In vivo metabolism of apolipoproteins A-I and E in patients with abetalipoproteinemia: implications for the roles of apolipoproteins B and E in HDL metabolism

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Abstract The metabolism of high density lipoproteins (HDL) is tightly linked to the metabolism of apoB-containing lipoproteins through the exchange and transfer of lipids and apolipoproteins within the plasma compartment. Abetalipoproteinemia (ABL), a genetic disease in which apoB is absent from the plasma and HDL are the sole plasma lipoproteins, is a model for the investigation of HDL metabolism without modification by apoB-containing lipoproteins. Apolipoproteins A-I and E are two of the major apolipoproteins in HDL. Plasma apoA-I levels, but not apoE levels, have been reported to be decreased in patients with ABL. Furthermore, HDL from ABL patients is enriched in apoE compared with normal subjects. The purpose of the present study was: *1)* to elucidate the metabolic basis of the low apoA-I levels in ABL; *2)* to determine whether in vivo apoE production rates are normal in the absence of apoB-lipoprotein secretion; and *3)* to test the hypothesis that apoE influences apoA-I and HDL catabolism in ABL. ^{131}I-labeled apoA-I and ^{125}I-labeled apoE were reassociated with autologous lipoproteins and injected into two unrelated ABL patients and control subjects. The mean residence time of apoA-I in ABL (2.4 days) was significantly decreased by nearly 50% compared with control subjects (4.7 ± 0.6 days). ApoA-I production rates were also significantly decreased by 40% in ABL (7.1 mg/kg-d) compared with control subjects (11.8 ± 1.7 mg/kg-d). The mean residence time of apoE in ABL (0.50 days) was somewhat shorter than that of control subjects (0.66 ± 0.15 days), whereas the mean apoE production rate in ABL (2.14 mg/kg-d) was not substantially different from that of control subjects (1.55 ± 0.62 mg/kg-d). HDL subfractions LpA-I and LpA-I:A-II were isolated using immunoaffinity chromatography. In contrast to the normal metabolism, apoA-I in LpA-I:A-II particles was catabolized at a faster rate than apoA-I in LpA-I, accounting for the greater decrease of plasma LpA-I:A-II relative to LpA-I in the ABL patients. HDL subfractions with and without apoE were also isolated using anti-apoE immunoaffinity chromatography. Labeled apoA-I in apoE-containing HDL was catabolized faster than that in HDL without apoE. Among the three different forms of apoE, the apoE monomer was catabolized at the fastest rate, the apoE homodimer at an intermediate rate, and the apoE-A-II heterodimer had the slowest rate of catabolism. In conclusion, decreased apoA-I levels in ABL are due to both a decreased rate of apoA-I production as well as an increased rate of apoA-I catabolism, in particular that of apoA-I in LpA-I:A-II. Despite the lack of secretion of apoB-containing lipoproteins, apoE production is not impaired in ABL. These results provide insight into the role of apoB and apoE in HDL metabolism.—**Ikewaki, K., D. J. Rader, L. A. Zech, and H. B. Brewer, Jr.** In vivo metabolism of apolipoproteins A-I and E in patients with abetalipoproteinemia: implications for the roles of apolipoproteins B and E in HDL metabolism. *J. Lipid Res.* 1994. **35:** 1809–1819.

Supplementary key words apoA-I • apoE • kinetics • radiotracer • high density lipoproteins

High density lipoproteins (HDL) have been proposed to be anti-atherogenic, based on multiple epidemiologic studies that have demonstrated an independent inverse correlation of HDL cholesterol levels with the incidence of coronary heart disease (CHD) (1, 2). However, the exact mechanisms by which HDL may protect against the development of premature CHD are not completely understood. Although HDL are heterogenous in apolipoprotein composition, apoA-I is a major apolipoprotein constituent; apoA-I levels, like HDL-C, have an inverse correlation with risk for CHD (3).

Abbreviations: ABL, abetalipoproteinemia; HDL, high density lipoprotein; LDL, low density lipoprotein; VLDL, very low density lipoprotein; FCR, fractional catabolic rate; RT, residence time; PR, production rate; CHD, coronary heart disease; TG, triglyceride; FC, free cholesterol; CE, cholesteryl ester; PL, phospholipid; LCAT, lecithin:cholesterol acyltransferase; CETP, cholesteryl ester transfer protein.

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HDL functions as a reservoir for excess cholesterol, phospholipid, and apolipoproteins, including apoC-II, apoC-III, and apoE released from the surface of triglyceride (TG)-rich lipoproteins during lipolysis (4, 5). HDL may also accept excess cholesterol from cells (6), and transport it back to the liver through a process termed reverse cholesterol transport (7). Lecithin:cholesterol acyltransferase (LCAT) is responsible for esterification of cholesterol within the plasma compartment. In humans, most cholesteryl ester in HDL is transferred to apoB-containing lipoproteins via the cholesteryl ester transfer protein (CETP) and is ultimately taken up by the liver via the LDL receptor (8). Alternative pathways proposed for the delivery of HDL cholesteryl ester to the liver are those mediated by the putative HDL receptor (9, 10) and apoE receptor (11, 12). However, the existence of this apoE-mediated pathway has not been confirmed in humans, in part because of the exchangeable nature of apoE between HDL and apoB-containing lipoproteins. Thus, HDL metabolism is linked to that of apoB-containing lipoproteins through active transfer of lipids and apolipoproteins between these two classes of plasma lipoproteins.

Abetalipoproteinemia (ABL) is an autosomal recessive disease characterized by the absence in the plasma of the apoB-containing lipoproteins: chylomicrons, VLDL, and LDL (13). HDL are the sole plasma lipoproteins in ABL, but even levels of HDL and apoA-I are substantially lower than normal (13). The few kinetic studies of HDL in ABL patients have shown that either decreased apoA-I production with normal catabolism (14, 15) or increased apoA-I catabolism (16) accounts for the decreased plasma apoA-I levels. Previous in vitro studies have suggested that apoE-containing HDL particles may play an important role in the delivery of cholesterol to tissues in ABL (17, 18). The present study was undertaken in order to: *1)* elucidate the metabolic basis of the low apoA-I levels in ABL; *2)* determine whether in vivo apoE production rates are normal in the absence of apoB-lipoprotein secretion; and *3)* test the hypothesis that apoE influences apoA-I and HDL catabolism in ABL.

METHODS

Study subjects

Two unrelated ABL patients were studied. Both patients had the spinocerebellar ataxia, degenerative pigmentary retinopathy, acanthocytosis, and absence of plasma apoB characteristic of ABL. Both ABL patients were on medication (vitamin A 50,000 IU, vitamin E 14,400 IU, and vitamin K 10 mg per day). Control subjects were healthy young adults who were admitted as normal volunteers to the Clinical Center of the National Institutes of Health. All study subjects had normal fasting glucose levels and normal thyroid, liver, and renal function, and were on no medications affecting lipid metabolism. They all gave informed consent to the study protocol approved by the Clinical Research Subpanel of the National Heart, Lung, and Blood Institute.

Isolation and iodination of apolipoproteins

ApoA-I was isolated from normal HDL by gel permeation and ion exchange chromatography as previously described (19). ApoE-3 was isolated from the VLDL of an apoE3/3 homozygote by heparin affinity and gel permeation chromatography as previously reported (20). Lyophilized apolipoproteins were solubilized in a buffer of 6 M guanidine-HCl and 1 M glycine (pH 8.5) and iodinated with ^{131}I or ^{125}I by a modification of the iodine monochloride method (21, 22). Iodination efficiency was 15–26% with incorporation of approximately 0.5 mol iodine per mol protein. ^{131}I-labeled apoA-I was reassociated with autologous whole plasma and free iodine was removed by extensive dialysis against PBS/0.01% EDTA. ^{125}I-labeled apoE was incubated with autologous plasma for 15 min at 37°C, then the plasma was adjusted to d 1.21 g/ml with solid KBr and ultracentrifuged in a 60 Ti rotor at 59,000 rpm for 24 h at 4°C. The d 1.21 g/ml lipoprotein supernatant was recovered by tube-slicing and free iodide was removed by dialysis. Human serum albumin was added to both injection materials at a final concentration of 1%. The injection samples were sterile-filtered through a 0.22 μm Millipore filter and tested for pyrogens prior to simultaneous injection into study subjects.

Study protocol

Three days before injection, the subjects were placed on an isoweight diet containing 47% carbohydrate, 37% fat (5% as polyunsaturated fat, 11% as monounsaturated fat, 19% as saturated fat), 16% protein, 200 mg of cholesterol per 1000 kcal, and a polyunsaturated to saturated fat ratio of approximately 0.3. Meals were given three times per day and the diet was continued during the metabolic study. One day prior to the study, the subjects were started on potassium iodide (900 mg) in divided doses and this was continued throughout the study period. After a 12-h fast, the subjects were injected with up to 25 μCi of ^{131}I-labeled apoA-I and 40 μCi of ^{125}I-labeled apoE. Blood samples were obtained 10 min after injection and then at 1, 3, 6, 12, 18, 24, and 36 h, daily through 5 days, and 7, 9, 11, 14 days post injection. Urine was collected continuously throughout the study.

Blood samples (20 ml) were drawn into tubes containing EDTA at a final concentration of 0.1%. The blood was kept on ice and the plasma was immediately separated by centrifugation at 2300 rpm for 30 min at 4°C. Sodium azide and aprotinin were added to the plasma at a final concentration of 0.05% and 200 KIU/ml, respectively. Radioactivities in plasma, urine, and isolated lipoprotein fractions were quantitated in a Packard Cobra gamma

counter (Packard Instrument Co., Downers Grove, IL).

Plasma radioactivity decay curves were constructed as the fraction of injected dose using the 10-min plasma counts as the initial time and were fitted using a computer-assisted curve fitting technique (23). The residence times (RT, days) were obtained from the area under the curves. The production rates (PR, mg/kg-d) were calculated from the following formula: PR = (plasma apolipoprotein concentration) × (volume of distribution of the apolipoprotein)/(residence time). The volume of distribution of apolipoprotein was determined using the 10-min plasma counts.

Isolation of HDL subfraction particles by immunoaffinity chromatography

Monoclonal antibodies against apoA-I, apoA-II, and apoE were prepared by intraperitoneal immunization of Balb/C mice with intact HDL_3 for apoA-I and apoA-II and with VLDL for apoE and characterized as previously reported (24). A mixture of three monoclonal antibodies for each apolipoprotein (2 mg/ml gel) was covalently bound to Affi-Gel 15 (Bio-Rad, Richmond, CA) according to the manufacturer's recommendations. A polyclonal antisera against apoB (Boehringer-Mannheim, Mannhein, Germany) was coupled to CNBr-Sepharose (5 mg/g gel).

The isolation procedure for LpA-I and LpA-I:A-II has been previously described (21). Briefly, plasma (0.5 ml) was applied to the anti-apoB immunoaffinity column equilibrated with the PBS/0.05% EDTA buffer and eluted at a flow rate of 10 ml/h. The retained fraction was eluted with 3 M sodium thiocyanate (NaSCN) at a flow rate of 30 ml/h. The non-retained fraction was then applied to the anti-apoA-II immunoaffinity column and separated into two fractions in the same manner. Finally, the non-retained fraction of the anti-apoA-II immunoaffinity column was applied to the anti-apoA-I immunoaffinity column. All the non-retained and the retained fractions were collected into 4-ml tubes and immediately measured for radioactivity. Greater than 90% of the radioactivity in the original sample was recovered using this system. Plasma from the ABL patients was directly applied to the anti-apoA-II affinity column followed by the anti-apoA-I immunoaffinity column for the isolation of LpA-I and LpA-I:A-II.

In addition, plasma (0.5 ml) from the ABL patients was applied to the anti-apoE immunoaffinity column and was separated into the two fractions, apoE-containing HDL and HDL without apoE, using the same procedure described above. Average recoveries of radioactivity were greater 90% for all three immunosorbents.

Isolation of ^{125}I-labeled apoE monomer, homodimer, and heterodimer

In the ABL patients, "HDL_1" ($d < 1.063$ g/ml), HDL_2, and HDL_3 fractions were isolated at selected time points by sequential ultracentrifugation as previously described (25). Isolated lipoprotein fractions were dialyzed against 10 mM ammonium bicarbonate and delipidated with ethanol–ether 3:1 (v/v). ApoE monomer, homodimer, and E-A-II heterodimer were separated by nonreducing sodium dodecyl sulfate polyacrylamide electrophoresis ($NaDodSO_4$-PAGE) according to the method previously reported (26). Before Coomassie blue staining, a polyvinylidene difluoride membrane (Immobilon PVDF, Millipore, Bedford, MA) was briefly blotted on the gels, and the bands corresponding to apoE monomer, homodimer, and E-A-II heterodimer were identified by incubating the membranes with anti-apoE and anti-apoA-II monoclonal antibodies. ApoE-containing bands were cut and ^{125}I-labeled apoE radioactivity was quantitated as reported previously (27). The counts at each time point were expressed as the fraction of the activity at 10 min as the initial activity.

Analytical methods

Agarose gel electrophoresis was performed using preformed 0.6% agarose plates (Helena Laboratories, Beaumont, TX). Plasma cholesterol and triglycerides were quantitated by automated enzymic techniques on an Abbott VPSS analyzer (Abbott Labs, North Chicago, IL). HDL cholesterol was determined by the dextran sulfate precipitation method (28). Plasma apoA-I and apoA-II were quantitated by immunoturbitometric assay (Boehringer-Manheim, Mannheim, Germany), apoB by competitive enzyme-linked immunosorbent assays (ELISA) (29), and apoE by radioimmunoassay (RIA) (20). Plasma apoA-I concentration in LpA-I was measured by differential electroimmunoassay using the method described by Parra et al. (24). The apoA-I concentration in LpA-I:A-II was obtained by subtracting the LpA-I value from the total plasma apoA-I concentration. ApoE and apoC-III isoforms were analyzed by isoelectric focusing followed by immunoblotting using anti-apoE or anti-apoC-III antibodies (30). Neuraminidase treatment was performed using the method previously reported (30).

Statistical analysis

Statistical significance of the difference of the means was determined by the Mann-Whitney U test using SPSS statistical package.

RESULTS

Characterization of study subjects

Clinical characteristics and plasma lipid and apolipoprotein values of the ABL patients are provided in **Table 1.** Body weights as well as lipid and apolipoprotein levels remained in steady state in all subjects throughout the study period. Values are the means of five fasting meas-

TABLE 1. Characterization of patients with abetalipoproteinemia (ABL)

Subject	Age	BMI	TC	TG	HDL	ApoA-I	ApoA-II	ApoE	LpA-I	LpA-I:A-II	% LpA-I[a]
	yr	*kg/m²*				*mg/dl*					*%*
ABL											
#1 (M)	50	26.3	35	4	30	47	12	3.3	24	23	51
#2 (F)	38	20.3	22	6	18	34	14	2.1	20	14	59
Control (n = 55)[b]											
Mean ± SD	22 ± 2	22.4 ± 2.7	160 ± 28[c]	82 ± 52[c]	50 ± 13[c]	136 ± 15[c]	33 ± 5[c]	2.8 ± 1.1	47 ± 8[c]	89 ± 15[c]	35 ± 4[c]

[a] The percentage of total plasma apoA-I present in LpA-I.
[b] Control subjects include 33 for apoA-I studies and 22 for apoE studies. Values for apoA-I, apoA-II, LpA-I, LpA-I:A-II, and % LpA-I are given as mean ± SD of 33 controls, and values for apoE are given as mean ± SD of 22 controls.
[c] Different from the means of the ABL patients by the Mann-Whitney U test, $P < 0.05$.

urements made while the subjects were on the metabolic diet. Plasma apoB was not detectable in the ABL patients. Plasma apoA-I levels were significantly decreased by 70% in ABL patients compared with controls, whereas plasma apoE levels were relatively normal in the ABL patients. Although concentrations of both LpA-I and LpA-I:A-II were significantly decreased in ABL, LpA-I:A-II levels were decreased to a greater extent relative to LpA-I, thus resulting in an significantly increased fraction of apoA-I in LpA-I in ABL compared with controls. Shown in **Fig. 1** are agarose electophoresis patterns of total plasma, lipoproteins of density ranges between 1.006 and 1.063 g/ml, and HDL_2 from the ABL patient #1. Lipoproteins (d 1.006–1.063 g/ml) migrated to the α position, but slightly slower than that of HDL_2. This lipoprotein fraction as well as total plasma showed no visible lipid staining band at the β position. Based on these observations, we designated the lipoproteins of density range between 1.006 and 1.063 g/ml as "HDL_1." **Figure 2** illustrates the apoE and apoC-III isoform patterns visualized by immunoblotting in one of the ABL patients. In contrast to the normal plasma, apoE in the apoE1 position was the dominant form in the ABL plasma; this band shifted to the apoE3 position after the treatment with neuraminidase, indicating that apoE3 is highly sialated in ABL. Increased sialation of apoC-III was also found in the ABL patients.

Metabolism of apoA-I and apoE

The plasma decay curves for ^{131}I-labeled apoA-I in the two ABL patients are shown in **Fig. 3.** In both ABL patients, labeled apoA-I was catabolized faster than in control subjects. The apoA-I plasma curves were fit to two ex-

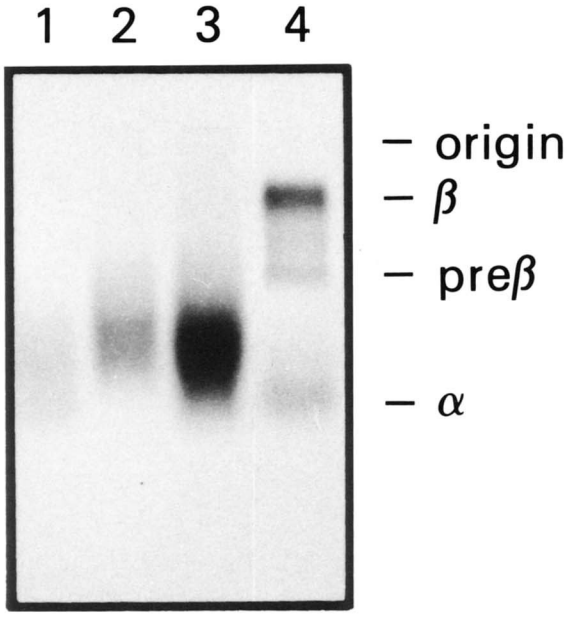

Fig. 1. Agarose gel electrophoresis of the plasma (lane 1), HDL_1 (lane 2), and HDL_2 (lane 3) in the ABL patient #1, and a control plasma (lane 4).

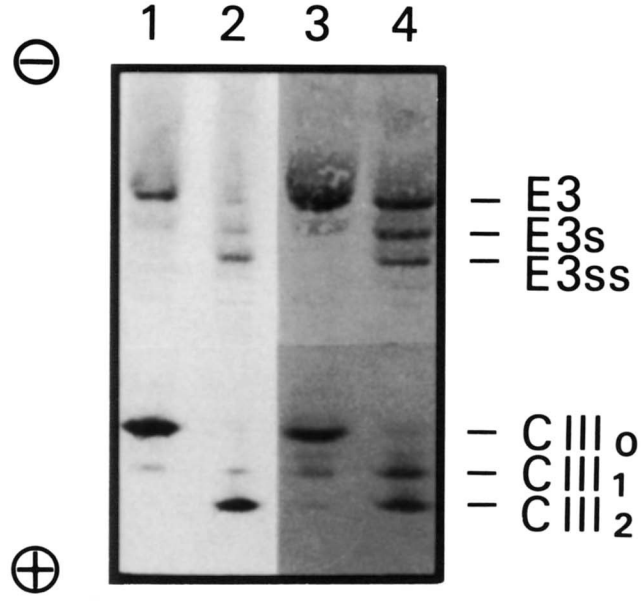

Fig. 2. Isoelectric focusing of apoE and apoC-III in the ABL patient #2 (lanes 1, 2) and a control subject (lanes 3, 4). Neuraminidase treatment was performed on the samples in lanes 1 and 3. ApoE and apoC-III isoproteins were visualized by using specific anti-apoE antibodies and anti-apoC-III antibodies.

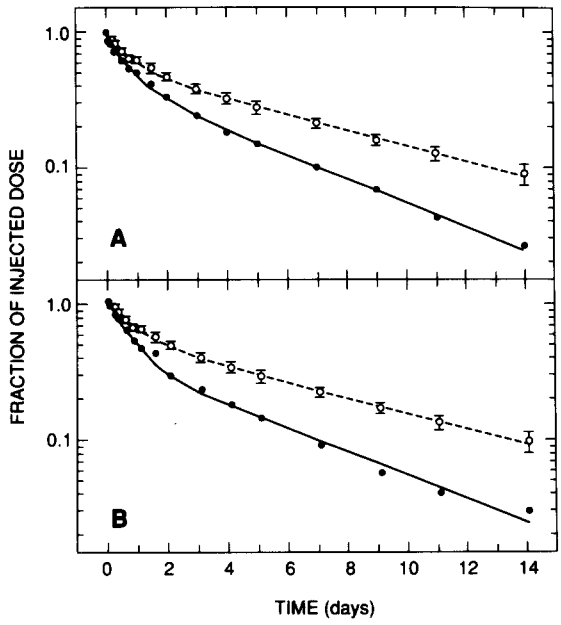

Fig. 3. Metabolism of ^{131}I-labeled apoA-I in the ABL patient #1 (panel A, solid circles) and patient #2 (panel B, solid circles) with the mean of control subjects (n = 33, open circles with dotted line). Data from controls subjects are given as the mean ± SD.

ponential functions in both ABL patients. The first component (a pool with a relatively rapid turnover rate) had similar rate constants in the ABL patients compared with controls. The second component (a pool with a relatively slow turnover rate) had faster rate constants in the ABL patients. The kinetic parameters of apoA-I are summarized in **Table 2.** The mean residence time of apoA-I in the ABL patients was significantly decreased by approximately 50% compared with control subjects. In addition, the production rates of apoA-I were also significantly decreased by 40%, indicating that both increased catabolism and decreased production of apoA-I account for the low plasma apoA-I concentrations in ABL. The increased apoA-I catabolism was confirmed by the urine/plasma (U/P) radioactivity ratios, which reflect fractional catabolic rates (FCR). U/P radioactivity ratios were increased to 0.38 ± 0.08 for the ABL patient #1 and 0.41 ± 0.08 for the ABL patient #2 compared with controls (0.20 ± 0.02), and these U/P ratios were comparable with the FCRs (0.40 and 0.44 day^{-1}, respectively) calculated from the plasma radioactivity curves.

The plasma decay curves for ^{125}I-labeled apoE in the two ABL patients are illustrated in **Fig. 4.** Radiolabeled apoE was catabolized at a similar rate in the ABL patients as in control subjects for several days after the injection, then at a somewhat faster rate in both ABL patients for the reminder of the study period. The mean residence time of apoE was significantly decreased by 25% in the ABL patients (Table 2). The apoE production rates in ABL patients, however, were not significantly different from controls.

Metabolism of LpA-I and LpA-I,A-II

The decay curves for ^{131}I-labeled apoA-I in LpA-I:A-II and LpA-I in ABL patient #2 with those from the mean of ten controls are shown in **Fig. 5.** ApoA-I in LpA-I:A-II was catabolized at a much faster rate in the ABL patient than that in controls, whereas the catabolism of apoA-I in LpA-I in ABL was similar to that in controls.

Metabolism of apoE-containing lipoproteins

At 10 min after the injection, the percentage distribution of total ^{131}I-labeled apoA-I in HDL with apoE was 13% in ABL patient #1 and 9% in ABL patient #2, about twofold increased compared with controls (mean 5.1%). Plasma decay curves of ^{131}I-labeled apoA-I in HDL with and without apoE in the ABL patients are shown in **Fig. 6.** ApoA-I in HDL with apoE was catabolized at a much faster rate than apoA-I in HDL without apoE in both ABL patients. At 24 h after injection, the fractions of initial activity remaining of labeled apoA-I with apoE were only 0.23 and 0.13 of initial radioactivities, while those of apoA-I without apoE were 0.55 and 0.48. These data were comparable with the fractions remaining of plasma total ^{125}I-labeled apoE (0.16 and 0.11) and total ^{131}I-labeled apoA-I (0.51 and 0.45).

TABLE 2. Kinetic parameters of apoA-I and apoE in abetalipoproteinemia (ABL)

	ApoA-I			ApoE		
Subjects	Conc.	RT	PR	Conc.	RT	PR
	mg/dl	*days*	*mg/kg-d*	*mg/dl*	*days*	*mg/kg-d*
ABL						
#1	47	2.5	8.3	3.3	0.52	2.55
#2	34	2.3	6.0	2.1	0.48	1.72
Controls		(n = 33)			(n = 22)	
Mean ± SD	136 ± 15	4.7 ± 0.6[a]	11.8 ± 1.7[a]	2.8 ± 1.1	0.67 ± 0.14[a]	1.55 ± 0.62

RT, residence time; PR, production rate.
[a] Different from the mean of the ABL patients by the Mann-Whitney U test, $P < 0.05$.

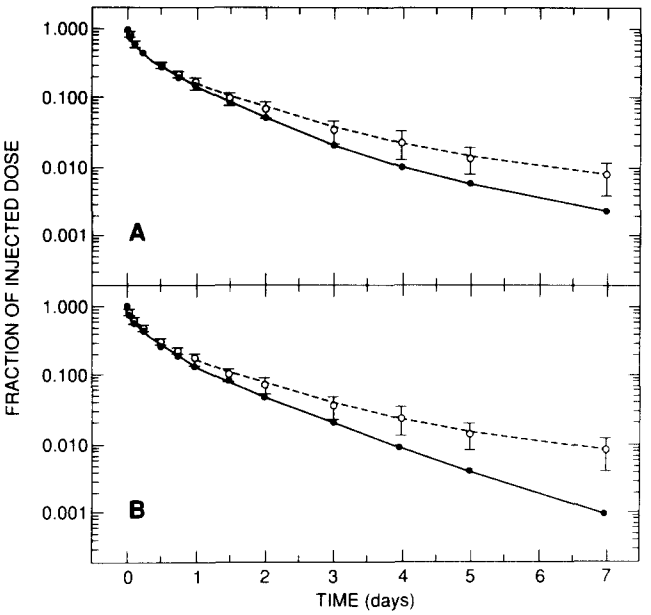

Fig. 4. Metabolism of ^{125}I-labeled apoE in the ABL patient #1 (panel A, solid circles) and patient #2 (panel B, solid circles) with the mean of control subjects (n = 22, open circles with dotted line). Data from controls subjects are given as the mean ± SD.

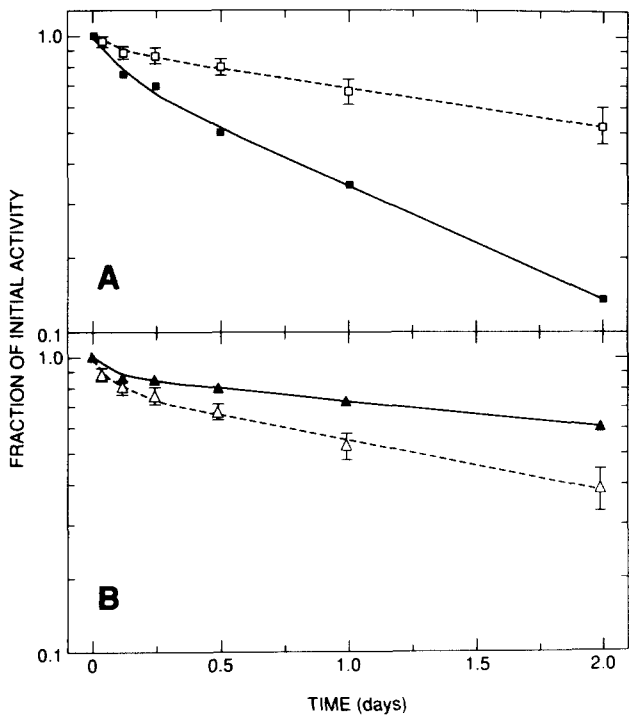

Fig. 5. Metabolism of ^{131}I-labeled apoA-I in LpA-I:A-II particles (panel A) and LpA-I particles (panel B) from the ABL patient #2 (solid lines) with the mean of control subjects (dotted lines). Data from control subjects are given as the mean ± SD.

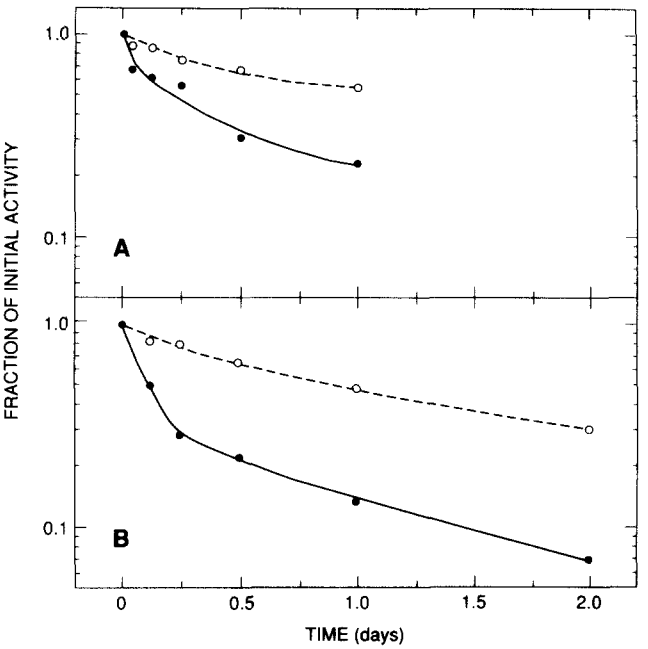

Fig. 6. Metabolism of ^{125}I-labeled apoE in HDL with apoE (solid circles) and HDL without apoE (open circles with dotted lines) in the ABL patient #1 (panel A) and #2 (panel B).

Metabolism of apoE monomer, homodimer, and heterodimer

Table 3 summarizes the distribution of ^{125}I-labeled apoE among monomer, apoE homodimer, and apoE-A-II heterodimer in "HDL_2" and HDL_2 from 10-min samples in ABL patients. In both HDL fractions, apoE monomer was present as the dominant form, in particular in "HDL_1." While only 4 to 6% of total apoE radioactivity was associated with the apoE-A-II heterodimer in "HDL_1," 15 to 27% was associated with heterodimer in HDL_2. Distribution of ^{125}I-labeled apoE in HDL_3 was almost identical to that in HDL_2. We determined the metabolism of the three apoE forms and the results in ABL patient #2 are included in **Fig. 7.** The apoE monomer was catabolized at the fastest rate, while the apoE-A-II heterodimer had the slowest rate of catabolism in both "HDL_1" and HDL_2 fractions. Identical results were obtained with the other ABL patient (data not shown).

DISCUSSION

Factors regulating HDL metabolism in humans remain incompletely understood. Remodeling of the lipid and apolipoprotein components of HDL is modulated by the presence of apoB-containing lipoproteins (7). Lipolysis of TG-rich lipoproteins is associated with the transfer of lipid and apolipoproteins to HDL (4, 5). Influx of free cholesterol from LDL facilitates LCAT-mediated esterification in HDL, in particular, LpA-I:A-II (31). CETP

TABLE 3. Distribution of ^{125}I-labeled apoE among monomer and dimers in plasma from abetalipoproteinemia (ABL)

	ABL Subject	Monomer	Homodimer	E-A-II heterodimer
			%	
HDL_1	#1	89.2	4.9	5.9
HDL_1	#2	90.7	5.6	3.7
HDL_2	#1	65.3	7.7	27.0
HDL_2	#2	68.8	15.8	15.4
HDL_3	#2	71.7	15.9	12.3

Values are expressed as a percentage distribution of the total apoE radioactivities.

facilitates the transfer of cholesteryl ester into apoB-containing lipoproteins with a reciprocal transfer of TG. Therefore, ABL provides a unique model for the study of human HDL metabolism in the absence of apoB-containing lipoproteins.

ApoE is also believed to influence HDL metabolism. ApoE-containing HDL particles are selectively abundant in species with low CETP activity such as the rat (32) and dog (33), especially those fed cholesterol-rich diets (34). In humans, relatively high CETP activity prevents the formation of apoE-rich HDL particles (35). In contrast, apoE-containing HDL accumulate in humans with CETP deficiency (36, 37) and in conditions, such as ABL (38) and the neonate (15), in which apoB-containing lipoproteins are absent or markedly decreased. ABL is a particularly interesting model for the investigation of the role of apoE in HDL metabolism, as there can be no apoE transfer between HDL and apoB-containing lipoproteins.

In this study, we found that ABL patients had both an increased catabolic rate as well as decreased production of apoA-I. Two previous studies of in vivo HDL kinetics in ABL, each in a single patient, reported normal apoA-I catabolism with decreased apoA-I production (14, 15). However, in one study (14), the ABL patient had a plasma apoA-I level substantially higher than most ABL patients, and therefore may not have been representative of ABL. In the other study, the U/P ratio (another method of assessing fractional catabolic rate) was higher in the ABL patient compared with controls, consistent with our findings. Furthermore, in both studies HDL were isolated by ultracentrifugation prior to injection. Given that HDL from ABL patients have abnormal chemical composition (39, 40), abnormal morphology (41), and a relative increase in small dense HDL (38), the ultracentrifugation may have dissociated some labeled apoA-I or excluded some small dense HDL, thus resulting in the injection of HDL particles not completely representative of the HDL spectrum in ABL. In contrast, our studies were performed using reassociation of the radiolabeled apoA-I with whole plasma prior to injection. Finally, supporting our results is the preliminary report by Schaefer and Ordovas (16) that apoA-I catabolic rates were increased in two ABL patients.

The decreased apoA-I production rates we found in these ABL patients are consistent with previous studies. Some previous studies (42, 43), but not all (44), observed a reduction of HDL in orotic acid-treated rat, suggesting decreased apoA-I secretion in this model as well. Although decreased apoA-I production in ABL could indicate that apoA-I secretion may be associated with the secretion of apoB-containing lipoproteins, it is also possible that the absence of apoB-containing lipoproteins may have indirect effects on apoA-I production.

Increased apoA-I catabolism is usually associated with decreased HDL particle size (21, 37, 45). However, the mean HDL particle size in ABL is increased (41, 46) compared with normal HDL; this increased HDL particle size was confirmed in the present study using gel filtration chromatography, in which the majority of apoA-I in the ABL subjects eluted earlier than that in controls (data not shown). Therefore, the increased apoA-I catabolism in ABL is not due to smaller HDL particle size. However, it is possible that alteration of the chemical composition (PL, FC-rich relative to CE) (14, 39, 47) or of particle morphology (square-packing) (14, 41) of HDL in ABL may result in a faster rate of catabolism independent of the particle size. Another explanation may be the increased apoE content of ABL HDL. At 10 min after the injection, the distribution of labeled apoA-I in apoE-containing HDL in ABL was increased twofold compared

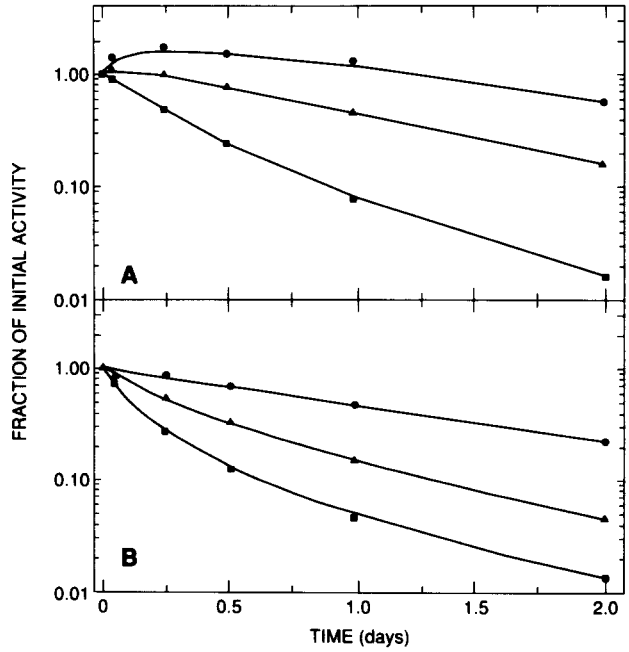

Fig. 7. Metabolism of ^{125}I-labeled apoE among monomer (squares), homodimer (triangles), and apoE-A-II heterodimer (circles) in HDL_1 (panel A) and HDL_2 (panel B) from ABL patient #1.

with controls. This data, together with the fact that apoA-I in apoE-containing HDL was catabolized at a faster rate than apoA-I in HDL without apoE, suggests that enrichment of apoE in ABL HDL may be at least one mechanism for the increased apoA-I catabolism.

HDL are heterogenous in apolipoprotein composition and comprise several subclasses including particles that contain both apoA-I and apoA-II (LpA-I:A-II) and those that contain apoA-I but not apoA-II (LpA-I) (48, 49). These two HDL subclasses have different physiologic functions and in vivo metabolism (21, 50). Cheung, Wolf, and Illingworth (40) previously reported that in ABL patients levels of LpA-I were 30% lower and levels of LpA-I:A-II were 60% lower than in controls, thus resulting in a greater fraction of apoA-I in LpA-I in ABL (52%) compared with controls (39%). In normal subjects, apoA-I on LpA-I has a faster catabolic rate than apoA-I on LpA-I:A-II (21). In contrast, we found that apoA-I on LpA-I:A-II was catabolized at a faster rate than LpA-I in the ABL patients. This metabolic alteration accounted for the greater decrease of plasma LpA-I:A-II relative to LpA-I in our ABL patients.

The observation in HepG2 medium that relatively less apoA-I was found in LpA-I:A-II than LpA-I (51) suggests that the transfer of apoA-I from LpA-I to LpA-I:A-II may occur after the secretion into the plasma. This process may be accelerated by the presence of apoB-containing lipoproteins (52). As there are no apoB-containing lipoproteins in ABL plasma, apoA-I may not be transferred from LpA-I to LpA-I:A-II to the same extent as in normal plasma, resulting in faster catabolism of apoA-I in LpA-I:A-II. Cheung and Wolf (31) reported that small LpA-I:A-II require LDL for transformation by LCAT into larger HDL particles, whereas LpA-I can be converted in the absence of LDL. HDL particles in ABL plasma are PL-rich and have increased FC/CE ratio, probably due to a relative reduction in LCAT activity (40, 41, 47, 53) as a result of the lack of apoB-containing lipoproteins (54). We recently demonstrated that decreased LCAT activity results specifically in rapid catabolism of apoA-I on LpA-I:A-II compared with LpA-I (55). Therefore, the rapid catabolism of apoA-I in LpA-I:A-II in ABL may be due to the relative decrease in LCAT activity.

Plasma apoE levels in ABL have been reported to be normal or slightly increased (14). In the present study, the rates of apoE production in the ABL patients were not different from the control subjects, indicating that apoB secretion is not required for normal apoE secretion. Supporting this observation is the report by Fazio et al. (56) that the synthesis and secretion of apoE are independent of the production of apoB-containing lipoproteins in HepG2 cells.

The overall catabolism of apoE in ABL was 25% faster than controls. In normal apoE3 homozygotes, 70% to 80% of total apoE in HDL is found in a E-A-II heterodimer (57, 58). In contrast, we found in the ABL patients that apoE was predominantly present as the monomer, with only 4–27% as the E-A-II heterodimer and 5–16% as the homodimer (Table 3). This lower percentage of the E-A-II heterodimer in ABL is consistent with the finding by Blum et al. (17) in which about 30% of total apoE was present as the E-A-II heterodimer in ABL. Furthermore, the rate of catabolism of apoE was directly associated with the form in which apoE was present. The apoE monomer was catabolized at the fastest rate, whereas the apoE-A-II heterodimer had the slowest catabolic rate. This in vivo finding is consistent with the reports by Borghini et al. (61) and Innerarity et al. (59) that reduced HDL bound to the LDL receptor on human fibroblasts more efficiently than non-reduced HDL, indicating that the apoE monomer interacts with the LDL receptor better than the E-A-II heterodimer. We found an increased amount of sialated apoE in both ABL patients as has been previously observed for apoA-II and apoC-III (41, 49, 60). This pattern is also observed when VLDL secretion from the liver is inhibited by orotic acid (13), suggesting that the processing of sialated apolipoproteins is altered in the absence of apoB-containing lipoproteins. It is possible that highly sialated apoE may not form the E-A-II complex as well, thus resulting in less heterodimer formation and therefore increasing the overall rate of apoE catabolism.

Finally, a major objective of the current study was to investigate the role of apoE in apoA-I metabolism in ABL. ApoE-rich HDL isolated from ABL plasma (14, 15, 38, 61) have high affinity for the LDL receptor (15, 61), and increase the cholesteryl ester content in fibroblasts (15), suggesting that these particles may deliver cholesterol to cells. Total body synthesis of cholesterol in ABL (62, 63) and cholesterol synthesis in lymphocytes isolated from ABL patients (64) have been reported to be normal despite the lack of VLDL and LDL. Thus, apoE-containing HDL in ABL, like those in neonates (65, 66) and many lower animal species (34), have been considered to play a major role in delivering cholesterol to the peripheral tissues. In the present study, we found that apoA-I in apoE-containing lipoproteins was catabolized at a much faster rate than that in HDL without apoE. This is the first demonstration in humans of accelerated catabolism of apoE-containing HDL particles. Supporting this finding is our previous observation in CETP-deficient subjects that although overall apoA-I catabolism was markedly delayed compared with normal HDL, apoA-I in apoE-rich HDL_1 was catabolized faster relative to that in HDL_2 (37). In addition, a similar effect of apoE on HDL metabolism has been observed in baboons (67). Unlike other situations in which the effect of apoE in HDL metabolism may be significantly modified by the transfer of apoE between HDL and apoB-containing

lipoproteins, in the ABL patients there can be no transfer of apoE, given the absence of apoB in the plasma.

In summary, the present study establishes that in ABL: *1)* the decreased apoA-I levels are due to both increased catabolic and decreased production rates of apoA-I; *2)* apoA-I on LpA-I:A-II is more rapidly catabolized than apoA-I on LpA-I, resulting in a greater decrease of plasma levels of apoA-I in LpA-I:A-II; *3)* apoE-containing HDL is catabolized at a faster rate relative to HDL without apoE; and *4)* the overall catabolic and production rates of apoE are relatively normal despite the lack of secretion of apoB-containing lipoproteins. These data provide new insights into the role of apoB-containing lipoproteins and apoE in HDL metabolism.

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