

Effects of reconstituted HDL on charge-based LDL subfractions as characterized by capillary isotachopheresis

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Abstract Modified LDL in human plasma including small, dense LDL (sdLDL) and oxidized LDL carries a more negative charge than unmodified LDL and is atherogenic. We examined the effects of apolipoprotein A-I (apoA-I)/POPC discs on charge-based LDL subfractions as determined by capillary isotachopheresis (cITP). Three normal healthy subjects and seven patients with metabolic disorders were included in the study. LDL in human plasma was separated into two major subfractions, fast- and slow-migrating LDL (fLDL and sLDL), by cITP. Normal LDL was characterized by low fLDL, and mildly oxidized LDL in vitro and mildly modified LDL in human plasma were characterized by increased fLDL. Moderately oxidized LDL in vitro and moderately modified LDL in a patient with hypertriglyceridemia and HDL deficiency were characterized by both increased fLDL and a new LDL subfraction with a faster mobility than fLDL [very-fast-migrating LDL as determined by cITP (vLDL)]. cITP LDL subfractions with faster electrophoretic mobility (fLDL vs. sLDL, vLDL vs. fLDL) were associated with an increased content of sdLDL. Incubation of a plasma fraction with $d > 1.019$ g/ml (depleted of triglyceride-rich lipoproteins) in the presence of apoA-I/POPC discs at 37°C greatly decreased vLDL and fLDL but increased sLDL. Incubation of whole plasma from patients with an altered distribution of cITP LDL subfractions in the presence of apoA-I/POPC discs also greatly decreased fLDL but increased sLDL. ApoA-I/POPC discs decreased the cITP fLDL level, the free cholesterol concentration, and platelet-activating factor acetylhydrolase activity in the sdLDL subclasses ($d = 1.040$ – 1.063 g/ml) and increased the size of LDL. ApoA-I/POPC discs reduced charge-modified LDL in human plasma by remodeling cITP fLDL into sLDL subfractions.—Zhang, B., Y. Uehara, S. Hida, S-i. Miura, D. L. Rainwater, M. Segawa, K. Kumagai, K-A. Rye, and K. Saku. Effects of reconstituted HDL on charge-based LDL subfractions as characterized by capillary isotachopheresis. *J. Lipid Res.* 2007. 48: 1175–1189.

Supplementary key words charge-based low density lipoprotein subfractions • plasma density > 1.019 g/ml fraction • small, dense low density lipoprotein subclasses • low density lipoprotein size

Qualitatively modified LDL in plasma is characterized by an increased negative charge and has been shown to be atherogenic (1). Mildly modified LDL with an increased negative charge is from multiple origins and could contribute to atherogenesis via several mechanisms, as a result of its proinflammatory, proapoptotic, and antiangiogenesis properties [see review by Sanchez-Quesada, Benitez, and Ordonez-Llanos (1)]. Currently, two techniques are used to separate and quantify charge-based LDL subfractions: ion-exchange chromatography (2–4) and capillary isotachopheresis (cITP) (5–7). The ion-exchange chromatography technique separates LDL isolated from plasma by ultracentrifugation into electronegative LDL [LDL(–)] and electropositive LDL [LDL(+)], whereas the cITP technique separates LDL into fast-migrating LDL (fLDL)

Abbreviations: apoA-I, apolipoprotein A-I; apoB, apolipoprotein B; CETP, cholesteryl ester transfer protein; cITP, capillary isotachopheresis; FC, free cholesterol; fHDL, fast-migrating HDL as determined by cITP; fLDL, fast-migrating LDL as determined by cITP; fTRL, fast-migrating triglyceride-rich lipoprotein as determined by cITP; GGE, gradient gel electrophoresis; HC, hypercholesterolemia; HDL, HDL deficiency; HDL-PAF-AH, HDL-associated platelet-activating factor acetylhydrolase; HTG, hypertriglyceridemia; idLDL, intermediate density LDL; iHDL, intermediate-migrating HDL as determined by cITP; lbLDL, large, buoyant LDL; LDL(–), electronegative LDL as separated by ion-exchange chromatography; LDL(+), electropositive LDL as separated by ion-exchange chromatography; LDL-PAF-AH, LDL-associated platelet-activating factor acetylhydrolase; NL, normolipidemia; OxLDL Ab, oxidized LDL antibody; PL, phospholipid; sdLDL, small, dense LDL; sHDL, slow-migrating HDL as determined by cITP; sLDL, slow-migrating LDL as determined by cITP; sTRL, slow-migrating triglyceride-rich lipoprotein as determined by cITP; TC, total cholesterol; TG, triglyceride; TRL, triglyceride-rich lipoprotein; vLDL, very-fast-migrating LDL as determined by cITP; vsTRL, very-slow-migrating triglyceride as determined by cITP.

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and slow-migrating LDL (sLDL) directly in plasma (5, 6). We previously showed that fLDL represents an electro-negative LDL subfraction and that levels of fLDL and sLDL in ultracentrifugally isolated LDL were proportional to the protein content of LDL (7). We also showed that levels of apolipoprotein B (apoB)-containing lipoprotein subfractions that were directly determined in plasma by cITP are proportional to the amount of apoB-containing lipoproteins (8).

HDL therapy through the infusion of reconstituted HDL or synthetic apolipoprotein A-I (apoA-I)/phospholipid (PL) complexes is attracting attention as a therapeutic approach for acute coronary syndromes (9). A recombinant apoA-I_{Milano}/PL complex administered intravenously in five doses at weekly intervals has been shown to produce a significant regression of coronary atherosclerosis in patients with acute coronary syndrome (10). Infusion of reconstituted HDL containing apoA-I and POPC rapidly normalized endothelium-dependent vasodilation in patients with hypercholesterolemia (HC) (11). Also, reconstituted HDL inhibited the C-reactive protein-induced expression of inflammatory adhesion molecules much more potently than plasma HDL (12). The ability of apoA-I/POPC discs to increase pre β -migrating HDL (13) and enhance the removal of lipids from arteries (14) is one of the mechanisms by which apoA-I/POPC discs cause a regression of atherosclerosis. We previously showed that *in vitro* incubation of apoA-I/POPC discs with human plasma HDL rapidly remodeled fast-migrating HDL (fHDL) and intermediate-migrating HDL (iHDL) into slow-migrating HDL (sHDL) as characterized by cITP (15), providing a mechanism for the rapid increase in pre β -migrating HDL observed in human subjects (13).

Nanjee et al. (13), who studied the acute effects of apoA-I/POPC discs on plasma lipoproteins in human subjects, observed a significant decrease in apoB during the infusion of apoA-I/POPC discs (4 h). Koizumi et al. (16) also found that apoHDL/PL complexes reduced non-HDL cholesterol in hyperlipidemic rabbits at 3 to 6 h after infusion of apoA-I/POPC discs for 30 min. Although the mechanism by which apoA-I/POPC discs decrease non-HDL cholesterol is not fully understood, LDL has been shown to interact with apoA-I/POPC discs and to take up PLs to increase the LDL PL/protein weight ratio and change the size distribution of LDL (17). Whether or not some interaction between apoA-I/POPC discs and LDL may change the charge distribution of LDL particles has not been examined. Because LDL(-) from both normolipidemic subjects and patients with familial HC has been shown to have impaired binding affinity to LDL receptors compared with LDL(+) as a result of increased NEFA and lysophosphatidylcholine (18), it is possible that an interaction between apoA-I/POPC discs and LDL may cause a change in the distribution of charge-based LDL subfractions and thus an increase in the clearance of LDL, resulting in decreased apoB levels.

Navab et al. (19) demonstrated that apoA-I/POPC discs can remove lipid hydroperoxides from human LDL and drastically reduce the inflammatory properties of LDL, as

assessed by the ability of LDL to induce lipid hydroperoxide formation and monocyte chemotactic activity in human artery wall cells. These findings also encouraged us to test the hypothesis that apoA-I/POPC discs remodel LDL to decrease its electronegative mobility. Because LDL(-) separated by chromatography techniques from both normal subjects (3) and patients with familial HC (20) has been shown to be inflammatory, it is possible that a drastic reduction in the inflammatory properties of LDL within 6 h after the infusion of apoA-I/POPC discs into human subjects (19) may be attributable to a reduction of modified LDL with increased negative charge.

In this study, the effects of apoA-I/POPC discs on charge-based LDL subfractions as determined by cITP were examined 1) by incubating plasma depleted of triglyceride-rich lipoproteins (TRLs) from subjects who had different distributions of cITP LDL subfractions and 2) by incubating whole plasma from subjects who had increased cITP fLDL from different origins in the presence of apoA-I/POPC discs. We also examined the effects of apoA-I/POPC discs on the lipid and enzyme composition of LDL, the distributions of cITP LDL subfractions, lipids, and LDL-associated enzyme platelet-activating factor acetylhydrolase (LDL-PAF-AH) in LDL density subclasses, including large, buoyant LDL (lbLDL; $d = 1.019\text{--}1.035$ g/ml), intermediate density LDL (idLDL; $d = 1.035\text{--}1.040$ g/ml), and small, dense LDL (sdLDL; $d = 1.040\text{--}1.063$ g/ml) subclasses (21), and the size of LDL.

METHODS

Subjects

Three volunteer subjects and seven outpatients at Fukuoka University were included in the study. Volunteer subjects were normal healthy subjects with normolipidemia (NL) or HC. Patients were randomly selected based on increased sdLDL cholesterol level (43 mg/dl), hypertriglyceridemia (HTG), HDL deficiency (HDL), increased oxidized LDL antibody (OxLDL Ab) concentration (764 mU/ml), increased LDL-PAF-AH activity (657 IU/l), diabetes mellitus, or being on hemodialysis. Increased OxLDL Ab level and LDL-PAF-AH activity were defined as greater than the respective 75th percentiles (378 mU/ml and 591 IU/l) in 60 outpatients (37 females and 23 males) who were entered in a clinical trial for HC. This study was approved by the Ethics Committee of Fukuoka University Hospital, and written informed consent was obtained from each patient. Overnight fasting blood was drawn on EDTA-Na₂, and butylated hydroxytoluene at a final concentration of 20 μ M was added to the isolated plasma. Aliquots of plasma for cITP analysis were protected with nitrogen gas and snap-frozen with liquid nitrogen and preserved as described previously (7). Plasma for the isolation of LDL and the $d > 1.019$ g/ml plasma fraction for incubation with apoA-I/POPC discs were used immediately without storage.

Measurement of lipid parameters

Plasma levels of total cholesterol (TC), triglyceride (TG), HDL-cholesterol, LDL-cholesterol, and free cholesterol (FC) were measured by enzymatic methods on an autoanalyzer (Hitachi 7600-020S; Hitachi High-Technologies, Tokyo, Japan) using reagent kits from Daiichi Pure Chemicals Co., Ltd. (Tokyo,

Japan). OxLDL Ab concentration was measured by enzyme immunoassay using a commercially available kit (OLAB; Biomedica, Vienna, Austria). LDL-PAF-AH activity was measured using an Azwell Auto PAF-AH reagent kit (Alfresa, Osaka, Japan) as described previously (22). The lipoprotein [a] level was measured by an agglutination turbidimetric method using the lipoprotein [a] Latex Daiichi kit (Daiichi Pure Chemicals).

Separation of plasma lipoprotein fractions

ApoB-depleted plasma, which contained plasma proteins and HDL fraction, was prepared by the precipitation of apoB-containing lipoproteins including TRL and LDL with phosphotungstate acid/MgCl₂, as described previously (23). LDL was isolated from EDTA plasma by sequential ultracentrifugation (7), and 10 μ l of the isolated LDL (0.5 mg/ml) was added to 6 μ l of apoB-depleted plasma for cITP analysis (7). Mildly and moderately oxidized LDL were prepared by incubating LDL (0.5 mg/ml) with CuSO₄ (2.5 μ M) at 37°C for 20 and 80 min, respectively.

TRL-depleted plasma, containing plasma proteins, HDL, and LDL, was prepared by isolating the plasma $d > 1.019$ g/ml fraction from TRL including VLDL and intermediate density lipoprotein by ultracentrifugation, as described previously (8). The density of the plasma was adjusted to 1.019 g/ml with solid KBr and overlaid with $d = 1.019$ g/ml KBr solution at a volume ratio of 7:3. The plasma $d > 1.019$ g/ml fraction (bottom 7/10th fraction) was collected using a tube slicer so that the volume of the isolated plasma fraction was comparable to that of the whole plasma. Plasma $d > 1.035$, 1.040, and 1.063 g/ml fractions were separated similarly. All of the plasma fractions were used for cITP analysis and the measurement of lipid parameters directly without excluding KBr by dialysis.

Preparation of apoA-I/POPC discs

ApoA-I/POPC discs, at a molar ratio of apoA-I to POPC of 1:92.5, were prepared by the cholate dialysis method (24) as described previously (25). Aliquots of apoA-I/POPC discs at apoA-I concentrations of 2.40 or 4.23 mg/ml were frozen at -80°C until use and were only thawed immediately before use.

In vitro incubation experiment procedures

The effects of apoA-I/POPC discs on cITP LDL subfractions were examined by incubating the plasma $d > 1.019$ g/ml fraction and whole plasma in the presence of apoA-I/POPC discs at apoA-I concentrations of 0.5 mg/ml and 0.8 or 1.2 mg/ml, respectively, at 37°C for the indicated times. Incubation was performed in a water bath under the protection of N₂ gas and was stopped by cooling the samples on ice. Nanjee et al. (13) and Navab et al. (19) administered apoA-I/POPC discs to male subjects as an intravenous infusion at a dose of 40 mg apoA-I/kg body weight (26), which is equivalent to 0.8–1.0 mg apoA-I/ml plasma in men. In this study, the amounts of apoA-I/POPC discs (0.5, 0.8, and 1.2 mg/ml apoA-I) were equivalent to those used in human studies.

Plasma LCAT activity was inhibited by DTNB, an LCAT inhibitor (final concentration, 2 mM). Plasma cholesteryl ester transfer protein (CETP) activity was inhibited by JTT-705, a CETP inhibitor (final concentration, 50 μ M) (23). An excess of JTT-705 was used to ensure total CETP inhibition.

Separation and quantification of lipoprotein subfractions by cITP

cITP of plasma lipoproteins was performed on a Beckman P/ACE MDQ system (Beckman-Coulter, Inc., Tokyo, Japan) according to the method of Böttcher et al. (5) with modifications

as described previously (7, 8, 15, 22, 23, 27–31). Levels of cITP LDL subfractions were expressed as peak areas relative to the internal marker 5-carboxyfluorescein.

Measurement of LDL particle diameter by gradient gel electrophoresis

LDL size was determined by native nondenaturing gradient gel electrophoresis (GGE) using composite gradient gels as described previously (32–34). Sudan Black B was used for lipid staining. LDL size was estimated by measuring median LDL particle diameter (defined as the diameter at which half of the LDL absorbance was on larger, and half on smaller, particles).

Analysis of LDL particles by negative stain electron microscopy

For transmission electron microscopy, the LDL fraction was separated by sequential ultracentrifugation and dialyzed against saline containing 1 mM EDTA (pH 8.0) overnight at 4°C to remove KBr. Then, LDL was dialyzed against a 20 μ M EDTA solution for 2 h at 4°C and negatively stained on formvar-coated grids using 1% uranium acetate. Electron micrographs were taken with a computer-controlled JEOL 1200EX electron microscope (JEOL, Inc., Tokyo, Japan). Images at a final magnification of 150,000 \times were acquired using a high-resolution digital camera. The average diameter of LDL particles was measured by the image-processing software iTEM (Transmission Electron Microscopy Imaging Platform; Olympus Soft Imaging Solutions GmbH, Berlin, Germany).

Measurement of LDL subclass concentrations by NMR

LDL subclass concentrations and mean LDL particle diameter were measured in EDTA plasma with an automated NMR spectroscopic assay as described previously (35). In brief, the particle concentrations of LDL subclasses of large (21.2–23.0 nm) and small (18.0–21.2 nm) LDL particles are derived from the measured amplitudes of the distinct lipid methyl group NMR signals they emit. Weighted-average LDL particle sizes (nm) were calculated from the subclass levels.

Statistical data analysis

Statistical analysis was performed using the SAS (Statistical Analysis System) Software Package (version 9.1; SAS Institute, Inc., Cary, NC) at Fukuoka University. Median values and upper and lower quartiles for the lipid and enzyme composition of LDL were calculated using the Univariate procedure (36). Changes in lipid parameters in LDL fractions before incubation of whole plasma in the absence of apoA-I/POPC discs and after incubation of whole plasma in the presence of apoA-I/POPC discs were examined by the Wilcoxon signed-rank test (36). Significant differences in the frequency distribution of the diameter of LDL particles measured by electron microscopy between plasma without incubation and after incubation in the presence of apoA-I/POPC discs were examined by Mantel-Haenszel Chi-square analysis using the FREQ procedure. All *P* values are two-tailed. The significance level was considered to be 5%.

RESULTS

A schematic diagram of the experimental procedures and the characteristics of the study subjects is shown in Fig. 1.

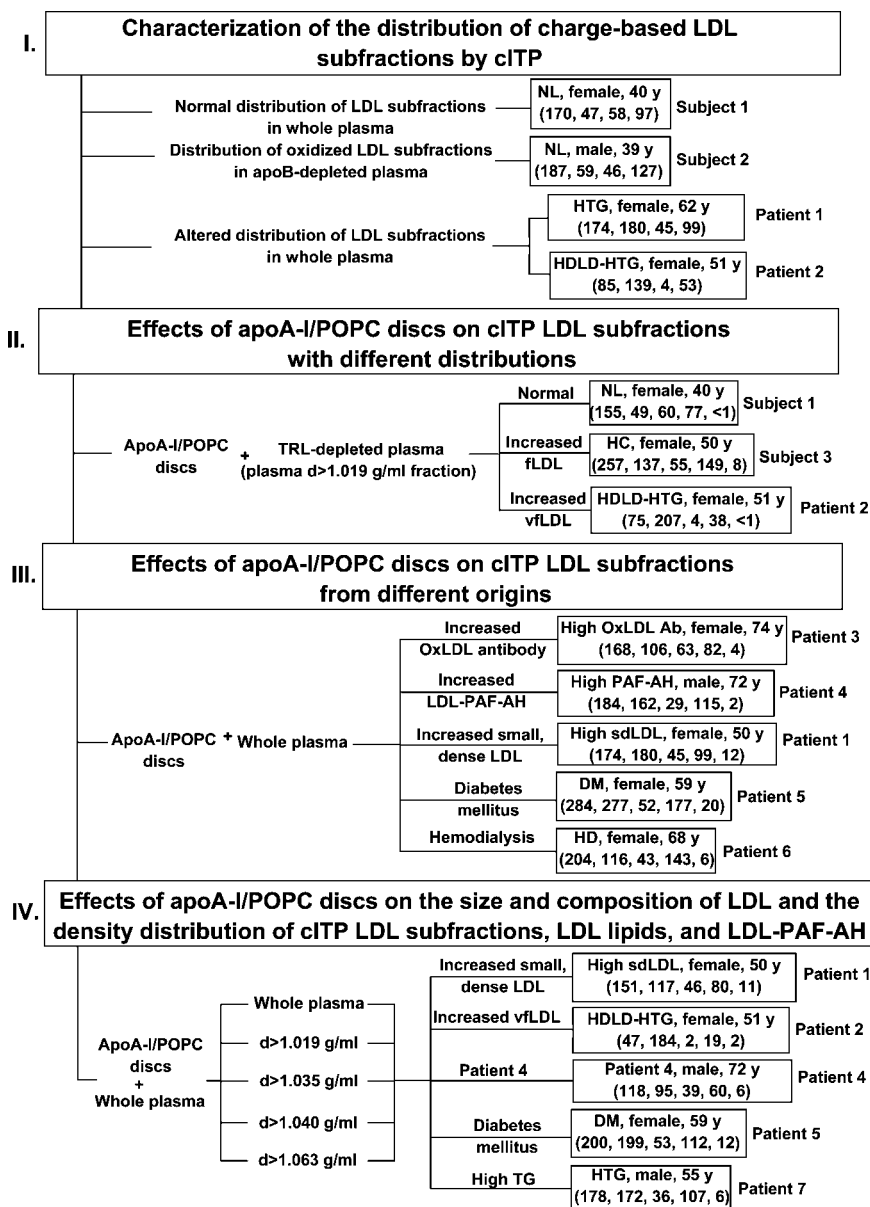


Fig. 1. Schematic diagram of the experiments and the characteristics of the volunteer subjects and outpatients included in this study. Values in parentheses indicate (from left to right) plasma levels of total cholesterol (TC), triglyceride, HDL-cholesterol, LDL-cholesterol, and lipoprotein [a] in mg/dl. apoA-I, apolipoprotein A-I; cITP, capillary isotachopheresis; DM, diabetes mellitus; fLDL, fast-migrating LDL as determined by cITP; HC, hypercholesterolemia; HD, hemodialysis; HDLD, HDL deficiency; HTG, hypertriglyceridemia; LDL-PAF-AH, LDL-associated platelet-activating factor acetylhydrolase; NL, normo-lipidemia; OxLDL Ab, oxidized LDL antibody; sdLDL, small, dense LDL; TG, triglyceride; TRL, triglyceride-rich lipoprotein; vLDL, very-fast-migrating LDL as determined by cITP.

Charge distributions of modified LDL subfractions in vitro and in vivo as characterized by cITP

To examine the effects of apoA-I/POPC discs on modified LDL with increased negative charge, we first examined the charge distributions of LDL in human plasma and LDL oxidatively modified in vitro. **Figure 2A** shows the typical lipoprotein profile in a normal healthy subject with NL (Fig. 1, subject 1) as analyzed by cITP. Plasma lipoproteins were separated into three HDL subfractions (peaks 1–3: fHDL, iHDL, and sHDL), two TRL subfractions [peaks 4

and 5: fast-migrating triglyceride-rich lipoprotein (fTRL) and slow-migrating triglyceride-rich lipoprotein (sTRL)], two major LDL subfractions (peaks 6 and 7: fLDL and sLDL), and a very minor LDL subfraction (peak 8). In the NL subject, cITP fLDL was a minor fraction and sLDL was a major fraction (Fig. 2A).

LDL isolated from a normal healthy subject (Fig. 1, subject 2) by ultracentrifugation was analyzed by cITP using plasma depleted of apoB-containing lipoproteins from the same subject as a plasma protein matrix (30). As shown in

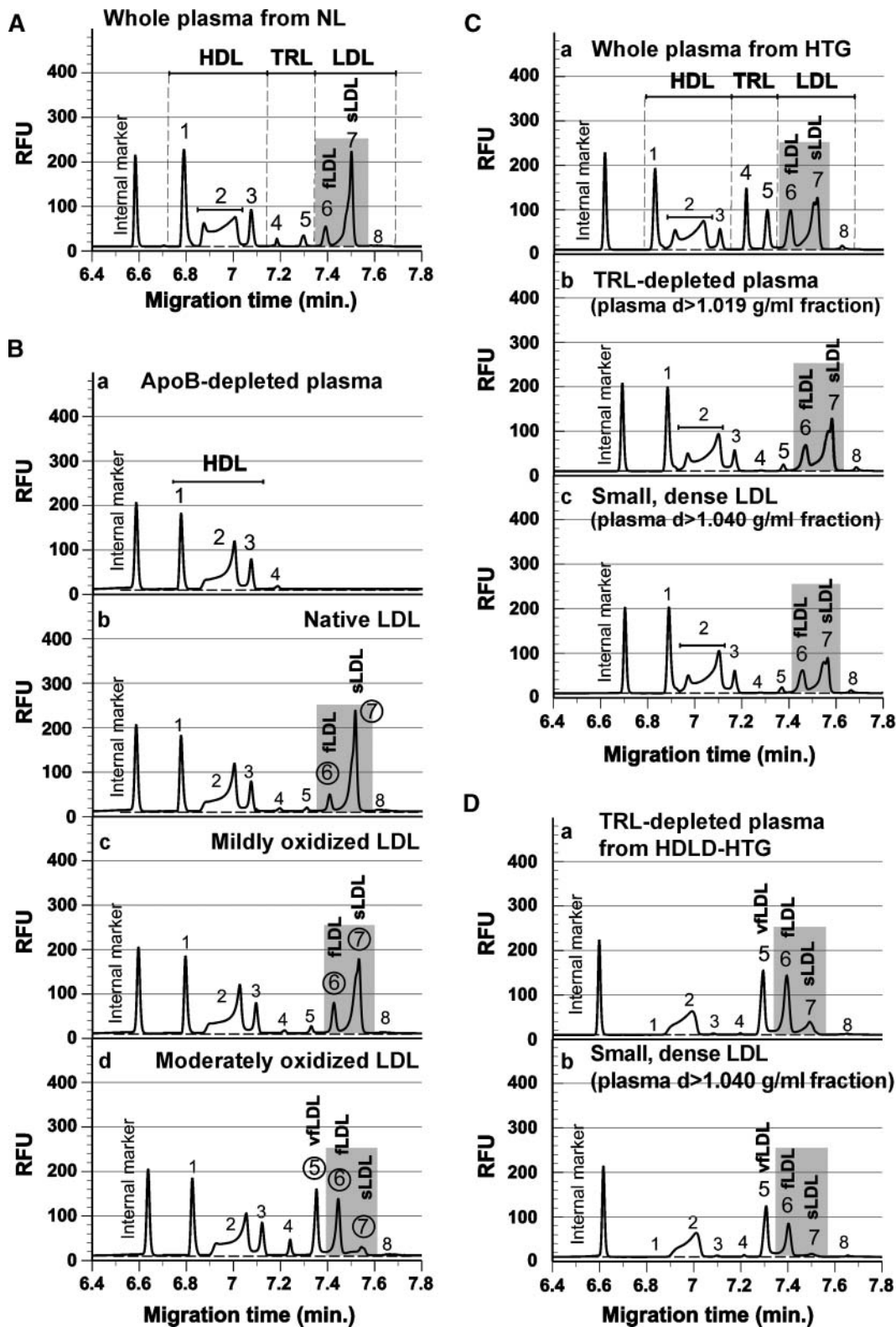


Fig. 2. A: Lipoprotein subfractions from a normal healthy subject with NL as characterized by cITP. Peaks 1–3, fast-migrating, intermediate-migrating, and slow-migrating HDL (fHDL, iHDL, and sHDL); peaks 4 and 5, fast-migrating and slow-migrating triglyceride-rich lipoproteins (fTRLs and sTRLs); peaks 6 and 7, fLDL and slow-migrating LDL (sLDL); peak 8, a very minor LDL subfraction. Lipoprotein was stained by a lipophilic dye, and detection was achieved by laser-induced fluorescence at 488 nm (7). RFU, relative fluorescence units. B: cITP lipoprotein subfractions in apolipoprotein B (apoB)-depleted plasma (a), native LDL (b), mildly oxidized LDL (c), and moderately oxidized LDL (d) in the presence of apoB-depleted plasma from a normal healthy subject with NL. Peak 5, vLDL. C: Lipoprotein subfractions in whole plasma (a) and the plasma $d > 1.019$ g/ml (b) and $d > 1.040$ g/ml (c) fractions in a patient with HTG. D: Lipoprotein subfractions in the plasma $d > 1.019$ g/ml (a) and $d > 1.040$ g/ml (b) fractions in an HTG patient with HDLD.

Fig. 2Ba, HDL fractions (peaks 1–3) and a negligible amount of apoB-containing lipoprotein (peak 4) were detected in apoB-depleted plasma. As shown in Fig. 2Bb, native LDL (LDL not subjected to *in vitro* oxidation) contained fLDL (peak 6) as a minor fraction and sLDL (peak 7) as a major fraction when analyzed by cITP in the presence of apoB-depleted plasma. A very minor fraction (peak 5) was also detected in native LDL (Fig. 2Bb). In mildly oxidized LDL (Fig. 2Bc), fLDL (peak 6) was increased with a decrease in sLDL (peak 7) compared with native LDL (Fig. 2Bb). In moderately oxidized LDL (Fig. 2Bd), the fLDL (peak 6) was further increased with a drastic decrease in sLDL (peak 7). However, the very minor fraction in native LDL (Fig. 2Bb, peak 5) was greatly increased in the moderately oxidized LDL (Fig. 2Bd, peak 5). This new fraction (peak 5) had faster electrophoretic mobility than fLDL (peak 6) and was defined as very-fast-migrating LDL (vfLDL).

A typical plasma lipoprotein profile from an HTG patient (Fig. 1, patient 1) with an altered distribution of LDL subfractions is shown in Fig. 2Ca. cITP fTRL (peak 4) and sTRL (peak 5) subfractions in this patient (Fig. 2Ca) were apparently higher than those in the NL subject (Fig. 2A) as a result of an increased TG level. Figure 2Cb shows cITP lipoprotein subfractions in the plasma $d > 1.019$ g/ml fraction (depleted of TRL). As shown, fTRL (peak 4) was not present and sTRL (peak 5) was present at only a negligible amount, as expected. However, the fLDL (peak 6) in the plasma $d > 1.019$ g/ml fraction (Fig. 2Cb) was apparently lower than that in whole plasma (Fig. 2Ca), indicating that a TRL fraction, defined as very-slow-migrating triglyceride (vsTRL), comigrates with cITP fLDL in whole plasma in this patient. We previously showed that the cITP vsTRL fraction was present in postprandial plasma from a volunteer subject (8). As shown in Fig. 2Cb, A, this patient had higher fLDL and lower sLDL than the NL subject despite similar LDL-cholesterol levels (Fig. 1, subject 1, 97 mg/dl, patient 1, 99 mg/dl). This distribution of LDL subfractions was similar to that of mildly oxidized LDL *in vitro* (Fig. 2Bc), indicating that LDL in this patient was mildly modified.

To examine whether or not moderately modified LDL is present in human plasma *in vivo*, we isolated the plasma $d > 1.019$ g/ml fraction from an HTG patient with HDL deficiency (HDL-D-HTG) (Fig. 1, patient 2) who had very low levels of sLDL but increased fLDL and sTRL in whole plasma (data not shown). Figure 2Da shows that both fLDL (peak 6) and vfLDL (peak 5) were increased in the plasma $d > 1.019$ g/ml fraction in the HDLD-HTG patient compared with the NL subject (Fig. 2A) and the HTG patient (Fig. 2Cb). The distribution of cITP LDL subfractions (peaks 5–7) in this patient (Fig. 2Da) was very similar to that of moderately oxidized LDL *in vitro* (Fig. 2Bd), indicating that moderately modified LDL was present in human plasma *in vivo*, although not uniformly.

Next, we examined whether or not LDL subfractions with different electrophoretic mobilities may differ with regard to the content of sdLDL. As shown in Fig. 2Cc, b, fLDL (peak 6) in the plasma $d > 1.040$ g/ml fraction

(depleted of TRL, lbLDL, and idLDL) from the HTG patient (Fig. 2Cc) was only slightly reduced compared with that in the plasma $d > 1.019$ g/ml fraction (Fig. 2Cb), whereas a reduction in sLDL (peak 7) was apparent, indicating that fLDL contained mostly sdLDL and the sLDL subfraction contained not only sdLDL but also lbLDL and idLDL in this HTG patient. Similarly, as shown in Fig. 2Db, a, vfLDL (peak 5) contained largely sdLDL, whereas fLDL (peak 6) contained more lbLDL and idLDL than sLDL in the HDLD-HTG patient. These results indicate that cITP LDL subfractions with different electrophoretic mobilities differ with respect to the content of sdLDL.

Effects of apoA-I/POPC discs on cITP LDL subfractions with different distributions

Because TRL interacts with apoA-I/POPC discs (8) and vsTRL comigrates with fLDL (Fig. 2Ca, b), the effects of apoA-I/POPC discs on LDL subfractions with different distributions were examined in TRL-depleted plasma (plasma $d > 1.019$ g/ml fraction). As shown in Fig. 3Aa–c, the NL subject (Fig. 1, subject 1), HC subject (Fig. 1, subject 3), and HDLD-HTG patient (Fig. 1, patient 2) had distributions of cITP LDL subfractions similar to native LDL (Fig. 2Bb), mildly modified LDL (Fig. 2Bc), and moderately modified LDL (Fig. 2Bd), respectively. The NL subject and HDLD-HTG patient were the same as those in experiment 1, but blood was drawn at different periods. As shown in Fig. 3A, B, incubation of plasma $d > 1.019$ g/ml fractions at 37°C in the presence of apoA-I/POPC discs produced significant changes in both HDL (peaks 1–3) and LDL (peaks 6 and 7) subfractions. cITP fHDL (peak 1) and iHDL (peak 2) were drastically decreased and sHDL (peak 3) was drastically increased after the plasma $d > 1.019$ g/ml fractions from the NL (Fig. 3Ba, Aa) and HC (Fig. 3Bb and Ab) subjects were incubated in the presence of apoA-I/POPC discs. This result confirms our previous finding that apoA-I/POPC discs remodeled cITP fHDL and iHDL into sHDL (15). Peak 4 in Fig. 2Ba–c is a fraction of apoA-I/POPC discs (data not shown). Incubation of the plasma $d > 1.019$ g/ml fractions from the NL (Fig. 3Ba) and HC (Fig. 3Bb) subjects in the presence of apoA-I/POPC discs markedly decreased cITP fLDL and increased sLDL (Fig. 3B, A). This result indicates that apoA-I/POPC discs also remodeled cITP fLDL into sLDL in the NL and HC subjects.

As shown in Fig. 3Bc, Ac, lipoprotein fractions with the mobility of cITP sHDL (peak 3) and the fraction of apoA-I/POPC discs (peak 4) were increased in the plasma $d > 1.019$ g/ml fraction from the HDLD-HTG patient after incubation in the presence of apoA-I/POPC discs. cITP vfLDL (peak 5) and fLDL (peak 6) were greatly reduced in plasma $d > 1.019$ g/ml fraction after incubation in the presence of apoA-I/POPC discs (Fig. 3Bc) compared with those in the absence of apoA-I/POPC discs (Fig. 3Ac). Surprisingly, the cITP sLDL subfraction (peak 7) that was present in negligible levels in the plasma $d > 1.019$ g/ml fraction in the absence of apoA-I/POPC discs (Fig. 3Ac) appeared in plasma $d > 1.019$ g/ml fraction after incubation in the presence of apoA-I/POPC discs (Fig. 3Bc, peak

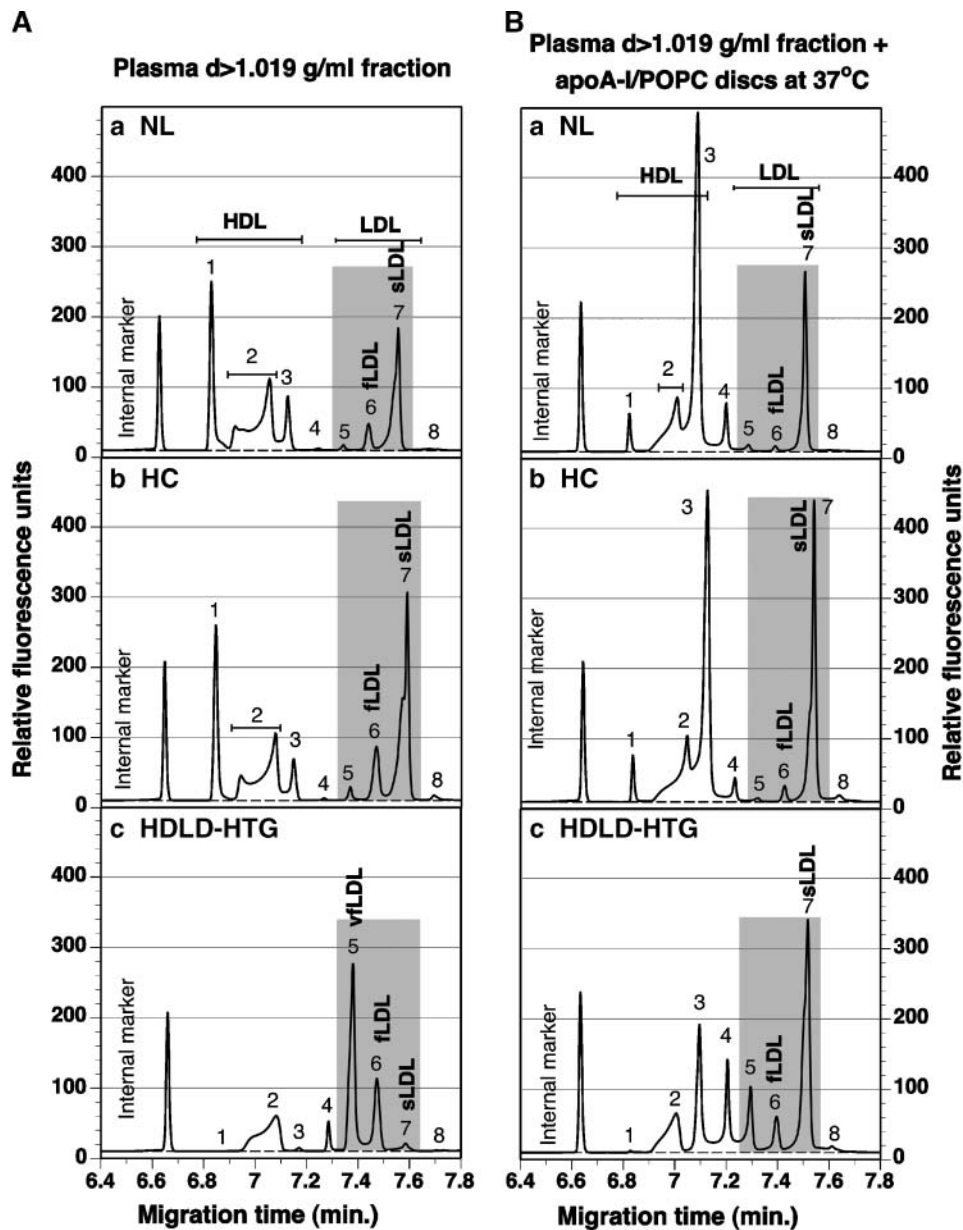


Fig. 3. cITP lipoprotein subfractions in the plasma $d > 1.019$ g/ml fractions from subjects with NL (a) and HC (b) and a patient with HDLD and HTG (HDLD-HTG) (c) in the absence of apoA-I/POPC discs (A) and after incubation at 37°C for 30 min (a, b) and 60 min (c) in the presence of apoA-I/POPC discs at an apoA-I concentration of 0.5 mg/ml (B). Peaks 1–3, fHDL, iHDL, and sHDL; peaks 4 and 5 in A, fTRL and vLDL; peak 4 and 5 in B an apoA-I/POPC disc fraction; peaks 6 and 7, fLDL and sLDL; peak 8, a very minor LDL fraction.

7). These results indicate that apoA-I/POPC discs remodeled cITP fLDL and vLDL into sLDL to normalize the altered distribution of LDL subfractions in this HDLD-HTG subject.

Incubating plasma from the NL, HC, and HDLD-HTG subjects that contained 2.0 mM DTNB, an LCAT inhibitor, or 50 μ M JTT-705, a CETP inhibitor, in the presence of apoA-I/POPC discs produced similar changes in cITP lipoprotein subfractions in plasma $d > 1.019$ g/ml fraction (data not shown). This result indicates that changes in cITP LDL subfractions caused by apoA-I/POPC discs were

not mediated by LCAT activity or CETP activity because the inhibition of LCAT activity or CETP activity did not affect the process.

Effects of apoA-I/POPC discs on cITP LDL subfractions from different origins

Because LDL(–) has been shown to be of multiple origins (1), the effects of apoA-I/POPC discs on altered cITP LDL subfractions were examined in whole plasma from patients with increased OxLDL Ab concentrations (Fig. 1, patient 3), increased LDL-PAF-AH activity (Fig. 1, patient 4),

increased sdLDL (Fig. 1, patient 1), or diabetes mellitus (Fig. 1, patient 5) and a patient on hemodialysis (Fig. 1, patient 6). As shown in Fig. 4Aa–e, all five patients had increased cITP fLDL (peak 6) compared with the normal healthy NL subject (Fig. 2A). As shown in Fig. 4B, A, lipoprotein subfractions in whole plasma from the five patients changed similarly to those in the plasma $d > 1.019$ g/ml fraction from the NL and HC subjects (Fig. 3) after whole

plasma from these patients was incubated at 37°C in the presence of apoA-I/POPC discs. ApoA-I/POPC discs apparently reduced the cITP fLDL subfraction (peak 6) and increased sLDL (peak 7) in all five patients (Fig. 4B, A). A slight increase in the very minor LDL subfraction (peak 8) was also observed (Fig. 4). Because peak 4 in the plasma $d > 1.019$ g/ml fraction after incubation in the presence of apoA-I/POPC discs (Fig. 4B) was a fraction of apoA-I/

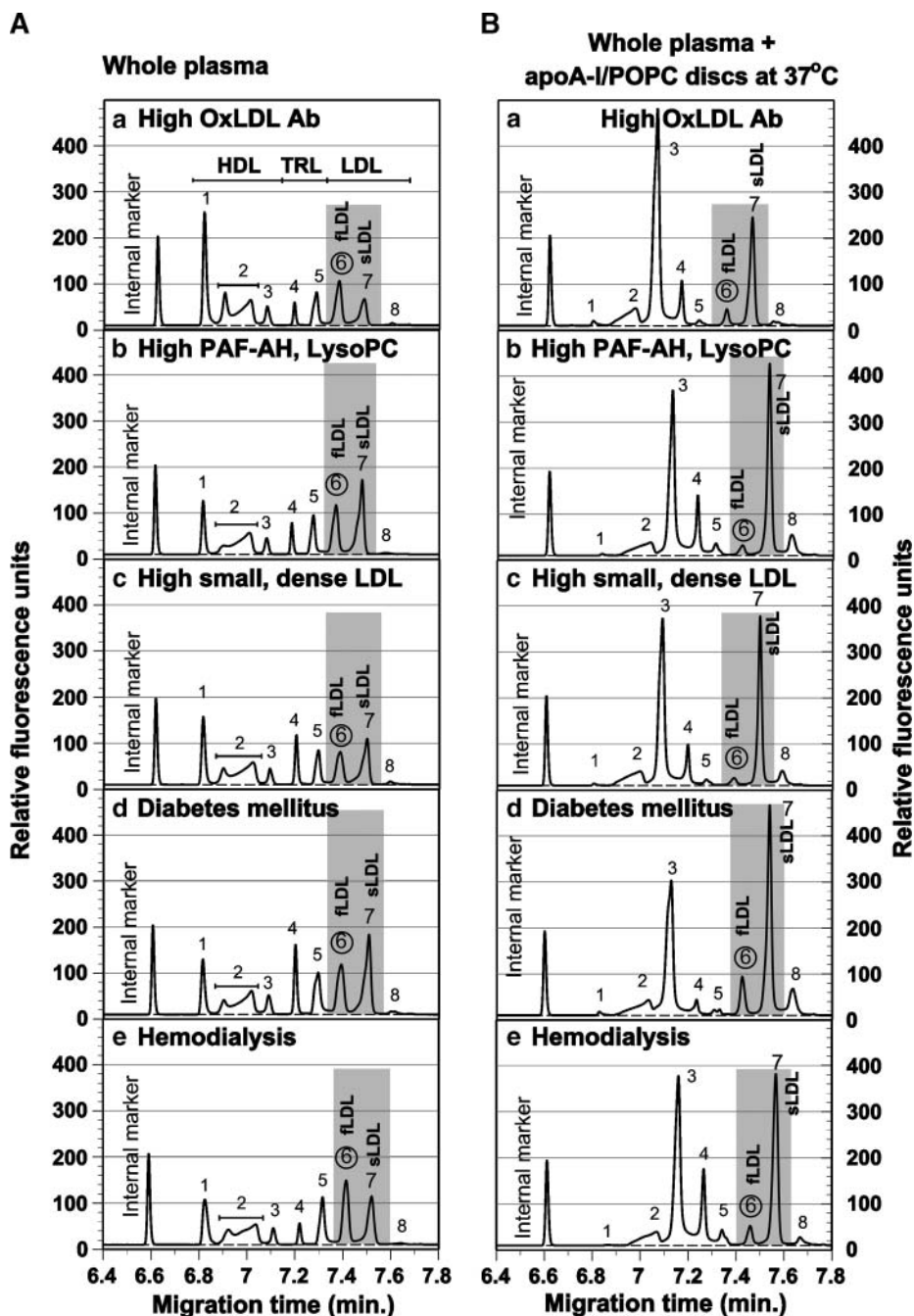


Fig. 4. cITP lipoprotein subfractions in whole plasma from patients with increased OxLDL Ab (a), increased LDL-PAF-AH (b), increased sLDL (c), and diabetes mellitus (d) and on hemodialysis (e) in the absence of apoA-I/POPC discs (A) and after incubation at 37°C for 90 min in the presence of apoA-I/POPC discs at an apoA-I concentration of 0.8 mg/ml (B). Peaks 1–3, fHDL, iHDL, and sHDL; peaks 4 and 5 in A, fTRL and sTRL; peaks 4 and 5 in B, an apoA-I/POPC disc fraction; peaks 6 and 7, fLDL and sLDL; peak 8, a very minor LDL fraction.

POPC discs (data not shown), it is apparent that TRL subfractions (peaks 4 and 5) were also greatly reduced (Fig. 4B, A).

Effects of apoA-I/POPC discs on the lipid and enzyme composition of LDL, the density distribution of cITP LDL subfractions, LDL lipids, and LDL-associated enzyme, and the size of LDL

To clarify whether or not the charge modification of LDL by apoA-I/POPC discs reduces the atherogenic properties of LDL, we examined the effects of apoA-I/POPC discs on the lipid and enzyme composition of LDL, the density distribution of cITP LDL subfractions, LDL lipid contents, and LDL-PAF-AH activity, and the size of LDL.

A quantitative ultracentrifugation technique was used to measure cITP LDL subfractions in lbLDL, idLDL, and sdLDL subclasses and lipid concentrations and PAF-AH activity in LDL fraction and subclasses of lbLDL, idLDL, and sdLDL. Because apoB-containing lipoproteins are susceptible to modification after isolation from HDL and plasma proteins, lipid parameters in LDL fraction and lbLDL, idLDL, and sdLDL subclasses were measured by comparing plasma fractions with and without depletion of these lipoproteins (HDL and plasma proteins not depleted). **Figure 5A** shows the cITP lipoprotein profile in whole plasma from a HTG patient (Fig. 1, patient 7) and those in the $d > 1.019$, 1.035 , 1.040 , and 1.063 g/ml fractions of the same plasma. As shown in Fig. 5Ab, the plasma $d > 1.019$ g/ml fraction contained HDL and LDL fractions, because the TRL fraction was depleted from the whole plasma (Fig. 5Aa). As shown in Fig. 5Ac, the plasma $d > 1.035$ g/ml fraction contained HDL, idLDL, and sdLDL, because both TRL and lbLDL were depleted from the whole plasma. The plasma $d > 1.040$ g/ml fraction contained HDL and sdLDL (Fig. 5Ad), and the plasma $d > 1.063$ g/ml fraction contained only HDL (Fig. 5Ae). Therefore, LDL fraction and lbLDL, idLDL, and sdLDL subclasses were quantified as the differences between the plasma $d > 1.019$ and 1.063 g/ml fractions, the plasma $d > 1.019$ and 1.035 g/ml fractions, the plasma $d > 1.035$ and 1.040 g/ml fractions, and the plasma $d > 1.040$ and 1.063 g/ml fractions, respectively.

Figure 5B shows the cITP lipoprotein profile of whole plasma from the same HTG patient (Fig. 1, patient 7) after incubation in the presence of apoA-I/POPC discs and those in the plasma $d > 1.019$, 1.035 , 1.040 , and 1.063 g/ml fractions. As shown in Fig. 5Aa, Ba, apoA-I/POPC discs caused significant changes in cITP lipoprotein subfractions in this HTG patient, similar to those in other subjects, as shown in Fig. 4. As shown in Fig. 5Bb, cITP sLDL in the plasma $d > 1.019$ g/ml fraction was apparently reduced compared with that in whole plasma (Fig. 5Ba). This result indicates that the incubation of whole plasma in the presence of apoA-I/POPC discs produced new particles that comigrated with sLDL. Because the new particles were lighter than LDL, they could originate from TRL.

Table 1 shows changes in the levels of cITP LDL subfractions, concentrations of TC, FC, TG, and PL, and PAF-AH activity in the LDL fraction and lbLDL, idLDL, and

sdLDL subclasses of plasma from five patients (Fig. 1, part IV, patients 1, 2, 4, 5, and 7) before incubation in the absence of apoA-I/POPC discs and after incubation in the presence of apoA-I/POPC discs. Median values and upper and lower quartiles (in parentheses) are given to show the distribution of continuous variables. Incubation of plasma in the presence of apoA-I/POPC discs did not significantly change cITP sLDL levels or TC, TG, or PL concentrations in the LDL fraction but significantly reduced cITP fLDL levels, FC concentration, and PAF-AH activity in the LDL fraction. As also shown in Table 1, cITP fLDL level, FC concentration, and PAF-AH activity in the sdLDL subclass were significantly reduced by incubation of plasma with apoA-I/POPC discs.

Figure 6A shows cITP fLDL levels, FC concentration, and PAF-AH activity in the lbLDL, idLDL, and sdLDL subclasses of plasma from each individual patient (Fig. 1, part IV, patients 1, 2, 4, 5, and 7) before incubation in the absence of apoA-I/POPC discs and after incubation in the presence of apoA-I/POPC discs. As shown in Fig. 6Aa, cITP fLDL was distributed in the sdLDL subclass of plasma before incubation. After the incubation of plasma in the presence of apoA-I/POPC discs, cITP fLDL in the sdLDL subclass was markedly reduced (Fig. 6Aa, right panel). This result indicates that apoA-I/POPC discs altered the density distribution of cITP fLDL. Because lipids were detected in cITP analysis, a reduction in fLDL indicates reduced lipid contents in the sdLDL subclass.

As shown in Fig. 6Ab, c, before the incubation of plasma in the absence of apoA-I/POPC discs, FC concentration and PAF-AH activity were distributed in the sdLDL subclass. After the incubation of plasma in the presence of apoA-I/POPC discs, FC concentration (Fig. 6Ab) and PAF-AH activity (Fig. 6Ac) in the sdLDL subclass were markedly reduced, in parallel with the changes in cITP fLDL (Fig. 6Aa). Parallel changes in cITP fLDL, FC, and PAF-AH in the sdLDL subclass indicate that the charge modification of LDL by apoA-I/POPC discs was associated with changes in the composition of LDL.

Because reductions in LDL-FC and LDL-PAF-AH by apoA-I/POPC discs were observed by *in vitro* incubation of human plasma (Table 1), acceptors of FC and PAF-AH should be present. Therefore, we examined changes in TC concentration and PAF-AH activity in plasma $d > 1.063$ g/ml fraction (HDL fraction) from the same patients (Fig. 1, part IV) before incubation of plasma in the absence of apoA-I/POPC discs and after incubation of plasma in the presence of apoA-I/POPC discs. As shown in Fig. 6Ba, b, incubation of plasma with apoA-I/POPC discs increased TC concentration and PAF-AH activity in plasma HDL fraction from all five patients. These results indicate that apoA-I/POPC discs caused the movement of FC and PAF-AH in the sdLDL subclass to the HDL fraction.

The effects of apoA-I/POPC discs on the size of LDL were examined by native nondenaturing composite GGE in plasma from three patients with HDL-D-HTG, increased sdLDL, and high TG concentration (Fig. 1, part IV, patients 2, 1, and 7, respectively) and by negative stain electron microscopy after LDL was separated by ultracentrifugation.

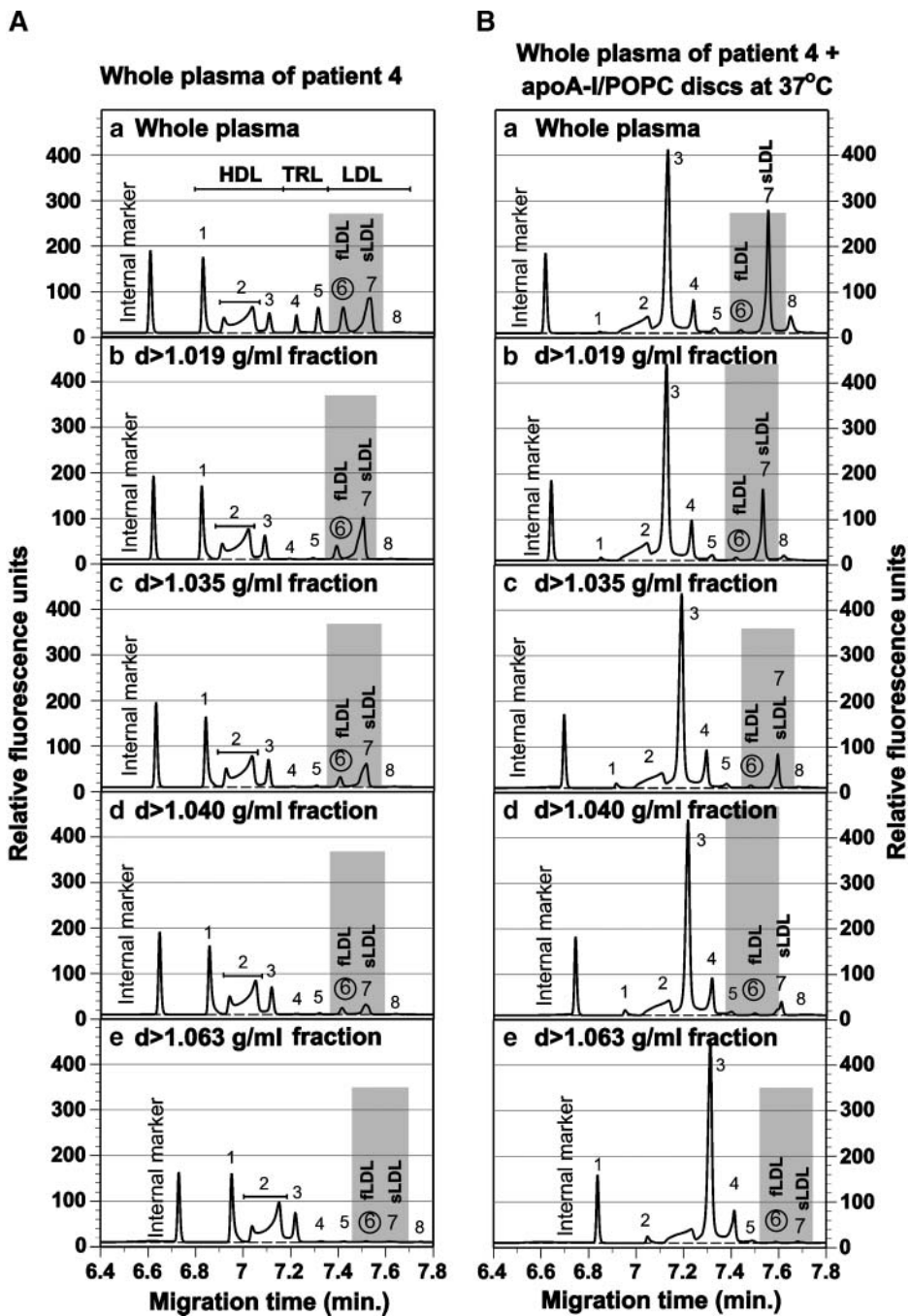


Fig. 5. cITP lipoprotein subfractions in whole plasma (a) and plasma $d > 1.019$ (b), 1.035 (c), 1.040 (d), and 1.063 g/ml (e) fractions from patient 7 (Fig. 1, part IV). A, B: Whole plasma without incubation in the absence of apoA-I/POPC discs (A) and after incubation at 37°C for 2 h in the presence of apoA-I/POPC discs at an apoA-I concentration of 1.2 mg/ml (B). Peaks 1–3, fHDL, iHDL, and sHDL; peaks 4 and 5 in A, fTRL and sTRL; peaks 6 and 7 in B, an apoA-I/POPC disc fraction; peaks 6 and 7, fLDL and sLDL; peak 8, a very minor LDL fraction.

trifugation from plasma of a hypercholesterolemic volunteer subject (Fig. 1, subject 3). As shown in Fig. 7A, LDL of plasma from all three patients migrated a shorter distance on the composite gradient gel after incubation in the presence of apoA-I/POPC discs than before incubation in the absence of apoA-I/POPC discs. Figure 7B shows the median diameters of LDL determined from the gradient gel.

As shown, apoA-I/POPC discs increased the median diameter of LDL in all three patients as determined by GGE.

Figure 8A, B shows electron micrographs of negatively stained LDL separated from plasma before incubation in the absence of apoA-I/POPC discs (Fig. 8A) and after incubation in the presence of apoA-I/POPC discs (Fig. 8B). As shown in Fig. 8C, the frequency distribution of the di-

ameter of LDL particles was shifted toward a larger size after plasma from the HC subject was incubated in the presence of apoA-I/POPC discs. Plasma after incubation in the presence of apoA-I/POPC discs contained significantly ($P < 0.01$) more large particles than before incubation in the absence of apoA-I/POPC discs. These results indicated that apoA-I/POPC discs increased the size of LDL particles.

To clarify how apoA-I/POPC discs increase the size of LDL, the effects of apoA-I/POPC discs on LDL subclass concentrations were examined by NMR spectroscopy in plasma from four patients with sLDL, increased LDL-PAF-AH, diabetes mellitus, and high TG concentration (Fig. 1, part IV, patients 1, 4, 5, and 7, respectively). As shown in Fig. 9, mean LDL diameters (left panel) and large LDL particle concentrations (right panel) in plasma from the four patients before incubation in the absence of apoA-I/POPC discs were increased after incubation in the presence of apoA-I/POPC discs, whereas small LDL particle concentrations did not appear to be affected (data not shown). This result suggests that apoA-I/POPC discs increased the size of LDL by increasing large LDL particles.

Therefore, composition and size analyses of LDL indicated that in vitro incubation of plasma with apoA-I/POPC discs caused favorable changes in LDL particles: a reduction of FC and PAF-AH in the sLDL subclass and an increase in LDL particle size.

DISCUSSION

A low HDL-cholesterol level is an established risk factor for coronary heart disease, and HDL therapy is emerging as an approach for the acute treatment of atherosclerosis (9, 10). ApoA-I/POPC discs have been shown to increase pre β HDL and enhance reverse cholesterol transport (9, 14, 26). However, the effects of apoA-I/POPC discs on LDL have also been reported (16, 17, 19, 26). ApoA-I/POPC discs decreased apoB levels in human subjects (26) and non-HDL cholesterol in hyperlipidemic rabbits (16). Also, apoA-I/POPC discs reduce the inflammatory properties of LDL by removing lipid hydroperoxides from human LDL (19).

Modified LDL in plasma that had an increased negative charge has been separated by ion-exchange chromatography and shown to have impaired binding affinity to the LDL receptor and to have inflammatory properties (1, 18, 20, 37). Therefore, this study tested the hypothesis that apoA-I/POPC discs reduce charge-modified LDL.

We used cITP to characterize the distribution of charge-based LDL subfractions in human plasma. Normally, LDL is separated as two major subfractions (fLDL and sLDL) (5–8, 28, 30). In normal healthy subjects with NL, cITP fLDL is a minor subfraction and sLDL is the major LDL subfraction (Fig. 2). We found that mildly modified LDL was characterized by increased fLDL (Fig. 2). Moderately modified LDL, which is not commonly seen in human plasma, was characterized with both increased cITP fLDL and vLDL (Fig. 2).

TABLE 1. Lipid and enzyme composition of LDL and the distribution of cITP LDL subfractions, LDL lipids, and LDL-PAF-AH in LDL density subclasses in whole plasma before incubation in the absence of apoA-I/POPC discs and after incubation at 37°C for 2 h in the presence of apoA-I/POPC discs

Parameter	Before Incubation in the Absence of apoA-I/POPC Discs				After Incubation in the Presence of apoA-I/POPC Discs			
	LDL Fraction	LDL Density Subclasses		sLDL	LDL Fraction	LDL Density Subclasses		sLDL
		fbLDL	idLDL			fbLDL	idLDL	
cITP fLDL ^a	0.27 (0.24, 0.56)	0.05 (0.02, 0.10)	0.06 (0.05, 0.06)	0.19 (0.18, 0.33)	0.04 ^b (0.02, 0.06)	0.00 (0.00, 0.00)	0.01 (0.00, 0.01)	0.06 ^b (0.02, 0.10)
cITP sLDL ^a	1.02 (0.90, 1.59)	0.27 (0.10, 0.33)	0.34 (0.25, 0.36)	0.56 (0.25, 0.97)	1.44 (1.11, 1.50)	0.22 (0.19, 0.41)	0.37 (0.35, 0.38)	0.55 (0.37, 0.83)
Total cholesterol (mg/dl)	68 (53, 91)	13 (12, 17)	19 (14, 21)	42 (13, 57)	66 (42, 87)	20 (14, 26)	13 (12, 20)	33 (10, 40)
Free cholesterol (mg/dl)	18 (15, 23)	5 (4, 5)	5 (4, 6)	10 (4, 13)	10 ^b (6, 11)	3 (3, 4)	2 (1, 2)	4 ^b (1, 5)
Triglyceride (mg/dl)	11 (11, 22)	5 (3, 7)	3 (2, 5)	6 (5, 10)	12 (12, 28)	5 (4, 13)	3 (3, 5)	5 (5, 9)
Phospholipid (mg/dl)	40 (35, 50)	11 (8, 14)	8 (7, 13)	24 (14, 33)	46 (37, 53)	16 (15, 19)	10 (9, 11)	20 (13, 28)
PAF-AH (IU/l)	265 (257, 287)	22 (17, 27)	25 (14, 42)	245 (229, 254)	118 ^b (75, 143)	14 (10, 33)	8 (4, 36)	57 ^b (10, 57)

apoA-I, apolipoprotein A-I; cITP, capillary isotachopheresis; fLDL, fast-migrating LDL as determined by cITP; fbLDL, large, buoyant LDL; idLDL, intermediate density LDL; sLDL, small, dense LDL; LDL-PAF-AH, LDL-associated platelet-activating factor acetylhydrolase; sLDL, slow-migrating LDL as determined by cITP.

^a Expressed as peak area relative to that of the internal marker 5-carboxyfluorescein. Data are presented as median values (25th percentile, 75th percentile).

^b $P < 0.01$, versus plasma without incubation in the absence of apoA-I/POPC discs, assessed by the Wilcoxon signed-rank test.

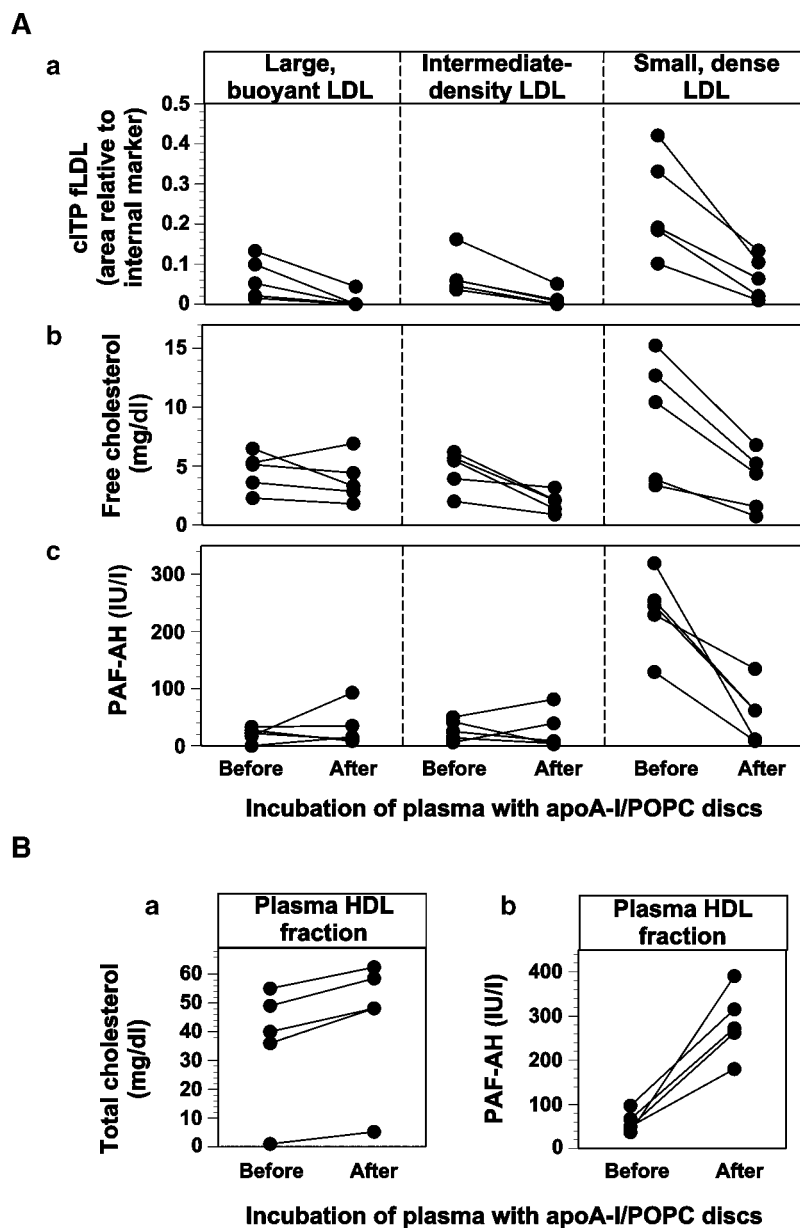


Fig. 6. Distributions of cITP fLDL level (a), free cholesterol concentration (b), and PAF-AH activity (c) in large, buoyant LDL ($d = 1.019\text{--}1.035$ g/ml), intermediate density LDL ($d = 1.035\text{--}1.040$ g/ml), and sdLDL ($d = 1.040\text{--}1.063$ g/ml) subclasses measured in the plasma $d > 1.019, 1.035, 1.040,$ and 1.063 g/ml fractions (A) and TC concentration (a) and PAF-AH activity (b) in HDL fraction measured in the plasma $d > 1.063$ g/ml fraction (B) of whole plasma from five patients (Fig. 1, part IV, patients 1, 2, 4, 5, and 7) before and after incubation at 37°C for 2 h in the presence of apoA-I/POPC discs at an apoA-I concentration of 0.8 (patient 2) or 1.2 mg/ml.

We also found that LDL subfractions with faster electrophoretic mobility (fLDL vs. sLDL, vLDL vs. fLDL) as determined by cITP contained more sdLDL than lbLDL and idLDL (Fig. 2). Because sdLDL is known to be atherogenic, this finding suggests that cITP LDL subfractions differ in their atherogenic properties. This finding agrees with those of other authors that LDL(-) is atherogenic (1, 20, 37).

We are the first to show that apoA-I/POPC discs rapidly (<2 h) remodeled both cITP fLDL and vLDL into sLDL in TRL-depleted plasma (Fig. 3), independent of LCAT or

CETP activity (data not shown). Surprisingly, apoA-I/POPC discs also remodeled both cITP TRL (fTRL and sTRL) and fLDL into sLDL in whole plasma from patients with various metabolic disorders (Fig. 4). Therefore, it is possible that the rapid reduction of charge-modified LDL by apoA-I/POPC discs may contribute to the effects of apoA-I/POPC discs on the quantity and quality of LDL observed in human subjects (13, 19).

In cITP analysis, lipoproteins are stained with a lipophilic dye [6-((N-(nitrobenz-2-oxa-1,3-diazol-4-yl)amino)-

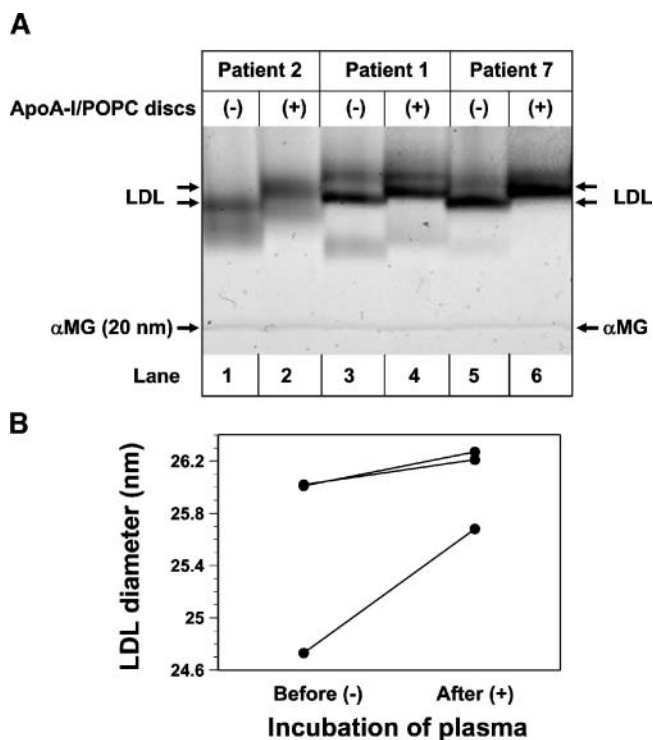


Fig. 7. A: Native nondenaturing composite gradient gel electrophoresis of plasma LDL followed by Sudan Black B staining. Plasma from a patient with HDLD-HTG (Fig. 1, part IV, patient 2; lanes 1, 2), a patient with increased sLDL (patient 1; lanes 3, 4), and a patient with a high TG concentration (patient 7; lanes 5, 6) was run before incubation in the absence of apoA-I/POPC discs (lanes 1, 3, 5) and after incubation in the presence of apoA-I/POPC discs (lanes 2, 4, 6). For reference, the arrows point to the locations of \sim 24.8 and 25.8 nm in the LDL size interval and to the faint refractile band labeled α MG (for α -macroglobulin; \sim 20 nm). B: Median diameter of LDL determined from the composite gradient gel.

hexanoyl)sphingosine (NBD C₆-ceramide)] and the separated zones are monitored by laser-induced fluorescence (5–8). Therefore, this analytical technique has the advantage that lipoprotein subfractions can be determined directly in plasma and the limitation that the compositions of lipoprotein subfractions cannot be determined. Therefore, to verify that modification of the LDL charge distribution by apoA-I/POPC discs is indeed associated with reduced atherogenic properties of LDL, we examined the effects of apoA-I/POPC discs on lipid and enzyme composition of LDL, the density distribution of cITP LDL subfractions, lipids, and PAF-AH in lLDL, idLDL, and sLDL subclasses, and the size of LDL particles (Table 1, Figs. 6–9). Our finding that cITP fLDL was distributed in the sLDL subclasses agrees with our previous finding (7) and that of Sanchez-Quesada et al. (4) that LDL(-) separated by chromatography was distributed in sLDL subclasses in normolipidemic subjects. Our finding that PAF-AH activity was distributed in the sLDL subclasses also agrees with those of other authors (38, 39).

We found a marked reduction of cITP fLDL level, FC concentration, and PAF-AH activity in the sLDL subclasses after incubation of plasma in the presence of apoA-

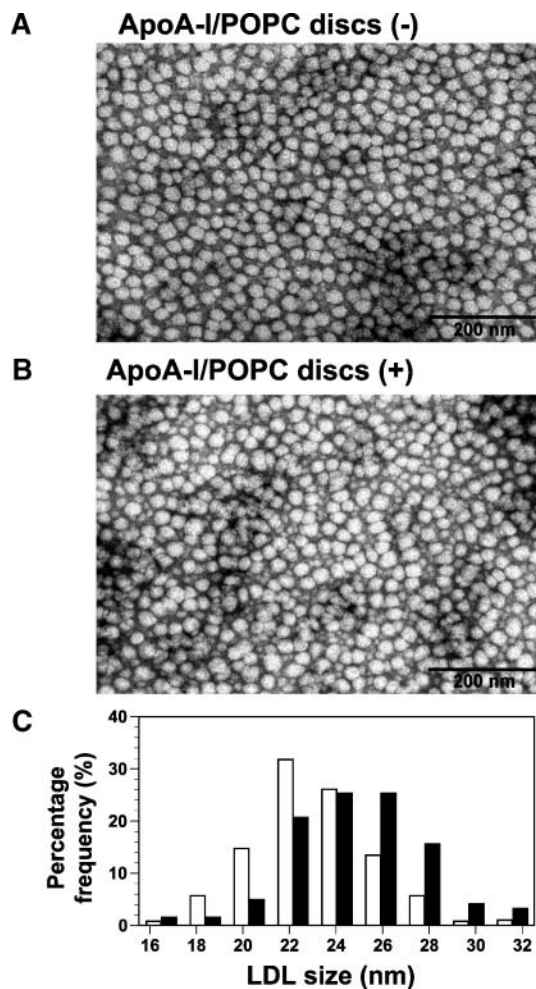


Fig. 8. A, B: Electron micrographs of negatively stained LDL separated by serial ultracentrifugation from plasma from a hypercholesterolemic volunteer subject (Fig. 1, subject 3) before incubation in the absence of apoA-I/POPC discs (A) and after incubation in the presence of apoA-I/POPC discs (B). Two measurements were made for the diameter of each LDL particle, and the mean diameter was used to calculate the size frequency. Magnification, 150,000 \times . Bars = 200 nm. C: Size frequency distribution of negatively stained LDL before incubation in the absence of apoA-I/POPC discs (open bars; n = 757) and after incubation in the presence of apoA-I/POPC discs (closed bars; n = 520).

I/POPC discs (Fig. 6A). We have previously shown that an increase in LDL-PAF-AH activity during cholesterol feeding in rabbits was attributable to the increased secretion of PAF-AH from macrophages (22). Tsimihodimos et al. (38) reported increased LDL-PAF-AH activity in patients with familial HC. We also found an increase in the size of LDL as determined by composite gradient gel (Fig. 7), electron microscopy (Fig. 8), and NMR spectroscopy (Fig. 9) after plasma was incubated with apoA-I/POPC discs. Therefore, a reduction of FC and PAF-AH in sLDL subclasses and an increase in LDL size by apoA-I/POPC discs indicate a reduced atherogenicity of LDL particles. We found that apoA-I/POPC discs caused a significant increase in FC and PAF-AH in the HDL fraction (Fig. 6B). Because plasma was incubated in vitro, this finding indicates the movement

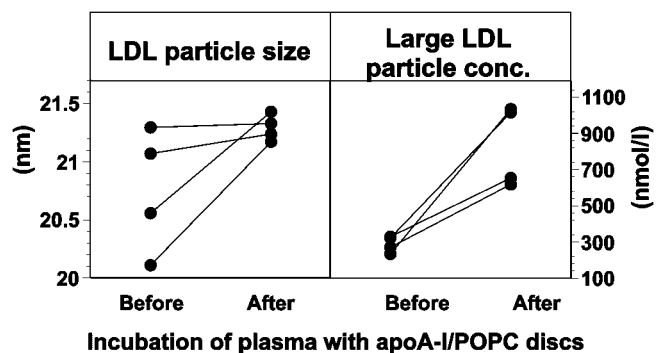


Fig. 9. NMR spectroscopic analysis of mean LDL particle diameters and large LDL particle concentrations in plasma from patients with sdLDL, increased LDL-PAF-AH activity, diabetes mellitus, and high triglyceride TG concentrations (Fig. 1, part IV, patients 1, 4, 5, and 7) before incubation in the absence of apoA-I and after incubation at 37°C for 2 h in the presence of apoA-I/POPC discs at an apoA-I concentration of 1.2 mg/ml.

of FC and PAF-AH from LDL to HDL induced by apoA-I/POPC discs. Therefore, the removal of LDL-FC and LDL-PAF-AH could be a mechanism by which apoA-I/POPC discs reduce cITP fLDL. Navab et al. (19) showed that apoA-I/POPC discs removed lipid hydroperoxides from LDL.

Shahrokh and Nichols (17) showed that LDL takes up PLs from apoA-I/POPC discs by incubating purified LDL and apoA-I/POPC discs for 6 and 24 h. However, we found that PL concentrations were increased markedly in the HDL fraction (data not shown) but only slightly in the LDL fraction (Table 1). This discrepancy could be attributable to the different incubation conditions: our incubation experiments were performed in whole plasma rather than in the purified LDL in their report (17).

Our results with NMR spectroscopy indicated that the size of LDL was increased by an increase in large LDL particles (Fig. 9). The increase in the size of LDL by apoA-I/POPC discs was not attributable to the incubation of plasma itself: we observed a decrease in the size of LDL after the plasma from a volunteer subject (Fig. 1, subject 3) was incubated at 37°C for 2 h in the absence of apoA-I/POPC discs, as examined by electron microscopy (data not shown). The increase in LDL size was also not the result of an increase in cholesteryl ester by LCAT activity or lipid transfer by CETP activity during the incubation of plasma, because we consistently found an increase in the size of LDL as examined by GGE after the plasma from 20 volunteer subjects with and without dyslipidemia was incubated with apoA-I/POPC discs at a final apoA-I concentration of 0.83 mg/ml in the presence of an LCAT inhibitor (DTNB; 2 mM) and a CETP inhibitor (JTT-705; 40 μM) (data not shown). It is not clear how apoA-I/POPC discs increase large LDL particles. We found that an increase in the size of LDL by apoA-I/POPC discs in 20 volunteer subjects could not be explained by the chemical composition of LDL; rather, it strongly depended on the FC concentration in the HDL fraction as assessed by

analysis of covariance (data not shown). It is possible that particle fusion could have occurred to cause the drastic changes in lipoprotein subfractions as characterized by cITP, an increase in LDL size as determined by GGE, electron microscopy, and NMR, and an increase in large LDL particles as determined by NMR, because the process was rapid: apparent changes in cITP lipoprotein subfractions were observed after only 15 min of incubation (data not shown).

Our finding that apoA-I/POPC discs rapidly reduce the cITP fLDL subfraction suggests that apoA-I/POPC discs could be an efficient way to decrease charge-modified LDL. The increased cITP sLDL can be effectively reduced by treatment with statins (27, 40). Because the cITP technique is quick (separation is complete within minutes) and sensitive (only a drop of plasma is required), it could be useful for monitoring the effects of lipid-modifying therapy.

In conclusion, the novel findings of this study are as follows: 1) apoA-I/POPC discs rapidly remodeled cITP vLDL and fLDL into sLDL independent of LCAT or CETP activity; 2) apoA-I/POPC discs reduced plasma fLDL from different origins; and 3) the mechanism by which apoA-I/POPC discs reduce cITP fLDL involves the removal of FC and PAF-AH from sdLDL subclasses to the HDL fraction. Further investigations are needed to clarify whether or not changes in the distribution of charge-based LDL subfractions caused by apoA-I/POPC discs are related to clinical outcomes. **■**

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