Abnormal high density lipoproteins of abetalipoproteinemia: relevance to normal HDL metabolism

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Abstract We investigated high density lipoprotein (HDL) subfractions in abetalipoproteinemia (ABL) using rate zonal ultracentrifugation. In ABL, HDL_2 is the major subfraction, 65% of total mass compared to less than 10% in normal subjects with similar HDL levels. HDL_2 and HDL_3 in ABL (n = 3) are larger and lighter than in normals (n = 3), with mean diameters of 136 ± 19 Å and 100 ± 12 Å, respectively (as compared to 113 ± 12 Å and 86 ± 11 Å), and contained more apoprotein E. ABL-HDL_2 and HDL_3 particles contain 2- to 2.5-fold more cholesteryl ester molecules than normals. ABL-HDL can be modified towards normal HDL by allowing VLDL triglycerides to exchange for ABL-HDL cholesteryl esters, followed by addition of lipoprotein lipase and hydrolysis of the triglycerides. In addition, ABL plasma contains a previously undescribed small and spherical (61 ± 8 Å) protein-rich (63% by weight) HDL fraction, which we call ABL-HDL_4. Our data suggest that absence of cholesteryl ester transfer to triglyceride-rich lipoprotein in ABL causes accumulation of abnormally large cholesteryl ester-rich particles.—**Deckelbaum, R. J., S. Eisenberg, Y. Oschry, M. Cooper, and C. Blum.** Abnormal high density lipoproteins of abetalipoproteinemia: relevance to normal HDL metabolism. *J. Lipid Res.* 1982. **23:** 1274–1282.

Supplementary key words plasma lipoprotein metabolism • apoE • HDL_2 • HDL_3 • cholesteryl esters

Attention has recently been focused on HDL, in particular HDL_2, in view of its proposed role in delaying atherosclerotic disease (1, 2). In spite of this important role of HDL in health and disease, little is known about pathways of formation and intravascular metabolism and catabolism of the lipoprotein. Discoidal lipoproteins of HDL density have been observed in eluates of liver perfusions (3) and intestinal lymph (4). These discoidal structures are believed to represent nascent HDL particles. Another major source of HDL precursors is "surface remnants" of triglyceride-rich lipoproteins (5–8). These "surface remnants" represent redundant surface components that separate from the shrunken core of triglyceride-rich lipoproteins during lipolysis and may form disc-like nascent HDL particles (5, 8) or become associated with pre-existing spherical HDL particles (9). Discoidal HDL precursors are transformed to spheres as cholesterol is converted to hydrophobic cholesteryl ester by the plasma enzyme lecithin:cholesterol acyltransferase (LCAT) (3). The source of HDL subpopulations (HDL_2, HDL_3) is poorly understood (9).

Abetalipoproteinemia (ABL) is a rare genetic disease characterized by absence of apoprotein B (apoB) and of the lipoproteins that contain apoB (10). As a consequence, ABL patients have extremely low levels of plasma cholesterol and triglycerides, and they almost completely lack lipoproteins in the density ranges of chylomicrons and very low density and low density lipoproteins (VLDL and LDL) (11, 12). Plasma lipids are carried almost entirely in the high density lipoproteins. HDL levels are low in this disease and the HDL is qualitatively abnormal (11, 13–15). In normal subjects, the smaller, denser HDL_3 particle accounts for most of the HDL. In ABL, the larger and less dense HDL_2 population is the major subfraction while HDL_3 is present in reduced amounts (13, 14). The cause of this qualitative abnormality in ABL-HDL is unknown. Data described here demonstrate that lack of interactions of HDL with triglyceride-rich lipoproteins, which we suggest occurs in normal subjects, is responsible for the HDL abnormalities in ABL. These studies indicate that major remodeling of HDL occurs in normal humans

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Abbreviations: ABL, abetalipoproteinemia; VLDL, plasma very low density lipoproteins; LDL, plasma low density lipoproteins; HDL, plasma high density lipoproteins; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; LCAT, lecithin:cholesterol acyltransferase.

and that this remodeling is associated with exchange of core lipids between HDL and the triglyceride-rich lipoproteins.

METHODS

Patients

Three patients with ABL were studied. They ranged in age from 21 to 33 years. Plasma cholesterol levels were 41, 45, and 35 mg/dl; triglyceride levels were 13, 5, and 5 mg/dl, respectively. ApoB was absent from plasma when assayed by a specific and sensitive (10 ng) radioimmunoassay. Plasma lipids in each set of parents of these patients were entirely normal, ruling out the possibility of homozygous familial hypobetalipoproteinemia (11). All three patients had gastrointestinal, ophthalmological, neurological, and hematological abnormalities typical of abetalipoproteinemia (10, 11).

Isolation of lipoproteins

Informed consent was obtained from patients and normal controls. Fasting blood was collected in plastic syringes containing disodium EDTA (1 mg/ml). Plasma was immediately separated at 4°C by low-speed centrifugation. Samples were used immediately for zonal ultracentrifugation or were stored in the dark at 4°C under N_2 for a maximum of 48 hr. Zonal ultracentrifugation was performed at 15°C using a Beckman Ti-14 zonal rotor in a Beckman L5-50 ultracentrifuge. HDL was isolated and subfractionated by zonal ultracentrifugation at 41,000 rpm in a discontinuous gradient of NaBr, spanning the density range 1.00–1.40 g/ml (16–18). Runs lasted exactly 22 hr and the rotor effluent was monitored continuously at 280 nm; 25-ml fractions were collected. For analysis, fractions under a given peak were pooled and concentrated by reverse dialysis. Because the smallest HDL particles (ABL-HDL_4) (see below) eluted just prior to the major plasma proteins, the fractions in which HDL_4 appeared were centrifuged in a solution of KBr (1.21 g/ml) in a Beckman 50 Ti rotor at 48,000 rpm for 48 hr. This procedure separated HDL_4 from other plasma proteins. Before analyses, all lipoprotein fractions were extensively dialyzed at 4°C in the dark for 24 hr against normal saline (0.15 M NaCl–1 mM EDTA, pH 8.5, 100:1(v/v)). Lipoproteins were then overlayered with N_2 and stored in the dark at 4°C.

Analyses

Lipids were extracted with chloroform–methanol 2:1 (v/v) (19). Total phospholipid content and individual phospholipid subclasses, total free and esterified cholesterol, and triglyceride contents were determined using procedures previously employed in our laboratories (8). Protein was measured by the method of Lowry et al. (20) with bovine serum albumin as a standard. Sodium dodecyl sulfate (0.1%) polyacrylamide gel (10%) electrophoresis (SDS-PAGE) of HDL apoproteins without prior delipidation was performed according to procedures previously described in detail (21, 22). Samples were run in duplicate with and without a prior 3-min incubation at 90°C in 5% 2-mercaptoethanol. Apoprotein bands were identified on the basis of their mobilities relative to purified apoproteins isolated by column chromatography.

Radioimmunoassay of apoproteins

Radioimmunoassays for apolipoproteins E, A-I, and A-II were performed as described in detail for apoE (23). ApoE was purified from the soluble apoproteins of pooled VLDL by a three-step procedure involving heparin-agarose affinity chromatography, DEAE cellulose chromatography, and preparative SDS-PAGE. ApoA-I and apoA-II were purified from HDL apoproteins by DEAE-cellulose chromatography. Specific antisera to these individual apoproteins were produced in rabbits. The apoproteins were radioiodinated by the chloramine-T procedure (23). Samples for assay were incubated overnight in 50 mM decyl sulfate, 50 mM sodium phosphate, 100 mM NaCl, 0.02% sodium azide, pH 7.4. The assays were performed with final concentration of 5 mM decyl sulfate, 50 mM sodium phosphate, 100 mM NaCl, 0.02% sodium azide, 0.4 μl of nonimmune rabbit serum/ml. After a 2-day incubation period, 1 μl of goat antirabbit serum was added to each assay tube, and the supply was centrifuged for 100,000 *g*-min the next day. Plasma and lipoprotein fractions gave identical displacement curves whether or not they had undergone prior delipidation with ethanol–ether or chloroform–methanol. Within assay coefficients of variation were 9%, 11%, and 5% for apoE, apoA-I, and apoA-II assays, respectively. Coefficients of variation for systematic between assay variability were 3%, 9%, and 4% for the apoE, apoA-I, and apoA-II assays, respectively.

Electron microscopy

Lipoproteins were negatively stained with 2% sodium phosphotungstate, pH 7.4, on collodion carbon-coated grids and sized as described previously (8). Electron micrographs were obtained with a Phillips 300 electron microscope, at instrument magnification of 90,000. Samples were examined at a concentration of 0.1 to 0.5 mg of protein/ml. For size determination, over 250 particles for each fraction were measured.

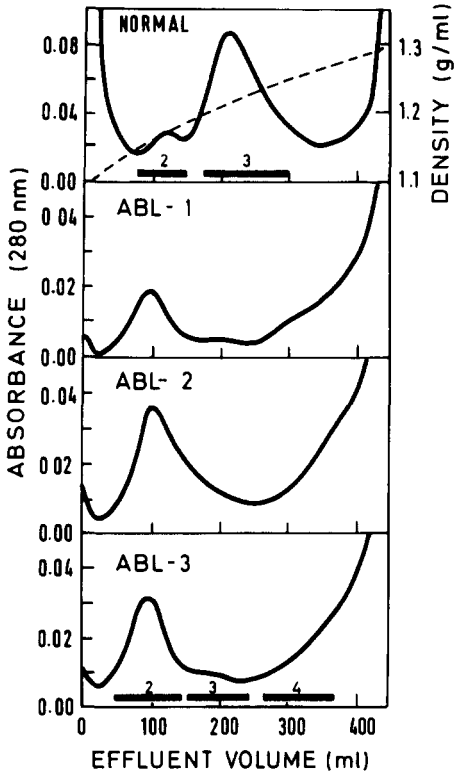

Fig. 1. Separation by rate zonal ultracentrifugation of HDL subfractions in three patients with abetalipoproteinemia and a normal control. Flotation is from right to left. In each frame the effluent pattern is shown as a continuous solid line after continuous measurement of absorbance at 280 nm. In the top frame the dotted line (-----) represents the salt density at different points across the effluent volume. The numbered solid bars (2 and 3) represent the fractions pooled to characterize HDL_2 and HDL_3 in normals; for the ABL patients (2, 3 and 4) represent HDL_2, HDL_3, and HDL_4, respectively. In ABL, HDL_2 is the major HDL subfraction in contrast to normals. In addition in ABL, HDL_2 and HDL_3 elute earlier than in normal controls. A separate HDL subfraction, HDL_4, can be separated in ABL. ABL-1, ABL-2, and ABL-3, are effluent patterns obtained with 10 ml of plasma from patients 1–3 with total plasma cholesterol of 41, 35, and 45 mg/dl, respectively. The normal effluent pattern was obtained with 20 ml of plasma from a normal subject with total plasma cholesterol of 171 mg/dl and HDL-cholesterol, 66 mg/dl.

Modification of ABL-HDL towards normal HDL

ABL-plasma was incubated with human VLDL and/or purified milk lipoprotein lipase; VLDL was then removed and the ABL-HDL was isolated by zonal ultracentrifugation as described above. Human VLDL was obtained from fasting control donors and isolated and washed in a Beckman 50.3 Ti rotor by salt density ultracentrifugation ($d < 1.006$ g/ml) (8). VLDL was added to 7.5 ml of ABL-plasma (patient 1) at a final VLDL triglyceride concentration of 5 mg/ml and a final volume of 9.2 ml. After 18 hr of incubation at 37°C under nitrogen, the $d < 1.006$ g/ml fraction was removed by ultracentrifugation. ABL-HDL was then isolated from the $d < 1.006$ g/ml fraction by zonal ultracentrifugation as outlined above.

In a parallel experiment, after removing the VLDL by density ultracentrifugation, 100 μl of purified bovine milk lipoprotein lipase was added to the ABL plasma and lipolysis was allowed to take place at 37°C for 60 min. Lipase was kindly provided by Dr. Thomas Olivecrona, Umea, Sweden, and used as previously described (8). ABL-HDL was then isolated by zonal ultracentrifugation.

In a third experiment, 7.5 ml of ABL-plasma was incubated at 37°C with 100 μl of lipase solution but no VLDL for 60 min, and ABL-HDL was isolated from the incubation mixture by zonal ultracentrifugation.

RESULTS

Characterization of lipoproteins

Zonal ultracentrifugation of normal and ABL plasma separates HDL into differing populations as shown in **Fig. 1**. Two major populations representing HDL_2 and HDL_3 are present in normals and in patients. In normals, HDL_3 is the major subpopulation, whereas the larger, less dense particles of HDL_2 are in great excess in ABL. Of total plasma (and thus HDL) cholesterol in ABL, 64.2 ± 5.2% (mean ± SD) is found in HDL_2, 28.1 ± 3.1% in HDL_3, with the remainder in HDL_4 (vide infra). The expected amount of HDL_2 in normal humans with similar HDL levels is only about 10–20% of total HDL (24). In ABL, both HDL_2 and HDL_3 elute from the rotor effluent earlier than do the corresponding subfractions of normal HDL. These data show not only that HDL_2 is the major lipoprotein in ABL, but also that both HDL_2 and HDL_3 in this disease are of lower density than their normal counterparts.

A consistent finding on HDL subfractionation in this disease is a broad low peak at 275–375 ml superimposed upon the ascending limb of the peak of the major plasma proteins. This broad, low peak is not seen when normal plasma is fractionated. Characterization of this peak confirmed the presence of a discrete lipoprotein particle smaller than either HDL_2 or HDL_3. Thus, patients with ABL have a previously undescribed subfraction of high density lipoprotein. In normal plasma, this discrete subfraction is either not present or is present in much lower concentration than in ABL. We refer to this particle as ABL-HDL_4.

In each ABL plasma sample studied, a small amount of material was eluted in the first 25 ml of the zonal effluent where normal VLDL and LDL would be eluted. This early fraction accounted for less than 3% of the cholesterol and less than 5% of triglyceride in ABL plasma.

The composition of the HDL subfractions of normal and ABL plasmas is given in **Table 1**. A lecithin to sphin-

TABLE 1. Composition analysis of HDL subfractions in abetalipoproteinemia and normal controls

Lipoprotein	Protein	Triglyceride	Free Cholesterol	Cholesteryl Ester	Phospholipid	Lecithin/ Sphingomyelin Molar Ratio
HDL_2-ABL	31.8 ± 2.1[a]	6.0 ± 3.1	7.7 ± 2.1	25.6 ± 4.2	29.0 ± 1.4	1.1 ± 0.2
HDL_2-Normal	37.4 ± 1.9	4.1 ± 1.3	5.2 ± 1.6	17.8 ± 3.2	35.5 ± 3.4	3.7 ± 1.0
HDL_3-ABL	45.2 ± 3.5	5.5 ± 2.2	4.7 ± 1.2	20.8 ± 3.2	23.7 ± 2.8	1.8 ± 0.2
HDL_3-Normal	48.9 ± 1.9	4.0 ± 0.4	2.2 ± 0.2	14.4 ± 2.5	30.5 ± 4.1	7.1 ± 0.7
HDL_4-ABL	63.2 ± 7.4	3.5 ± 3.3	2.6 ± 0.9	15.9 ± 3.4	14.7 ± 2.7	2.9 ± 1.3

[a] Composition is expressed as relative weight composition (% of total lipoprotein mass). Results are mean ± S.D. of HDL subfractions isolated by zonal ultracentrifugation of three patients with abetalipoproteinemia and three normolipemic controls. Since HDL_4 can be isolated only in ABL patients, no comparable values exist for normals.

gomyelin ratio of 1.1 in ABL-HDL_2 is among the lowest ever described for any human lipoprotein fraction and is much lower than that for normal HDL_2. HDL_3 shows similar differences between patients and normals but to a lesser degree. ABL-HDL_4 contained almost 64% protein by weight, a value greater than that of previously reported human high density lipoprotein populations in normolipemic subjects.

The ratio of surface constituents (cholesterol, phospholipids, and protein) to core constituents (cholesteryl esters and triglycerides) can be used to make inference on the size of spherical lipoprotein particles. Since the surface area of a sphere increases with the square of the radius, while the volume increases with the cube of the radius, a lower ratio of surface/core constituents would imply a population of larger particles. The ratio of surface to core constituents in ABL-HDL_2 and HDL_3 is 60% that of normals. This indicates that larger lipoproteins are present in these subfractions in ABL than in normals.

Electron microscopy of ABL plasma lipoprotein (**Fig. 2**) confirms that ABL-HDL_2 is larger than normal HDL_2 (diameter 136 ± 19 Å vs. 113 ± 12 Å). Similarly, ABL-HDL_3 is larger than normal HDL_3 (100 ± 12 Å vs. 86 ± 4 Å). These relatively small differences in diameter represent large differences in volume. The apolar core volume of ABL-HDL_2 is 2.3-times that of normal HDL_2 and ABL-HDL_3 has 2.2-times the core volume of its normal counterpart. ABL-HDL_4 contains a population of spherical particles, diameter 61 ± 8 Å. No discs or vesicles were observed in any HDL subfraction of ABL plasma.

Polyacrylamide gel electrophoresis patterns of HDL subfractions from patients with ABL and from normal volunteers are shown in **Fig. 3**. ABL-HDL_2 in every patient studied shows markedly increased apoE relative to normals. Above apoE a dark staining band, the apoE-A-II disulfide dimer (25), is identified. This complex is reduced in both ABL-HDL_2 and HDL_3 by preincubation of the apoproteins with mercaptoethanol (**Fig. 4**). Apoprotein profiles in HDL_3 and HDL_4 of ABL appear similar to normal HDL_3 although apoE is more prominent in ABL-HDL_3. These results confirm that the unusual apoprotein bands previously reported in ABL-HDL (14, 26) can now be clearly recognized as apoE and the E-A-II apoprotein complex.

Apoproteins A-I, A-II, and E were quantitated by radioimmunoassay in HDL subfractions in two patients and in normal volunteers (**Table 2**). These analyses

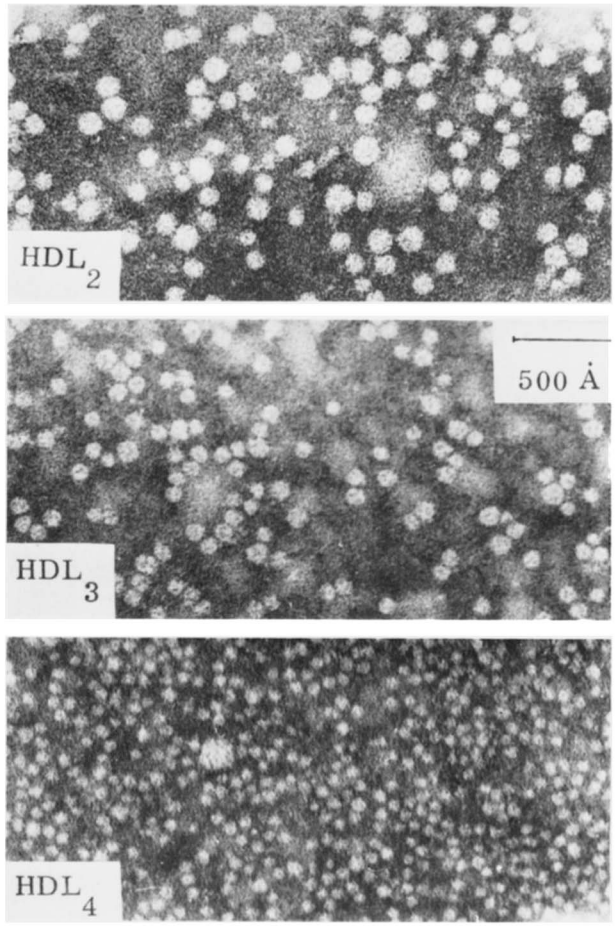

Fig. 2. Electron microscopy of HDL subfractions in abetalipoproteinemia.

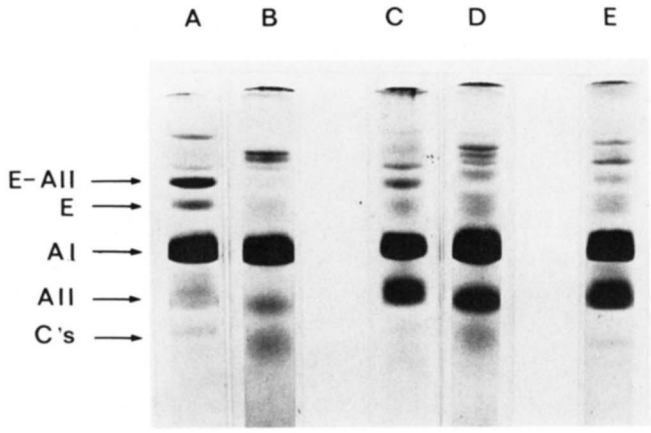

Fig. 3. Electrophoretic behavior of apoproteins in HDL subfractions in ABL compared to normal controls on SDS-PAGE. A) ABL-HDL_2, B) Normal HDL_2, C) ABL-HDL_3, D) Normal HDL_3, E) ABL-HDL_4. In each case 0.10 to 0.11 mg of total protein was applied to the gel.

demonstrate clear differences from normals in apoprotein profiles for ABL-HDL_2, HDL_3, and HDL_4, and confirm increased apoE in ABL lipoproteins.

Modification of ABL-HDL_2

In the previous section we demonstrated that ABL-HDL_2 and HDL_3 are at least twofold larger in core volume than normals. Thus, they carry a substantially increased quantity of cholesteryl ester.

To determine whether the large size of ABL-HDL is due to lack of triglyceride-rich lipoproteins, we incubated ABL-plasma with normal VLDL and subsequently with lipoprotein lipase (**Fig. 5**). Incubation of ABL-plasma with VLDL resulted in enrichment of the ABL-HDL_2 with triglyceride, a decrease in cholesteryl ester, and a fall in the cholesteryl ester:protein ratio showing net depletion of cholesteryl ester (**Table 3**). The zonal rotor elution profile of the triglyceride-enriched ABL-HDL however was identical to that of native ABL-HDL_2 indicating that particle size and density had not been modified although composition had been modified (Fig. 5A). Incubation of ABL-plasma with purified lipoprotein lipase alone had little effect on ABL-HDL_2 (Fig. 5C and Table 3). In contrast to these results, Fig. 5B demonstrates that after incubation of triglyceride-enriched ABL-HDL with purified lipoprotein lipase, the zonal elution profile of the ABL-HDL_2 is modified to resemble that of normal HDL_2 (compare Figs. 5B and 1A). Compositional analysis of the post-lipolysis, triglyceride-enriched ABL-HDL shows, indeed, a reduction in triglyceride and a net loss of cholesteryl ester relative to the original unincubated ABL-HDL_2 (Table 3).

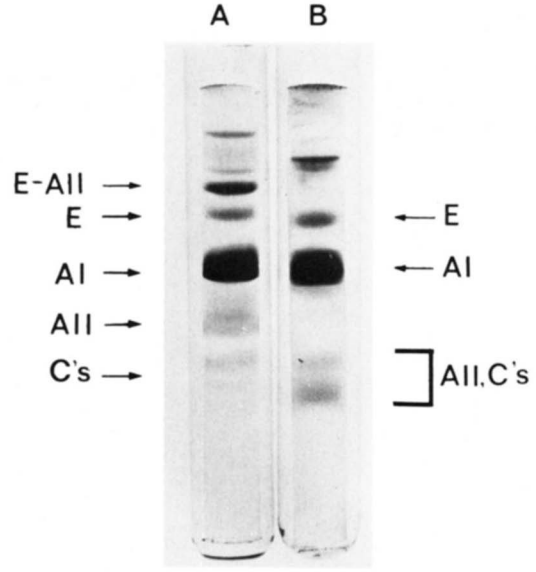

Fig. 4. Effect of mercaptoethanol reduction on the SDS-PAGE apoprotein mobilities of ABL-HDL_2. A) ABL-HDL_2 apoprotein without prior incubation with 5% 2-mercaptoethanol; B) ABL-HDL_2 apoproteins after incubation with mercaptoethanol. After mercaptoethanol incubation the E-A-II apoprotein complex and dimeric apoprotein A-II are reduced to their products, apoE and monomeric apoA-II.

TABLE 2. Apoprotein A-I, A-II, and E contribution to HDL subfractions in abetalipoproteinemia and normal controls

		A-I	A-II	E
HDL_2	Patient 1	81.1[a]	11.6	7.3
	Patient 2	84.5	10.4	5.1
	Normal	84.7 ± 4.9[b]	10.8 ± 2.1	4.5 ± 2.9
HDL_3	Patient 1	55.4	42.4	2.3
	Patient 2	56.8	41.3	1.9
	Normal	65.7 ± 6.4	33.6 ± 6.2	0.8 ± 0.4
HDL_4	Patient 1	66.4	32.9	0.7
	Patient 2	70.9	28.3	0.9

[a] Composition is expressed as relative weight composition (% of total of apoproteins A-I plus A-II plus E). Each value for patients 1 and 2 is the mean of two separate determinations. Since HDL_4 can be isolated only in ABL patients, no comparable values exist for normals.
[b] Mean ± S.D. from four normolipemic controls.

DISCUSSION

Abetalipoproteinemia is a disease in which apoB-containing lipoproteins (chylomicrons, VLDL, and LDL) are absent from plasma. This provides a unique situation in which formation of HDL cannot be modified by interactions with triglyceride-rich lipoproteins, and enables an investigation on the effects of such interactions on HDL. In doing so, we have elucidated pathways responsible for major remodeling of the HDL system. These pathways may provide adequate explanation for HDL variability in patients and normal humans.

Using zonal ultracentrifugation, which allows full isolation of lipoprotein subpopulations despite flotation changes that may cross density limits of standard salt density ultracentrifugation, we have observed new abnormalities in HDL of ABL: the presence of an HDL subpopulation lighter and larger than any known HDL (designated here ABL-HDL_2) and the presence of a small spherical protein-rich heavy HDL particle designated ABL-HDL_4. Almost two-thirds of the plasma and therefore of HDL cholesterol in ABL is associated with the ABL-HDL_2 population. We propose that the unique properties of these HDL subpopulations reflect the basic metabolic defect of the disease, absence of apoB-containing lipoproteins.

Spherical HDL particles, smaller and denser than normal HDL_3, have been previously observed in LCAT deficiency (27) and Tangier disease (28). Also, small HDL populations have been found in plasma of severely hypertriglyceridemic patients (17) especially subsequent to lipoprotein triglyceride lipolysis following heparin injection (29). In ABL, the HDL_4 lipoproteins cannot be formed from triglyceride-rich lipoproteins as they are absent from plasma.

In Tangier disease, HDL consists predominantly of large vesicles and discoidal particles (28, 30) probably reflecting hypercatabolism of apoA-I (31) and inability to form normal HDL (32). The source of the minor apoA-II-rich small-sized spherical HDL population (28) in this disease is unclear. In LCAT deficiency, most HDL is also of vesicular and discoidal shape, reflecting inability of these patients to form spherical particles by converting cholesterol to cholesteryl ester via the LCAT reaction.

The presence of these spherical protein-rich HDL particles containing cholesteryl ester both in ABL, where surface remnants of triglyceride-rich lipoproteins are not formed, and in LCAT deficiency, where surface remnants are not converted to spheres (an LCAT-dependent process), suggests that HDL_4-like particles are not catabolic products of triglyceride-rich lipoproteins. More likely, they are directly secreted into plasma. In ABL, we suggest the source of spherical HDL_4 to be intestine or liver, tissues rich with cholesterol-esterifying activity. However, the presence of apoproteins A-I and A-II—both largely of intestinal origin (33)— and the

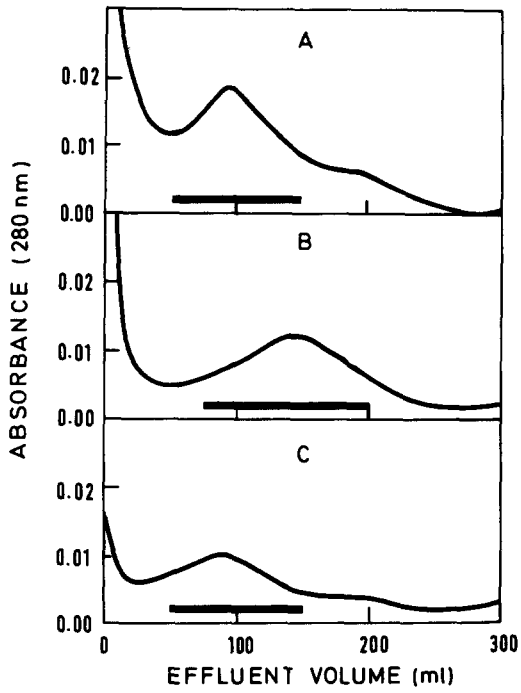

Fig. 5. Modifications of ABL-HDL_2 following incubations of 7.5 ml of ABL plasma with VLDL and lipoprotein lipase. Rate zonal ultracentrifugation analysis. A) Elution patterns of ABL-HDL_2 (peak at ~100 ml elution volume) and ABL-HDL_3 (peak at ~200 ml elution volume) were unchanged after incubation with human VLDL (compare with Fig. 1). B) After incubation of ABL-plasma with VLDL, and subsequent addition of purified lipoprotein lipase, the major lipoprotein peak, ABL-HDL_2 moved to a higher density in the rotor effluent. C) Incubation of ABL-plasma with lipoprotein lipase without prior incubation with VLDL had no effect on the ABL-HDL elution profile. The solid horizontal bars represent the fraction pooled to characterize HDL_2.

TABLE 3. Compositional changes in abetalipoproteinemia HDL_2 induced by incubation of abetalipoproteinemia plasma with normal human VLDL and/or purified lipoprotein lipase

	Protein	Triglyceride	Free Cholesterol	Cholesteryl Ester	Phospholipid	Cholesteryl Ester:Protein Ratio (w/w)
Unincubated ABL-plasma	30.7[a]	7.8	10.3	22.1	29.0	0.72
ABL-plasma + VLDL[b]	34.1	19.0	2.5	8.1	36.4	0.24
ABL-plasma + VLDL + lipoprotein lipase[c]	39.7	11.3	2.7	11.6	34.7	0.29
ABL-plasma + lipoprotein lipase[d]	31.8	6.8	7.8	23.8	29.8	0.75

[a] Composition is expressed as relative weight composition (% of total lipoprotein mass). Each value represents the mean of two separate determinations.

[b] ABL-plasma (7.5 ml) was incubated with human VLDL for 18 hr at 37°C under nitrogen. After incubation the VLDL fraction was removed by ultracentrifugation and ABL-HDL was isolated by zonal ultracentrifugation.

[c] After removal of VLDL, lipoprotein lipase was added to the ABL-plasma (see Methods).

[d] Lipoprotein lipase only was added to ABL-plasma.

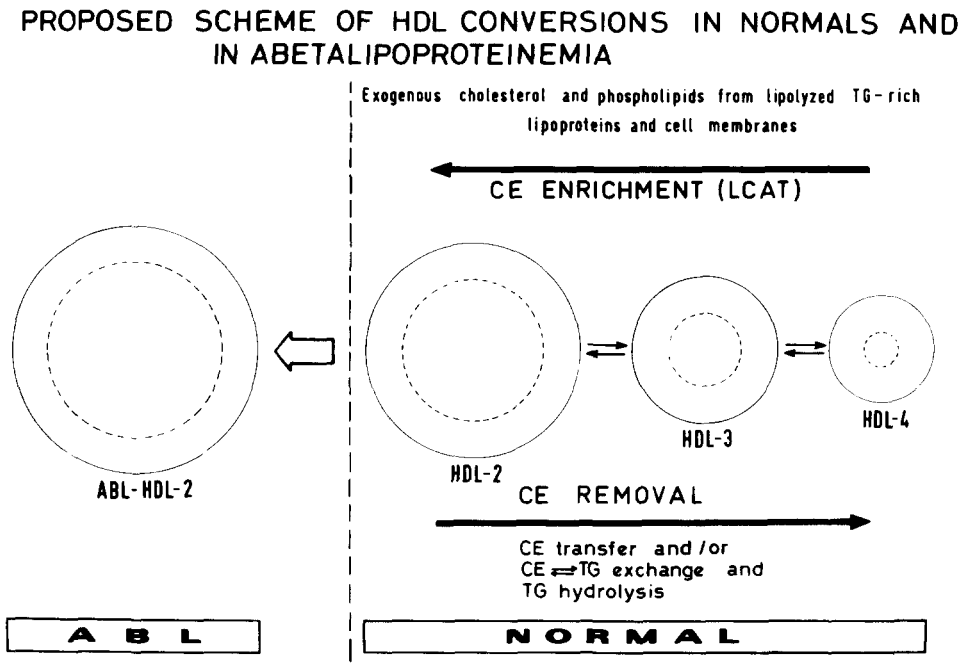

Fig. 6. Proposed scheme of HDL conversions in normal and in abetalipoproteinemic subjects. The scheme describes a pathway for enrichment of HDL with cholesteryl ester via the LCAT reaction. The source of free cholesterol and phospholipids for the LCAT reaction is from surface constituents of lipolyzed chylomicrons and VLDL (in normals) and from cell membranes (normals and ABL). HDL precursors are the smallest particles, designated here as HDL_4 or HDL_4-like particles. Accumulation of cholesteryl esters leads to increase in size of the particles to form first HDL_3 and then HDL_2. Concomitantly, a pathway for cholesteryl ester removal from HDL is operative. This path operates through direct transfer of cholesteryl esters to lower density lipoproteins (chylomicrons, VLDL, and LDL) and/or cholesteryl ester ↔ triglyceride exchange followed by hydrolysis of the HDL triglycerides. With either mechanism of cholesteryl ester removal, a limitation of the HDL size is achieved. In normals, the dynamic equilibrium of cholesteryl ester enrichment and removal results in HDL subpopulation distribution of a predominant HDL_3 pattern. In ABL, a disease where acceptor lipoprotein for excess cholesteryl esters is absent from plasma, the equilibrium is shifted to the left, resulting in the appearance of the abnormally large-sized cholesteryl ester enriched ABL-HDL_2 and ABL-HDL_3 particles.

paucity of apoE—an apoprotein of hepatic origin (21)—provide evidence for an intestinal origin of ABL-HDL_4. Despite its spherical shape, HDL_4 may indeed be an "intestinal nascent" HDL in ABL and LCAT deficiency as well as in normals. Although small-sized and heavy HDL particles were recently described in normal plasma as part of the far tail of HDL_3 peaks (18), they do not form a discrete fraction. Our attempts to identify such tiny HDL particles in normal plasma as a *discrete* lipoprotein subpopulation discernible from the main HDL_3 peak (as seen in ABL) have been unsuccessful so far.[2] Therefore, in normal humans, HDL_4-like populations may be obscured by the large amounts of plasma HDL_3 whereas in ABL when HDL_3 levels are extremely low, they become prominent.

It has previously been suggested that HDL_2 in ABL floats at lower density than normal HDL_2 (15). The ability of each ABL-HDL_2 particle to carry more than twice the cholesteryl ester of normal HDL_2 has not been, however, previously appreciated. The estimated core volume (see results) of ABL-HDL_2 is at least 2.3-times that of normal HDL_2, and for ABL-HDL_3 2.2-times that of normal HDL_3. This increased core volume is reflected by the higher cholesteryl ester content of ABL-HDL and points to accumulation of cholesteryl esters in ABL-HDL_2 (and HDL_3), much above that observed in normal HDL_2. The source of HDL cholesteryl esters is esterification of free cholesterol (acquired from other lipoproteins and cell membranes) via the LCAT reaction. If these cholesteryl esters remain in HDL, then HDL_3 will grow to larger HDL_2 as recently suggested by Barter, Ha, and Calvert (34). In normal humans, maximum HDL size is less than that acquired in ABL, suggesting that ABL patients are missing a pathway that contributes to limitation of HDL volume. Such a pathway would be the activity of the plasma cholesteryl ester transfer protein(s) (34–36). It has been suggested that as much as 80% of the LCAT-derived cholesteryl esters in normal humans are transferred by this reaction(s)

[2] Oschry, Y., S. Eisenberg, and R. J. Deckelbaum. Unpublished observation.

from the HDL to lower density lipoproteins (37). We have recently demonstrated that cholesteryl ester loss from LDL (38) and HDL[3] occurs in exchange for triglycerides originating from the triglyceride-rich lipoproteins, a pathway originally described by Nichols and Smith (39). The triglyceride exchanged into the HDL core is susceptible to hydrolysis by lipoprotein lipase. The resulting HDL particle will be somewhat smaller and poorer in cholesteryl ester than at the start of this process. Triglyceride-rich lipoproteins are needed in this pathway both as cholesteryl ester acceptors and triglyceride donors. In the absence of triglyceride-rich lipoproteins in ABL, exchanges of cholesteryl ester for triglyceride as well as cholesteryl ester transfer cannot occur and cholesteryl esters would accumulate in HDL particles in supranormal amounts. Our ability to modify ABL-HDL towards normal HDL by incubation with triglyceride-rich lipoproteins and lipoprotein lipase is strong evidence that this postulated pathway is potentially present in ABL plasma and is important in the formation of a normal population of HDL particles.

The considerations discussed above suggest that major remodeling of HDL occurs in normal plasma as schematically represented in **Fig. 6**. HDL according to the scheme is affected by two opposing metabolic pathways, one causing cholesteryl ester accumulation and the other, removal. The initial HDL particle is a small sphere containing mainly surface constituents, i.e., apoprotein and phospholipid similar to or identical with ABL-HDL_4. This small protein-rich particle acquires lipids (phospholipids and free cholesterol) from lipolyzed lipoproteins (in normal individuals) and from cells (in both normals and ABL) and, through the activity of the LCAT system, increases in size to form HDL_3. With the continued generation of more cholesteryl esters and addition of apoproteins and surface lipids, an HDL_2 particle is formed. During this cycle, however, some—or much—of the LCAT-derived cholesteryl esters are transferred to other lipoproteins partly in exchange for triglyceride. Acquired triglyceride is then lost via lipolysis. Thus, the HDL system represents a dynamic equilibrium of several complex processes: generation of precursors (surface remnants, HDL_4-like and nascent discoidal particles), accumulation of free cholesterol and phospholipid derived from lipolysis and from cells, LCAT activity generating cholesteryl esters, and transfer of cholesteryl esters to other lipoproteins. This hypothesis explains the marked variation of HDL size, composition, density, and subpopulation variability as observed in numerous clinical situations as well as in ABL, a disease that might serve as an exceptionally suitable model for further testing of the validity of these concepts.

[3] Deckelbaum, R. J., S. Eisenberg, and Y. Oschry. Unpublished observations.

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