Evidence for electronegativity of plasma high density lipoprotein-3 as one major determinant of human cholesteryl ester transfer protein activity

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Abstract Plasma high density lipoprotein-3 (HDL_3) subfractions with different composition and electric charge properties were isolated by anion exchange chromatography; their ability to exchange cholesteryl esters with low density lipoproteins (LDL) in the presence of the human cholesteryl ester transfer protein (CETP) was studied. The rate of radiolabeled cholesteryl esters transferred between LDL and HDL_3 was progressively enhanced as the negative charge density of HDL_3 particles increased, until the maximal transfer value was reached with a charge density ranging between -2,200 and -2,250 esu/cm^2. Consistent data were obtained when cholesteryl ester transfer was measured either from radiolabeled LDL towards HDL_3 or from radiolabeled HDL_3 towards LDL. In both cases, a progressive decrease in the cholesteryl ester transfer rate was observed as the charge density increased above the optimal value. When HDL_3 particles were progressively enriched with apoA-II with no modification of their lipid content, the electronegative charge progressively decreased. In good agreement with data obtained with native HDL_3 subfractions isolated from human plasma, the rate of radiolabeled cholesteryl esters transferred from LDL towards apoA-II-enriched HDL_3 increased progressively as the density of negative charge increased, until an optimal surface charge density of approx. -2,200 esu/cm^2, was reached. As the charge density of apoA-II-enriched HDL_3 exceeded the optimal value, the cholesteryl ester transfer rate was substantially reduced. Consistent observations were made by substituting apoA-II for apoA-I either in immunopurified HDL_3 particles containing mainly apoA-I or in the plasma HDL_3 subfractions with the highest electronegativity. It is concluded that the charge density of plasma HDL_3 constitutes one major determinant of maximal CETP activity.—**Masson, D., A. Athias, and L. Lagrost.** Evidence for electronegativity of plasma high density lipoprotein-3 as one major determinant of human cholesteryl ester transfer protein activity. *J. Lipid Res.* 1996. **37:** 1579–1590.

Supplementary key words HDL_3 • electronegative charge • apoA-I • apoA-II

The cholesteryl ester transfer protein (CETP) promotes the exchange of neutral lipid species, i.e., cholesteryl esters and triglycerides, between plasma lipoprotein fractions (1). Despite the absence of consensus concerning the kinetic model of the CETP-mediated lipid transfer reaction (2, 3), it is clear that the direct interaction of CETP with the lipoprotein surface represents one key step of the transfer process (4). Indeed, the binding of CETP to lipoprotein substrates and the lipid transfer activity were shown to increase in parallel, whereas the disruption of CETP-lipoprotein complexes inhibits the lipid transfer reaction (5). Earlier studies by Pattnaik and Zilversmit (6) demonstrated that the interaction of CETP with lipoprotein particles is electrostatic in nature. More specifically, it would involve the interaction of one, or several, positively charged groups of the CETP molecule (7) with negative charges localized in the lipoprotein surface (6). That latter mechanism is supported by several experimental evidences. CETP-lipoprotein interactions are enhanced when the negative charge of lipoprotein particles is increased by acylation or succinylation of amino groups (6, 8), by digestion with either phospholipase A2 (6) or lipoprotein lipase (9, 10), or by addition of negatively charged non-esterified fatty acids (NEFA) (10–12). CETP-lipoprotein interactions are markedly decreased when reducing the negative charge at the lipoprotein surface by lowering the pH of the medium (6, 10), by conducting incubations in the presence of divalent cations (Ca^{2+}, Mn^{2+}) (6, 8), or by

Abbreviations: CETP, cholesteryl ester transfer protein; HDL, high density lipoproteins; HDL_3, high density lipoprotein-subfraction 3; LDL, low density lipoproteins; NEFA, non-esterified fatty acids; ^{3}H-CE-HDL_3, HDL_3 containing tritiated cholesteryl esters; ^{3}H-CE-LDL, LDL containing tritiated cholesteryl esters; HDL_3-A-I, HDL_3 containing mainly apolipoprotein A-I; PLTP, phospholipid transfer protein; LCAT, lecithin:cholesterol acyltransferase; TBS, Tris-buffered saline; FPLC, fast protein liquid chromatography; esu, electrostatic unit; IEF, isoelectric focusing.

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reducing the NEFA content of lipoprotein particles with fatty acid-poor albumin (10–12). In fact, an optimal number of lipoprotein negative charges would be required to preserve a maximal CETP-mediated lipid transfer rate. Indeed, recent in vitro studies with chemically modified lipoproteins revealed that an appropriate affinity of CETP for donor and acceptor lipoprotein substrates is needed, with both excessive and insufficient CETP-lipoprotein interactions reducing the lipid transfer reaction (8).

Although the electrostatic interaction of CETP with lipoprotein substrates is now considered as the initiating step of the lipid transfer reaction, the determinants of lipoprotein electronegativity, as well as the role of the electrostatic charge of native plasma lipoprotein fractions in determining plasma CETP activity, remain unclear. In the present study, human plasma HDL_3 were fractionated according to their electrostatic charge by using anion-exchange chromatography, and the influence of the lipid and apolipoprotein composition of isolated HDL_3 on both their surface potential and their ability to act as substrates for CETP was addressed.

MATERIALS AND METHODS

Isolation of HDL_3 particles

Fresh citrated plasma from normolipidemic subjects was provided by the Centre de Transfusion Sanguine (Hôpital du Bocage, Dijon, France). HDL_3 were isolated as the plasma fraction of density 1.13–1.21 g/ml by sequential ultracentrifugation at 55,000 rpm (223,000 *g*) in a 70-Ti rotor in an L7 ultracentrifuge (Beckman, Palo Alto, CA), with two 20-h spins at the lower density and one 30-h spin at the higher density. The HDL_3 fraction was subsequently washed with one 8-h spin at the density of 1.21 g/ml, at a speed of 80,000 rpm (561,000 *g*) in an NVT-90 rotor on a Beckman XL-90 ultracentrifuge. Densities were adjusted by the addition of solid KBr. The isolated lipoproteins were dialyzed overnight against a buffer of 10 mmol/l Tris, 3 mmol/l NaN_3, pH 7.4.

Fractionation of HDL_3 by anion exchange chromatography

Ultracentrifugally isolated HDL_3 were fractionated on a Mono Q HR 5/5 column (Pharmacia Biotech Inc.) which was connected to a Fast Protein Liquid Chromatography (FPLC) system (Pharmacia Biotech Inc.). Briefly, approximately 2 mg of HDL protein was injected into the column, and HDL_3 subfractions were eluted for 60 min at a flow rate of 1 ml/min, according to an NaCl gradient ranging from 0 to 0.4 mol/l. Protein-containing fractions, eluting from the column, were monitored at 280 nm by using a UV-1 detector (Pharmacia Biotech Inc.), and 2-ml fractions were collected.

Anti-apoA-II immunoaffinity chromatography

Ultracentrifugally isolated HDL_3 were fractionated on an anti-apoA-II column according to the procedure previously described (13). HDL_3 containing mainly apoA-I (HDL_3-A-I) were recovered in the fraction that did not bind to the anti-apoA-II immunoaffinity column.

Preparation of apoA-II-enriched HDL_3

ApoA-I in immunopurified HDL_3-A-I or in the plasma HDL_3 subfractions with the highest elecronegativity was progressively replaced by apoA-II upon the incubation of HDL_3 particles in the presence of increasing amounts of delipidated HDL apolipoproteins (13). ApoA-II-enriched HDL_3 were ultracentrifugally reisolated as the d < 1.13 g/ml fraction after incubation in the presence of delipidated HDL apolipoprotein in order to remove free apolipoproteins. Resulting apoA-II-enriched HDL_3 did not differ markedly in their lipid composition, and contained virtually only apoA-I and apoA-II in their protein moiety (13). In the present study, the HDL_3 to added apoHDL ratio ranged from 1:0 to 1:2, allowing us to obtain HDL_3 particles with apoA-II:apoA-I+apoA-II percentage mass ranging from 8 up to 88%.

Native polyacrylamide gradient gel electrophoresis

Apparent hydrodynamic diameters of HDL_3 were estimated by electrophoresis in 15–250 g/l polyacrylamide gradient gels according to the general procedure previously described (14). At the end of the electrophoresis, the gels were stained with Coomassie Brilliant Blue G, and the distribution profiles of HDL_3 were obtained by analysis of the gel on a Bio-Rad GS-670 imaging densitometer. The mean apparent diameters of HDL_3 subfractions were determined by comparison with globular protein standards (high molecular weight protein calibration kit, Pharmacia Biotech Inc.) that were submitted to electrophoresis together with the samples. The mean size of the heterogenous HDL_3 subfractions was calculated from the total area under the corresponding densitometric curve. Fifty percent of the total area were of smaller size and 50% of the total area were of larger size as compared with the mean size value.

Agarose gel electrophoresis

The electrophoretic mobility (U) of HDL_3 particles was determined by electrophoresis on 0.5% agarose gels (Paragon Lipo kit, Beckman) according to the method described by Sparks and Phillips (15). Briefly, the gels were cast in a Sebia Tank K20 system, and electrophoresis was performed for 45 min at 100 V in barbital buffer, pH 8.6. After electrophoresis, the gels were

successively fixed for 5 min in an ethanol–acetic acid–water 60:10:30 solution, dried, stained for 5 min with a 0.07% solution of Sudan Black B in ethanol–water 55:45, and destained for 10 min with a solution of ethanol–water 45:55. In parallel, gel portions containing purified bovine serum albumin, which was used as an internal standard, were stained with a 0.8 g/l solution of Coomassie Brilliant Blue G 250 in perchloric acid, 0.33 mol/l, and destained in a solution of methanol–acetic acid–water 35:25:40. Mean migration distances were obtained by analysis of the gel on a Bio-Rad GS-670 imaging densitometer.

Calculation of electrophoretic mobility and net charge of HDL_3 particles

Surface charges of HDL_3 were estimated by using the equations given by Sparks and Phillips (15). Electrophoretic mobilities (U) were calculated by dividing the electrophoretic velocity (mean migration distance (mm)/time(s)) by the electrophoretic potential (voltage (V)/gel distance (cm)). To correct the pI-dependent retardation effects, the following equation was applied (15):

$$U \text{ corrected} = (U \text{ agarose} - 0.136)/\ 1.211 \qquad \textit{Eq. 1)}$$

The net charge of particles (V) were estimated by this relationship:

$$V = (1.049 \times 10^7)\ U\ r\ (1 + kr + kr_i) / (f(1 + kr_i)) \qquad \textit{Eq. 2)}$$

where V is the number of excess positive or negative charges per particle; U is the electrophoretic mobility of the particle (mm V s^{-1} cm^{-1}); r is the particle hydrodynamic radius (cm); r_i is the counterion radius (Na: 2.5×10^{-8} cm); n is the coefficient of viscosity (0.0089 poise); k is the Debye-Huckel constant which is calculated from the electrolyte ionic strength (I) by the following equation:

$$k = I^{0.5} / (3.06 \times 10^{-8}) \qquad \textit{Eq. 3)}$$

and f is function of the particle size and the thickness of the ionic double layer surrounding it. For a solvent ionic strength of 0.05, the following equation gives the dependence of f on r:

$$f = (3.66 \times 10^5\ r) + (-1.74 \times 10^{11} r^2) + (3.54 \times 10^{16} r^3) + (-1.8 \times 10^{21} r^4) + 0.979 \qquad \textit{Eq. 4)}$$

The density of surface charge (Cd) of the particle in esu/cm^2 was estimated by the following equation:

$$Cd = V \times 4.8 \times 10^{-10} / 4\ \pi\ r^2 \qquad \textit{Eq. 5)}$$

Finally, the surface potentials of lipoproteins were calculated by using the Henry's equation (15):

$$S = U \times 6\ \pi\ n / D \qquad \textit{Eq. 6)}$$

where D is the solvent dielectric constant.

Preparation of radiolabeled LDL

LDL were biosynthetically labeled according to the procedure previously described (13). Briefly, a $d > 1.13$ g/ml plasma fraction obtained after ultracentrifugation of 20 ml of normolipidemic plasma was dialysed against TBS and then incubated for 24 h at 37°C with 10 nmol of [1α, 2α -^{3}H]cholesterol (sp act, 46 Ci/mmol; Amersham Corp.) to allow cholesterol esterification by lecithin:cholesterol acyltransferase. Subsequently, the $1.019 < d < 1.055$ g/ml fraction obtained from 10 ml of plasma was added to the incubated mixture. The incubation was then prolonged for 6 h to allow the transfer of radiolabeled cholesteryl esters from HDL_3 towards LDL. Finally, radiolabeled LDL (^{3}H-CE-LDL) were recovered by sequential ultracentrifugation.

Radiolabeling of HDL_3 subfractions

Individual HDL_3 subfractions isolated from the MonoQ column were radiolabeled by incubation in the presence of radiolabeled LDL and purified CETP. Briefly, HDL_3 subfractions (100 nmol of cholesterol), ^{3}H-CE-LDL (75 nmol of cholesterol), and CETP (25 µg of protein) were incubated for 24 h at 37°C in a final volume of 200 µl. Subsequently, ^{3}H-CE-HDL_3 subfractions were recovered by sequential ultracentrifugation. As observed by using agarose gel electrophoresis, radiolabeling of HDL_3 subfractions did not affect their electrostatic charge.

Purification of cholesteryl ester transfer protein

CETP was purified from 2500 ml of citrated, normolipidemic human plasma by using a combination of the procedures previously described (13, 16). Briefly, the plasma protein fraction precipitated with ammonium sulfate between 35 and 55% of saturation was subjected to ultracentrifugation at a density of 1.25 g/ml. The resulting $d > 1.25$ g/ml fraction was then subjected successively to hydrophobic interaction chromatography on a phenyl-Sepharose CL-4B column (Pharmacia, Uppsala, Sweden), to cation exchange chromatography on a carboxymethyl-cellulose column (Whatman, Kent, UK), to affinity chromatography on an heparin-Ultrogel A4R column, and to anion exchange chromatography on a Mono Q HR 5/5 column (Pharmacia). Chromatographic separations were performed on an FPLC system (Pharmacia) at 4°C, with the exception of anion-exchange chromatography which was conducted at room temperature. A linear gradient

ranging from 0 to 0.5 mol/l of NaCl was used to elute the cholesteryl ester transfer activity from the Mono Q column. The active fractions, which were eluted with a Tris 20 mmol/l, NaCl 150 mmol/l (pH 7.4) buffer were pooled, aliquoted, and stored at -80°C. The CETP preparation was deprived of both LCAT and phospholipid transfer protein (PLTP) (16).

Measurement of cholesteryl ester transfer activity

Cholesteryl ester transfer activity was determined by measuring the transfer of radiolabeled cholesteryl esters either from ^{3}H-CE-LDL to unlabeled acceptor HDL_3 or from ^{3}H-CE-HDL_3 to unlabeled acceptor LDL (13). Briefly, the radiolabeled lipoprotein donor (2.5 nmol of cholesterol) and the unlabeled lipoprotein acceptor (10 nmol of cholesterol) were incubated for 3 h at 37°C in the presence of partially purified CETP (4.5 μg) in a final volume of 50 μl. At the end of the incubation, the tubes were immediately placed on ice, and a 45-μl volume of each incubated mixture was added to 1.95 ml of a d 1.07 g/ml KBr solution in 2-ml Quickseal centrifugation tubes (Beckman). The tubes were then sealed and ultracentrifuged for 18 h at 35,000 rpm in a 50.4 Ti rotor, in an L7 ultracentrifuge (Beckman). At the end of the ultracentrifugation run, the d < 1.068 and the d > 1.068 g/ml fractions were recovered in 1-ml volumes and transferred into counting vials containing 2 ml of scintillation fluid. The radioactivity was assayed for 2 min in a Wallac 1410 liquid scintillation counter (Pharmacia). The recovery of total radioactivity in the d < 1.068 and in the d > 1.068 g/ml fractions was greater than 95%. In non-incubated controls containing the radiolabeled lipoprotein donor and the unlabeled lipoprotein acceptor, constantly less than 10% of the radioactivity was recovered in the unlabeled lipoprotein fraction. Cholesteryl ester transfer was expressed as the percentage of total radioactivity transferred from the lipoprotein tracer towards the d > 1.068 g/ml or d < 1.068 g/ml fractions, after deduction of blank values from control mixtures which were kept at 4°C. Control values in mixtures incubated at 37°C containing lipoprotein substrates but with no purified CETP added did not differ significantly from blank values in control mixtures maintained at 4°C. These data indicate, therefore, that isolated lipoprotein fractions were deprived of active endogenous CETP.

Protein and lipid analyses

All chemical assays were performed on a Cobas-Fara Centrifugal Analyzer (Hoffmann-La Roche). Total cholesterol, unesterified cholesterol, triglyceride, and phospholipid concentrations were measured by enzymatic methods using Boehringer Mannheim reagents. Concentrations of apoA-I and apoA-II were determined by immunoturbidimetry with anti-apoA-I and anti-apoA-II antibodies purchased from Behringwerke AG (Marburg, Germany). ApoA-I standard was purchased from Behringwerke AG. ApoA-II standard was purchased from Immuno AG (Vienna, Austria).

RESULTS

Fractionation and characterization of plasma HDL_3

HDL_3 ultracentrifugally isolated from human plasma were fractionated on the basis of their electrostatic charge by using anion exchange chromatography (see

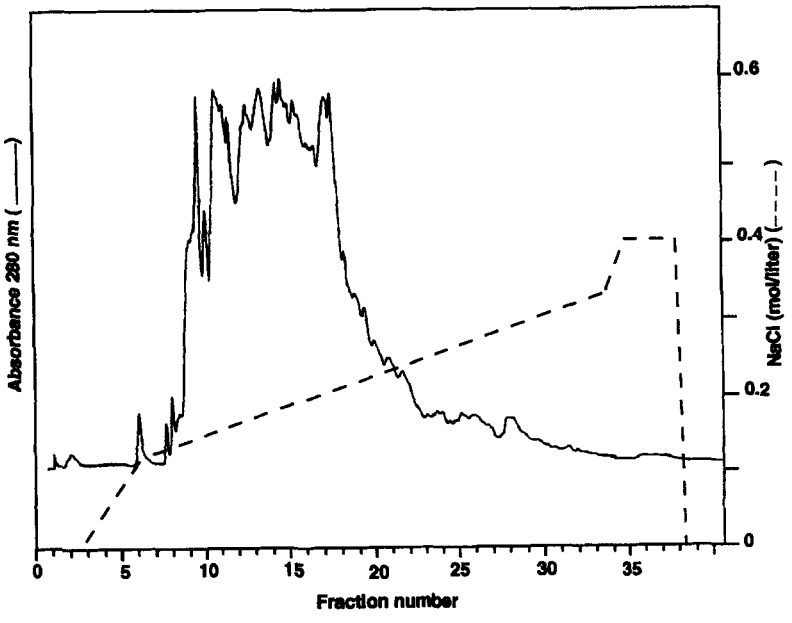

Fig. 1. Fractionation of plasma HDL_3 by anion exchange chromatography. The HDL_3 fraction isolated from normolipidemic human plasma was applied on a Mono Q HR 5/5 column. Bound proteins were then eluted by a continuous salt gradient ranging from 0 to 0.4 mmol/l of NaCl (see Materials and Methods). The chromatogram is representative of five similar experiments.

Materials and Methods). As shown in **Fig. 1,** which presents one typical elution profile, plasma HDL_3 eluted as a heterogenous broad peak in the 0.1–0.3 mol/l NaCl concentration range. The HDL_3 particles with the lowest electronegativity were eluted in the earlier subfractions, whereas the particles with the highest electronegativity were retained longer. Collected 2-ml fractions were pooled in order to obtain 9 distinct subfractions (subfractions 7–9, 10–11, 12–13, 14–15, 16–17, 18–20, 21–23, 24–26, and 27–30), each containing approximately 100 μg of total cholesterol.

Agarose gel electrophoresis revealed that total HDL_3 were constituted of a mixture of particles with a migration distance ranging between 17 mm and 29 mm, corresponding to electronegative charge densities ranging between -1,700 and -2,500 esu/cm^2, respectively. In support of the higher electronegativity of the long-retained particles, the elution delay of HDL_3 from the Mono-Q column and their migration distance on agarose gel increased in parallel. As shown in **Fig. 2,** the mean migration distance on agarose gel increased gradually from 18.9 mm for subfraction 7–9 to 26.5 mm for subfraction 27–30.

As described under Materials and Methods, the mean apparent diameter of HDL_3 was determined by comparison with protein standards of known size that were submitted to electrophoresis together with the sample. The densitometric analysis of polyacrylamide gradient gels revealed that total HDL_3 were constituted of a mixture of distinct subfractions with mean apparent diameters ranging from approx. 7.50 nm up to approx. 10.50 nm. Only 10% of total HDL_3 exhibited mean diameters greater that 10.00 nm. The analysis of HDL_3 on polyacrylamide gradient gels revealed that each isolated subfraction was constituted, in fact, of a mixture of several distinct subpopulations (**Fig. 3**). In spite of the heterogenous distribution of HDL_3 subfractions eluted from the MonoQ column, a mean apparent diameter was calculated for each Mono-Q fraction (see Materials and Methods). The mean apparent diameter of HDL_3 was 8.78 nm and a gradual increase in the mean size of HDL_3 from subfraction 7–9 to subfraction 27–30 was observed (**Table 1**).

The charge characteristics of HDL_3, i.e., the density of surface charges and the surface potentials, were calculated from both the electrophoretic mobility in agarose gel and from the Stoke's radius of the lipoprotein particles by using the equations reported by Sparks and Phillips (15) (see Materials and Methods). As shown in Table 1, the surface potential (expressed in mV), as well as the density of surface charge (expressed in electrostatic unit (esu) per cm^2) increased gradually from subfraction 7–9 to subfraction 27–30.

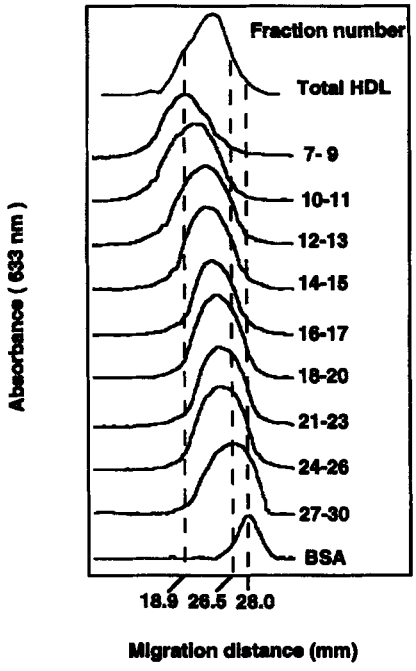

Fig. 2. Electrophoretic profiles of plasma HDL_3 subfractions in agarose gel. HDL_3 subfractions (5 μg of protein) isolated by anion exchange chromatography were submitted to electrophoresis in a 10-μl volume in 0.5% agarose gels (see Materials and Methods). Migration profiles were obtained by analysis of agarose gels on an imaging densitometer. Bovine serum albumin (BSA) was run on each gel as a standard.

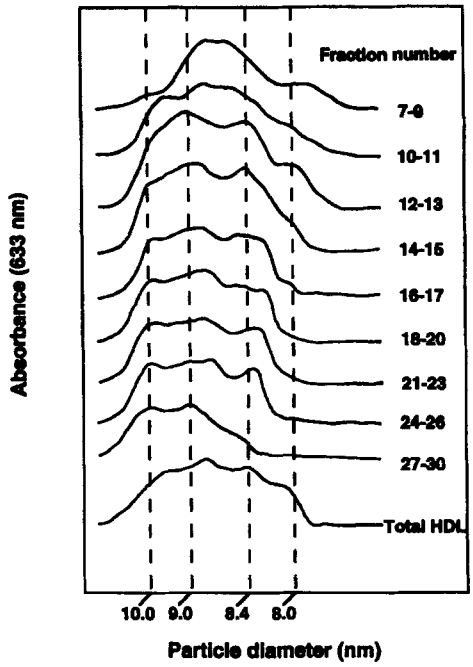

Fig. 3. Particle size distribution of HDL_3 subfractions separated by anion exchange chromatography. HDL_3 particles (5 μg of protein) were submitted to electophoresis on native 15–250 g/l polyacrylamide gradient gel in a 10-μl volume, and were analyzed by laser densitometric scanning, as described under Materials and Methods. These results are representative of four similar experiments.

TABLE 1. Size and charge characteristics of plasma HDL_3 subfractions isolated by anion exchange chromatography

Fraction Number	Particle Diameter	Agarose Migration	Surface Potential	Density of Surface Charge
	nm	mm	*-mV*	*-esu/cm²*
7-9	8.60	18.9	9.4	1,820
10-11	8.70	22.2	10.7	2,060
12-13	8.76	23.3	11.1	2,140
14-15	8.76	24.0	11.4	2,190
16-17	8.84	24.8	11.7	2,240
18-20	9.00	24.8	11.7	2,230
21-23	9.00	25.1	11.8	2,250
24-26	9.10	25.4	11.9	2,260
27-30	9.34	26.5	12.4	2,330

Ultracentrifugally isolated HDL_3 were separated on a MonoQ column, and their size and electric charge were determined by polyacrylamide gradient gel electrophoresis and agarose gel electrophoresis, respectively (see Materials and Methods). Surface potentials and charge densities were calculated as described under Materials and Methods.

The differences in the charge characteristics of various HDL_3 subfractions were associated with differences in their lipid and apolipoprotein composition. As presented in **Table 2,** the first eluted subfractions contained particles rich in apolipoproteins, mainly apoA-I, but relatively poor in core lipids, cholesteryl esters, and triglycerides. As the elution time progressively increased from subfraction 7-9 to subfraction 27-30, the total lipid content of HDL_3 particles tended to increase gradually, due mainly to a marked enrichment of the lipoprotein core with cholesteryl esters and triglycerides. In the meantime, the apolipoprotein content of HDL_3 particles was substantially reduced, and an approximately 2-fold decrease in the apoA-I content of subfraction 27-30 as compared with subfraction 7-9 was observed. In contrast, the apoA-II content tended to be higher in the long-retained HDL_3 subfractions than in subfraction 7-9 (Table 2). As a consequence, the A-II:A-I+A-II ratio was more markedly increased in the last eluted subfractions than in the first ones (Table 2).

The density of surface negative charges of HDL_3 correlated positively with their size and their relative content in cholesteryl ester, triglyceride, free cholesterol, and apoA-II, but negatively with their relative content in apoA-I (**Table 3**). Cholesteryl ester, free cholesterol, triglyceride, apoA-I, apoA-II, and HDL size, when combined in a multi-variable model accounted for 90% of the variability in the density of negative charges in HDL_3. In the multi-variable model, only cholesteryl ester, triglyceride, apoA-I, and particle size reached the significance level ($P = 0.013$, $P = 0.045$, $P = 0.002$, and $P = 0.013$, respectively; n = 25).

Effect of the electronegative charge of HDL_3 particles on cholesteryl ester transfer activity

In order to determine the influence of the electronegative charge of HDL_3 particles on the CETP-mediated neutral lipid transfer process, we compared the ability of the various HDL_3 subfractions eluted from the Mono-Q column to exchange radiolabeled cholesteryl esters with isolated LDL in the presence of purified CETP (see Materials and Methods). As shown in Figs. 4-6, significant differences appeared in the ability of the different HDL_3 subfractions to act as substrate in the CETP-mediated cholesteryl ester transfer reaction. With all the HDL_3 series studied, the rate of radiolabeled

TABLE 2. Composition (mass percent) of plasma HDL_3 subfractions isolated by anion-exchange chromatography

Fraction Number	Free Cholesterol	Phospholipids	Cholesteryl Esters	Triglycerides	ApoA-I	ApoA-II	ApoA-I+A-II	A-II:A-I+A-II%
				%				
7-9	1.6	22.8	13.4	3.4	47.7	11.2	58.9	19
10-11	2.2	25.4	18.1	4.3	37.6	12.4	50.0	25
12-13	2.5	26.2	18.8	4.8	35.2	12.5	47.7	26
14-15	2.6	26.6	20.5	4.8	33.1	12.4	45.5	27
16-17	2.6	26.2	20.9	4.9	29.5	15.9	45.4	35
18-20	2.5	23.9	21.0	4.9	29.3	18.3	47.6	38
21-23	3.0	26.4	23.4	5.0	25.3	16.9	42.2	40
24-26	3.1	25.3	24.6	5.4	25.4	16.3	41.7	40
27-30	3.4	27.7	25.6	5.7	23.3	14.3	37.6	38
Total HDL_3	2.7	25.6	21.2	5.0	31.7	13.8	45.5	30

Ultracentrifugally isolated HDL_3 particles were separated on a MonoQ column as described under Materials and Methods. Values are representative of five similar experiments.

TABLE 3. Correlation of the protein and lipid contents of HDL_3 with HDL_3, electronegativity

	Charge Density (-esu/cm^2)	
	r	*P*
Free cholesterol	0.531	0.0063
Phospholipids	-0.160	0.4458
Cholesteryl esters	0.402	0.0465
Triglycerides	0.436	0.0292
Total neutral lipids (cholesteryl esters + triglycerides)	0.504	0.0101
Total lipids	0.409	0.0426
ApoA-I	-0.713	0.0001
ApoA-II	0.678	0.0002
Total protein (apoA-I + apoA-II)	-0.403	0.0459
Size	0.449	0.0243

HDL_3 isolated by anion-exchange chromatography were analyzed for their charge density and their lipid and protein composition (see Materials and Methods); *r*, coefficient of correlation; *P*, significance level.

cholesteryl esters transferred from ^{3}H-CE-LDL towards HDL_3 was progressively enhanced as the negative charge density of HDL_3 particles increased, until the maximal transfer value was reached with a mean charge density of -2,230 esu/cm^2 (mean surface potential, -11.6 mV) (**Fig. 4**). Interestingly, as the charge density increased above the mean value of -2,230 esu/cm^2, a progressive decrease in the cholesteryl ester transfer rate was observed (Fig. 4). In spite of slight variations from one series of experiments to the other, concordant observations were made when HDL_3 subfractions were added to the incubation mixtures on the basis of either their protein, cholesterol, or phospholipid content, and in all cases the optimal charge densities corresponding to the maximal transfer value ranged between -2,200 and -2,250 esu/cm^2 (surface potential ranging between -11.5 and -11.8 mV) (**Fig. 5**). In addition, consistent data were obtained when cholesteryl ester transfer was measured not from ^{3}H-CE-LDL towards HDL_3 but in the opposite direction, from various ^{3}H-CE-HDL_3 subfractions towards LDL (**Fig. 6**). The maximal transfer rate of cholesteryl esters from ^{3}H-CE-HDL_3 towards LDL was reached with an optimal surface potential of -11.8 mV (Fig. 6).

Enrichment of immunopurified HDL_3-A-I particles with apoA-II: effect on charge characteristics and CETP activity

In order to determine the effect of alterations in the protein moiety of HDL_3 particles on their electronegative charge, HDL_3-A-I were isolated from human plasma and were progressively enriched with apoA-II as described under Materials and Methods. In accordance with previous studies from our laboratory (13, 17), the replacement of apoA-I by apoA-II, which allowed us to obtain five HDL_3 subfractions with AII:AI+AII percentage mass ranging from 8.3 to 88.0%, was not accompanied by marked changes in the mean size and lipid composition of the particles (results not shown). As shown in **Table 4**, the electronegative charge of HDL_3 particles decreased progressively from subfraction I to subfraction V as apoA-I was replaced by apoA-II.

In a second step, the ability of various apoA-II-enriched HDL_3 to exchange radiolabeled cholesteryl esters with LDL in the presence of purified CETP was compared. In accordance with previous data obtained with plasma HDL_3 subfractions (Figs. 4–6), the rate of cholesteryl ester transferred towards apoA-II-enriched

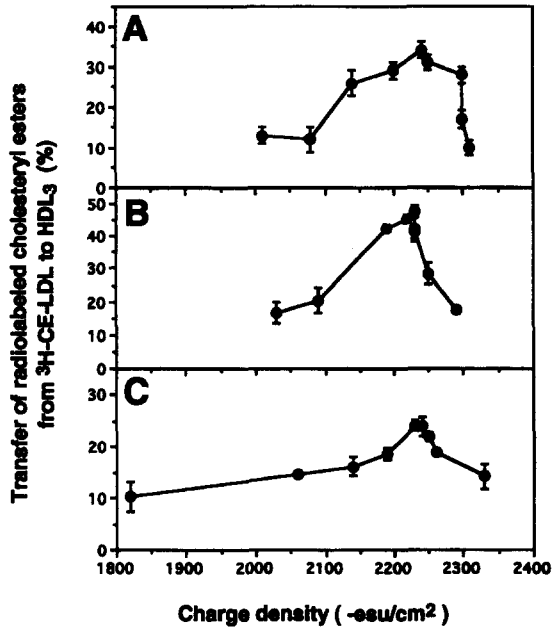

Fig. 4. Effect of the density of electronegative charges of plasma HDL_3 subfractions on the rate of radiolabeled cholesteryl esters transferred from LDL to HDL_3. Mixtures containing ^{3}H-CE-LDL (2.5 nmol of cholesterol), HDL_3 subfractions isolated by anion-exchange chromatography (10 nmol of cholesterol), and CETP were incubated for 3 h at 37°C. At the end of the incubation, the rate of radiolabeled cholesteryl esters transferred was determined after separation of LDL and HDL_3 fractions by ultracentrifugation as described under Materials and Methods. The figure shows results from three independent experiments (A, B, and C). Each point represents the mean ± SD of triplicate determinations.

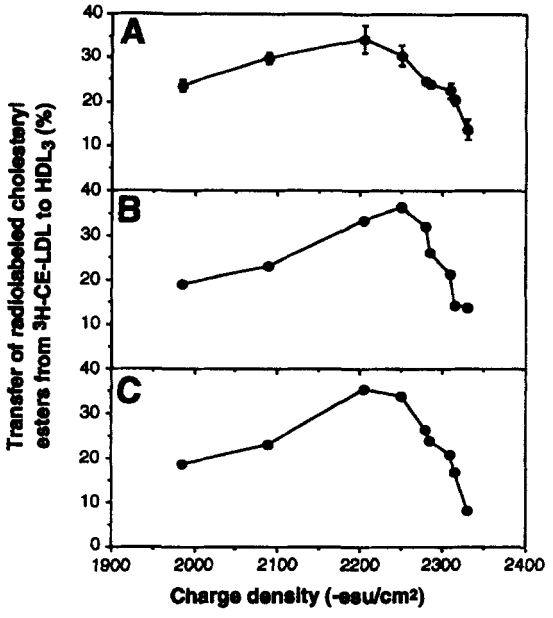

Fig. 5. Relationships between cholesteryl ester transfer activity and electronegativity of HDL_3 when added on the basis of their protein, cholesterol, or phospholipid contents. Experimental conditions were as described in the legend to Fig. 4, except that HDL_3 subfractions were added on the basis of either their protein content (final concentration, 10 μg of protein; panel A), their cholesterol content (final concentration, 10 nmol of cholesterol; panel B), or their phospholipid content (final concentration, 6 μg of phospholipid; panel C). Each point represents the mean ± SD of triplicate determinations in panel A, and the mean of duplicate determinations in panels B and C.

HDL_3 increased progressively as the density of negative charge increased (**Fig. 7**). An optimal charge value approximating -2,200 esu/cm^2 tended to be reached only in experiment 7A, while no maximal value was observed in experiment 7B.

Enrichment with apoA-II of the most electronegatively charged plasma HDL_3 subfractions: effect on charge characteristics and CETP activity

In order to confirm that a maximal transfer value corresponded to an optimal electronegative charge not only with plasma HDL_3 subfractions but also with apoA-II-enriched HDL_3, apoA-II was substituted for apoA-I in the most electronegatively charged plasma HDL_3 subfractions (mean surface potential, -12.2 mV; mean surface charge density, -2,290 esu/cm^2), corresponding to the last subfractions eluted from the Mono-Q column. As shown in **Table 5,** the electronegative charge of HDL_3 particles decreased progressively from subfraction I to subfraction VII, as the AII:AI+AII percentage mass increased from 29.6 up to 82.7%, respectively.

Again, as observed above with plasma HDL_3 subfractions (Figs. 4 and 6) and immunopurified HDL_3-A-I enriched with apoA-II (Fig. 7A), the rate of cholesteryl esters transferred from ^{3}H-CE-LDL towards the most electronegative plasma HDL_3 subfractions enriched with apoA-II increased up to a maximal value when an optimal electrostatic charge, approximating in that case -2,200 esu/cm^2 (surface potential, -11.6 mV) was reached (**Fig. 8**).

DISCUSSION

The role of electronegative charges in mediating the interaction of CETP with lipoprotein substrates has been demonstrated in previous studies by using lipoproteins that were modified in vitro with either lipolytic enzymes or chemical agents (6, 8, 10). In the present study, the use of anion-exchange chromatography, which allowed us to obtain several HDL_3 subfractions of distinct electronegative charge, presents several advantages over previous methods in which HDL were fractionated on the basis of their electrical properties. Indeed, with the previously used techniques of isoelectric focusing (IEF) (18, 19), isotachophoresis (20, 21), or chromatofocusing (22), the various lipoprotein subfractions were separated according to a wide pH gradient and their migration was dependent on their isoelectric point. In the present study, anion-exchange chromatography on MonoQ column allowed us to obtain the rapid fractionation of HDL_3 particles at a constant physiological pH of 7.4, which was based on the direct interaction of negatively charged groups of the lipoprotein substrates with the positively charged, quaternary amine groups of the MonoQ column. This latter method led to the separation of 9 distinct HDL_3 subfractions with different electrophoretic mobilities and composition.

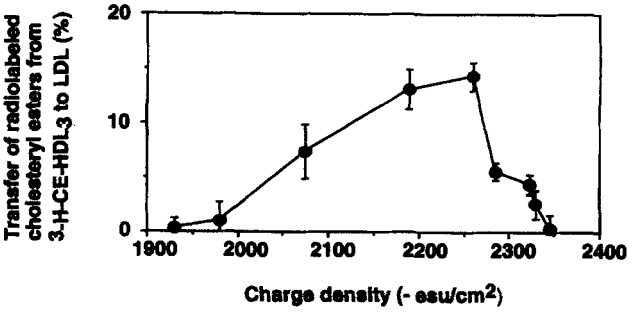

Fig. 6. Effect of the density of electronegative charges of plasma HDL_3 subfractions on the rate of radiolabeled cholesteryl esters transferred from HDL_3 to LDL. HDL_3 subfractions were radiolabeled as described under Materials and Methods. Mixtures containing ^{3}H-CE-HDL_3 subfractions (2.5 nmol of cholesterol), LDL (10 nmol of cholesterol), and CETP were incubated for 3 h at 37°C. At the end of the incubation, the rate of radiolabeled cholesteryl esters transferred was determined after separation of LDL and HDL_3 fractions by ultracentrifugation as described under Materials and Methods. Each point represents the mean ± SD of triplicate determinations.

TABLE 4. Physicochemical characteristics of immunopurified HDL_3-A-I after enrichment with apoA-II

Fraction Number	A-II:A-I+A-II%	Agarose Migration	Particle Diameter	Surface Potential	Density of Surface Charge
		mm	nm	*-mV*	*-esu/cm²*
I	8.3	25.0	9.4	11.8	2,210
II	29.1	24.8	9.6	11.7	2,190
III	49.4	24.4	9.7	11.6	2,150
IV	69.7	23.4	9.9	11.2	2,070
V	88.0	22.0	10.0	10.6	1,960

Immunopurified HDL_3-A-I were progressively enriched with apoA-II and their size and electric charge were determined as described under Materials and Methods.

Whereas the apolipoprotein A-I and A-II contents of MonoQ subfractions with a high and low electronegative potential were in quite good agreement with the apolipoprotein content of acidic and alkaline HDL fractions isolated by IEF (19), some discrepancies appeared while trying to connect the lipid composition of HDL subfractions obtained with either techniques. This latter point might be explained both by differences in the starting plasma HDL fraction, and by differences in the criteria used to fractionate HDL, i.e., either the isoelectric point with IEF or the number of negative charges with anion-exchange chromatography. In fact, we report in the present study that the density of negative charges at the HDL_3 surface correlated significantly with a number of parameters, including mainly the apoA-I content, the cholesteryl ester content, the triglyceride content, and the size of HDL_3. These observations were in good agreement with recent studies that demonstrated that the charge characteristics of HDL particles, as determined by the electrophoretic evaluation of HDL electronegativity on agarose gel (15), are determined both by the presence of acidic lipids and by the conformation of apolipoproteins at the lipoprotein surface (23). As the apolipoprotein conformation itself is dependent of the neutral lipid core and the shape of HDL particles (23), it results that the surface potential of HDL is mainly a function of their lipid content. In support of this latter view, unesterified cholesterol (24, 25) and neutral lipids (23) were demonstrated to alter markedly the electrical properties of HDL as the result of changes in the conformation of apoA-I. More precisely, detailed studies with both spherical and discoidal recombinant HDL revealed that the surface charge characteristics of apoA-I-containing particles is dependent directly on apoA-I α-helix stability (25, 26). In other words, increased charge potential of HDL would result from the destabilization of apoA-I α-helical structure, as induced by lipid components (25, 26).

When plasma HDL_3 subfractions with different electronegative charge were studied for their ability to act as substrates in the CETP-mediated cholesteryl ester transfer reaction, marked differences from one subfraction to another were observed. The most striking point of these studies was the requirement of an optimal charge value of HDL_3, probably reflecting an optimal interaction with CETP, to obtain the maximal transfer rate. One similar tendency has been reported in previous in vitro studies in which lipoprotein fractions were progressively enriched with negatively charged nonesterified fatty acids (8, 11). Indeed, the CETP-mediated transfer of radiolabeled cholesteryl esters among isolated lipoprotein fractions has been shown to be progressively activated with low concentrations of NEFA, but inhibited with high concentrations of NEFA (8, 11). As proposed by Nishida, Arai, and Nishida (8), the dual effect of NEFA on CETP activity, depending on its final concentration in the incubation mixtures, might be explained in terms of optimal electrostatic interactions

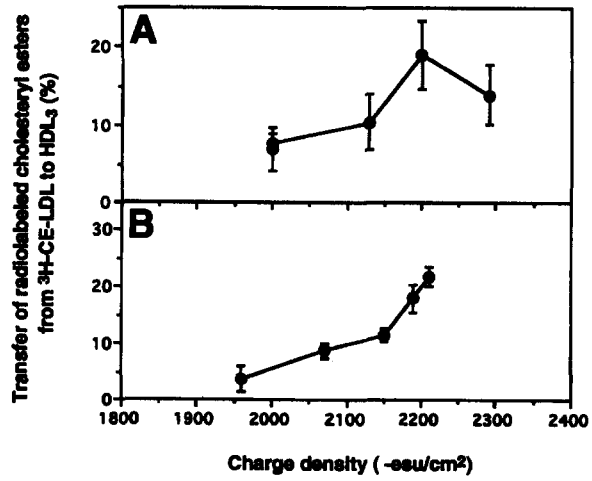

Fig. 7. Effect of the enrichment with apoA-II of immunopurified HDL_3-A-I on their electronegativity and on the rate of radiolabeled cholesteryl esters transferred from LDL to HDL_3. HDL_3-A-I were immunopurified, and progressively enriched with apoA-II as described under Materials and Methods (see Table 4). Mixtures containing ^{3}H-CE-LDL (2.5 nmol of cholesterol), HDL_3 (10 nmol of cholesterol), and CETP were then incubated for 3 h at 37°C. At the end of the incubation, the rate of radiolabeled cholesteryl esters transferred was determined after separation of LDL and HDL_3 fractions by ultracentrifugation as described under Materials and Methods. The figure shows results from two independent experiments (A and B). Each point represents the mean ± SD of triplicate determinations.

TABLE 5. Physicochemical characteristics of the most electronegative HDL_3 subfractions enriched with apoA-II

Fraction	Agarose Migration	Particle Diameter	Surface Potential	Density of Surface Charge	A-II:A-I + A-II
	mm	nm	*-mV*	*-esu/cm²*	
					%
I	26.0	9.35	12.2	2,290	29.6
II	25.8	9.25	12.1	2,280	31.6
III	25.5	9.35	12.0	2,255	34.0
IV	25.0	9.35	11.8	2,215	36.2
V	24.6	9.25	11.6	2,195	40.7
VI	23.8	9.35	11.3	2,150	48.3
VII	23.0	9.20	11.0	2,090	82.7

The most electronegatively charged HDL_3 subfractions, corresponding to the last subfractions eluted from the monoQ column, were pooled (fraction I) and then progressively enriched with apoA-II. Their size and electric charge were determined as described under Materials and Methods.

that are required for maximal cholesteryl ester transfer activity.

Although the lipid content of HDL constitutes the major determinant of the HDL electrostatic charge (23), the apolipoprotein content might also affect electrical properties. In order to investigate this latter hypothesis, we chose in the present study to replace apoA-I by apoA-II progressively either in immunopurified HDL_3 particles containing mainly apoA-I or in the plasma HDL_3 subfractions with the highest electronegativity corresponding to the last fractions eluted from the MonoQ column. These experimental approaches allowed us to obtain HDL_3 particles with similar size and lipid composition but with marked alteration in their apoA-I and apoA-II contents (13, 17). The replacement of apoA-I by apoA-II was characterized by a progressive decrease in the density of electronegative charges at the HDL_3 surface. As the substitution of apoA-II for apoA-I was not accompanied by substantial changes in the lipid composition of HDL_3, it can be concluded that the low electronegativity of apoA-II-enriched HDL_3 is directly linked to alterations in its apolipoprotein content. At least two mechanisms might account for this latter point. On the one hand, the replacement of apoA-I by a less negatively charged apolipoprotein, i.e., apoA-II, would directly account for the lower electronegativity of apoA-II-enriched HDL_3. In support of this hypothesis, Rye and Barter (27) demonstrated that lipid-free apoA-I has a higher electrophoretic mobility than apoA-II, and that recombinant particles containing only apoA-I have a higher electrophoretic mobility than recombinant particles containing only apoA-II. On the other hand, based on observations of Sparks and coworkers (23, 24) who demonstrated that alterations in the lipoprotein structure and composition can induce changes in the α-helicity and charge characteristics of apoA-I, subtle structural changes in the apoA-II-enriched HDL_3 as compared with non-enriched homologous particles might modify the conformation of proximal apoA-I molecules, and as a result might modify the overall charge properties of HDL_3. Davidson and coworkers (23) proposed that alterations in the charge properties of apoA-I might relate directly to changes in the ionization of acidic and basic amino acid residues.

In accordance with previous studies from our laboratory (13, 28), we observed that the replacement of apoA-I by apoA-II in plasma HDL_3 was associated with a significant and progressive decrease in the CETP-mediated exchange of radiolabeled cholesteryl esters between HDL_3 and LDL lipoprotein fractions. In contrast, by using recombinant HDL, Rye and Barter (27) did not find significant apoA-II-induced alterations in CETP activity. However, in that latter study, experiments were conducted with artificial particles that contained either only apoA-I or only apoA-II but not both apolipoproteins, and CETP activity was evaluated by using a less sensitive assay that measured the net mass of neutral lipids transferred from one lipoprotein substrate to the other. In fact, the precise role of apoA-I and apoA-II in determining CETP activity has been widely discussed during the past few years, and different conclusions arose from distinct studies. In particular, apoA-II has

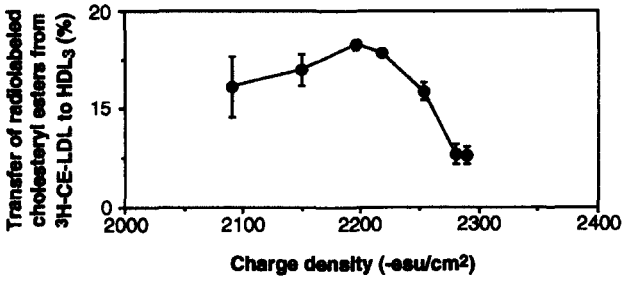

Fig. 8. Effect of the enrichment with apoA-II of the most electronegative plasma HDL_3 subfractions on their electronegativity and on the rate of radiolabeled cholesteryl esters transferred from LDL to HDL_3. The HDL_3 subfractions with the highest electronegativity, corresponding to the last fractions eluted from the MonoQ column, were pooled and progressively enriched with apoA-II (see Table 5). Mixtures containing ^{3}H-CE-LDL (2.5 nmol of cholesterol), HDL_3 (10 nmol of cholesterol), and CETP were then incubated for 3 h at 37°C. At the end of the incubation, the rate of radiolabeled cholesteryl esters transferred was determined after separation of LDL and HDL_3 fractions by ultracentrifugation as described under Materials and Methods. Each point represents the mean ± SD of triplicate determinations.

been described as an inhibitory (13, 28, 29), neutral (30), or activating (31) factor by using various experimental systems. In fact, Davidson and coworkers (23) demonstrated that the major determinant of HDL charge is the composition of the neutral lipid core, suggesting that the electronegative charge of plasma HDL_3 subfractions, and consequently their ability to interact with CETP, was dependent mainly on their neutral lipid content rather than on their apoA-II:apoA-I+apoA-II mass ratio.

Interestingly, striking similarities appeared when connecting cholesteryl ester transfer rates with the density of electronegative charges of HDL_3 subfractions that were either enriched in vitro with apoA-II or isolated directly from human plasma by using anion-exchange chromatography. Indeed, with all the experimental systems used in the present study, the maximal cholesteryl ester transfer rates tended to be observed with a surface potential of approximately -11.7 mV (mean density of electronegative charges ranging between -2,200 and -2,250 esu/cm^2). In other words, different alterations in the lipid and apolipoprotein composition of HDL_3, but leading to the similar optimal electronegative charge, could contribute to the maximal cholesteryl ester transfer rate. However, it is noteworthy that despite strong similarities in the shape of the transfer curves obtained with the various HDL_3 subfractions used in the present study, large fluctuations in the absolute cholesteryl ester transfer rates measured with each series were observed, suggesting that for one given electrostatic charge value other factors may modulate CETP activity. In addition, it is uncertain whether the maximal transfer activity occurring at -2,200/-2,250 esu/cm^2 is a general property of CETP rather than a unique property of the LDL/HDL_3 transfer system used in the present study. The role of lipoprotein electronegative charge in determining the maximal transfer rate among other plasma lipoprotein fractions deserves further investigation.

In conclusion, results of the present study demonstrated that the electronegative charge of plasma HDL_3 subfractions represents one major determinant of the maximal cholesteryl ester transfer rate. As plasma lipoproteins constitute a complex mixture of different subfractions with distinct composition and charge properties, the data discussed in the present manuscript may be of physiological interest. They suggest that the modulation of CETP activity by non-esterified fatty acids (9, 32, 33), free cholesterol (34, 35), phospholipids (6), and neutral lipids (36, 37) might, in fact, be directly mediated through alterations they induce in the electric charge properties of lipoprotein substrates.

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REFERENCES

1. Tall, A. R. 1993. Plasma cholesteryl ester transfer protein. *J. Lipid Res.* **34:** 1255–1274.
2. Ihm, J., D. M. Quinn, S. J. Busch, B. Chataing, and J. A. K. Harmony. 1982. Kinetics of plasma protein-catalyzed exchange of phosphatidylcholine and cholesteryl ester between plasma lipoproteins. *J. Lipid Res.* **23:** 1328–1341.
3. Barter, P. J., and M. E. Jones. 1980. Kinetic studies of the transfer of esterified cholesterol between human plasma low and high density lipoproteins. *J. Lipid Res.* **21:** 238–249.
4. Lagrost, L. 1994. Regulation of cholesteryl ester transfer protein (CETP) activity: review of in vitro and in vivo studies. *Biochim. Biophys. Acta.* **1215:** 209–236.
5. Morton, R. E. 1985. Binding of plasma-derived lipid transfer protein to lipoprotein substrates. *J. Biol. Chem.* **260:** 12593–12599.
6. Pattnaik, N. M., and D. Zilversmit. 1978. Interaction of cholesteryl ester exchange protein with human plasma lipoproteins and phospholipid vesicles. *J. Biol. Chem.* **254:** 2782–2786.
7. Wang, S., L. Deng, M. L. Brown, L. B. Agellon, and A. R. Tall. 1991. Structure function studies of human cholesteryl ester transfer protein by linker insertion scanning mutagenesis. *Biochemistry.* **30:** 3484–3490.
8. Nishida, H. I., H. Arai, and T. Nishida. 1993. Cholesteryl ester transfer mediated by lipid transfer protein is influenced by changes in the charge characteristics of plasma lipoproteins. *J. Biol. Chem.* **268:** 16352–16360.
9. Tall, A. R., D. Sammett, G. M. Vita, R. Deckelbaum, and T. Olivecrona. 1984. Lipoprotein lipase enhances the cholesteryl ester transfer protein-mediated transfer of cholesteryl esters from high density lipoproteins to very low density lipoproteins. *J. Biol. Chem.* **259:** 9587–9594.
10. Sammett, D., and A. R. Tall. 1985. Mechanisms of enhancement of cholesteryl ester transfer protein activity by lipolysis. *J. Biol. Chem.* **260:** 6687–6697.
11. Lagrost, L., and P. J. Barter. 1991. Effect of various non-esterified fatty acids on the transfer of cholesteryl esters from HDL to LDL induced by the cholesteryl ester transfer protein. *Biochim. Biophys. Acta.* **1085:** 209–216.
12. Barter, P. J., L. B. F. Chang, and O. V. Rajaram. 1990. Sodium oleate promotes a redistribution of cholesteryl esters from high to low density lipoproteins. *Atherosclerosis.* **84:** 13–24.
13. Lagrost, L., L. Perségol, C. Lallemant, and P. Gambert. 1994. Influence of apolipoprotein composition of high density lipoprotein particles on cholesteryl ester transfer protein activity. *J. Biol. Chem.* **269:** 3189–3197.
14. Blanche, P. J., E. L. Gong, T. M. Forte, and A. V. Nichols. 1981. Characterization of human high-density lipoproteins by gradient gel electrophoresis. *Biochim. Biophys. Acta.* **1085:** 209–216.
15. Sparks, D. L., and M. C. Phillips. 1992. Quantitative measurement of lipoprotein surface charge by agarose gel electrophoresis. *J. Lipid Res.* **33:** 123–130.
16. Lagrost, L., A. Athias, P. Gambert, and C. Lallemant. 1994. Comparative study of phospholipid transfer activi-

ties mediated by cholesteryl ester transfer protein and phospholipid transfer protein. *J. Lipid Res.* **35:** 825–835.
17. Lagrost, L., C. Dengremont, A. Athias, C. de Geitere, J-C. Fruchart, C. Lallemant, P. Gambert, and G. Castro. 1995. Modulation of cholesterol efflux from Fu5AH hepatoma cells by the apolipoprotein content of high density lipoprotein particles. *J. Biol. Chem.* **270:** 13004–13009.
18. Von Hodenberg, E., S. Heinen, K. E. Howell, C. Luley, K. Kübler, and H. M. Bond. 1991. Cholesterol efflux from macrophages mediated by high-density lipoprotein subfractions, which differ principally in apolipoprotein A-I and apolipoprotein A-II ratios. *Biochim. Biophys. Acta.* **1086:** 173–184.
19. Marcel, Y. L., P. K. Weech, T. Nguyen, R. W. Milne, and W. J. Mc Conathy. 1984. Apolipoproteins as the basis for heterogeneity in high-density lipoprotein. *Eur. J. Biochem.* **143:** 467–476.
20. Nowicka, G., T. Brüning, A. Böttcher, G. Kahl, and G. Schmitz. 1990. Macrophage interaction of HDL subclasses separated by free flow isotachophoresis. *J. Lipid Res.* **31:** 1947–1963.
21. Bittolo Bon, G., G. Cazzolato, and P. Avogaro. 1981. Preparative isotachophoresis of human plasma high density lipoproteins HDL_2 and HDL_3. *J. Lipid Res.* **22:** 998–1002.
22. Nestruck, A. C., P. D. Niedman, H. Wieland, and D. Seidel. 1983. Chromatofocusing of human high density lipoproteins and isolation of lipoproteins A and A-I. *Biochim. Biophys. Acta.* **753:** 65–73.
23. Davidson, W. S., D. L. Sparks, S. Lund-Katz, and M. C. Phillips. 1994. The molecular basis for the difference in charge between pre-β and α-migrating high density lipoproteins. *J. Biol. Chem.* **269:** 8959–8965.
24. Sparks, D. L., W. S. Davidson, S. Lund-Katz, and M. C. Phillips. 1992. Effect of cholesterol on the charge and structure of apolipoprotein A-I in recombinant high density lipoprotein particles. *J. Biol. Chem.* **268:** 23250–23257.
25. Sparks, D. L., G. M. Anantharamaiah, J. P. Segrest, and M. C. Phillips. 1995. Effect of the cholesterol content of reconstituted Lp A-I on lecithin:cholesterol acyltransferase activity. *J. Biol. Chem.* **270:** 5151–5157.
26. Sparks, D. L., S. Lund-Katz, and M. C. Phillips. 1992. The charge and structural stability of apolipoprotein A-I in discoidal and spherical recombinant high density lipoprotein particles. *J. Biol. Chem.* **267:** 25839–25847.
27. Rye, K-A., and P. J. Barter. 1994. The influence of apolipoproteins on the structure and function of spheroidal, reconstituted high density lipoproteins. *J. Biol. Chem.* **269:** 10298–10303.
28. Guyard-Dangremont, V., L. Lagrost, and P. Gambert. 1994. Comparative effects of purified apolipoproteins A-I, A-II, and A-IV on cholesteryl ester transfer protein activity. *J. Lipid Res.* **35:** 982–992.
29. Rye, K-A., K. H. Garrety, and P. J. Barter. 1992. Changes in the size of reconstituted high density lipoproteins during incubation with cholesteryl ester transfer protein: the role of apolipoproteins. *J. Lipid Res.* **33:** 215–224.
30. Moulin, P., M. C. Cheung, C. Bruce, S. Zhong, T. Cocke, H. Richardson, and A. R. Tall. 1994. Gender effects on the distribution of the cholesteryl ester transfer protein in apolipoprotein A-I-defined lipoprotein subpopulations. *J. Lipid Res.* **35:** 793–802.
31. Sparks, D., J. J. Frohlich, and P. H. Pritchard. 1991. Lipid transfer proteins, hypertriglyceridemia, and reduced high-density lipoproteins cholesterol (editorial). *Am. Heart J.* **122:** 601–607.
32. Lagrost, L., E. Florentin, V. Guyard-Dangremont, A. Athias, H. Ganjini, C. Lallemant, and P. Gambert. 1995. Evidence for nonesterified fatty acids as modulators of neutral lipid transfers in normolipidemic human plasma. *Arterioscler. Thromb. Vasc. Biol.* **15:** 1388–1396.
33. Braschi, S., L. Lagrost, E. Florentin, C. Martin, A. Athias, P. Gambert, M. Krempf, C. Lallemant, and B. Jacotot. 1996. Increased cholesteryl ester transfer activity in plasma from analbuminemic patients. *Arterioscler. Thromb. Vasc. Biol.* **16:** 441–449.
34. Tall, A. R., D. Sammett, and E. Granot. 1986. Mechanisms of enhanced cholesteryl ester transfer from high density lipoproteins to apolipoprotein B-containing lipoproteins during alimentary lipemia. *J. Clin. Invest.* **77:** 1163–1172.
35. Fielding, C. J., G. M. Reaven, G. Liu, and P. E. Fielding. 1984. Increased free cholesterol in plasma low and very low density lipoproteins in non-insulin dependent diabetes mellitus: its role in the inhibition of cholesteryl ester transfer. *Proc. Natl. Acad. Sci. USA.* **81:** 2512–2516.
36. Morton, R. E. 1988. Free cholesterol is a potent regulator of lipid transfer protein function. *J. Biol. Chem.* **263:** 12235–12241.
37. Sparks, D. L., and P. H. Pritchard. 1989. Transfer of cholesteryl ester into high density lipoprotein by cholesteryl ester transfer protein: effect of HDL lipid and apoprotein content. *J. Lipid Res.* **30:** 1491–1498.
38. Morton, R. E., and J. E. Steinbrunner. 1990. Concentration of neutral lipids in the phospholipid surface of substrate particles determines lipid transfer protein activity. *J. Lipid Res.* **31:** 1559–1567.