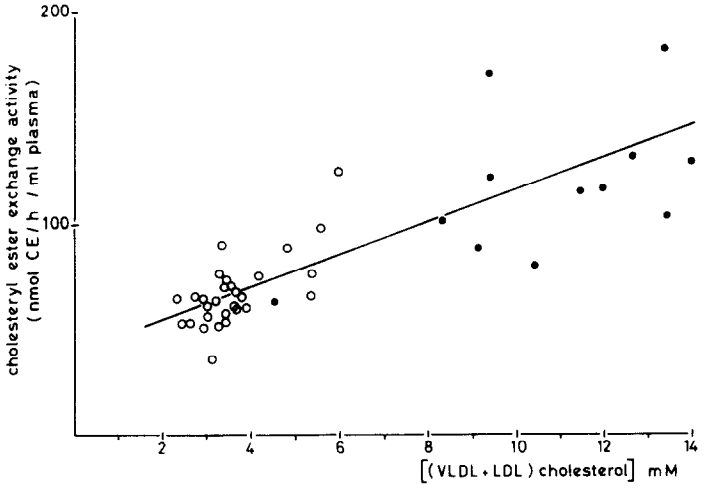

Fig. 3. Relationship between cholesteryl ester transfer activity and (VLDL + LDL) cholesterol in normolipidemic (○) and hyperlipidemic subjects (●). Experimental conditions as in Fig. 2. $y = 7.5x + 39.4$, $r = 0.80$ ($P < 0.005$).

Discussion

The present study provides evidence that more than half of the cholesteryl ester transfer activity is located in HDL_3 and that cholesteryl ester transfer activity is high in plasma of hyperlipidemic patients.

HDL_2 does not contain a significant amount of CETA. In agreement with other one-step gradient ultracentrifugation studies [24,25], two cholesterol peaks were found in the HDL density range, one at a density of 1.08–1.09 and the other at 1.12–1.13. Longer centrifugation times (48 h) did not affect the cholesterol, protein and/or density profile significantly. The area in which CETA was found was HDL_3.

Based on interaction studies of human cholesteryl ester transfer protein with isolated plasma lipoprotein, Pattnaik and Zilversmit [26] suggested that the transfer protein exists bound to HDL, in spite of the fact that the protein was normally found in the $d > 1.25$ fraction. HDL prepared by sequential flotation ultracentrifugation [27] contained a decreasing amount of cholesteryl ester transfer activity after successive spins, approaching zero after three spins at $d = 1.250$ g/ml. Cholesteryl ester transfer activity is probably separated from the HDL-CETA complex during the preparation of lipoproteins. This is also known for various apo-proteins and LCAT [28]. In a mathematical approach, Barter [29] calculated that the proportion of transfer protein bound to HDL will be more than twice that which is unbound to lipoprotein. The location of CETA in HDL_3 is not surprising, concerning the role of HDL in cholesterol metabolism. VHDL is involved in the removal of cholesterol from cells [30], and HDL_3 is the substrate for LCAT [31].

It was observed in this study that CETA differs among human subjects. A significantly higher CETA was found in hyperlipidemic patients than in normolipidemic controls. In whole plasma, transport rates of cholesteryl ester are well correlated with (VLDL + LDL) cholesterol [9]. In our study a positive correlation between CETA in delipidated plasma and (VLDL + LDL) cholesterol and an inverse correlation between CETA and HDL cholesterol was found, both within the normal and hyperlipidemic groups and in the groups combined. It should be noted that CETA was measured in delipidated plasma in an incubate containing fixed amounts of a standard mixture of LDL and HDL. The activity of the transfer is, therefore, measured independent of the cholesterol levels in and the characteristics of VLDL, LDL, and HDL of the subjects' plasmas. Obviously, we cannot discriminate between an increased activity of the cholesteryl ester transfer protein or a decreased activity of the inhibitor [32]. The findings might suggest an active role of the transfer activity in determining relative cholesterol ester distribution among HDL and (VLDL + LDL). An independent role of the concentrations and characteristics of VLDL, LDL, and HDL in the transfer of cholesteryl esters remains possible.

In a recent report, Orschy and Eisenberg [33] ascribe the difference between human and rat lipoproteins, at least in part, to the absence of CETA. The absence of CETA, as in rat, will cause accumulation of CE in HDL and a relative paucity of CE in other lipoproteins. In our report, a low CETA is found in plasma with a relatively high HDL to (VLDL + LDL) cholesterol ratio. High CETA is found in plasma with a low HDL to (VLDL + LDL) ratio. That the strongest correlation was found between CETA and (VLDL + LDL) and not VLDL or LDL separately, in combination between the known conversion of VLDL to LDL, does suggest that HDL-CE is transferred first to VLDL and subsequently to LDL.

Our technique measured an exchange, rather than a transfer activity, between HDL and LDL. It has to be verified whether exchange, as measured in vitro, is comparable to transfer in vivo and whether the same protein is responsible for both processes.

In conclusion, it appears that the protein(s) responsible for the cholesterol ester transfer activity is (are) loosely attached to HDL_3 and is removed from that

lipoprotein fraction after repeated ultracentrifugation. The finding of a high cholesterol ester transfer activity in delipidated hyperlipidemic plasma, positively correlated with (VLDL + LDL) cholesterol, suggests a role for the transfer activity in regulating the relative distribution of cholesteryl ester among the different lipoproteins. Further study and confirmation in subgroups with clearly defined hyperlipoproteinemia and dyslipoproteinemia is required.

References

1 Miller, G.J. and Miller, N.E., Plasma high-density lipoprotein concentration and development of ischaemic heart disease, Lancet, i (1975) 16.
2 Rhoads, G.G., Gulbrandsen, C.C. and Kagan, A., Serum lipoproteins and coronary heart disease in a population study of Hawaii Japanese men, N. Engl. J. Med., 294 (1976) 293.
3 Glomset, J.A. and Norum, K.R., The metabolic role of lecithin: cholesterol acyltransferase — Perspectives from pathology, Adv. Lipid Res., 11 (1973) 1.
4 Nichols, A.V. and Smith, L., Effect of very low density lipoproteins on lipid transfer in incubated serum, J. Lipid Res., 6 (1965) 206.
5 Zilversmit, D.B., Hughes, L.B. and Balmer, J., Stimulation of cholesterol ester exchange by lipoproteinfree plasma, Biochim. Biophys. Acta, 409 (1975) 393.
6 Barter, P.J. and Lally, J.I., The activity of an esterified cholesterol transfer factor in human and rat serum, Biochim. Biophys. Acta, 531 (1978) 233.
7 Barter, P.J. and Lally, J.I., In vitro exchanges of esterified cholesterol between serum lipoprotein fractions — Studies in humans and rabbits, Metabolism, 28 (1979) 230.
8 Sniderman, A., Teng, B., Vezina, C. and Marcel, Y.L., Cholesterol ester exchange between human plasma high and low density lipoproteins, mediated by a plasma protein factor, Atherosclerosis, 31 (1978) 327.
9 Chajek, T. and Fielding, C.J., Isolation and characterization of a human serum cholesteryl ester transfer protein, Proc. Nat. Acad. Sci. (USA), 75 (1978) 3445.
10 Pattnaik, N.M., Montes, A., Hughes, L.B. and Zilversmit, D.B., Cholesteryl ester exchange protein in human plasma, isolation and characterization, Biochim. Biophys. Acta, 530 (1978) 428.
11 Morton, R.E. and Zilversmit, D.B., The separation of apolipoprotein D from cholesteryl ester transfer protein, Biochim. Biophys. Acta, 663 (1981) 350.
12 Fielding, P.E. and Fielding, C.J., Cholesteryl ester transfer complex in human plasma, Proc. Nat. Acad. Sci. (USA), 77 (1980) 3327.
13 Ihm, J., Ellsworth, J.L., Chataing, B. and Harmony, J.A.K., Plasma protein-facilitated coupled exchange of phosphatidyl choline and cholesteryl ester in the absence of cholesterol esterification, J. Biol. Chem., 257 (1982) 4818.
14 Ha, Y.C., Calvert, G.D., McIntosh, G.H. and Barter, P.J.M., A physiologic role for the esterified cholesterol transfer protein: In vivo studies in rabbits and pigs, Metabolism, 30 (1981) 380.
15 Redgrave, T.G., Roberts, D.C.K. and West, C.E., Separation of plasma lipoproteins by density gradient ultracentrifugation, Anal. Biochem., 65 (1977) 42.
16 Cham, B.E. and Knowles, B.R., In vitro partial relipidation of apolipoproteins in plasma, J. Lipid Res., 17 (1976) 176.
17 Glomset, J.A., Norum, K.R. and King, W., Plasma lipoproteins in familial lecithin: cholesterol acyltransferase deficiency — Lipid composition and reactivity in vitro, J. Clin. Invest., 49 (1970) 1827.
18 Burstein, M., Scholnick, H.R. and Morfin, R., Rapid method for the isolation of lipoproteins from human serum by precipitation with polyanions, J. Lipid Res., 11 (1970) 583.
19 Bligh, E.G. and Dyer, W.J., A rapid method of total lipid extraction and purification of total lipids from animal tissues, Canad. J. Biochem. Physiol., 37 (1959) 911.
20 Barter, P.J. and Jones, M.E., Rate of exchange of esterified cholesterol between human plasma low and high density lipoproteins, Atherosclerosis, 34 (1979) 67.

21 Bradford, M.M., A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of dye binding, Anal. Biochem., 72 (1976) 248.
22 Weber, K. and Osborne, M., The reliability of molecular weight determinations by dodecylsulfate-polyacrylamide gel electrophoresis, J. Biol. Chem., 244 (1969) 4406.
23 Armitage, P., Statistical Methods in Medical Research, Blackwell, Oxford, 1973.
24 Terpstra, A.H.M., Woodward, C.J.H. and Sachez-Muñiz, F.J., Improved techniques for the separation of serum lipoproteins by density gradient ultracentrifugation, visualization by prestaining and rapid separation of serum lipoproteins from small volumes of serum, Anal. Biochem., 111 (1981) 149.
25 Cheung, M.C. and Albers, J.J., Distribution of cholesterol and apolipoprotein AI and AII in human high density lipoproteins separated by CsCl equilibrium gradient centrifugation — Evidence for HDL subpopulation with different AI/AII molar ratios, J. Lipid Res., 20 (1979) 200.
26 Pattnaik, N.M. and Zilversmit, D.B., Interaction of cholesteryl ester exchange protein with human plasma lipoproteins and phospholipid vesicles, J. Biol. Chem., 254 (1979) 2782.
27 Havel, R.J., Eder, H.A. and Bragdon, J.H., The distribution and chemical composition of ultracentrifugally separated lipoproteins in human serum, J. Clin Invest., 34 (1955) 1345.
28 Curry, M.D., Alaupovic, P. and Suenram, C.A., Determination of apolipoprotein A and its constituents AI and AII polypeptides by separate electroimmunoassay, Clin. Chem., 22 (1976) 315.
29 Barter, P.J., Hopkins, G.J., Gorjatschko, L. and Jones, M.E., A unified model of esterified cholesterol exchanges between human plasma lipoproteins, Atherosclerosis, 44 (1982) 27.
30 Oram, J.F., Albers, J.J., Cheung, M.C. and Bierman, E.L., The effects of subfractions of high density lipoprotein on cholesterol efflux from cultured fibroblasts — Regulation of low density lipoprotein receptors, J. Biol. Chem., 256 (1981) 8348.
31 Fielding, C.J. and Fielding, P.E., Purification and substrate specificity of lecithin:cholesterol acyltransferase from human plasma, FEBS Lett., 15 (1971) 355.
32 Morton, R.E. and Zilversmit, D.B., A plasma inhibitor of triglyceride and cholesteryl ester transfer activities, J. Biol. Chem., 256 (1982) 11992.
33 Oschry, Y. and Eisenberg, S., Rat plasma lipoproteins — Reevaluation of a lipoprotein system in an animal devoid of cholesteryl ester transfer activity, J. Lipid. Res., 23 (1982) 1099.