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J Clin Invest. 1990;**86**(3):688-695. <https://doi.org/10.1172/JCI114764>.

Research Article

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Accumulation of Apolipoprotein E-rich High Density Lipoproteins in Hyperalphalipoproteinemic Human Subjects with Plasma Cholesteryl Ester Transfer Protein Deficiency

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Abstract

This study characterized the plasma lipoproteins of familial hyperalphalipoproteinemic patients with or without deficiency of cholesteryl ester transfer protein (CETP) activity. The subjects with CETP deficiency have increased levels of apolipoprotein (apo) E. The increased concentration of apo E in these subjects was correlated to the appearance of apo E-rich high density lipoproteins (HDL). Sodium dodecyl sulfate-polyacrylamide gel analysis revealed that these lipoproteins contained predominantly the apo E (82%) and little amount of apo A-I (18%). These apo E-rich HDL displayed a much higher affinity than human LDL in binding to LDL receptors on human fibroblasts. Furthermore, 3.5 times fewer apo E-rich HDL than LDL were required to saturate the receptors on fibroblasts. These data indicated that the apo E-rich HDL in CETP-deficient human subjects contained multiple copies of apo E and bound to the LDL receptor through multiple interactions. The apo E-rich HDL, with similar properties as cholesterol-induced apo E HDL_c, were not detectable in normal human subjects or in hyperalphalipoproteinemic subjects with normal CETP activity. The apo E-containing HDL in the latter subjects were smaller and contained only small amounts of apo E (14%). The difference in apo E-containing HDL in these subjects suggests a correlation between CETP level and the appearance of apo E-rich HDL. (*J. Clin. Invest.* 1990; 86:688-695.) Key words: cholesterol ester transfer • protein • apolipoprotein E • hyperalphalipoproteinemia • HDL

Introduction

Epidemiological studies have demonstrated that elevated levels of high density lipoprotein (HDL)-cholesterol are negatively correlated to the incidence of coronary heart disease, suggesting a role for HDL in the prevention of atherosclerotic lesions (1). Although the precise mechanism by which HDL can be protective against atherosclerosis remains unknown, a current hypothesis favors a role for HDL in cholesterol efflux from peripheral tissues and in the transport of extrahepatic

cholesterol to the liver for reprocessing and excretion (2). Although many environmental and hormonal factors may contribute to the control of HDL levels, previous studies have also shown that HDL level may also be regulated by genetic factors (3).

Familial hyperalphalipoproteinemia has been reported to be inherited as an autosomal dominant trait characterized by increased plasma HDL-cholesterol (3). Although earlier studies have suggested that hyperalphalipoproteinemia is associated with a single major gene (3), it is likely that this condition can be a result of several different genetic abnormalities. Taskinen et al. (4) reported that members of five different families with high HDL-cholesterol levels frequently demonstrated increased lipoprotein lipase activity. In this particular case, the increase in HDL level may be derived from accelerated catabolism of triglyceride-rich lipoproteins. Matsuzawa et al. (5) and Yamashita et al. (6) reported two cases of hyperalphalipoproteinemia with massive corneal opacification, one of which was also accompanied by coronary heart disease. These two probands were shown to have a decreased activity of hepatic triglyceride lipase (6). More recently, Yamashita et al. (7) reported that, in three different families, a high level of HDL was associated with a dramatic decrease in the activity of plasma cholesteryl ester transfer protein (CETP).¹ These results suggest that plasma HDL level may also be correlated to CETP activity. The relationship between HDL level and CETP activity remains to be elucidated.

The correlation of CETP and HDL was also noted by characterization of lipoproteins in various animal models (8). It is interesting to note that animals with low levels of CETP also displayed high plasma HDL (8). These animals possess a unique subclass of HDL, called HDL₁, that contains apolipoprotein (apo) A-I and apo E and has a hydrated density of 1.02-1.063 g/ml (9). When these animals were fed a cholesterol-rich diet, the HDL₁ was found to be larger, containing more cholesteryl ester and apo E. These cholesterol-induced lipoproteins are termed HDL_c (9). Although apo E has been detected in HDL₁- and HDL₂-like particles isolated from human subjects (10-12), the presence of apo E-enriched HDL, with properties similar to HDL_c, has not been reported previously in fasted human subjects. It is possible that high CETP level in human subjects precludes the formation of these lipoproteins. This hypothesis was examined in the current study by characterization of the lipoproteins in hyperalphalipoproteinemic patients with or without deficiency of CETP. Results reported herein demonstrate that apo E-rich

A portion of this paper was presented at the American Heart Association 1989 Scientific Session.

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Received for publication 5 March 1990 and in revised form 9 May 1990.

J. Clin. Invest.

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0021-9738/90/09/0688/08 \$2.00

Volume 86, September 1990, 688-695

1. Abbreviation used in this paper: CETP, cholesteryl ester transfer protein.

HDL, with characteristics similar to the HDL_c, are present in human subjects with CETP deficiency.

Methods

Materials. Dulbecco's modified Eagle's medium (DME), trypsin-EDTA solution, and gentamycin sulfate were purchased from Gibco Laboratories, Grand Island, NY. Fetal calf serum was from Hazleton Research Products, Lenexa, KS. Tissue culture flasks (75 cm², 250 ml) and plates (35 × 20 mm, six wells) were obtained from Corning Glass Works, Corning, NY. Cholesterol and triglyceride quantitation kits were purchased from Kyowa Medex Co., Tokyo, Japan. Commercial kits for measuring plasma apo E were obtained from Daiichi Pure Chemicals Co., Ltd., Tokyo, Japan. Sodium [¹²⁵I]iodide (carrier-free) in NaOH and Bolton-Hunter reagents were obtained from Amersham Corp., Arlington Heights, IL. [¹⁴C]cholesteryl oleate was obtained from New England Nuclear, Boston, MA. Agarose films were obtained from Corning Medical, Palo Alto, CA. High-performance liquid chromatography (HPLC) columns were obtained from TOSOH, Tokyo, Japan. Molecular weight standards and bovine serum albumin were purchased from Sigma Chemical Co., St. Louis, MO. Diaflo ultrafilters (PM 30) were purchased from Amicon Corp., Danvers, MA. Pevikon was obtained from Mercer Consolidated Co., Deer Park, NY. Other analytical grade reagents were obtained from Fisher Scientific Co., Cincinnati, OH.

Human subjects. Plasma was obtained from two unrelated hyperalphalipoproteinemic patients (cases 1 and 2) with complete deficiency in CETP activity. The clinical data of patient 1 have been reported previously (7). Plasma was also obtained from two hyperalphalipoproteinemic subjects with normal CETP activity and from ten normolipidemic individuals for comparison. The levels of HDL-cholesterol, apo E, and CETP activity in the plasma of these subjects are shown in Table I.

Lipoprotein isolation and characterization. Blood samples were collected after an overnight fast into tubes containing EDTA. Protease inhibitors (100 kIU/ml of aprotinin and 1 mM benzamidine) were added to the samples immediately after collection. Total lipids and HDL-cholesterol were determined as described previously (5). Plasma apo E concentration was measured by the single radial immunodiffusion method (13) and isoelectric focusing of the apolipoproteins in VLDL was performed as described (14).

Table I. Clinical and Biochemical Data of the Human Subjects

	Age	Sex	Cholesterol		CETP activity (100 × kt)	Apo-E
			Plasma	HDL		
			mg/dl			mg/dl
Case 1*	35	M	313±29	226±5	<0.1	19.7±2.2
Case 2*	54	F	241±15	154±11	<0.1	10.8±1.6
Case 3‡	76	F	201, 248	136, 132	12.2, 13.8	5.5, 4.9
Case 4‡	63	F	250, 243	135, 138	14.9, 15.6	4.7, 4.8
Control§	NA	NA	183±24	54±15	18.0±6.1	4.3±1.1

Plasma apo E concentration was determined by single radial immunodiffusion method as described in Itakura et al. (13). CETP activity was determined in a 10-h incubation from [¹⁴C]cholesteryl ester-labeled HDL₃ (5 μg of cholesterol) to LDL (25 μg of cholesterol) with 10 μl of lipoprotein-deficient serum. The transfer of cholesteryl esters during this period was linear with time. NA, not applicable.

* Mean±SD of three separate samples.

‡ Values were determined from two separate samples.

§ Mean±SD of 10 normolipidemic subjects.

The distribution of cholesterol and apolipoproteins among the different classes of lipoproteins was determined by HPLC on gel permeation columns (7). The apolipoproteins in each fraction were analyzed with 10% SDS-PAGE (15). Proteins in each fraction were visualized by staining with Coomassie Blue. Although the staining intensity may vary among different apolipoproteins, their distribution among the different fractions could be determined by densitometric scanning of the stained gels.

Plasma lipoproteins were also prepared by ultracentrifugation method (16). The lipoproteins were stored at 4°C under sterile conditions in saline-EDTA (0.15 M NaCl, 0.01% EDTA, pH 7.4) until use. The α-migrating lipoproteins in the *d* = 1.019–1.063 fraction were separated from the α-migrating LDL by Pevikon block electrophoresis (17). Electrophoretic analysis of the lipoproteins was performed by electrophoresis in 1% agarose gels (18). The lipoproteins were visualized after electrophoresis by staining with Oil Red O. Protein concentration was estimated by the method of Lowry et al. (19). Lipid composition of the lipoproteins was determined as described above. Total cholesterol recovery from ultracentrifugation and Pevikon block electrophoresis was usually between 90% and 95%.

Cell culture and binding assays. The LDL was radiolabeled with ¹²⁵I using iodine monochloride and the apo E-containing HDL were iodinated by the Bolton-Hunter method as described (20). In a typical experiment, normal human fibroblasts were plated in 35-mm Petri dishes at a density of 3.5 × 10⁴ cells per dish. When the cell culture reached 75% confluency, the monolayers were washed three times with DME and were cultured for 48 h in medium containing 10% human lipoprotein-deficient serum. The binding assays were performed as described (20). Competitive binding studies were performed at 4°C on ice by the addition of DME containing 25 mM Hepes (pH 7.4), 10% lipoprotein-deficient serum, 1.0–1.5 μg/ml of ¹²⁵I-LDL, and various amounts of unlabeled lipoproteins to cells. After incubation for 3 h at 4°C, media were removed and the plates were rapidly washed at 4°C three times with 3-ml aliquots of cold phosphate-buffered saline (PBS) containing 2 mg/ml of bovine serum albumin. Each plate was then incubated twice for 10 min at 4°C with the same washing medium and once with PBS without bovine serum albumin. The cells were removed from the plates by dissolving in 1.5 ml of 0.1 N NaOH. The radioactivity of the total 1.5 ml of solution was counted in a gamma counter. Aliquots (200 μl) were taken for protein determination.

The affinity constants for specific binding of LDL and apo E-containing lipoproteins to the cells were measured by direct binding assays with iodinated lipoproteins. Varying concentrations of radiolabeled lipoproteins were incubated with the fibroblasts in the presence or absence of 600 μg/ml of LDL at 4°C. Specific binding of the lipoproteins to receptors was determined by subtracting the nonspecific binding, observed in the presence of excess unlabeled LDL, from total binding observed in the absence of unlabeled lipoproteins. The equilibrium dissociation constant (*K_d*) and maximum binding (*B_{max}*) were evaluated by Scatchard plot (21) as described (22).

Cholesteryl ester transfer activity. The activity of CETP was assayed as described (7) with minor modifications. Human plasma HDL₃ was radiolabeled with [¹⁴C]cholesteryl oleate (23) and used as the donor lipoprotein. Unlabeled LDL was used as the acceptor for the radiolabeled cholesteryl esters. 10 μl of lipoprotein-deficient plasma from each subject was used as the source of CETP. The assay was performed in a total volume of 600 μl containing 10 mM Tris-HCl, pH 7.4, and 150 mM NaCl for 5 h at 37°C. The reaction was terminated by incubation on ice and the LDL was precipitated by addition of 2,500 U/ml heparin and 1 M MnCl₂. Cholesteryl ester transfer activity was expressed as 100 × *kt*, where *k* is the fraction of lipid transferred per unit time *t* (24).

Results

Lipoprotein characterization. The clinical data presented in Table I clearly indicate that patients with CETP deficiency

have higher plasma levels of apo E. Isoelectric focusing analysis revealed that the apo E in these subjects are the E3/3 phenotype (data not shown). The increased amount of apo E along with hyperalphalipoproteinemia in these subjects suggested that apo E-rich HDL may be present in the plasma of these individuals. In view of the possibility that apo E could be transferred from VLDL to HDL during ultracentrifugation, the distribution of apo E among the patients' lipoproteins was compared with that of normal individuals initially by gel permeation chromatography. The distribution of plasma cholesterol, apo A-I, apo B, and apo E in each fraction was also determined for comparison (Fig. 1). Results indicated that the apo B-containing lipoproteins from CETP-containing and

CETP-deficient hyperalphalipoproteinemic subjects eluted from the column at a position similar to control LDL. The apo A-I-containing lipoproteins in CETP-deficient subjects were larger than those in subjects with normal CETP and in control subjects. In contrast, the elution profiles of apo E-containing lipoproteins were strikingly different between the various samples. Although apo E from normal subjects and CETP-containing hyperalphalipoproteinemic patients were found predominantly in the VLDL fraction, the apo E-containing lipoproteins of CETP-deficient patients eluted from the column between the LDL and HDL particles and were clearly separated from the VLDL fraction (Fig. 1). The column elution profile of these apo E-containing lipoproteins was similar to

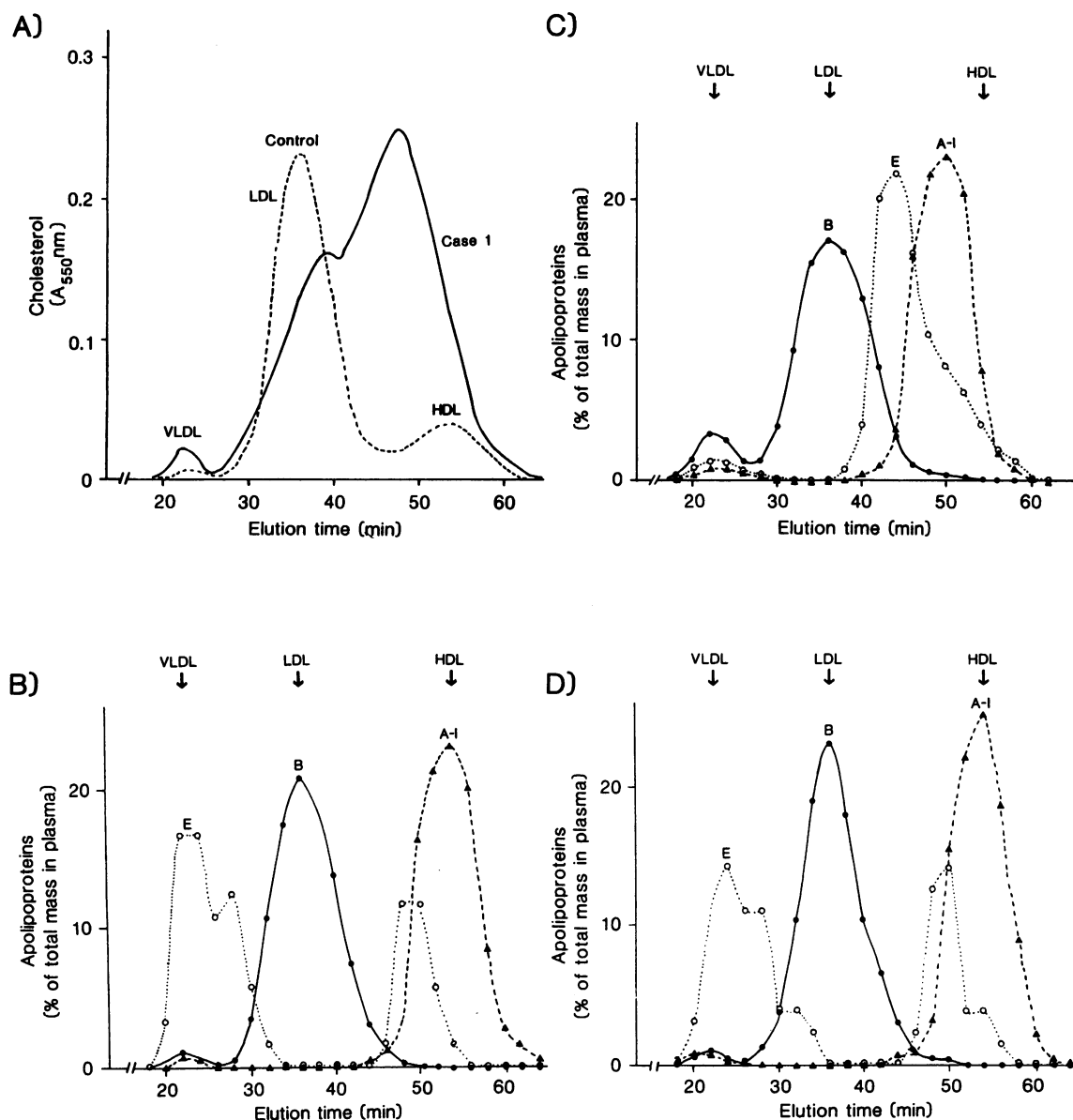


Figure 1. Distribution of cholesterol, apo A-I (▲), apo B (●), and apo E (○) in HPLC-separated plasma. Plasma (0.5 ml) of patient 1 and a control was applied to gel permeation column using G5000PW and G3000SW columns. (A) Cholesterol content of each fraction from the plasma of a control subject (---) and the CETP-deficient patient (—). (B–D) Apolipoprotein distribution in plasma of control, the CETP-deficient (subject 1), and normal CETP (subject 3) hyperalphalipoproteinemic patients, respectively. A fixed amount of each fraction was analyzed by SDS-PAGE. The apolipoprotein distribution was determined by densitometric scanning of stained gels.

Table II. Plasma Concentration of Cholesterol in Lipoproteins from Control and Hyperalphalipoproteinemic Subjects

Lipoproteins	Case 1	Case 2	Case 3	Case 4	Control (n = 10)
	mg/dl				
$d < 1.006$	28.6	5.6	1.5	2.4	10.2±4.2
$d = 1.006-1.019$	9.5	5.6	6.7	3.9	4.7±2.2
$d = 1.019-1.063$					
α -Lipoproteins	15.7	17.7	3.0	3.9	NA
β -Lipoproteins	36.2	86.6	49.8	105.1	110.4±26.6
$d = 1.063-1.125$	224.8	136.3	107.0	105.6	24.6±8.6
$d = 1.125-1.21$	25.2	23.0	33.0	31.1	25.8±5.2

Values are given as mean±SD (where applicable). NA, not applicable.

that of the HDL_c particles of cholesterol-fed animals. This observation suggests that the apo E-containing lipoproteins may be similar to the HDL_c.

The plasma lipoproteins of the various subjects were also prepared by ultracentrifugation method for characterization. The distribution of plasma cholesterol among the lipoprotein fractions from each subject was then determined. The results, as shown in Table II, indicated that most of the plasma cholesterol in the hyperalphalipoproteinemic subjects was associated with HDL₂-like particles in the $d = 1.063-1.125$ g/ml fraction. The level of HDL₃-cholesterol in the $d = 1.125-1.21$ g/ml fraction was similar between control and hyperalphalipoproteinemic subjects. To determine whether large HDL particles similar in sizes to HDL₁ and/or HDL_c were present in the patient groups, the lipoproteins in the $d = 1.019-1.063$ g/ml fraction were subjected to further characterization. The lipoproteins in this fraction were found to be different between the normolipidemic and hyperalphalipoproteinemic subjects. Although LDL with β -electrophoretic mobility were the only lipoproteins present in this fraction of normal plasma, the $d = 1.019-1.063$ g/ml fraction of hyperalphalipoproteinemic subjects was heterogeneous. Agarose gel electrophoresis revealed the presence of lipoproteins with a slow α mobility in addition to the β -migrating LDL (Fig. 2 A). The slow α -migrating $d = 1.019-1.063$ g/ml lipoproteins were especially evi-

dent from the two patients with CETP deficiency and appeared in trace amount in the hyperalphalipoproteinemic subject with normal CETP activity. Although these lipoproteins were not evident in the subjects with normal CETP activity, further fractionation of the $d = 1.019-1.063$ g/ml fraction revealed the presence of both slow α - and β -migrating lipoproteins in these subjects. The former lipoproteins were not detected from normal subjects. The slow α -migrating lipoproteins in the hyperalphalipoproteinemic subjects were then separated from the β -migrating lipoproteins in this density fraction by Pevikon block electrophoresis before further characterization. The resulting lipoproteins appeared to be homogeneous and migrated as a single band with a slow α mobility on agarose gels (Fig. 2 B).

The α - and β -migrating lipoproteins in the $d = 1.019-1.063$ g/ml fraction of the hyperalphalipoproteinemic subjects were further characterized by compositional analysis. Results indicated that the β -migrating lipoproteins contained more triglyceride and less cholesteryl ester than the normal LDL. The α -migrating lipoproteins were cholesteryl ester-rich and were depleted of triglyceride (Table III). Moreover, SDS-PAGE of the apolipoproteins showed that the β -lipoproteins were LDL and contained apo B100 as the only apolipoprotein constituent (data not shown). In contrast, the α -lipoproteins contained apo E and apo A-I without any detectable apo B (Fig. 3). It is interesting to note that the α -lipoproteins isolated from the subjects with CETP deficiency contained predominantly apo E. Based on densitometric scanning of the Coomassie Blue-stained gels, 82% of the apolipoproteins in this fraction was apo E and 18% was apo A-I. Therefore, the lipoproteins in this fraction displayed a composition similar to those of HDL_c in hypercholesterolemic animals (9). In contrast to the results of the CETP-deficient hyperalphalipoproteinemic subjects, the α -lipoproteins in the subjects with normal CETP resembled the HDL₁ and contained mostly apo A-I (~86%) with small amount (14%) of apo E. The apo E-enriched HDL detected in the plasma of CETP-deficient hyperalphalipoproteinemic subjects accounted for 6-10% of the total HDL cholesterol in these subjects. In contrast, the HDL₁-like particles accounted for 2-3% of HDL cholesterol in patients with normal CETP (Table II).

Receptor binding characteristics of the apo E-rich HDL. The demonstration of the apo E-rich HDL in CETP-deficient

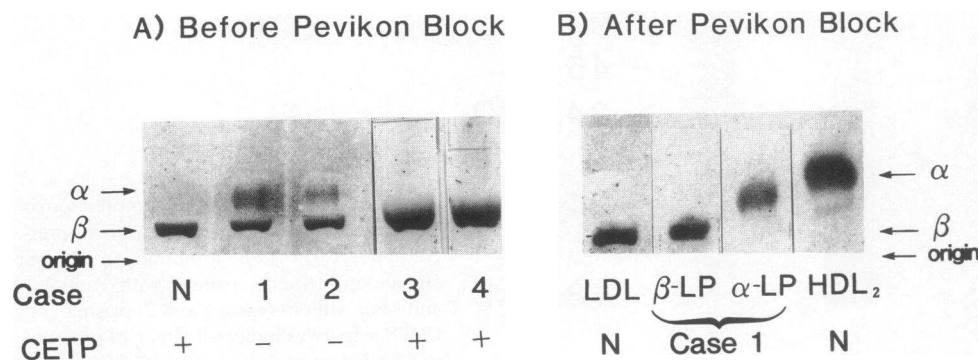


Figure 2. Agarose gel electrophoresis of the $d = 1.019-1.063$ lipoproteins from a normal (N), and four hyperalphalipoproteinemic subjects (1-4). Lipoproteins were isolated at the density range of 1.019-1.063 g/ml by ultracentrifugation. The samples were analyzed (A) before and (B) after Pevikon block electrophoretic separation of the α - and β -migrating lipoproteins. The presence or absence of CETP activity in the plasma of the subjects was indicated for identification. Electrophoresis was performed in 75 mM bar-

bital buffer, pH 8.6. The gel was stained with Oil Red O to visualize the lipoproteins. The origin of sample application and the α and β positions of migration are indicated.

Table III. Composition of the Lipoproteins in $d = 1.019-1.063$ Fraction of Control and Hyperalphalipoproteinemic Subjects

	Free cholesterol	Cholesteryl ester	Triglyceride	Phospholipid	Protein
	<i>percent by weight</i>				
β-Lipoproteins					
Control*	11.2 \pm 0.9	30.4 \pm 0.8	6.5 \pm 0.7	27.1 \pm 1.1	24.8 \pm 1.8
Case 1	10.6	26.7	10.3	25.8	26.6
Case 2	10.9	27.6	9.7	25.5	26.2
Case 3	10.2	23.6	10.5	26.6	29.1
Case 4	10.9	28.5	6.0	27.3	27.2
α-Lipoproteins					
Case 1	11.1	25.0	1.0	35.6	27.4
Case 2	11.2	25.1	0.9	35.8	27.0
Case 3	7.8	16.8	2.1	37.1	36.1
Case 4	4.2	18.6	5.7	33.6	37.9

* Values reported were the mean \pm SD of lipoproteins from 10 normolipidemic subjects. The $d = 1.019-1.063$ fraction from control subjects invariably contained only the β -migrating LDL.

subjects warranted further characterization of these lipoproteins. The receptor binding activity of these lipoproteins were tested by their ability to compete with 125 I-LDL for binding to the LDL receptor on human fibroblasts. Fifty percent inhibition of 125 I-LDL binding to the fibroblasts was observed at 2.26 μ g/ml of LDL. Similar inhibition was observed with 9.0 μ g/ml of the apo E-containing HDL from the CETP-containing hyperalphalipoproteinemic patients. In contrast, the apo E-rich HDL, isolated from the CETP-deficient subjects, bound avidly to the LDL receptor on fibroblasts and inhibited 50% of 125 I-LDL binding at concentrations of 0.25 and 0.30 μ g/ml (data not shown).

The interaction of the different lipoproteins with the LDL receptor was further investigated by direct binding experiments using radiolabeled lipoproteins. The apo E-rich HDL from cases 1 and 2 were shown to have the highest affinity for the LDL receptor and bound to the fibroblasts with a K_d of 0.32 and 0.40 μ g/ml, respectively (Fig. 4). In contrast, normal LDL and apo E-containing HDL from normal CETP subjects bound to the fibroblasts with much lower affinity ($K_d = 2.6$ and 5.5 μ g/ml, respectively). Interestingly, at receptor saturation,

approximately equal number of LDL and apo E-containing HDL were bound to the cells. In contrast, 3.5-fold less apo E-rich HDL, from CETP-deficient subjects, were required for saturation of the LDL receptor on fibroblasts. These results indicated that the apo E-containing HDL from subjects with normal CETP were similar to LDL and interacted with the LDL receptor with a 1:1 stoichiometry (21). The higher affinity and lower capacity of the apo E-rich HDL from CETP-deficient subjects for the LDL receptor suggested that these lipoproteins may be similar to the HDL_c in hypercholesterolemic animals and interacted with the LDL receptor through multiple receptor binding domains (21).

Discussion

The current study indicates that there are at least two types of hyperalphalipoproteinemia. One of these may be related to defective CETP activity and the appearance of large apo E-rich HDL. The second form of hyperalphalipoproteinemia may be related to increased number of HDL₁- and HDL₂-like

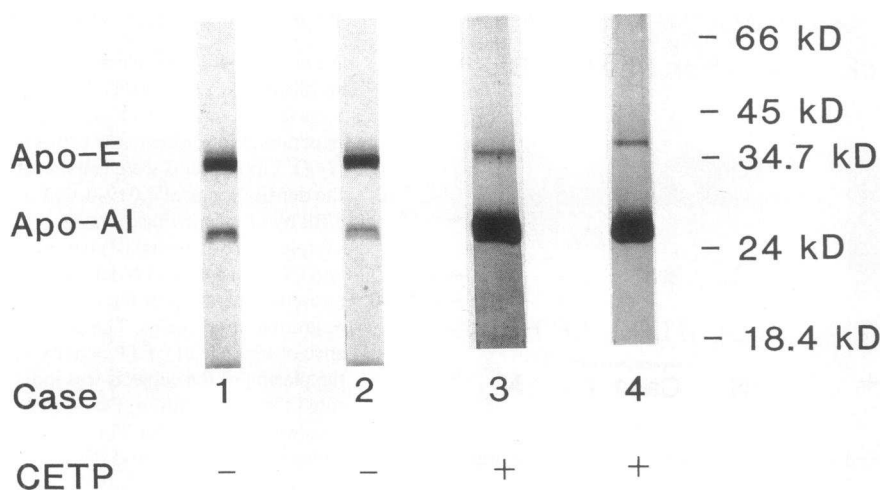


Figure 3. SDS-PAGE of apolipoproteins associated with the α -migrating lipoproteins present in $d = 1.019-1.063$ g/ml fraction of hyperalphalipoproteinemic patients with (cases 3 and 4) or without (cases 1 and 2) plasma CETP activity as indicated. 20 μ g of protein was loaded on each lane of a 10% SDS-polyacrylamide gel. The apo E and apo A-I bands were identified by comparison with standard apolipoproteins.

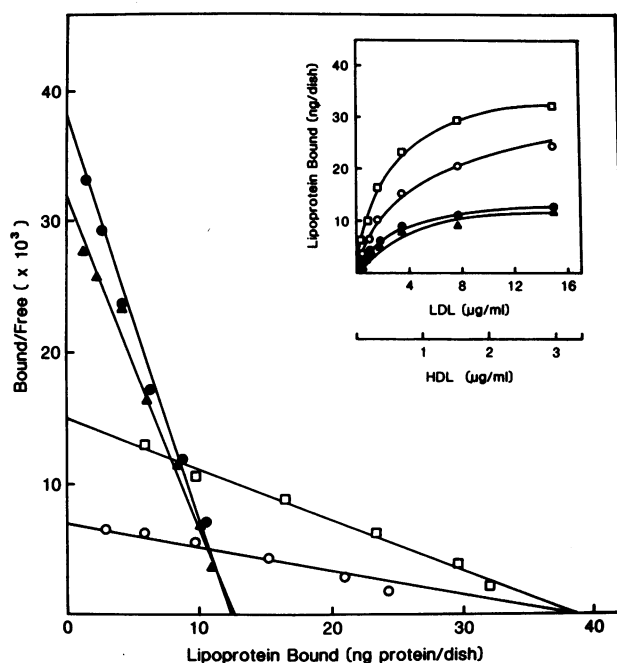


Figure 4. Scatchard analysis of the binding of human lipoproteins to LDL receptors on fibroblasts. The receptor binding characteristics of apo-E-rich HDL from subject 1 (●) and subject 2 (▲) were compared with the apo E-containing HDL from subject 3 (○) and with normal LDL (□). (Inset) Direct binding data from which the Scatchard plot was derived. Each data point represents the average of duplicate determinations.

particles with normal CETP activity. The precise genetic and biochemical basis for these hyperalphalipoproteinemia remains unknown and requires further investigations. Nevertheless, the presence of the unique apo E-rich HDL in CETP-deficient subjects, but not in other hyperalphalipoproteinemic subjects is an intriguing observation. The apo E-rich HDL was a prominent lipoprotein in the plasma of CETP-deficient patients accounting for 6–10% of the total HDL-cholesterol. These lipoproteins resembled the HDL_c particles found in animals with a moderate level of hypercholesterolemia. However, they were different from the apo E HDL_c (HDL_c containing only apo E) found in animals with severe form of hypercholesterolemia (25). The human apo E-rich HDL had a hydrated density of 1.019–1.063 g/ml, displayed an α migration upon electrophoresis, and contained 82% apo E and 18% apo A-I. These apo E-rich HDL displayed a higher affinity than LDL in binding to the LDL receptors on human fibroblasts. Moreover, at receptor saturation, less lipoproteins than LDL were bound to the human fibroblasts. These observations suggested that these human lipoproteins were also similar to the cholesterol-induced HDL_c and contained multiple copies of apo E capable of binding to the LDL receptor via multiple interactions (22).

The presence of apo E-rich HDL was well documented in cholesterol-fed animals (9). Weisgraber and Mahley (11) and Marcel et al. (12) have also shown the existence of a subfraction of human HDL containing the E apolipoprotein. Mahley et al. (10) have shown that human subjects consuming a cholesterol-rich diet possessed higher levels of these apo E con-

taining HDL. However, the HDL in these subjects were smaller than the apo E-rich HDL_c, floated at a higher density ($d = 1.095\text{--}1.21\text{ g/ml}$) in ultracentrifugation, and displayed a lower affinity than LDL for binding to the LDL receptor. The lower affinity of these HDL for the LDL receptor suggests that the lipoproteins contained one or less copy of apo E per particle (22, 26). Therefore, the apo E-containing HDL in human subjects described thus far were similar to HDL₁ and HDL₂, and were quite different from the cholesteryl ester-rich and apo E-rich HDL_c. The latter lipoproteins have been shown to display a much higher affinity than LDL in binding to the LDL receptor (22). Since apo E-rich HDL_c was only detected in animals with low CETP level after cholesterol feeding, it is possible that the high level of CETP in human subjects may inhibit the formation of these apo E-rich HDL. The results of the current study, showing the accumulation of apo E-rich HDL in CETP-deficient human subjects, are supportive of this hypothesis. The human apo E-rich HDL reported in this study was observed as a prominent lipoprotein in familial hyperalphalipoproteinemic subjects with deficiency of CETP. The apo E-rich HDL was not observed in the plasma of hyperalphalipoproteinemic subjects with normal CETP activity.

The presence of the unique apo E-rich HDL in CETP-deficient subjects is most likely a direct result of defective cholesteryl ester transfer from HDL to VLDL. An inverse relationship between CETP and the accumulation of large HDL was suggested previously in animal studies and in *in vitro* experiments. Gavish et al. (27) showed that the conversion of HDL₃ to large HDL in the rat could be delayed by infusion of partially purified CETP. In more recent studies by Tall and his associates (28, 29), inhibition of CETP activity *in vitro* with a neutralizing antibody resulted in the redistribution of apo E to larger lipoproteins and the inhibition of cholesteryl ester transfer from HDL to VLDL. The observation that apo E-enriched HDL_c was found only in cholesterol-fed animals which have low levels of CETP activity (9) is supportive of this hypothesis.

The apo E-rich, cholesteryl ester-rich HDL was also detected in neonates (30) and in abetalipoproteinemic patients (31, 32). Although the level of CETP in these subjects was unknown, it is interesting to note that the plasma of neonates contained only small amounts of VLDL (33). The abetalipoproteinemic subjects also contained little, if any, VLDL owing to defects in the synthesis of triglyceride-rich lipoproteins. The accumulation of apo E-rich lipoproteins in these subjects was probably due to the absence of an acceptor lipoprotein for the cholesteryl ester transfer pathway (31). In the current study, we showed directly that CETP deficiency resulted in the accumulation of the cholesteryl ester-rich HDL with an enrichment of apo E. The increased core volume of the HDL, owing to continuous acquisition and esterification of cholesterol, required the continuous addition of apo E (34). The accumulation of these apo E-rich and cholesteryl ester-rich HDL in CETP-deficient subjects must then be due to the inability to transfer excess cholesteryl ester and possibly apo E to lower density lipoproteins. Additionally, the accumulation of these apo E-rich lipoproteins, which have a high affinity for the LDL receptor, suggests that the accumulation of apo E-rich and cholesteryl ester-rich HDL results in a slower catabolic rate due to saturation of the hepatic LDL receptor.

The results of this study, together with those of other investigators, suggested that low CETP level may increase cholesteryl ester-rich HDL concentrations in plasma. A corollary to this observation would be the increased formation of cholesteryl ester-rich VLDL in the presence of high CETP level. Since cholesteryl ester-rich VLDL have been shown to induce cholesteryl ester accumulation and foam cell formation in macrophages (2, 34, 35), a high CETP level may indirectly contribute to the atherogenic process. Future clinical studies in patients with known premature vascular disease would be valuable in order to assess the validity of this theory.

Acknowledgments

We thank Drs. John R. Wetterau and Judith A. K. Harmony for valuable discussions.

This work was supported in part by grants from the National Institutes of Health (HL-22619) and the Biomedical Research Support Grant Program; the Japanese Ministry of Education (No. 6040268 and 01480289); a Grant-in-Aid for Special Project Research from the Japanese Ministry of Education, Science and Culture; a Research Grant for Adult Disease, the Japanese Heart Association; the American Heart Association, Southwestern Ohio Affiliate (SW-88-21 and SW-89-44F); and an Established Investigatorship award to Dr. Hui (85-237).

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