Polymorphism of human plasma apolipoprotein C-III at birth and in early neonatal life

Walter J. McConathy, Mary A. McCaffree, and Daniel M. Lane

Oklahoma Medical Research Foundation, University of Oklahoma Health Sciences Center, and Presbyterian Hospital, Oklahoma City, OK

Abstract Studies were carried out to investigate potential changes in apolipoprotein C-III (apoC-III) polymorphism from birth through the first month of life. Maternal serum at term and neonatal serum at birth, 3, 14, and 28 days were analyzed for the three principal polymorphic forms of apoC-III: apoC-III-0, apoC-III-1, and apoC-III-2. A two-dimensional electrophoretic procedure combining isoelectric focusing and electroimmunoassay was developed for this purpose. In maternal serum (n = 28), apoC-III-1 to apoC-III-2 ratios (1.45) were typical of normolipidemic adult serum, while the corresponding cord sera (n = 32) had a reversal of the apoC-III-1 to apoC-III-2 ratio (0.55). Basic polyacrylamide gel electrophoretic patterns of isolated cord sera lipoprotein density classes confirmed the reversal of the apoC-III-1 to apoC-III-2 ratio in each class. Following birth, the apoC-III-1 to apoC-III-2 ratio reached unity (1.04) at 3 days and did not change significantly thereafter. It can be concluded that the apoC-III-1 to apoC-III-2 ratio in the normal newborn increases shortly after birth primarily due to an increase in apoC-III-1, and this change appears to be associated with oral feeding, suggesting that intestinal factors may play a role in controlling the ratio of plasma apoC-III-1 to apoC-III-2.—**McConathy, W. J., M. A. McCaffree, and D. M. Lane.** Polymorphism of human plasma apolipoprotein C-III at birth and in early neonatal life. *J. Lipid Res.* 1987. **28:** 1193–1198.

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A number of human plasma apolipoproteins have been shown to exist in several different polymorphic forms (1, 2). Apolipoprotein C-III (apoC-III) is a human plasma apolipoprotein whose polymorphism is due to the degree of glycosylation. ApoC-III has been shown to exist almost exclusively in three forms differing only in the degree of sialyation (3, 4): apoC-III-2 (2 moles of sialic acid), apoC-III-1 (one mole of sialic acid), and apoC-III-0 (no sialic acid). Other variants of apoC-III have also been reported (5) but no definitive evidence for the nature of the chemical difference has been provided.

Apolipoprotein C-III in human plasma lipoproteins is distributed across the spectrum of ultracentrifugally defined lipoproteins (6) but is found predominantly in high density lipoproteins (HDL) in adult normotriglyceridemic plasma and in very low density lipoproteins (VLDL) in hypertriglyceridemic states (5, 6). Studies on the polymorphism of apoC-III in VLDL of hypertriglyceridemic plasma by scanning of focused apolipoproteins have indicated that in some subjects the ratio of the polymorphic forms of apoC-III-1 and apoC-III-2 differ from normals (5, 7). Such findings have led to the hypothesis that this reversed ratio (apoC-III-2 > apoC-III-1) may play a role in the accompanying hypertriglyceridemia. In addition, a number of other potential functions for apoC-III have been reported, including inhibition of uptake of lipoproteins by the liver (8), noncompetitive inhibition of lipoprotein lipase activity (9), and intracellular activation of sphingomyelinase (10).

Based on our observations on cord sera where apoC-III-2 was present in larger amounts than apoC-III-1, early neonatal life seemed an appropriate area to examine the potential changes in apoC-III polymorphism under the impact of exogenous fat. The purpose of the present communication is to describe an analytical procedure to measure the principal forms of apoC-III in small volumes of whole plasma and apply this procedure to monitor how these forms change in the first month of life under the impact of oral feeding.

MATERIALS AND METHODS

Subject recruitment

Prior to their admission to the labor and delivery suite, mothers-to-be were recruited from the practice of members of the Department of Obstetrics at a local hospital only when they were to have their infant subsequently followed by a member of the Department of Pediatrics at the same

Abbreviations: HDL, high density lipoprotein; VLDL, very low density lipoprotein; TC, total cholesterol; C, free cholesterol; CE, cholesteryl ester; TG, triglyceride.

hospital. Permission to participate in the study was obtained in the delivery suite. Following delivery, when the newborn infant met the requirements for admission to the study, informed consent was obtained from the mother. Criteria for admission included: *1*) cephalic vaginal delivery, *2*) a birth weight between 2500 and 4000 g, *3*) a gestation period of 39–41 weeks, and *4*) no perinatal disorder.

Sample collection

Maternal blood of full-term infants was collected before delivery as a venous sample in the labor and delivery suite. Cord blood was obtained from the placenta immediately following the infant's birth. All subsequent samples were obtained from the infants by heel-stick using a standard collection system at 2–3 days, 12–14 days, and 26–28 days following delivery.

Laboratory methods

Analyses of apoC-III polymorphism used a procedure combining isoelectric focusing of whole serum run under denaturing conditions in polyacrylamide gels combined with a modification of the electroimmunoassay conditions previously described for apoC-III (6). Isoelectric focusing was performed in 7% polyacrylamide gels (80 × 2.5 mm) containing 6 M urea and 2% pH 4–6 Ampholine (LKB Produktur) in a manner similar to that previously described (11). Maternal sera (5 μl) or neonatal sera (30 μl) were applied to the top of the gel and mixed with 25 μl of tetramethylurea and 1 μl of mercaptoethanol. Samples were initially focused for 1 hr at 100 V, overnight at 200 volts, and for 2 hr at 300 V. After completion of focusing, the gel was removed from the tube and soaked in Tris–Veronal buffer for 10 min. This gel was positioned on Cronar film previously placed on a glass plate; following this, the top glass plate was positioned, clamped to form the sandwich, and the agarose gel containing anti-apoC-III at 58°C was added. After cooling, the sandwich was kept at 6°C for several hours and then electrophoresed at a field strength of 7 V/cm for 16 hr. The 16-hr electrophoresis period was based on the observed height and area of the rockets using several different time intervals; 16 hr was found to yield the maximal levels for the polymorphic forms of apoC-III. After completion of the electrophoresis, the gel was washed and stained with Coomassie Brilliant Blue G as previously described (6). The areas under rockets were determined with a Hewlett Packard digitizer as previously described (12). The total integrated area in arbitrary units was converted to mg/dl of apoC-III by dividing the total area by the levels of apoC-III previously reported for cord serum (13).

Neutral lipid analyses for triacylglycerol, cholesterol, and cholesteryl ester were performed by gas–liquid chromatography as previously described (14). Electroimmunoassays were used to determine apolipoproteins A-I and B, as previously described (14). ApoC-III-1 and apoC-III-2 used as markers were isolated from hypertriglyceridemic VLDL as previously described (6). Immunoreactivity of the anti-apoC-III used in this study was assessed by electroimmunoassay of apoC-III-1 and apoC-III-2 of known protein content as previously outlined (6). The two polymorphic forms were indistinguishable using this anti-apoC-III with respect to both appearance of the rocket and their protein content. Lipoprotein density classes were isolated and basic polyacrylamide gel electrophoresis was performed according to Olofsson, McConathy, and Alaupovic (11). Statistical analyses for significant differences between two groups at each sampling interval were performed by the *t*-test and the Duncan Multiple Range test for comparison of mother and infant sera at each collection interval. Pearson correlation coefficients were calculated using the Statistical Analysis System (SAS Institute, Inc., Carey, NC).

RESULTS

Analyses of isolated cord sera density classes by basic polyacrylamide gel electrophoresis demonstrated a ratio of apoC-III-1 to apoC-III-2 not typically seen in adult plasma because, in each lipoprotein density class, apoC-III-2 was the predominant polymorphic form of apoC-III **(Fig. 1).** To investigate how this ratio changes under the impact of oral feeding, the combined procedure using isoelectric focusing and the electroimmunoassay was developed to monitor these changes in polymorphism in small volumes of neonatal serum. As illustrated **(Fig. 2)**, electrophoresis of the imbedded, focused gel into an agarose gel containing antibodies to apoC-III yielded

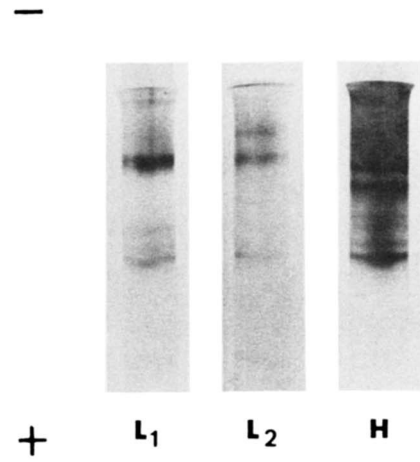

Fig. 1. Basic polyacrylamide gel pattern of lipoprotein density classes of cord sera. Most acidic component (+) is apoC-III-2. L_1, very low and low density lipoproteins ($d < 1.019$ g/ml); L_2, low density lipoproteins (d 1.019–1.063 g/ml); and H, high density lipoproteins (d 1.063–1.21 g/ml).

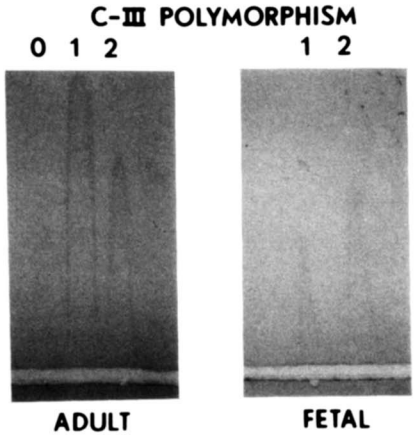

Fig. 2. Comparison of apoC-III pattern of adult (maternal sera) and fetal (cord sera) in whole sera by two-dimensional crossed-technique. 0, apoC-III polymorphic form without sialic acid; 1, apoC-III form with 1 mole of sialic acid; 2, apoC-III form with 2 moles of sialic acid; 1st dimension, isoelectric focusing; 2nd dimension, agarose gel containing antibodies to apoC-III.

three peaks for adult sera, corresponding to apoC-III-0, apoC-III-1, and apoC-III-2. The identity of apoC-III-1 and apoC-III-2 was established by adding either purified apoC-III-1 or apoC-III-2 to either the adult serum or the cord serum and monitoring the change in the corresponding peaks compared to the unmodified serum. In each case, the appropriate change in either apoC-III-1 or apoC-III-2 was observed. The presence in adult sera of only one additional rocket in the less acidic pI range and its spacing indicated that this corresponded to apoC-III-0. The absence of any additional areas of reactivity supports previous findings that these are the principal polymorphic forms of apoC-III (3, 4) in maternal, cord, and neonatal sera.

This method was applied to a population of mothers and their normal infants to monitor the potential changes of apoC-III levels in the first month of life. In **Table 1**, maternal, cord, and neonatal levels of apoC-III polymorphic forms are presented. The maternal ratio of apoC-III-1 to apoC-III-2 was similar to that seen in normolipidemic adult VLDL (5, 7, 15). Total apoC-III levels in maternal sera were significantly higher ($P < 0.05$) than the levels seen in cord, 3-day, 14-day and 28-day neonatal sera. In cord sera, total apoC-III levels were significantly lower than the 3-day levels ($P < 0.01$) but not different from the 14- and 28-day samples. Comparisons of levels of the polymorphic forms of apoC-III-1 and apoC-III-2 between each group were similar to those seen for the total apoC-III except that apoC-III-1 levels in cord sera were significantly lower ($P < 0.03$) than all the other groups. ApoC-III-0 was detected in 75% of the maternal sera, in less than 25% of the cord and neonatal sera, and in none of the 3-day sera. In addition, major qualitative differences were found between maternal, cord, and neonatal sera. ApoC-III-1 was the predominant form in maternal samples, whereas apoC-III-2 was the predominant form in cord samples. From 3 days of age on, equal amounts of apoC-III-1 and apoC-III-2 were found during normal newborn life.

In **Table 2**, neutral lipids, apoA-I, apoB, gestational age, and birth weight are given for each study group. To gain some insights into the possible relationships between the various polymorphic forms for apoC-III and plasma lipoprotein parameters in full-term neonates and their mothers, correlational analyses of apoC-III-1 and apoC-III-2 with these lipoprotein parameters were performed **(Table 3)**. Maternal sera demonstrated a negative relationship between apoC-III-0% and apoB, while the apoC-III-1% was negatively correlated with both TC and

TABLE 1. Apolipoprotein C-III polymorphism in maternal sera, cord sera, and the neonatal period (3, 14, and 28 days)

	ApoC-III	ApoC-III-0	ApoC-III-1	ApoC-III-2	ApoC-III-1/ ApoC-III-2	ApoC-III-0	ApoC-III-1	ApoC-III-2
		mg/dl					*%*	
Mothers	21.4 (7.5)[a,b]	1.9 (1.0)[c]	11.7 (4.2)[b]	8.2 (3.1)[b]	1.45 (0.30)[b]	7.9 (3.9)	54.4 (5.3)[c]	38.3 (5.7)[b]
n =	28	21	28	28				
Cord sera	5.0 (1.5)[c]	0.3[c]	1.7 (0.6)[b]	3.3 (1.0)	0.55 (0.31)[b]		31.0 (8.4)[b]	67.8 (8.6)[b]
n =	32	2	32	32				
Neonatal								
3-Day	9.0 (3.3)[c]	ND[d]	4.4 (1.6)	4.6 (1.8)	1.02 (0.25)		49.7 (10.4)	49.3 (10.4)
n =	19		19	19			26	26
14-Day	7.7 (4.3)	0.8 (0.4)	3.4 (1.7)	4.1 (2.6)	0.96 (0.42)	6.4 (1.8)	46.2 (9.2)[c]	50.7 (9.0)
n =	16	4	16	16		5	17	17
28-Day	6.8 (2.7)	0.6 (0.1)	3.4 (1.4)	3.3 (1.3)	1.07 (0.22)	7.7 (0.6)	49.7 (5.8)	47.9 (6.0)
n =	13	3	13	13		3	15	15

[a] Mean (SD).
[b] Duncan multiple range, significantly different from other groups ($P < 0.05$).
[c] Significant difference between groups ($P < 0.05$).
[d] Not detected.

TABLE 2. Lipoprotein parameters

	TG	TC	C	CE	CE/C	ApoA-I	ApoB
			mg/dl			*mg/dl*	
Mothers	140.3 (57.2)[a]	213.6 (50.4)	62.0 (21.4)	256.1 (61.7)	4.50 (1.84)	189.1 (60.9)	169.1 (63.0)
n =	27					24	23
Cord blood	13.7 (9.0)	60.4 (14.0)	22.1 (6.1)	64.7 (17.1)	3.02 (0.85)	86.5 (26.2)	35.3 (18.8)
n =	30					28	28
3-Day	59.9 (34.4)	77.4 (18.8)	32.3 (7.0)	76.2 (21.7)	2.36 (0.42)	88.0 (52.7)	67.5 (20.0)
n =	24					11	15
14-Day	71.5 (45.9)	139.5 (40.8)	49.2 (17.5)	152.5 (43.7)	3.21 (0.68)	146.4 (70.4)	78.4 (27.1)
n =	15	15	15	15	15	4	8
28-Day	67.0 (42.8)	108.7 (32.1)	39.2 (11.4)	117.6 (37.3)	3.02 (0.56)	90.6 (70.8)	44.6 (13.5)
n =	14	15	15	15	15	6	7

[a]Mean (SD).

CE. In addition, the levels of apoC-III-2 were directly related to TG, CE, and TC. In cord sera, apoC-III-1 correlated with TG, while apoC-III-2 correlated with TC and CE but not TG. At 3 days, apoC-III-1 correlated with C while no significant correlations at 14 days were found. At 28 days, apoC-III-1 and apoC-III correlated with TG, as was seen in cord sera for apoC-III-1. In addition, both apoC-III-2 and apoC-III-2% were positively correlated with the CE/C ratio.

DISCUSSION

Application of a crossed immunoelectrophoretic system combining isoelectric focusing and an adaptation of the apoC-III electroimmunoassay revealed that shortly after the birth of a full-term infant, the ratio of apoC-III-1 to apoC-III-2 changed from a system with apoC-III-2 the dominant form in cord blood to one at 3 days of age containing essentially equal amounts of apoC-III-1 and apoC-III-2. This ratio of apoC-III-1 to apoC-III-2 remained unchanged throughout the first month of life.

These changes in apoC-III polymorphism suggest several possibilities but are most consistent with either the induction of apoC-III-1 synthesis in the intestinal mucosa/liver or conversion of apoC-III-2 to apoC-III-1 in response to oral fat-feeding. Such an induction seems plausible since apoC-III has been detected in intestinal cells (16), though the preponderance of evidence indicates that apoC-III would primarily stem from the liver (17). Additional support for the intestinal origin of a portion of apoC-III stems from recent studies using cDNA probes. Such evidence has indicated that the majority of mRNA for apoC-III is found in the fetal liver, though the intestine has levels of mRNA for apoC-III that are 60% of the liver level (18). Thus, it appears that it is possible that the appearance of apoC-III-1 is due to the induction of its mRNA and direct synthesis in the intestinal tract. In addition, whether all polymorphic forms or only one form, e.g. apoC-III-2, are secreted from the liver remains unknown, though recent evidence has indicated both apoC-III-1 and apoC-III-2 can be secreted by a human hepatoma cell line (19). With respect to the conversion of apoC-III-2 to apoC-III-1, little evidence is available to support this particular processing event in the metabolism of apoC-III. However, the removal of sialic acid by either an extra- or intracellular neuraminidase could account for this conversion as suggested for the sialo-forms of apoE (19). As for other plasma glycoproteins (20), the complete removal of sialic acid might lead to the removal and proteolytic degradation of apoC-III-0. Thus, the absence of

TABLE 3. Correlation analyses of polymorphic forms of C-III in mother and full-term neonate sera

	n	r^a	P
Mothers			
ApoC-III-0% vs. apoB	18	−0.499	0.0351
ApoC-III-1% vs. CE	27	−0.389	0.0450
ApoC-III-1% vs. TC	27	−0.389	0.0450
ApoC-III-1 vs. apoB	23	0.603	0.0023
ApoC-III-2 vs. TG	27	0.497	0.0084
ApoC-III-2 vs. apoB	27	0.688	0.0003
ApoC-III-2 vs. TC	27	0.470	0.0133
ApoC-III-2 vs. CE	27	0.430	0.0254
ApoC-III vs. apoB	23	0.634	0.0012
Cord			
ApoC-III-1 vs. TG	30	0.340	0.0657
ApoC-III-2 vs. TC	30	0.449	0.0128
ApoC-III-2 vs. CE	30	0.389	0.0335
3-Day			
ApoC-III-1 vs. C	19	0.484	0.0357
28-Day			
ApoC-III-1 vs. TG	12	0.694	0.0123
ApoC-III-2% vs. CE/C	12	0.564	0.0357
ApoC-III-2 vs. CE/C	12	0.637	0.0258
ApoC-III vs. TG	12	0.618	0.0312

[a]Pearson correlation coefficients.

sialyated apoC-III on complex lipoprotein particles (those containing two or more distinct apolipoproteins), such as lipoprotein B:C-III-0 or B:C-III-0 E (21), could lead to their increased uptake and degradation by the liver (8).

The maternal ratio of apoC-III-1 to apoC-III-2 fell within the range reported for both gravid and nongravid adult apoC-III-1/apoC-III-2 ratios seen by isoelectric focusing of stained VLDL samples (5, 7, 15). In addition, these levels and ratios of apoC-III polymorphic forms were significantly different from their corresponding infant's cord sera. Although apoC-III-2 crossing the placenta and leading to the altered ratio of apoC-III polymorphic forms in cord sera cannot be ruled out, this possibility appears unlikely. This conclusion is based, in part, on the difference in the concentration gradient for apoC-III-1 and apoC-III-2 between the maternal and fetal compartments, where one would expect apoC-III-1 should cross in at least equal quantities. Since the half-life of apoC-III in normal subjects with similar plasma TG levels is 28 hr (22), any maternal contribution of apoC-III at 3 days should be minimal and not be a major contributor to the observed apoC-III levels or percentages.

The availability of this assay also offered the possibility to examine the relationship between the apoC-III polymorphic forms and other plasma lipoprotein parameters. Previous studies have shown several potential functional roles (8–10) for apoC-III and demonstrated that plasma apoC-III is directly correlated with plasma triglyceride levels (6, 23). However, as shown in the present study, having apoC-III-2 levels greater than apoC-III-1 levels is not necessarily associated with elevated TG. To explore possible relationships of each polymorphic form of apoC-III, Pearson correlational coefficients were examined (Table 3). In maternal sera, the major forms of apoC-III were positively correlated with apoB. The inhibition of uptake by apoC-III-1 and/or apoC-III-2 of apoB-containing lipoproteins at the placenta by receptor-mediated events (24) could be analogous to those reported for the liver (8). The correlations also indicate a distinction between cord sera levels of apoC-III-1, the major form in adult plasma, and apoC-III-2 with respect to their relationship to TG and cholesterol moieties. ApoC-III-1 is correlated with TG while apoC-III-2 is correlated with CE and TC.

In summary, the application of this assay to differentiate the polymorphic forms of apoC-III in small volumes of whole plasma has shown that the apoC-III-1/apoC-III-2 ratio in cord sera changes in the first few days of life, apparently in response to oral feeding. This finding suggests that the stimulation of the intestine by fat absorption leads to a reversal of the apoC-III-1/apoC-III-2 ratio seen in cord sera primarily due to an increase in apoC-III-1. In addition, the correlational analyses indicate different relationships between the polymorphic forms of apoC-III and other lipoprotein parameters. Studies in refined systems may lead to further insights into the role of apoC-III polymorphic forms in triglyceride and cholesterol metabolism in the fetal, neonatal, and adult states.

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