

Elevated levels of apolipoprotein E in the high density lipoproteins of human cord blood plasma

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Abstract The concentrations and lipoprotein distributions of apolipoprotein E (apoE) in normal human umbilical cord blood plasma were determined. The mean plasma apoE level of 95 neonates was considerably higher than that of 49 normal adults (58.1 vs 35.8 $\mu\text{g/ml}$). This elevation of apoE levels was in striking contrast to the lower than adult levels of cholesterol (72 mg/dl vs 185 mg/dl), triglyceride (37.8 mg/dl vs 97.6 mg/dl), and LDL cholesterol (25 mg/dl vs 110 mg/dl) in neonatal plasma. For the group of 95 neonates, the plasma apoE concentration correlated significantly with total plasma cholesterol concentration ($r = 0.60$), with LDL cholesterol concentration ($r = 0.27$) and with HDL cholesterol concentration ($r = 0.50$). Among the neonates, 87% of plasma apoE was associated with a less dense subfraction of high density lipoprotein compared to a mean of 58% for 30 normal adults. Thus, for neonates, despite hypolipidemia, the absolute concentration of apoE in HDL (50 $\mu\text{g/ml}$) was 2.5 times that of adults (20 $\mu\text{g/ml}$). ■ We speculate that the very low level of neonatal VLDL, providing limited substrate for lipolysis, may result in retarded removal of apoE from plasma and the observed high level of apoE in neonatal HDL. We hypothesize that in the fetus and neonate, as has been demonstrated in abetalipoproteinemia, apoE-rich HDL may functionally substitute for LDL in delivering cholesterol to cells. — Blum, C. B., P. A. Davis, and T. M. Forte. Elevated levels of apolipoprotein E in the high density lipoproteins of human cord blood plasma. *J. Lipid Res.* 1985. 26: 755–760.

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Apolipoprotein E (apoE), a 35,000 molecular weight glycoprotein which is a major protein constituent of very low density lipoprotein (VLDL) and a minor constituent of high density lipoprotein (HDL), plays a key functional role in lipoprotein metabolism (1–4). ApoE is a recognition marker for receptor-mediated cellular uptake of lipoproteins, particularly of chylomicron remnants (4). A unique feature of this recognition and subsequent internalization is that it is mediated by two distinct types of cell surface receptors, the apoB,E receptor and the apoE receptor (3). These two receptor systems appear to have different modes of regulation since the apoB,E receptor is rapidly downregulated upon entry of cholesterol into cells, while the apoE receptor is not.

Plasma levels of apoE are modulated by dietary factors that affect plasma lipid levels (5, 6). Diets high in saturated fatty acids increase plasma apoE levels in humans, as well as increasing plasma cholesterol and triglyceride levels. In several species, diets high in saturated fat, cholesterol, and taurocholate, when given along with propylthiouracil, cause not only elevated levels of plasma cholesterol and apoE, but also the appearance of two new lipoprotein species, B-VLDL and HDL_c (7, 8). These lipoproteins are present only in trace amounts in normal plasma and may be involved in atherogenesis. Although apoE may take part in this pathological process, in some circumstances it seems to have beneficial effects. For instance, in abetalipoproteinemia apoE-rich HDL is a functional substitute for low density lipoprotein (LDL) in delivering cholesterol to tissues (9). Given the low plasma LDL levels of the fetus (10–14) along with its high requirement for cholesterol to enable rapid growth, an analogy to abetalipoproteinemia seemed possible; perhaps in the normal fetus, apoE is quantitatively important in delivering cholesterol to cells. This hypothesis is consistent with our previous work which suggested high levels of apoE in the LDL and HDL of cord blood plasma (15, 16). This report extends those observations with measurements of the total apoE content of cord blood plasma and with quantitation of the lipoprotein distribution of apoE in human umbilical cord blood.

Some of this work has been reported previously in abstract form (17).

METHODS

Blood samples

Cord blood plasma samples were obtained from 95

Abbreviations: VLDL, very low density lipoprotein; LDL, low density lipoprotein; HDL, high density lipoprotein.

normal neonates as described by Davis and Forte (18). All samples were analyzed for total cholesterol and triglyceride concentration, and samples in which either exceeded 100 mg/dl were excluded from cord blood plasma pools. The cord blood plasma samples were then either pooled or were analyzed individually. All samples were stored at 4°C. Adult plasma samples were obtained from 45 normal adult volunteers and were also analyzed for total triglyceride and cholesterol content. For analysis of apoE content by radioimmunoassay, 20- μ l aliquots of plasma were pipetted into plastic tubes, frozen, lyophilized, and shipped from the Donner Laboratory to Columbia University for radioimmunoassay.

Lipoprotein fractionation

Cord blood plasma lipoproteins were fractionated by sequential preparative density ultracentrifugation according to Lindgren, Jensen, and Hatch (19), except that all centrifugation was performed at 4°C. The ultracentrifugal fractions including VLDL ($d < 1.006$ g/ml), LDL ($d 1.006$ – 1.063 g/ml), HDL ($d 1.063$ – 1.21 g/ml), and the nonlipoprotein fraction ($d > 1.21$ g/ml) were either pipetted into screw-capped vials or lyophilized and stored at 4°C until analysis.

Cord blood plasma lipoproteins were fractionated by gel filtration through 6% agarose. Whole plasma was applied to a 0.8 \times 100 cm Bio-Gel A-5m column and eluted with phosphate-buffered saline as described previously (20). Two-ml fractions were collected and stored at 4°C until analyzed.

Radioimmunoassay of apoE

Assays of apoE were performed as previously described (20). Samples were treated overnight in 50 mM Na decylsulfate and the assay was carried out with final concentrations in the incubation mixture of 5 mM Na decylsulfate, 50 mM Na phosphate, 100 mM NaCl, 0.02% Na azide, 0.04% nonimmune rabbit serum, 0.011% spe-

cific antiserum to apoE, and 30,000 cpm of 125 I-labeled apoE. After 48 hr incubation at 4°C, goat antirabbit serum was added, and the assay was harvested by centrifugation on the following day. Calibrated plasma pools stored at -80°C were used to prepare standard curves (each containing 15 different apoE concentrations) and pipetted in duplicate. The plasma was calibrated against a primary standard of purified apoE. The within-assay coefficient of variation was 9%, while the coefficient of variation for systematic between-assay variability was 3%.

Analytical procedures

Plasma cholesterol and triglyceride concentrations in cord blood plasma were determined enzymatically (18). Cholesterol and triglyceride concentrations in adult blood plasma were determined with a Technicon AutoAnalyzer I (21, 22). The cholesterol concentrations in the fractions obtained from agarose column chromatography were measured by the method of Chiamori and Henry (23). Protein concentration was determined by the method of Lowry et al. (24) as modified by Markwell et al. (25) using a bovine serum albumin standard. HDL cholesterol was determined by the differential precipitation method of Steele et al. (26).

RESULTS

The concentrations in plasma of triglyceride, cholesterol, HDL cholesterol, and apoE are presented in **Table 1**. For each of these, the neonatal levels differed substantially from adult levels. Triglyceride and total cholesterol levels of cord blood plasma were approximately 40% the average adult levels. LDL levels were reduced to an even greater extent, to 23% of adult levels. HDL cholesterol was also reduced, but to a lesser extent, as the level in cord blood plasma was three-fourths the value obtained from normal adult plasma.

TABLE 1. Plasma concentrations (mean \pm SD) of apoE, cholesterol, triglyceride, HDL cholesterol, and LDL cholesterol

	Normal Neonates			
	Total (n = 95)	Male (n = 41)	Female (n = 55)	Adults (n = 49)
ApoE (μ g/ml)	58.1 \pm 26.5	57.4 \pm 27.3	58.7 \pm 26.2	35.8 \pm 10.4
Chol (mg/dl)	72.1 \pm 21.3	69.4 \pm 20.7	74.1 \pm 21.7	185.1 \pm 33.0
Trig (mg/dl)	37.8 \pm 16.9	40.7 \pm 22.4	35.7 \pm 10.6	97.6 \pm 36.1
HDL (mg/dl)	39.7 \pm 12.4	36.5 \pm 10.1	42.1 \pm 13.6	54.7 \pm 11.2
LDL (mg/dl)	24.8 \pm 13.6	24.8 \pm 14.9	24.9 \pm 12.8	110.4 \pm 29.7

Abbreviations: ApoE, Chol, Trig, HDL, and LDL represent the plasma concentrations of apolipoprotein E, cholesterol, triglycerides, HDL cholesterol, and LDL cholesterol, respectively. LDL cholesterol in cord blood plasma and in adult plasma was estimated from cholesterol, triglyceride, and HDL determinations by the method of Friedewald, Levy, and Fredrickson (34). This has been validated by Hardell and Carlson for use with neonatal plasma (12).

TABLE 2. Correlation (r)^a of plasma apoE, cholesterol, triglyceride, HDL cholesterol, and LDL cholesterol in samples from 95 neonates and 49 adults

	Chol	Trig	LDL	HDL	ApoE
Neonates					
Chol	1.00				
Trig	0.054	1.000			
LDL	0.830	-0.065	1.000		
HDL	0.787	-0.101	0.342	1.000	
ApoE	0.603	-0.135	0.269	0.497	1.000
Adults					
Chol	1.000				
Trig	0.451	1.000			
LDL	0.918	0.309	1.000		
HDL	0.215	-0.130	-0.102	1.000	
ApoE	0.603	0.276	0.557	0.110	1.000

^aFor 95 neonates, $P = 0.05$ for $r = 0.17$, $P = 0.025$ for $r = 0.20$, $P = 0.01$ for $r = 0.23$; for 49 adults, $P = 0.05$ for $r = 0.24$, $P = 0.025$ for $r = 0.28$, $P = 0.01$ for $r = 0.33$.

The values obtained for cord blood levels of apoE also differed dramatically from average adult levels. However, in contrast to the cholesterol, triglyceride, and HDL levels, which were lower in cord blood plasma, the neonatal levels of apoE were approximately double adult levels.

There were only small differences between male and female neonates with respect to the concentrations of apoE, total plasma cholesterol and triglyceride, and LDL and HDL cholesterol. With the exception of HDL cholesterol ($P < 0.025$), none of these small sex differences was statistically significant.

The simple linear correlations of the lipid and lipoprotein parameters with one another are shown in Table 2. Significant relationships with apoE are seen for total plasma cholesterol, LDL cholesterol, and HDL cholesterol. However, the relationship with LDL cholesterol is quantitatively weak, variation in LDL cholesterol describing only 7% (r^2) of the variation in apoE. The relationships of total plasma cholesterol and of HDL cholesterol with apoE are stronger, accounting for 36% and 25%, respectively, of the variation in apoE. The strength of the relationship between apoE and total plasma cholesterol in these neonates ($r = 0.60$) is similar to that in 49 normal

adults ($r = 0.60$). However, the relationship of apoE with HDL cholesterol in neonates ($r = 0.50$) was much stronger than that found in the adults ($r = 0.11$). Furthermore, the relationship of apoE with LDL cholesterol in neonates ($r = 0.27$) was much weaker than that found in adults ($r = 0.56$).

The distribution of apoE among the plasma lipoproteins was investigated both by ultracentrifugation (Table 3) and by 6% agarose column chromatography (Table 4 and Fig. 1). The ultracentrifugal experiments demonstrated a relative redistribution of apoE from the $d < 1.006$ g/ml fraction of plasma to the $d 1.063$ - 1.21 g/ml fraction in cord blood plasma compared to adult plasma. The absolute concentration of apoE in cord blood plasma (58.1 $\mu\text{g/ml}$) was also greater than that in adult plasma (35.8 $\mu\text{g/ml}$). Therefore, these experiments imply a major increase in the absolute concentration of apoE in the cord blood HDL fraction compared to the adult HDL fraction (28.8 $\mu\text{g/ml}$ vs 12.2 $\mu\text{g/ml}$ according to the data from these ultracentrifugation studies).

Since ultracentrifugation can cause certain artifactual alterations in the distribution of apoE in plasma (20, 27), similar studies of the lipoprotein distribution of apoE were performed using 6% agarose column chromatography (Table 4, Fig. 1). In cord blood plasma, as well as in adult plasma, all apoE eluted in fractions associated with lipoproteins. Thus, the apoE found in the non-lipoprotein fraction in the ultracentrifugation studies was an artifact caused by ultracentrifugation. In this respect, the findings for cord blood plasma correspond to previous reports for adult plasma (20, 27). However, there were major relative and absolute increases in apoE in the HDL fraction in cord blood plasma compared to adult plasma. The concentration of apoE in the HDL fraction as determined by column chromatography was 49.5 $\mu\text{g/ml}$ in cord blood plasma compared to 20.3 $\mu\text{g/ml}$ in normal adult plasma.

DISCUSSION

In previous investigations, human cord blood lipoproteins differed from those of the adult in several respects. The plasma lipids (28, 29) in the neonate were character-

TABLE 3. Lipoprotein density distribution of apoE in cord blood and normal adult human plasma

	Distribution of Lipoprotein-Associated ApoE in Density Ranges			% of ApoE in $d > 1.21$ g/ml
	< 1.006 g/ml	1.006-1.063 g/ml	1.063-1.21 g/ml	
Cord blood ^a	4.7	27.2	68.1	27.3
Adult blood ^a	26.0	26.9	47.2	27.3

^aData for cord blood plasma are the mean of two experiments, each using pooled plasma. Data from nine normal adults are recalculated from reference 20.

TABLE 4. Lipoprotein fractionation of apoE in cord blood and adult human plasma by agarose column chromatography

	Triglyceride-Rich Lipoproteins		High Density Lipoprotein	
	%	$\mu\text{g/ml}$	%	$\mu\text{g/ml}$
Cord blood ^a	13	7.4	87	49.5
Adult blood ^a	42	15.0	58	20.3

^aData for cord blood plasma are the mean of two experiments, each using pooled plasma. Data for normal adult plasma are the means obtained from 30 individuals.

ized by marked reductions in total triglyceride and cholesterol levels. This reduction in lipid content was most pronounced in VLDL, was substantial in LDL, and was least pronounced in HDL (10-14). This non-uniform reduction in neonatal levels resulted in a lipoprotein distribution with HDL as the predominant species.

In contrast to the lower lipid levels of neonatal plasma, the present studies demonstrate a level of apoE approximately twice that seen in adult plasma. Thus, there was a 6-fold enrichment of apoE per total cholesterol relative to that seen in adults. This finding was unexpected in light of our previous observation of a positive correlation of plasma cholesterol levels with apoE levels in normal adults.

In cord blood as in adult blood, the apoE level correlated with the cholesterol level ($r = 0.60$ for both cord blood and adult blood plasma). In cord blood, there was also a weak, but significant, correlation of apoE with HDL cholesterol ($r = 0.50$), while no significant correlation existed in adult blood plasma ($r = 0.11$). Another difference between the neonatal and adult samples is that the neonatal samples showed no correlation of apoE levels with total plasma triglyceride concentration ($r = -0.14$), while the adult samples showed a significant correlation ($r = 0.28$). Perhaps this lack of relationship of apoE with triglycerides in the cord blood samples was due to the smaller variation in neonatal triglyceride concentration than in adult levels (SD = 17 mg/dl for cord blood samples, 36 mg/dl for adult blood samples).

In our previous work, the isolation and characterization of the lipoprotein species present in cord blood plasma revealed that, within the lipoprotein density classes, marked differences between neonatal lipoprotein and adult lipoprotein particles were evident (15, 16). Cord blood LDL, when compared to adult LDL, had reduced size-heterogeneity, increased triglyceride content, and larger amounts of apoA-I and apoE. The apoE and apoA-I of cord blood LDL were carried on particles that were intermediate in size between adult LDL and HDL and probably represented a continuation of the spectrum of particles present in the lower density fractions of HDL. When the HDL was isolated, subfractionated, and characterized, a shift toward larger sized HDL particles

was discerned. HDL₂ was the major HDL species present, in contrast to the adult, where HDL₃ is the predominant species. This larger HDL was also enriched in apoE and apoA-I.

These findings then led to questions about the distribution of apoE among lipoproteins in neonatal cord blood plasma. These questions were the focus of the present work. Fractionation of the plasma lipoproteins by both

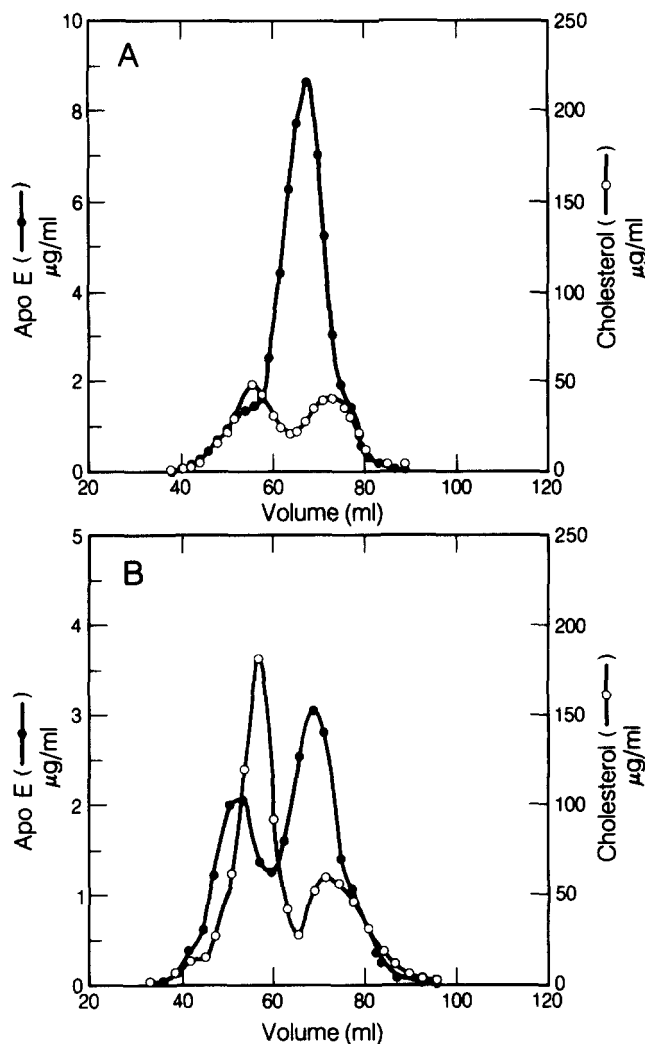


Fig. 1. Agarose column chromatography of neonatal (A) and adult (B) human plasma. Plasma was applied and eluted as indicated in the text. ApoE and cholesterol concentrations were measured in the eluate. This experiment utilized pooled neonatal plasma with the following characteristics: apoE, 56 $\mu\text{g/ml}$; cholesterol, 64 mg/dl; triglycerides, 40 mg/dl; and HDL cholesterol, 33 mg/dl; 86% of apoE eluted in the major HDL peak. The adult plasma chromatogram most closely representing the mean of all adults studied was selected for this figure: apoE, 35.6 $\mu\text{g/ml}$; cholesterol, 173 mg/dl; triglycerides, 95 mg/dl; HDL, 57 mg/dl; 59% of apoE eluted in the major HDL peak. The first peak of cholesterol represents LDL and the second peak represents HDL. For the chromatogram of adult plasma, a shoulder on the ascending limb of the first peak of the cholesterol profile represents VLDL cholesterol. Measured recovery of apoE from the column was 101% for the neonatal plasma sample and 94% for the adult plasma sample.

ultracentrifugation and agarose column chromatography revealed an approximately 2.4-fold increased concentration of apoE in the HDL fraction of neonatal plasma compared to adult plasma.

Thus, our studies have shown a major increase in cord blood apoE levels and a redistribution of cord blood apoE to an HDL fraction. This is the first report of the lipoprotein distribution of apoE in cord blood plasma. The only previous reports of apoE levels in cord blood plasma, by Dolphin et al. (30) and by McConathy and Lane (31), did not identify substantially higher total plasma apoE levels in neonates than in adults. For those reports, apoE was measured by electroimmunoassay. The reason for the discrepancy between our results and those of Dolphin et al. (30) and of McConathy and Lane (31) is not clear. Differences related to the immunoassays are a possible explanation. For instance, the report of Dolphin et al. (30) does not indicate whether prior delipidation of plasma with organic solvents affected the measured apoE levels. Thus, it seems possible that not all of the apoE in plasma is measured in that electroimmunoassay. In our radioimmunoassay, as has been reported previously (20), prior delipidation of plasma did not alter the measured apoE levels.

The experiments reported here do not define the mechanism responsible for the elevated apoE level of neonatal plasma. However, it seems possible that the extremely low VLDL levels of neonatal plasma may be responsible for this phenomenon. We had reported previously (32) that apoE levels fall promptly with the initiation of post-heparin lipolysis. This suggested a potential role for lipolysis of triglyceride-rich lipoproteins in triggering the removal of apoE from plasma. Thus, the extremely low neonatal VLDL levels may cause a retarded rate of removal of apoE from plasma with consequent development of the observed elevation of plasma apoE concentrations.

The most extreme model of hypocholesterolemia, abetalipoproteinemia, demonstrates a pattern of apoE levels and lipoprotein distribution very analogous to that of normal neonates (9). In abetalipoproteinemia, total plasma levels of apoE are elevated and all apoE in plasma is present in a less dense subfraction of HDL. In the normal neonate, total plasma levels of apoE are elevated and there is a relative and absolute redistribution of apoE to a less dense subfraction of HDL. HDL particles with apoE account for 31% of the total cholesterol of neonatal HDL as calculated from the concentrations reported here and from analyses of composition reported previously (33). The apoE-rich HDL of patients with abetalipoproteinemia and of normal neonates can deliver cholesterol to cells very effectively by interaction with apoB,E receptors (9, 33). Thus, we postulate that the normal human neonate is analogous to the patient with abetalipoproteinemia, in that apoE-rich HDL functionally

substitutes for LDL in delivering cholesterol to tissue. It seems likely that apoE-rich HDL is an important supplier of cholesterol for very active synthesis of membranes and cell growth of the fetus and neonate. ■

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