Characterization of human plasma apolipoprotein E-containing lipoproteins in the high density lipoprotein size range: focus on pre-β_1-LpE, pre-β_2-LpE, and α-LpE

Larbi Krimbou, Michel Tremblay, Jean Davignon, and Jeffrey S. Cohn[1]

Hyperlipidemia and Atherosclerosis Research Group, Clinical Research Institute of Montreal, 110 Pine Avenue West, Montreal, Quebec, Canada H2W 1R7

Abstract We have used two-dimensional gel electrophoresis to separate and characterize human plasma apolipoprotein (apo) E-containing lipoproteins in the high density lipoprotein (HDL) size range. Lipoproteins were separated from whole plasma by electrophoresis (according to charge) in a 0.75% agarose gel, and then in the second dimension (according to size) in a 2–15% non-denaturing polyacrylamide gradient gel. ApoE-containing lipoproteins were detected by radiography after electrotransfer of lipoproteins to nitrocellulose membranes and incubation with ^{125}I-labeled affinity-purified polyclonal apoE antibody. ApoE-containing lipoproteins in the HDL size range had a particle size ranging from 9 to 18.5 nm in diameter and could be characterized as having either γ, pre-β_1-, pre-β_2- or α-electrophoretic mobility (designated γ-LpE, pre-β_1-LpE, pre-β_2LpE, and α-LpE respectively). γ-LpE and a substantial proportion of pre-β_1- and pre-β_2-LpE did not co-migrate with apoA-I, apoA-II, apoC-III, or apoB-100. Subsequent experiments focused on the pre-β_1-LpE, pre-β_2LpE, and α-LpE subfractions, which represented >95% of apoE in HDL-sized lipoproteins. Storage of plasma at 4°C or in vitro incubation of plasma at 37°C caused a relative decrease in pre-β_1-LpE and increase in α-LpE. Normolipidemic patients with an apoE 2/2 phenotype tended to have increased levels of α-LpE, whereas apoE 4/4 subjects tended to have a greater proportion of HDL-apoE as pre-β_1-LpE. Decrease in plasma HDL apoE concentration after an oral fat load was associated with a reduction in the plasma concentration of all HDL-apoE subfractions. These results demonstrate that: *1*) apoE-containing HDL are heterogeneous in size and charge; *2*) pre-β_1-LpE is a relatively labile HDL subfraction; *3*) HDL-apoE subfraction distribution is dependent on apoE phenotype; and *4*) all apoE-containing HDL subfractions participate in the plasma transfer of apoE during the postprandial period.—**Krimbou, L., M. Tremblay, J. Davignon, and J. S. Cohn.** Characterization of human plasma apolipoprotein E-containing lipoproteins in the high density lipoprotein size range: focus on pre-β_1-LpE, pre-β_2-LpE, and α-LpE. *J. Lipid Res.* 1997. **38:** 35–48.

Supplementary key words apoE phenotype • FPLC • coronary artery disease

Epidemiological studies have consistently demonstrated that the incidence of coronary artery disease (CAD) is inversely related to the plasma concentration of high density lipoprotein (HDL) (1). It is generally believed that HDL inhibits atherogenesis by promoting the efflux of excess cholesterol from lipid-laden macrophages (2) and by mediating the plasma transport of excess cholesterol to the liver for eventual excretion in the bile (a process referred to as reverse cholesterol transport, RCT) (3, 4). HDL has, however, been shown to mediate other potentially anti-atherogenic functions, such as modulating the plasma clearance of triglyceride-rich lipoproteins (TRL) (5), inhibiting low density lipoprotein (LDL) oxidation (6), and inhibiting the expression of endothelial cell adhesion molecules (7).

In order to gain a better understanding of the mechanism by which HDL provides protection against CAD, numerous studies (reviewed in refs. 8 and 9) have investigated the structural and functional heterogeneity of plasma lipoproteins in the HDL fraction. Human HDL has been separated by ultracentrifugation into two major density subfractions, designated HDL_2 (density (d): 1.063–1.125 g/ml) and HDL_3 (d: 1.125–1.21 g/ml) (10). Non-denaturing polyacrylamide gradient gel electrophoresis has been used to separate HDL on the basis of particle size into five subfractions, namely HDL_{3c}

Abbreviations: apo, apolipoprotein; CAD, coronary artery disease; d, density; ELISA, enzyme-linked immunosorbent assay; EDTA, ethylenediamine-tetraacetate; FPLC, fast protein liquid chromatography; HDL, high density lipoprotein; IDL, intermediate density lipoprotein; LDL, low density lipoprotein; LRP, LDL receptor-related protein; PBS, phosphate-buffered saline; RCT, reverse cholesterol transport; TRL, triglyceride-rich lipoprotein; VLDL, very low density lipoprotein.

[1]To whom correspondence should be addressed.

(smallest), HDL_{3b}, HDL_{3a}, HDL_{2a}, and HDL_{2b} (largest), ranging in size from 7.2 to 12 nm (11), where the first three subfractions fall within the HDL_3 and the latter two subfractions fall within the HDL_2 density range. In terms of net particle charge, HDL has been separated by agarose gel electrophoresis into pre-β-(more positive) and α-migrating (more negative) subfractions (12), and by combining agarose gel electrophoresis with non-denaturing polyacrylamide gradient gel electrophoresis (two-dimensional electrophoresis), 6 subfractions (13) and more recently 12 subfractions (14) of HDL have been identified. Finally, HDL can be characterized on the basis of apolipoprotein (apo) composition (15), and immunochemical separation techniques have revealed the existence of an apoA-I-containing HDL subclass that also contains apoA-II (LpAI-AII) and one that contains apoA-I only (LpAI) (16). The presence of different apoA-IV-containing HDL subclasses has also been documented (17).

ApoE is a glycoprotein of 34,200 Da that plays an essential role in the movement of lipid between cells (18). It mediates the cellular uptake of plasma TRL by being a ligand for both the low density lipoprotein (LDL) receptor (19) and the LDL receptor-related protein (LRP) (20). In hypertriglyceridemic individuals, a large proportion of plasma apoE (70–90%) is associated with TRL; however, in normolipidemic subjects, the majority of apoE (>60%) in plasma is associated with HDL (21). It has been suggested that HDL-apoE is involved in a number of aspects of plasma lipoprotein metabolism, including: *1*) receptor-mediated delivery of HDL cholesterol to the liver (22); *2*) hepatic lipase-catalyzed hydrolysis of HDL phospholipid (23); *3*) plasma cholesterol esterification (24, 25); *4*) plasma cholesteryl ester transfer (26); *5*) postprandial triglyceride metabolism (27); and *6*) efflux of cell-derived cholesterol (28, 29).

In order to further investigate the nature of apoE-containing HDL in human plasma, we have in the present study used two-dimensional gel electrophoresis with immunological detection of apoE to identify and characterize different HDL-sized lipoprotein subfractions. Experiments have demonstrated that both in vivo and in vitro factors are able to affect the distribution of apoE between these HDL subfractions.

MATERIALS AND METHODS

Blood sampling

Blood samples were obtained from male and female subjects (Table 1), who had fasted overnight. Blood was drawn from an arm vein into evacuated tubes containing ethylenediamine-tetraacetate (EDTA, final concentration: 1.5 mg/ml). Collection tubes were immediately placed in ice before being centrifuged (3000 rpm, 15 min). Plasma was separated from red blood cells by aspiration and was stored at 4°C for chromatographic or lipid analysis. Separation of apoE-containing HDL subfractions by two-dimensional gel electrophoresis was routinely carried out within 30 min of plasma isolation, except in those experiments where storage conditions were purposely modified.

Separation of plasma lipoproteins by two-dimensional gel electrophoresis

The separation of plasma lipoproteins by two-dimensional non-denaturing gel electrophoresis was based on the electrophoretic system of Laemmli (30). Plasma samples (200 μl) were diluted with 100 μl of sample buffer (62.5 mM Tris-HCl, 10% glycerol, pH 6.8) and were separated by agarose gel electrophoresis (100 V, 8 h, 4°C) in 0.75% agarose using 25 mM Tris-glycine buffer (pH 8.3). Agarose gels did not contain albumin (14). Two agarose strips (approximately 7 cm in length) containing electrophoretically separated lipoproteins were then positioned at the top of each 2–15% polyacrylamide concave gradient gel and were sealed into position with agarose (0.75%). A high molecular weight protein standard (7.1 nm to 17.0 nm) (Pharmacia, Piscataway, NJ) was iodinated using IODO-GEN® Iodination Reagent (1,3,4,6-tetrachloro-3α-6α-diphenylglycouril, Pierce Chem. Co., Rockford, IL) (31) and was incorporated into 0.5-cm pieces of agarose (approximately 100,000 cpm per piece). This pre-cast agarose containing radioactive molecular weight standard was placed on the top (right- and left-hand side) of each gradient gel. Electrophoresis in the second dimension was carried out for 20 h (4°C) at 80 V.

Detection of lipoproteins after electrophoresis

Electrophoretically separated lipoproteins were electrotransferred (20 h, 30 V, 4°C), using a Trans-Blot transfer cell (Bio-Rad Laboratories, Hercules, CA) onto nitrocellulose membranes (Hybond ECL, Amersham Life Science, Buckinghamshire, England). Sudan Black lipid staining of one-dimensional and two-dimensional gels after electrotransfer confirmed that transfer of HDL-sized lipoproteins was essentially 100%. Transfer of larger lipoproteins was less complete. Membranes were incubated for 30 min in phosphate-buffered saline (PBS) containing 5% non-fat milk powder. For the detection of apoE-containing lipoproteins, gels were incubated (3 h) with immunopurified polyclonal apoE antibody (Genzyme Corp, Cambridge, MA) that had been labeled with ^{125}I (31). For the detection of apoA-I, apoA-II, apoC-III, and apoB-100, the following antibodies

(^{125}I-labeled) were used: affinity-purified goat polyclonal anti-human apoA-I antibody, monoclonal apoA-II antibody (clone no.: 4A2.2D), affinity-purified goat polyclonal anti-human apoC-III antibody (Biodesign International, Kennebunk, ME), and monoclonal apoB-100 antibody MB47 (32). After incubation with antibodies, membranes were washed three times (30 min) with PBS containing 0.05% (v/v) Tween-20 and the presence of labeled antibodies was detected by autoradiography using XAR-2 Kodak film. In some experiments, films exposed to labeled anti-apoE antibody were scanned with an IS-1000 Digital Imaging System (Alpha Innotech Corp., San Leandro, CA) and apoE-containing HDL subpopulations were quantitated by densitometry by expressing detected apoE in each subfraction as a percentage of total.

Separation of plasma lipoproteins by automated gel filtration chromatography

Plasma lipoproteins were separated by automated gel filtration chromatography on a Pharmacia (Pharmacia LKB Biotechnology, Uppsala, Sweden) fast protein liquid chromatography (FPLC) system, as described previously (21). Plasma samples (1 ml) were manually transferred to a 2-ml sample loop with two washes of 0.5 ml saline solution. They were programmed (Liquid Chromatography Controller LCC-500 Plus) to be loaded and separated on a 50-cm column (16 mm internal diameter) packed with cross-linked agarose gel (Superose 6 prep grade, Pharmacia). The column was eluted with 0.15 mol/l NaCl (0.01% EDTA, 0.02% sodium azide, pH 7.2) at a rate of 1.0 ml/min, and 25 min after addition of sample, 90 × 1-ml fractions were collected sequentially. Sample elution was monitored spectrophotometrically at optical density 280 nm.

Lipid and lipoprotein analyses

Cholesterol and triglyceride concentrations were determined enzymatically on an autoanalyzer (Cobas Mira, Roche). HDL cholesterol concentration was determined by measuring cholesterol in the supernate after heparin–manganese precipitation of apoB-containing lipoproteins in the $d > 1.006$ g/ml fraction of plasma prepared by ultracentrifugation. Plasma apoB and apoA-I concentrations were measured by nephelometry (Behring Nephelometer 100 Analyzer), and apoE was determined by enzyme-linked immunosorbent assay (21). ApoE phenotypes were determined by immunoblotting of plasma separated by minigel electrophoresis (33). Agarose gel analysis of FPLC elution fractions was carried out on 0.5% Paragon agarose gels using a Beckman Paragon Electrophoresis System (Beckman Instruments, Inc., Fullerton, CA). FPLC fractions were concentrated before electrophoresis using Minicon-CS15 concentrators (Amicon, Inc., Beverley, MA). Electrophoretically separated lipoproteins were detected with Sudan Black stain. ApoE was detected by immunoblotting using horseradish peroxidase-labeled polyclonal apoE antibody and enhanced chemiluminescence (ECL Western Blotting detection reagents, Amersham).

RESULTS

Using the present two-dimensional gel electrophoretic system to characterize apoE-containing lipoproteins in human plasma, we have been able to consistently identify a number of different apoE-containing lipoprotein subfractions in the HDL size range. The separation of plasma apoE-containing lipoproteins for two normolipidemic male subjects is shown in **Fig. 1.** Total plasma and HDL apoE concentrations for these two subjects (A and B (sample 1), left and right, respectively, in Fig. 1) are shown in **Table 1,** together with other plasma lipid and lipoprotein concentrations. Plasma samples were obtained after an overnight fast and were immediately separated by electrophoresis. They were kept in ice before separation, and electrophoresis was carried out in a cold room (4°C) to avoid in vitro modification of lipoproteins and/or transfer of apoE between lipoproteins. In order to show the separation of lipoproteins (according to charge) in the first dimension, agarose gels were stained for lipid or immunoblotted with ^{125}I-labeled apoE antibody, as shown at the top of Fig. 1. The β-, pre-β-, and α-migrating regions of the gel were identified according to the position of the lipid-stained LDL, VLDL, and HDL bands, respectively. ApoE immunoreactivity was observed between the β- and α-regions of the gel. Gamma (γ)-migrating apoE (between the origin and the β-region of the gel) was not detectable after one-dimensional agarose gel electrophoresis. Separation of lipoproteins in a second dimension (according to size) resulted in the consistent and reproducible resolution of a number of apoE-containing lipoprotein subspecies. A significant amount of apoE-immunoreactive material remained at the top of the gels (as indicated by the small horizontal arrows in Fig. 1). This material had a β- to pre-β-mobility and represented apoE associated with triglyceride-rich lipoproteins (very low density (VLDL) and intermediate density lipoproteins (IDL)) and their remnants, which were too large to enter the gel. ApoE-containing lipoproteins with a size similar to HDL migrated with either γ-, pre-β-, or α-mobility. The majority of apoE in HDL-sized lipoproteins migrated with pre-β mobility and only a small proportion co-localized with α-migrating HDL

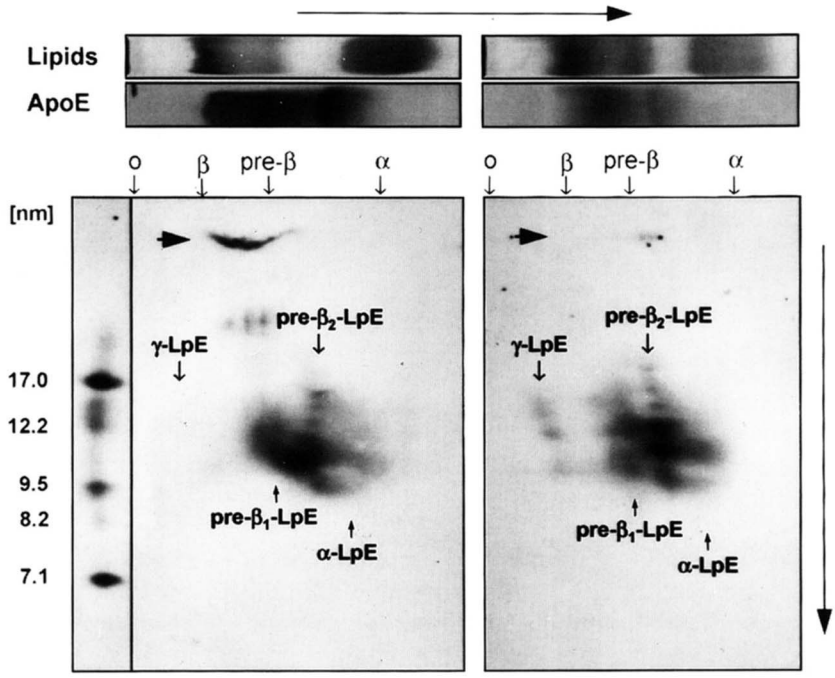

Fig. 1. Two-dimensional gel electrophoretic separation of apoE-containing lipoproteins from the plasma of two normolipidemic subjects with an apoE 3/3 phenotype. Lipid and apolipoprotein concentrations for the two plasma samples (from subjects A and B, left and right, respectively: sample 1 for subject B) are given in Table 1. Plasma was separated in the first dimension by agarose gel electrophoresis and lipoproteins were detected with lipid stain or with ^{125}I-labeled apoE antibody (top of figure). The electrophoretic mobility of lipoproteins in the first dimension is indicated (O = origin). Lipoproteins were separated in the second dimension by size in a 2–15% polyacrylamide gradient gel, and were immunodetected with ^{125}I-labeled apoE antibody. Molecular size markers are indicated on the left. The presence of apoE at the top of the gradient gel (indicated by small horizontal arrows) represents apoE-containing triglyceride-rich lipoproteins that were restricted by their size from entering the gel. Different apoE-containing HDL subpopulations are indicated with vertical arrows. γ-LpE is barely visible in the gel on the left.

TABLE 1. Plasma lipid and apolipoprotein concentrations of study subjects

			Plasma Concentration							
Subject	Age	Gender	Cholesterol	Triglyceride	HDL Cholesterol	HDL ApoE	ApoB	ApoA-I	ApoE	ApoE Phenotype
	yr		*mmol/l*	*mmol/l*	*mmol/l*	*mg/dl*		*mg/dl*		
A	34	M	3.96	0.71	1.05	2.24	87	138	3.63	3/3
B	34	M								3/3
Sample 1: 23/11/95			4.27	1.75	0.91	1.30	141	118	3.72	
Sample 2: 13/12/95			3.97	1.22	0.91	1.79	103	130	3.79	
Sample 3: 18/12/95			4.53	1.16	1.07	1.72	106	129	3.53	
Sample 4: 30/12/95			4.66	1.05	0.90	2.02	124	113	4.17	
Sample 5: 22/04/96			5.06	2.27	0.92	n.d.	119	106	n.d.	
C	37	M	4.51	0.62	1.72	3.20	84	158	4.14	3/3
D	36	M	6.33	1.47	0.75	2.66	160	107	4.58	3/3
E	30	M	3.41	1.48	0.65	5.52	49	166	8.91	2/2
F	59	M	6.14	3.25	1.35	2.60	79	168	13.6	2/2
G	50	M	4.52	3.98	0.86	1.58	121	120	3.21	3/3
H	64	F	5.80	2.62	1.49	0.67	125	193	3.90	4/4
I	41	M	5.79	3.30	0.95	0.57	136	133	5.38	4/4

Patient B was sampled on five different occasions as indicated; n.d., not determined.

lipid. ApoE-containing HDL routinely had a diameter between 9 and 18.5 nm, as determined by comparison with molecular standards. This size range was confirmed by running samples and standards to equilibrium (3,000 volt hours) in 3–24% or 3–35% gradient gels. The presence in plasma of a γ-migrating apoE-rich lipoprotein with a size between 12 and 16 nm has been reported previously and has been termed γ-LpE (28). We have subsequently classified the other apoE-containing HDL subpopulations as pre-β_1-LpE, pre-β_2-LpE, and α-LpE, corresponding to their electrophoretic migration in the first dimension (Fig. 1). Within each of these subfractions, a number of different-sized subpopulations were evident, though their number and resolution varied from one subject to another. At least five or six apoE-reactive spots were identifiable in the pre-β_2-LpE fraction.

We have assumed that the presence of different apoE-containing HDL-sized subfractions after two-dimensional gel electrophoresis is an accurate reflection of the presence of different subpopulations in vivo. It is possible, however, that the heterogeneity of the LpE fraction was in part due to the separation procedure itself (i.e., during the 8-h agarose gel separation, transfer of apoE or lipid led to the formation of particular apoE-containing particles which were not normally present in circulating plasma). Although this possibility cannot be totally excluded, experiments with agarose gels containing 0.3% albumin (designed to stabilize particles during electrophoresis) and experiments involving a separation of 1 h 45 min (at 170 V) rather than 8 h (at 100 V) did not result in significant alteration of the LpE subpopulation distribution (data not shown).

In order to establish the relationship of apoE-containing HDL subpopulations with lipoproteins containing apoA-I, apoA-II, or apoC-III, the same plasma sample was separated in four separate gels. After electrophoresis, proteins were transferred to nitrocellulose and each membrane was immunoblotted with a different ^{125}I-labeled affinity-purified antibody (shown for subject B (sample 3) in **Fig. 2**). Reactivity to the four apolipoproteins in HDL-sized lipoproteins was compared by superimposing films according to the position of radioactively labeled standards on each side of the gel. The position and two-dimensional pattern of apoA-I-containing lipoproteins was comparable to that reported previously by others (13, 14). Pre-β_1- and pre-β_2-LpA-I fractions were clearly identifiable (as indicated in Fig. 2). Pre-β_3-LpA-I (normally seen above pre-β_2-LpA-I (34)) was not detected in the plasma of subject B in Fig. 2. Pre-β_3-LpA-I was, however, observed in two other subjects, and this lipoprotein fraction co-migrated with the largest-sized subfractions of pre-β-LpE. ApoA-II was found in the same region of the gel as apoA-I, and co-migrated with pre-β_2-LpA-I. ApoC-III also had a pattern of distribution similar to apoA-I and two bands of apoC-III co-migrated with pre-β_2-LpA-I. When the apoA-I, apoA-II, and apoC-III-containing regions of the gel were compared to that of apoE (shown diagramatically in **Fig. 3**), the apoE-containing subfractions tended to have a slower electrophoretic mobility (the majority of HDL apoE had pre-β migration) and tended to be larger in size (migrated above α-LpA-I subfractions). The most intensely stained pre-β_2-LpE subpopulation (the fifth pre-β_2-LpE spot from the top in Fig. 2) corresponded to the second band (containing two spots) of (smaller sized) pre-β_2-LpA-I. The first spot of (larger sized) pre-β_2-LpA-I corresponded to the fourth band of pre-β_2-LpE. In addition, apoA-I, apoA-II, and apoC-III were detected in the same region of the gel as small pre-β_2-LpE and α-LpE (Fig. 3). No apoB-100 (as detected with monoclonal antibody MB47 (32)) was found in association with apoE-containing HDL-sized subfractions (data from two separate experiments not shown).

As previous studies had investigated human plasma γ-LpE (28, 29), subsequent experiments focused on pre-β_1-LpE, pre-β_2-LpE, and α-LpE, which represented >90% of apoE in HDL-sized lipoproteins (as assessed by densitometric scanning of radiographic films). HDL containing apoE had previously been described as having α-electrophoretic mobility in agarose gels (28, 35); however, we consistently observed that the majority of apoE in HDL-sized lipoproteins migrated with pre-β mobility and only a small proportion co-migrated with α-migrating HDL lipid (Fig. 1). This was substantiated by separating lipoproteins by automated gel filtration chromatography (on an FPLC system), a separation technique which, unlike ultracentrifugation (UTC), does not cause dissociation of apoE from lipoprotein particles (36). FPLC-isolated apoE-containing HDL (21) had fast pre-β or slow α-mobility in one-dimensional agarose gels (data not shown).

During the course of the present studies, it became apparent that the HDL-apoE subfraction distribution of plasma samples stored at 4°C was not stable over time. Experiments were therefore carried out to determine the effect of time and temperature of storage on plasma HDL-apoE subfraction distribution. As shown for plasma from subject C (Table 1) in **Fig. 4 (a-d)**, keeping the sample at room temperature for 150 min or storing the sample at 4°C for 4 days resulted in a reduction of pre-β_1-LpE (compared to plasma separated immediately). Almost complete disappearance of pre-β_1-LpE was observed when the plasma sample was incubated for 90 min at 37°C (Fig. 4d). These results showed that storage of plasma at 4°C caused the disappearance of pre-β_1-LpE, but did not result in the appearance of HDL-apoE particles different from those that were pres-

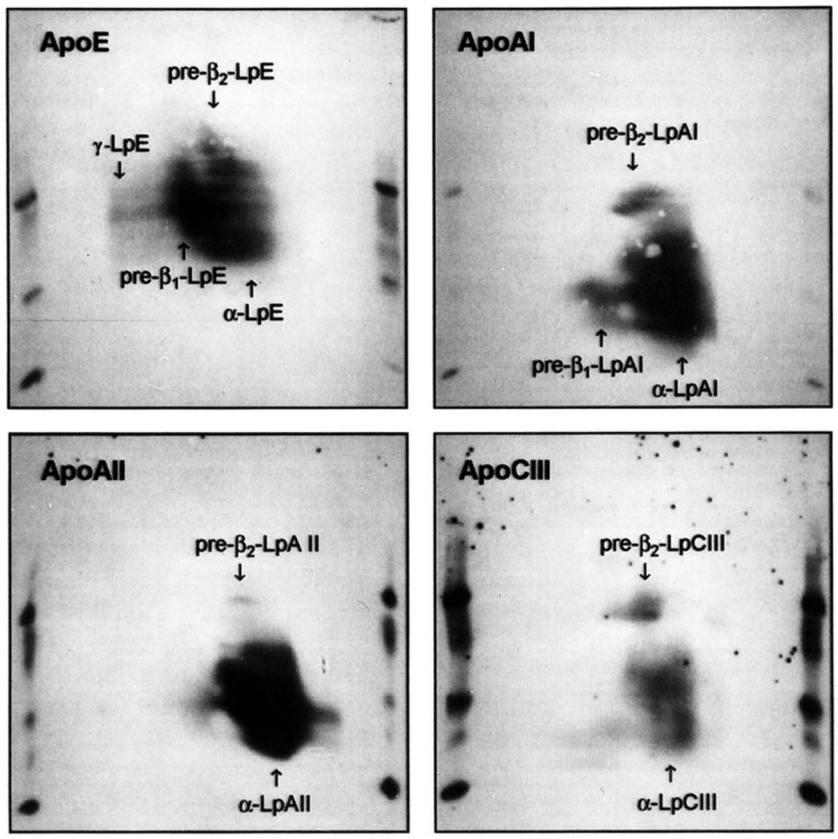

Fig. 2. Presence of apoE in HDL-sized lipoproteins separated by two-dimensional electrophoresis from the plasma of subject B (sample 3, Table 1), in comparison with the presence of apoA-I, apoA-II, and apoC-III (as indicated). On the left- and right-hand sides of each gel are molecular standards, as shown in Fig. 1. Different HDL subpopulations are indicated with arrows.

ent in freshly separated plasma. This, however, was not the case for all subjects, and the characteristic pattern of changes for these latter individuals (n = 3) is exemplified by the plasma of subject B (sample 5, Table 1), which was stored at 4°C with and without preservatives (Fig. 4 e–g). Partial disappearance of pre-β_1-LpE was associated with the appearance of smaller-sized α-migrating fractions (indicated with horizontal arrows in Fig. 4f and 4g), in addition to the appearance of smaller pre-β_1-LpE particles (~9 nm). The appearance of these subfractions could not be inhibited by the addition of a cocktail of preservatives at the time of plasma separation, which included an LCAT inhibitor (dithiobis-2-nitrobenzoic acid, DTNB), a preservative (sodium azide), and a protease inhibitor (aprotinin) (Fig. 4g). Decrease in pre-β_1-LpE (10–13 nm) and increase in smaller pre-β_1-LpE (~9 nm) was also observed after plasma was frozen for periods of 2 weeks to 3 months (data not shown). The effect of freezing was not, however, as great as the effect of storage for 4 days at 4°C, and it is unclear whether this effect was a result of the freezing process itself or was caused by thawing of samples by bringing them to room temperature over a 40-min period.

In order to determine whether the lability (disappearance) of pre-β_1-LpE during plasma incubation at 37°C was dependent on cholesterol esterification, plasma samples (from three individuals) were incubated at 37°C in the presence or absence of dithiobis-2-nitrobenzoic acid (DTNB), an inhibitor of lecithin: cholesterol acyltransferase (LCAT)-mediated cholesterol esterification. As shown for subject D (Table 1) in **Fig. 5,** plasma incubation resulted in almost complete disappearance of pre-β_1-LpE, and DTNB did not inhibit this effect. In two of the three subjects, less than 10% of pre-β_1-LpE remained after incubation with DTNB (as exemplified in Fig. 5), and for the remaining subject there was complete disappearance of pre-β_1-LpE. Reduction in pre-β_1-LpE during plasma incubation is apparently a consequence of apoE redistribution from HDL to less dense fractions, as total plasma HDL apoE concentration decreased 30–40% during incubation, as assessed by measuring apoE concentration in HDL isolated by FPLC (21). DTNB was (as expected from the

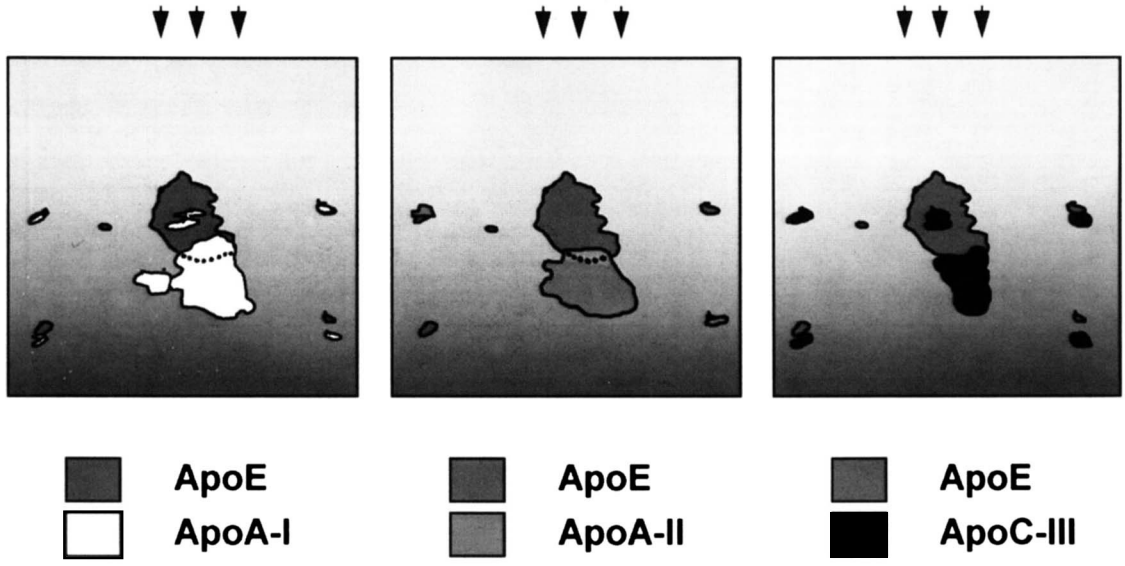

Fig. 3. Diagram showing the position of apoE-containing HDL in relation to HDL apoA-I, apoA-II, and apoC-III after two-dimensional gel electrophoresis. The arrows at the top of each diagram represent (from left to right) the pre-β_1-, pre-β_2-, and α-regions of the gel. The spots on either side of each diagram represent the high and low molecular size standards which were used to superimpose regions of apolipoprotein reactivity.

work of Miida et al. (37)) able to totally prevent the disappearance of pre-β-LpA-I (the band of pre-β-migrating apoA-I in lane A but not lane B in the gel at the bottom right of Fig. 5), indicating that DTNB was indeed active in these experiments.

The potential of HDL-apoE distribution to be affected by in vitro modification of plasma samples during storage is clearly evident from the aforementioned experiments. When samples were handled carefully, however (i.e., blood was immediately placed in ice and plasma was kept below 4°C before prompt separation by electrophoresis), a very reproducible pattern of HDL-apoE distribution could be obtained for the same individual sampled on different occasions. This is illustrated by the data in **Table 2** showing the relative distribution of apoE between HDL subpopulations, as determined by densitometric scanning of radiographic films. Results are given for three subjects (A, B, and C, Table 1), who were sampled on three occasions in the case of subject A and C, and on five occasions (spanning a 5-month period) in the case of subject B. HDL-apoE separations for subject B appear in Figs. 1, 2, 4, 6, and 7. For all three subjects, the majority of HDL-apoE was found in pre-β-LpE (~30% associated with pre-β_1-LpE and 40–50% associated with pre-β_2-LpE), about 25% was associated with α-LpE, and less than 10% was found as γ-LpE. Results for samples obtained on different occasions were reproducible, as exemplified by the plasma analyzed on five different occasions (from subject B), where the standard deviation was 11% or less for the major electrophoretic subpopulations. Results for γ-LpE were less consistent. The number of different-sized fractions in each electrophoretic region (indicated by numbers in parentheses in Table 2) also differed to some extent, which may have been due to physiological or methodological variability. A larger number of different-sized lipoprotein subfractions were consistently observed in the pre-β_2-LpE fraction compared to pre-β_1-LpE or α-LpE.

During the course of screening the plasma of different subjects, we noted that the distribution of apoE-containing HDL subpopulations was in part dependent on apoE phenotype. This effect is demonstrated in **Fig. 6,** in which the separation of apoE-containing HDL is shown for two subjects with an apoE 2/2 phenotype, two subjects with an apoE 3/3 phenotype, and two subjects with an apoE 4/4 phenotype. On the left-hand side are gels for normolipidemic subjects and on the right are gels for mildly hypertriglyceridemic individuals (as documented in Table 1). In general, subjects with an apoE 2/2 phenotype had a greater proportion of α-LpE, whereas individuals with an apoE 4/4 phenotype had a greater proportion of pre-β_1-LpE. Heterozygotic subjects (with an apoE 3/2 or apoE 4/3 phenotype) tended to have an LpE distribution intermediate between those of respective homozygotes, although some exceptions were observed. These data therefore suggested that the presence of a more basic apoE phenotype (apoE 2) caused more HDL-apoE to migrate as α-LpE, and a more acidic apoE phenotype (apoE 4)

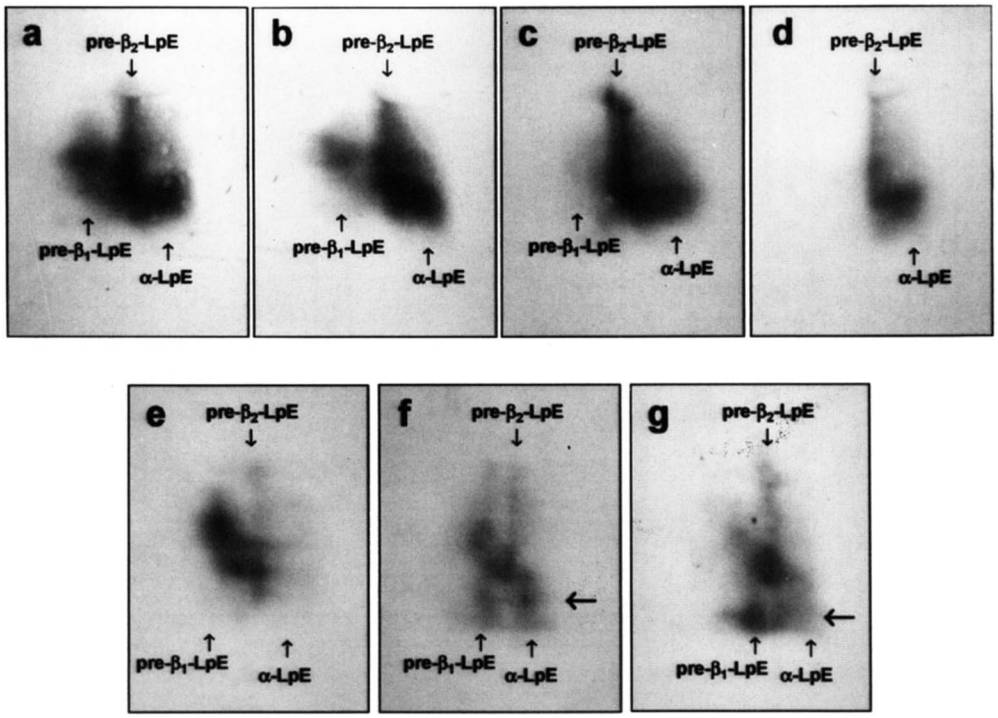

Fig. 4. Effect of time and temperature of plasma storage on the distribution of apoE between HDL-sized lipoprotein subfractions. Top panel: plasma from subject C was (a) kept in ice and electrophoretically separated within 30 min, or (b) maintained at room temperature (22°C) for 150 min, (c) stored for 4 days at 4°C, or (d) incubated for 90 min at 37°C, before being separated by two-dimensional electrophoresis. Decrease in pre-β_1-LpE is evidenced by the reduction of apoE immunoreactivity in the pre-β_1-LpE region of each membrane (indicated with arrows). Bottom panel: plasma from subject B (sample 5, Table 1) was (a) kept in ice and separated within 30 min or was stored for 4 days at 4°C in the (b) presence or (c) absence of a preservative cocktail containing an LCAT inhibitor (dithiobis-2-nitrobenzoic acid, DTNB), a preservative (sodium azide), and a protease inhibitor (aprotinin), before being separated by two-dimensional electrophoresis. Pre-β_1-LpE in freshly separated plasma (indicated in panel e) was less evident in stored plasmas, and in this sample disappearance of pre-β_1-LpE was associated with the appearance of smaller-sized pre-β_2- and α-migrating subfractions (indicated by horizontal arrows in f and g).

caused more HDL-apoE to migrate as pre-β_1-LpE; however, apoE phenotype was clearly not the sole determinant of apoE subfraction distribution.

In order to study different HDL apoE subpopulations in a more physiological context, HDL-apoE distribution was determined in three subjects after an overnight fast and then at 2-h intervals for 8 h after the ingestion of an oral fat load (liquid cream). The aim of these experiments was to determine which subfraction of apoE-containing HDL was most active in donating and/or accepting apoE during postprandial plasma triglyceride lipolysis. Results for two subjects (B (sample 2) and D, Table 1) are shown in **Fig. 7.** Plasma HDL apoE concentration decreased significantly after the fat load and returned to fasting levels within 8 h. HDL-apoE subpopulation distribution was qualitatively very similar in plasma obtained before and 8 h after the fat load. In general, a postprandial decrease and then increase in apoE was observed for all HDL subfractions, and we were unable to identify a specific subfraction that was quantitatively more important in donating or accepting apoE during the postprandial period.

DISCUSSION

We have identified different plasma apoE-containing HDL-sized lipoprotein subpopulations separated on the basis of charge and size by two-dimensional gel electrophoresis. HDL-apoE subfractions had either γ-, pre-β_1-, pre-β_2-, or α-electrophoretic mobility (designated γ-LpE, pre-β_1-LpE, pre-β_2-LpE, and α-LpE, respectively), which varied in size from 9 to 18.5 nm in diameter. In most subjects, pre-β-migrating LpE was the predominant apoE-containing HDL subpopulation and in general 5 to 6 different-sized lipoprotein species were detected in this fraction.

The two-dimensional electrophoretic separation of plasma apoE-containing lipoproteins in the HDL size range has been documented previously (29, 35, 38). The majority of this work has focused on γ-LpE, a lipoprotein fraction which has been proposed to be an important initial acceptor of cell-derived cholesterol (28). We have focused in the present study on the remaining HDL-sized apoE subfractions, which account for >90% of HDL apoE (Table 2). These subfractions have an

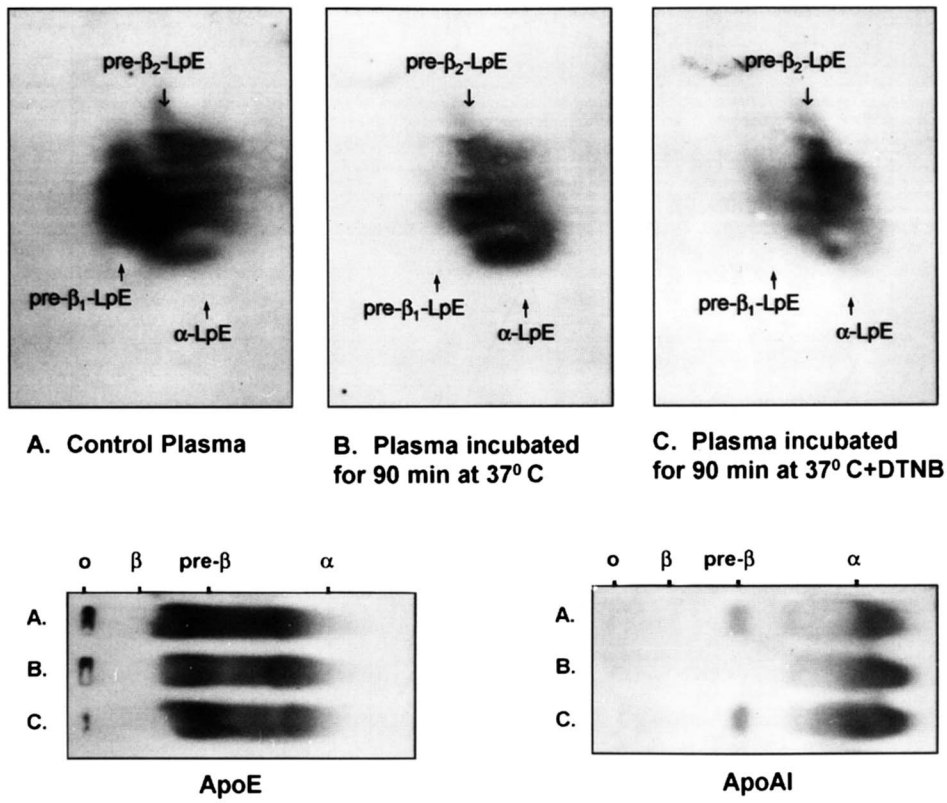

Fig. 5. Effect of plasma incubation on the distribution of apoE in HDL subpopulations in the presence and absence of an LCAT inhibitor. A: control plasma which was kept in ice for 90 min; B: plasma incubated for 90 min at 37°C in the absence of enzyme inhibitor; C: plasma incubated for 90 min at 37°C in the presence of an LCAT inhibitor (dithiobis-2-nitrobenzoic acid, DTNB). The plasma sample was obtained from subject D (Table 1). Top panels show the presence of apoE-containing HDL subpopulations after two-dimensional gel electrophoresis. The bottom two panels show the presence of apoE (left-hand side) and apoA-I (right-hand side) in lipoproteins from the same plasma samples separated by one-dimensional agarose gel electrophoresis. Plasmas were applied at the origin (labeled "O").

agarose gel electrophoretic mobility similar to VLDL lipid and VLDL apoE (Fig. 1) and are thus predominantly pre-β-migrating lipoproteins. A fraction of HDL apoE migrates with slow α-mobility (referred to as α-LpE) and in subjects with an apoE 3/3 phenotype this represents ~25% of HDL apoE (Table 2).

A number of factors are probably responsible for differences in charge (and hence agarose gel electrophoretic mobility) of different HDL-apoE subfractions. Davidson et al. (39) have studied the molecular basis for the difference in agarose electrophoretic mobility of pre-β- and α-migrating apoA-I-containing HDL, and have shown that their net charge is determined by: *1*) the presence of negatively charged phosphatidylinositol molecules on the surface of HDL, and *2*) the conformation of constituent apolipoproteins, determined by the presence of neutral core lipid esters and the subsequent shape of HDL particles (i.e., the disc and spherical shape of pre-β- and α-migrating HDL, respectively). γ-LpE lipoproteins have previously been shown to be spherical (28), but the shape of other LpE subpopulations has not been determined. The shape and lipid composition of LpE lipoproteins probably influences their overall charge, as does the number and/or conformation of apoE molecules (40) on each particle. The phenotype of apoE is also able to influence the agarose electrophoretic mobility of LpE (Fig. 6), and the presence of a more basic apoE phenotype (apoE 2) causes more HDL-apoE to migrate as α-LpE, and a more acidic apoE phonotype (apoE 4) results in more HDL-apoE as pre-β_1-LpE.

It is well recognized that apoE is an apolipoprotein that readily exchanges between different lipoproteins in the circulation (18). We have, however, been able to obtain a reproducible pattern of HDL-apoE subfraction distribution (Table 2), provided that the processing of blood and plasma samples was carefully controlled (i.e., blood was immediately placed in ice, and plasma was maintained below 4°C before prompt separation by electrophoresis). This protocol was necessary in order to avoid the tendency for pre-β_1-LpE to disappear after

TABLE 2. Relative distribution of apoE between HDL subpopulations as determined by densitometric scanning of radiographic films used to detect the presence of apoE in lipoproteins separated by two-dimensional gradient gel electrophoresis

Subject Sample No.	Relative Intensity (%)			
	γ-LpE	Pre-β_1-LpE	Pre-β_2-LpE	α-LpE
A				
1	4.1 (2)[a]	29.0 (2)	38.3 (5)	28.6 (2)
2	8.4 (1)	24.8 (1)	44.6 (6)	22.1 (3)
3	nd	23.9 (1)	51.5 (6)	24.5 (2)
	4.2 ± 4.2[b]	25.9 ± 2.7	44.8 ± 6.6	25.1 ± 3.3
B				
1	10.6 (3)	29.5 (3)	39.5 (6)	20.4 (3)
2	1.4 (2)	30.3 (3)	42.6 (6)	25.6 (4)
3	7.1 (2)	30.7 (3)	41.5 (7)	20.7 (3)
4	nd	32.1 (1)	43.9 (5)	24.1 (4)
5	5.3 (2)	34.1 (3)	38.7 (5)	21.9 (2)
	4.9 ± 4.3	31.3 ± 1.6	41.2 ± 1.9	22.5 ± 2.3
C				
1	nd	24.0 (2)	48.6 (6)	27.3 (2)
2	nd	22.5 (3)	50.2 (6)	27.3 (2)
3	nd	30.0 (2)	42.3 (6)	27.7 (2)
		25.5 ± 4.0	47.0 ± 4.2	27.5 ± 0.2

[a]Numbers in parentheses represent the number of apoE spots detectable in each subfraction; nd, not detectable.

[b]Mean values (± SD) are shown for each subpopulation.

plasma isolation. The lability of pre-β_1-LpE was evidenced by a reduction of pre-β_1-LpE in plasma maintained at room temperature, in plasma stored for 4 days at 4°C, or in plasma incubated in vitro (90 min at 37°C) (Fig. 4). The disappearance of pre-β_1-LpE in these cases could have been due to: *a*) conversion of pre-β_1-LpE lipoproteins to other HDL or non-HDL fractions; *b*) transfer of apoE to other HDL or non-HDL fractions; *c*) modification of apoE (e.g., dimerization) resulting in a change in pre-β_1-LpE electrophoretic mobility; or *d*) proteolytic degradation of apoE. Disappearance of pre-β_1-LpE was not, however, prevented by protease or LCAT inhibition (Figs. 4 and 5), suggesting that neither proteolysis nor cholesteryl ester esterification was responsible. As the sulfhydryl reagent DTNB (used in the present experiments to inhibit LCAT) also inhibits dimerization, this mechanism can also be excluded. Evidence for redistribution of apoE to lower density lipoproteins was obtained when plasma was incubated at 37°C, and in storage experiments conducted at 4°C evidence was obtained for the appearance of apoE-containing HDL particles that were not present in freshly separated plasma (Fig. 4). The lability of plasma pre-β_1-LpE, therefore, appears to be associated with a redistribution of apoE to less dense lipoproteins or in certain cases, results in the production of HDL species not present in freshly separated plasma. The factors responsible for the lability of pre-β_1-LpE deserve further study, and this phenomenon clearly needs to be carefully controlled in studies (28, 29) that are carried out to assign a physiological role to electrophoretically separated apoE-containing HDL-sized lipoproteins.

We have found that after electrophoretic separation, a significant proportion of HDL-sized lipoproteins containing apoE did not co-migrate with apoA-I, apoA-II, apoC-III, or apoB-100 (Figs. 2 and 3), suggesting that these lipoproteins (i.e., large pre-β-LpE) contain apoE as their major apolipoprotein component. Castro and Fielding (36) have shown, using affinity chromatography, that all apoE in plasma of normolipidemic individuals is associated with either apoA-I or apoB. However, in diabetic and LCAT-deficient patients, apoE unassociated with apoA-I and apoB can be detected (41, 42). Furthermore, γ-LpE (28) and apoE HDL_c (cholesterol-enriched HDL induced by cholesterol feeding (43)) have apoE as their only apolipoprotein component. The balance of evidence thus suggests that plasma apoE-containing lipoproteins exist that do not contain apoA-I or apoB. These lipoproteins have a size similar to HDL; however, it is a misnomer to call these particles "high density lipoproteins" as they do not float in the typical HDL density range (i.e., $1.063 < d < 1.21$ g/ml), nor do they contain apoA-I, the characteristic structural apolipoprotein of HDL. They nevertheless appear to have a metabolic function similar to HDL by playing a role in reverse cholesterol transport.

After the ingestion of a fat-rich meal, circulating HDL is a source of apoE (and other apolipoproteins including apoC-II) necessary for the efficient clearance of plasma triglyceride-rich chylomicrons. Initially, apoE is

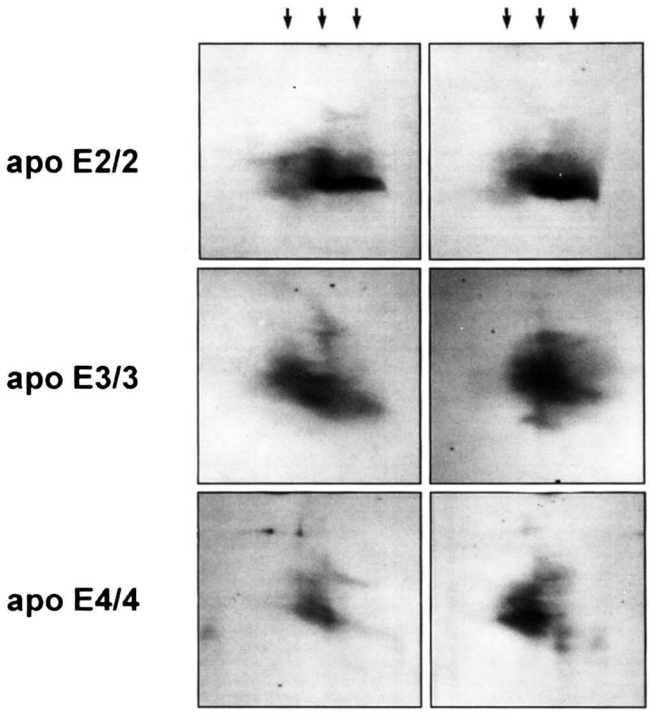

Fig. 6. Effect of apoE phenotype on HDL-apoE subfraction distribution. Representative gels are shown for six individuals, having an apoE 2/2 (top panels, subjects E and F), apoE 3/3 (middle panels, sample 4, subject B and subject G) or apoE 4/4 phenotype (bottom panels, subjects H and I) (Table 1). The arrows at the top of each diagram represent (from left to right) the pre-β_1-, pre-β_2-, and α-regions of the gel. Plasma triglyceride concentration was elevated to a similar extent in subjects F, G, and I (right-hand panels).

transferred from HDL to chylomicrons (27), and during triglyceride hydrolysis apoE is transferred back to HDL (44). This transfer of apoE is associated with an initial postprandial increase in the plasma concentration of TRL apoE and decrease in the concentration of HDL apoE. Reciprocal changes occur 4 to 8 h after meal-feeding (27). In order to establish the relative importance of HDL apoE subfractions in either donating or accepting apoE during the postprandial period, the HDL apoE subfraction distribution of healthy normolipidemic subjects was determined in the fasted and fed state (Fig. 7). The apoE content of all HDL subfractions decreased postprandially in parallel with the initial decrease in HDL apoE concentration. Similarly, at later postprandial time points, the apoE content of all HDL subfractions increased as plasma HDL apoE concentration returned to its fasting level. We have, therefore, been unable to identify an HDL-apoE subfraction that plays a quantitatively more or else less significant role in lipoprotein transfer of apoE in the postprandial state. These results also provide evidence that under normal physiological conditions, relative distribution of apoE among HDL subpopulations is maintained constant.

Although apoE has been implicated in a number of different aspects of HDL metabolism, the physiological roles of HDL apoE need to be further defined. HDL containing essentially apoE alone (apoE HDL_c) have been isolated from the plasma of cholesterol-fed dogs (43). These lipoproteins are rapidly cleared from circulating plasma by the liver, due to efficient uptake by hepatic parenchymal cells through specific receptor-mediated recognition of apoE (22). It has therefore been proposed that apoE-containing HDL play an important role in reverse cholesterol transport (45), by acting as specific vehicles for direct cholesterol delivery to the liver. The direct interaction of apoE-HDL with hepatic cells is supported by the observation that hepatic lipase-catalyzed hydrolysis of phospholipid monolayers is activated by apoE (46) and that hepatic lipase preferentially hydrolyzes the phospholipids of apoE-rich HDL (23). ApoE-containing HDL also appear to play a role in the process of plasma cholesterol esterification, as evidenced by the accumulation of apoE-rich HDL in the plasma of subjects with familial lecithin: cholesterol acyltransferase (LCAT) deficiency (24) and by the ability of discoidal lipid complexes containing apoE to activate LCAT in vitro (25). ApoE-rich HDL also accumulate in the plasma of hyperalphalipoproteinemic subjects with plasma cholesterol ester transfer protein (CETP) deficiency (26) and in hamsters with reduced plasma CETP activity caused by subcutaneous injection of CETP neutralizing monoclonal antibody (47). In vitro experiments have not, however, supported a direct role of HDL apoE in CETP-mediated cholesteryl ester transfer (48, 49). In addition (and as already mentioned), HDL-apoE is involved in mediating the efficient metabolism of postprandial TRL (27) and in mediating the efflux of cell-derived cholesterol (28, 29). Clearly, additional studies are required to define how the distribution of apoE among HDL subfractions is affected by these processes. It is also of interest to know how HDL-apoE subfraction distribution influences the putative ability of HDL-apoE to protect against the development of atherosclerosis (50, 51).

In conclusion, the present results have shown that: *1*) apoE-containing HDL are heterogeneous in size and charge; *2*) pre-β_1-LpE is a relatively labile HDL subfraction; *3*) HDL-apoE subfraction distribution is dependent on apoE phenotype; and *4*) all apoE-containing HDL subfractions participate in the plasma transfer of apoE during the postprandial period. In addition, the present experiments point out the importance of using fresh and carefully prepared plasma samples for accurate two-dimensional electrophoretic analysis of apoE-containing subfractions.

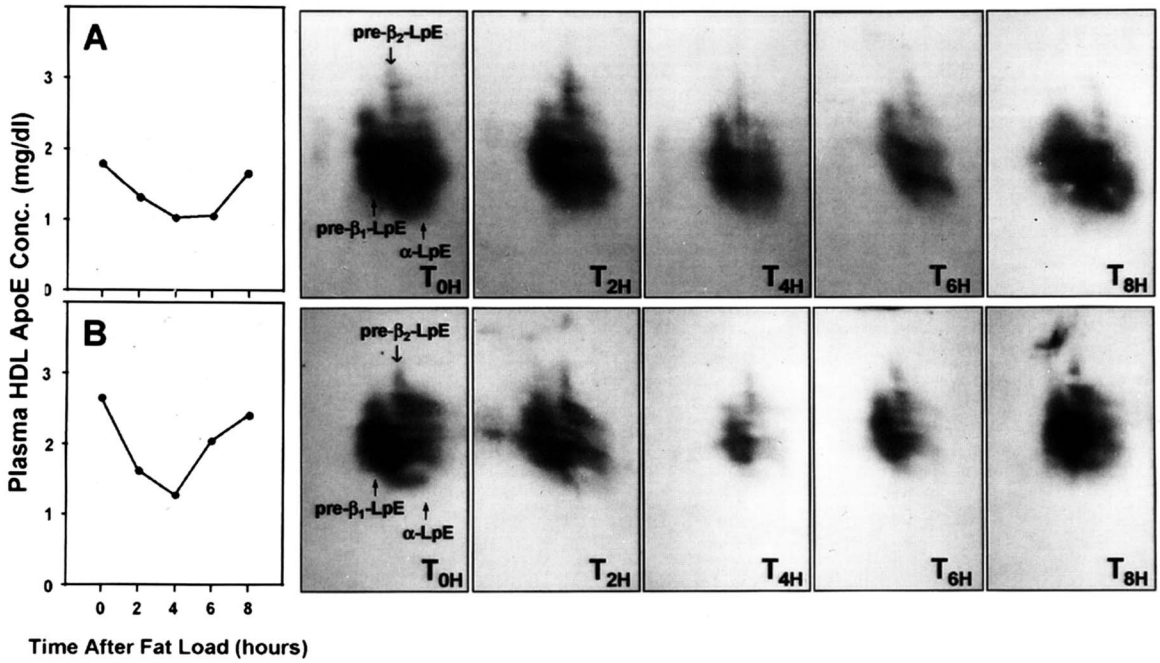

Fig. 7. Plasma HDL-apoE concentration and HDL-apoE subpopulation distribution in two normolipidemic subjects fed an oral fat load (A: subject B, sample 2 and B: subject D (Table 1)). After an overnight fast, subjects were given a liquid cream drink containing 1 gram fat per kg body weight. Blood samples were obtained in the fasting state (0 h) and then at 2-hour intervals for 8 h. ApoE-containing HDL were isolated by gel filtration chromatography (FPLC) and plasma concentration of apoE in this fraction was determined (see Methods). ApoE-containing HDL subpopulations were separated from plasma at each time point and are identified with arrows for plasma obtained in the fasting state (T_{0H}).

The technical assistance of Hélène Jacques and Mireille Amiot is gratefully acknowledged. We would like to thank Dr. Jose Ordovas and Dr. Ernst Schaefer for providing immunopurified polyclonal human apoE antibody prepared by Genzyme Corp. The generous gift of MB47 monoclonal antibody from Dr. Linda Curtiss was also appreciated. We would like to thank the head nurse of the IRCM primary prevention lipid clinic, Denise Dubreuil, for her assistance in obtaining blood samples. This work was supported by a joint University-Industry grant from the Medical Research Council of Canada and Ciba-Geigy Canada Ltd (UI-11407), and by La Succession J.A. De Sève.

Manuscript received 12 June 1996 and in revised form 9 September 1996.

REFERENCES

1. Gordon, D., and B. M. Rifkind. 1989. Current concepts: high density lipoproteins: the clinical implications of recent studies. *N. Engl. J. Med.* **321:** 1311–1315.
2. Brown, M. S., and J. L. Goldstein. 1983. Lipoprotein metabolism in the macrophage: implications for cholesterol deposition in atherosclerosis. *Annu. Review Biochem.* **52:** 223–261.
3. Tall, A. R. 1990. Plasma high-density lipoproteins: metabolism and relationship to atherogenesis. *J. Clin. Invest.* **86:** 379–384.
4. Fielding, C. J., and P. E. Fielding. 1995. Molecular physiology of reverse cholesterol transport. *J. Lipid Res.* **36:** 211–228.
5. Patsch, J. R., J. B. Karlin, L. W. Scott, L. C. Smith, and A. M. Gotto, Jr. 1983. Inverse relationship between blood levels of high density lipoprotein subfraction 2 and magnitude of postprandial lipemia. *Proc. Natl. Acad. Sci. USA.* **80:** 1449–1453.
6. Parthasarathy, S., J. Barnett, and L. G. Fong. 1990. High-density lipoprotein inhibits the oxidative modification of low-density lipoprotein. *Biochim. Biophys. Acta.* **1044:** 275–283.
7. Cockerill, G. W., K-A. Rye, J. R. Gamble, M. A. Vadas, and P. J. Barter. 1995. High density lipoproteins inhibit cytokine-induced expression of endothelial cell adhesion molecules. *Arterioscler. Thromb. Vasc. Biol.* **15:** 1987–1994.
8. von Eckardstein, A., Y. Huang, and G. Assman. 1994. Physiological role and clinical significance of high-density lipoprotein subclasses. *Curr. Opin. Lipidol.* **5:** 404–416.
9. Leroy, A., J. Dallongeville and J-C. Fruchart. 1995. Apolipoprotein A-I-containing lipoproteins and atherosclerosis. *Curr. Opin. Lipidol.* **6:** 281–285.
10. De Lalla, O. F., and J. W. Gofman. 1954. Ultracentrifugal analysis of serum lipoproteins. *Methods Biochem. Anal.* **1:** 459–478.
11. Blanche, P. J., E. L. Gong, T. M. Forte, and A. V. Nicols. 1981. Characterization of human high-density lipoproteins by gradient gel electrophoresis. *Biochim. Biophys. Acta.* **665:** 408–419.
12. Kunitake, S. T., K. J. La Sala, and J. P. Kane. 1985. Apolipoprotein A-I-containing lipoproteins with pre-beta electrophoretic mobility. *J. Lipid Res.* **26:** 549–555.

13. Castro, G. R., and C. J. Fielding. 1988. Early incorporation of cell-derived cholesterol into pre-β-migrating HDL. *Biochemistry.* **27:** 25–29.
14. Asztalos, B. F., C. H. Sloop, L. Wong, and P. S. Roheim. 1993. Two-dimensional electrophoresis of plasma lipoproteins: recognition of new apoA-I-containing subpopulations. *Biochim. Biophys. Acta.* **1169:** 291–300.
15. Marcel, Y. L., P. K. Weech, T. D. Nguyen, R. W. Milne, and W. J. McConathy. 1984. Apolipoproteins as the basis for heterogeneity in high density lipoprotein$_2$ and high density lipoprotein$_3$. *Eur. J. Biochem.* **143:** 467–476.
16. Cheung, M., and J. J. Albers. 1984. Characterization of lipoprotein particles by immunoaffinity chromatography: particles containing A-I and A-II and particles containing A-I but no A-II. *J. Biol. Chem.* **259:** 12201–12209.
17. von Eckardstein, A., Y. Huang, S. Wu, A. S. Sarmadi, S. Schwartz, A. Steinmetz, and G. Assmann. 1995. Lipoproteins containing apolipoprotein A-IV but not apolipoprotein A-I take up and esterify cell-derived cholesterol in plasma. *Arterioscler. Thromb. Vasc. Biol.* **15:** 1755–1763.
18. Mahley, R. W. 1988. Apolipoprotein E: cholesterol transport protein with expanding role in cell biology. *Science.* **240:** 622–630.
19. Bersot, T. P., R. W. Mahley, M. S. Brown, and J. L. Goldstein. 1976. Interaction of swine lipoproteins with the low density lipoprotein receptor in human fibroblasts. *J. Biol. Chem.* **251:** 2395–2398.
20. Kowal, R. C., J. Herz, J. L. Goldstein, V. Esser, and M. S. Brown. 1979. Low density lipoprotein receptor-related protein mediates uptake of cholesteryl esters derived from apoprotein E-enriched lipoproteins. *Proc. Natl. Acad. Sci. USA.* **86:** 5810–5814.
21. Cohn, J. S., M. Tremblay, M. Amiot, D. Bouthillier, M. Roy, J. Genest, Jr., and J. Davignon. 1996. Plasma concentration of apolipoprotein E in intermediate-sized remnant-like lipoproteins in normolipidemic and hyperlipidemic subjects. *Arterioscler. Thromb. Vasc. Biol.* **16:** 149–159.
22. Mahley, R. W., T. L. Innerarity, K. H. Weisgraber, and S. Y. Oh. 1979. Altered metabolism (in vivo and in vitro) of plasma lipoproteins after selective chemical modification of lysine residues of the apoproteins. *J. Clin. Invest.* **64:** 743–750.
23. Thuren, T., K. H. Weisgraber, P. Sisson, and M. Waite. 1992. Role of apolipoprotein E in hepatic lipase-catalyzed hydrolysis of phospholipid in high-density lipoproteins. *Biochemistry.* **31:** 2332–2338.
24. Mitchell, C. D., W. C. King, K. R. Applegate, T. Forte, J. A. Glomset, K. R. Norum, and E. Gjone. 1980. Characterization of apolipoprotein E-rich high density lipoproteins in familial lecithin:cholesterol acyltransferase deficiency. *J. Lipid Res.* **21:** 625–634.
25. Zorich, N., A. Jonas, and H. J. Pownall. 1985. Activation of lecithin:cholesterol acyltransferase by human apolipoprotein E in discoidal complexes with lipids. *J. Biol. Chem.* **260:** 8831–8837.
26. Yamashita, S., D. L. Sprecher, N. Sakai, Y. Matsuzawa, S. Tarui, and D. Y. Hui. 1990. Accumulation of apolipoprotein E-rich high density lipoproteins in hyperalphalipoproteinemic human subjects with plasma cholesteryl ester transfer protein deficiency. *J. Clin. Invest.* **86:** 688–695.
27. Blum C. B. 1982. Dynamics of apolipoprotein E metabolism in humans. *J. Lipid Res.* **23:** 1308–1316.
28. Huang, Y., A. von Eckardstein, S. Wu, N. Maeda, and G. Assmann. 1994. A plasma lipoprotein containing only apolipoprotein E and with γ mobility on electrophoresis releases cholesterol from cells. *Proc. Natl. Acad. Sci. USA.* **91:** 1834–1838.
29. Huang, Y., A. von Eckardstein, S. Wu, and G. Assmann. 1995. Effects of the apolipoprotein E polymorphism on uptake and transfer of cell-derived cholesterol in plasma. *J. Clin. Invest.* **96:** 2693–2701.
30. Laemmli, U. K. 1970. Cleavage of structural protein during asembly of the head of bacteriophage T_4. *Nature.* **227:** 680–685.
31. Salacinski, P. R., C. McLean, J. E. Sykes, V. V. Clement-Jones, and P. J. Lowry. 1981. Iodination of proteins, glycoproteins, and peptides using a solid-phase oxidizing agent, 1,3,4,6-tetrachloro-3 alpha, 6 alpha-diphenyl glycoluril (Iodogen). *Anal. Biochem.* **117:** 136–146.
32. Young, S. C., J. L. Witztum, D. C. Casal, L. K. Curtiss, and S. Bernstein. 1986. Conservation of the low density lipoprotein-receptor domain of apoprotein B demonstrated by new monoclonal antibody, MB47. *Arteriosclerosis.* **6:** 178–188.
33. Hill, J. S., and P. H. Pritchard. 1990. Improved phenotyping of apolipoprotein E: application to population frequency distribution. *Clin. Chem.* **36:** 1871–1874.
34. Francone, O. L., A. Gurakar, and C. Fielding. 1989. Distribution and functions of lecithin:cholesterol acyltransferase and cholesteryl ester transfer protein in plasma lipoproteins. Evidence for a functional unit containing these activities together with apolipoproteins A-I and D that catalyzes the esterification and transfer of cell-derived cholesterol. *J. Biol. Chem.* **264:** 7066 - 7072.
35. von Eckardstein, A., Y. Huang, S. Wu, H. Funke, G. Noseda, and G. Assmann. 1995. Reverse cholesterol transport in plasma of patients with different forms of familial HDL deficiency. *Arterioscler. Thromb. Vasc. Biol.* **15:** 691–703.
36. Castro, G. R., and C. J. Fielding. 1984. Evidence for the distribution of apolipoprotein E between lipoprotein classes in human normocholesterolemic plasma and for the origin of unassociated apolipoprotein E (Lp-E). *J. Lipid Res.* **25:** 58–67.
37. Miida, T., M. Kawano, C. J. Fielding, and P. E. Fielding. 1992. Regulation of the concentration of preβ high-density lipoprotein in normal plasma by cell membranes and lecithin-cholesterol acyltransferase activity. *Biochemistry.* **31:** 11112–11117.
38. Francone, O. L., C. J. Fielding, and P. E. Fielding. 1990. Distribution of cell-derived cholesterol among plasma lipoproteins: a comparison of three techniques. *J. Lipid Res.* **31:** 2195–2200.
39. 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.
40. Lund-Katz, S., K. H. Weisgraber, R. W. Mahley, and M. C. Phillips. 1993. Conformation of apolipoprotein E in lipoproteins. *J. Biol. Chem.* **268:** 23008–23015.
41. Fielding, C. J., G. R. Castro, C. Donner, P. E. Fielding, and G. M. Reaven. 1986. Distribution of apolipoprotein E in the plasma of insulin-dependent and non-insulin-dependent diabetics and its relation to cholesterol net transport. *J. Lipid Res.* **27:** 1052–1061.
42. Fielding, C. J., J. Frohlich, K. Moser, and P. E. Fielding. 1982. Promotion of sterol efflux and net transport by apolipoprotein E in lecithin:cholesterol acyltransferase deficiency. *Metabolism.* **31:** 1023–1028.
43. Mahley, R. W., T. L. Innerarity, K. H. Weisgraber, and D. L. Fry. 1977. Canine hyperlipoproteinemia and athero-

sclerosis. Accumulation of lipid by aortic medial cells in vivo and in vitro. *Am. J. Pathol.* **87:** 205–225.
44. Gibson, J. C., and W. V. Brown. 1988. Effect of lipoprotein lipase and hepatic triglyceride lipase activity on the distribution of apolipoprotein E among the plasma lipoproteins. *Atherosclerosis.* **75:** 45–55.
45. Mahley, R. W., T. L. Innerarity, and K. H. Weisgraber. 1980. Alterations in metabolic activity of plasma lipoproteins following selective chemical modification of the apoproteins. *Ann. NY Acad. Sci.* **348:** 265–277.
46. Thuren, T., R. W. Wilcox, P. Sisson, and M. Waite. 1991. Hepatic lipase hydrolysis of lipid monolayers. Regulation by apolipoproteins. *J. Biol. Chem.* **266:** 4853–4861.
47. Evans, G. F., W. R. Bensch, L. D. Apelgren, D. Bailey, R. F. Kauffman, T. F. Bumol, and S. H. Zuckerman. 1994. Inhibition of cholesteryl ester transfer protein in normocholesterolemic and hypercholesterolemic hamsters: effect on HDL subspecies, quantity, and apolipoprotein distribution. *J. Lipid Res.* **35:** 1634–1645.
48. Marcel, Y. L., C. Vézina, D. Emond, R. B. Verdery, and R. W. Milne. 1981. High density lipoprotein subfractions isolated by heparin-Sepharose affinity chromatography and their role in cholesteryl ester transfer to very low density lipoproteins. *J. Lipid Res.* **22:** 1198–1205.
49. Marcel, Y. L., C. Vézina and R. W. Milne. 1983. Cholesteryl ester and apolipoprotein E transfer between human high density lipoproteins and chylomicrons. *Biochim. Biophys. Acta.* **750:** 411–417.
50. Saxena, U., E. Ferguson, and C. L. Bisgaier. 1993. Apolipoprotein E modulates low density lipoprotein retention by lipoprotein lipase anchored to the subendothelial matrix. *J. Biol. Chem.* **268:** 14812–14819.
51. Wilson, H. M., J. C. Patel, D. Russell, and E. R. Skinner. 1993. Alterations in the concentration of an apolipoprotein E-containing subfraction of plasma high density lipoprotein in coronary heart disease. *Clin. Chim. Acta.* **220:** 175–187.