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High-Density Lipoprotein Subclass Distribution And Human Cord Blood Lipid Levels

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ABSTRACT. The high-density lipoprotein (HDL) subclass distribution was examined by gradient gel electrophoresis (gge) in 154 human cord blood samples, and determinations of triglyceride, total cholesterol, and HDL-cholesterol levels were performed. Three distinct gge patterns were recognizable. The first pattern, termed the normal (gge) pattern, was distinguished by a prominent double peak in the $(HDL_{2a})_{gge}$ region and a pronounced peak in the $(HDL_{3b})_{gge}$ region. Minor peaks, or shoulders, were also seen in the $(HDL_{2b})_{gge}$ and $(HDL_{3c})_{gge}$ regions, and a valley was present in the $(HDL_{3a})_{gge}$ region. This pattern was associated with normal lipid levels for cord blood plasma (mean triglycerides: 30–42 mg/dl; mean total cholesterol 62–85 mg/dl; mean HDL-cholesterol: 34–41 mg/dl).The second pattern, termed the 2b(gge) pattern, contained a major peak in the $(HDL_{2b})_{gge}$ region rather than the shoulder seen in the normal (gge) pattern, while the $(HDL_{2a})_{gge}$, $(HDL_{3b})_{gge}$, and $(HDL_{3c})_{gge}$ regions were less pronounced. This pattern was associated with elevated total cholesterol and HDL-C levels (means 85–102 and 49–56 mg/dl, respectively). The third pattern, termed the 3b(gge) pattern, was characterized by a paucity of material in the $(HDL_{2b})_{gge}$ region, a single peak in the $(HDL_{2a})_{gge}$ region, and either a relative increase in the $(HDL_{3b})_{gge}$ region, or a simultaneous increase in both $(HDL_{3b})_{gge}$ and $(HDL_{3c})_{gge}$. This pattern was associated with elevated triglyceride levels (means 78–88 mg/dl) and decreased HDL-C levels (means 20–30 mg/dl). Only two infants had a simultaneous elevation of triglycerides and total cholesterol and both cases exhibited the 3b(gge) pattern. Our study demonstrates that although the triglyceride and cholesterol levels in the newborn are much lower than those in adults, they are the important factors associated with the HDL subclass distribution. Elevated cholesterol was related to increased particles in the $(HDL_{2b})_{gge}$ region while elevated triglyceride levels were associated with a decrease in $(HDL_{2b+2a})_{gge}$ particles and a concomitant increase in $(HDL_{3b})_{gge}$ particles. (*Pediatr Res* 20: 487–491, 1986)

Abbreviations

HDL, high-density lipoproteins
TG, triglyceride
TC, total cholesterol
HDL-C, HDL-cholesterol
gge, gradient gel electrophoresis
CB, cord blood

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There is growing evidence that HDL, and in particular the HDL_2 subclass, have a protective role in the process of atherogenesis (1–3). In contrast to adults, in whom low density lipoproteins predominate, HDL is the predominant lipoprotein species in human CB (4–10). Davis *et al.* (4) found that in healthy term infants with lipoprotein levels below 100 mg/dl, cord blood HDL differed from that of adult in size distribution and in apolipoprotein content. They noted that the less dense HDL components of CB were larger, enriched in unesterified cholesterol, and contained the bulk of apolipoprotein E; the latter was principally in the apolipoprotein (E-AII) complex form. The more dense HDL components were smaller and enriched in total protein and cholesteryl ester. Evaluation of HDL particle size distribution by gradient gel electrophoresis revealed that the CB HDL distribution was broader than that reported for adult HDL by Blanche *et al.* (11) and was deficient in $(HDL_{3a})_{gge}$, the major peak in adult HDL. Several reports indicate that prematurity and pre- and perinatal complications elevate the cholesterol and TG levels of cord blood (12–22). However, little is known about the effect of elevated lipid levels on the HDL subclass distribution in the newborn. The purpose of this study is to evaluate the relationship between the HDL particle size distribution and levels of cholesterol and TG in CB.

MATERIALS AND METHODS

Cord blood samples. Umbilical CB was collected into tubes containing EDTA (1 mg/ml) and refrigerated immediately. Red cells were separated from plasma by centrifugation at 4000 × *g* for 20 min at 4° C. TC and TG were quantitated on 880 CB samples over an 8-month period. Of the total CB plasmas examined, 154 samples with plasma volume of 2 ml or more were selected for determination of HDL-cholesterol and for evaluation by gradient polyacrylamide gel electrophoresis. Since prematurity and perinatal complications have been shown to be associated with elevated CB lipid levels (12–22), emphasis was placed on inclusion of infants with pre- and perinatal complications or lipid levels above 100 mg/dl.

Determination of the lipid levels. Total plasma TG and cholesterol values were determined by means of enzymatic kits (TG with kits from Gilford Diagnostics, Cleveland, OH; TC with kits from Worthington Diagnostics, Freehold, NJ). Assays require a total of 0.1 ml CB plasma. HDL-C was determined by the precipitation technique of Steele *et al.* (23). Briefly, heparin and $MnCl_2$ were added to 0.5 ml plasma and incubated for 30 min. After incubation, the samples were centrifuged for 30 min (1500 × *g* at 4° C) and the supernatant pipetted. The supernatant was then analyzed for cholesterol (= HDL-C).

Lipoprotein isolation and gradient gel electrophoresis. CB lipoproteins were isolated at d 1.21 g/ml in a single ultracentrifugal step in a Beckman 40.3 rotor (100,000 × *g* 24 h at 4° C) essentially as described by Lindgren *et al.* (24). To isolate lipoproteins, 2 ml of cord plasma were underlaid with 4 ml of NaCl-NaBr of d 1.310; cord plasmas with volumes of 1.5 ml were adjusted to 2.0 ml with 0.154 M NaCl prior to centrifugation. After centrifugation, the top 1.0 ml of d ≤ 1.21 g/ml was harvested by pipetting.

The d < 1.21 g/ml fractions were analyzed by gradient polyacrylamide gel electrophoresis in order to determine HDL size distribution. This is a nondenaturing electrophoretic analysis that shows good reproducibility of particle size; the coefficient of variation with 4–30% gels is 0.5–1.7% (25). Total pattern area and the relative distribution of area among the subpopulations is reproducible, on average, to within 10%. Electrophoresis was carried out on precast Pharmacia 4–30% polyacrylamide slab gels (Pharmacia, Piscataway, NJ) according to the procedure of Nichols *et al.* (25). Reference proteins used to determine particle diameter consisted of thyroglobulin, apoferritin, lactate dehydrogenase, and bovine serum albumin. Gels were stained with Coomassie G-250 to identify protein bands, and densitometric scans were obtained with a Transidyne RFT densitometer (Transidyne Corp., Ann Arbor, MI).

To compare the HDL particle size distribution of individual CB lipoprotein samples, the d < 1.21 g/ml fractions were electrophoresed on 4–30% gels and were then scanned after protein staining. As illustrated in Figure 1, the height (in cm) of each peak and shoulder was measured and its relative value calculated as a percentage of the total of all heights in the scan. This value is then the relative optical density of that particular protein band. If no peak or shoulder was present, the relative optical density was considered to be 0. The terms $(HDL_{3a})_{gge}$, $(HDL_{3b})_{gge}$, and $(HDL_{3c})_{gge}$, according to the nomenclature of Nichols *et al.* (25), designate major particle size subpopulations observed by gradient gel electrophoresis within the ultracentrifugal HDL_3 fraction (d 1.125–1.20 g/ml) from adult plasma, while the terms $(HDL_{2b})_{gge}$ and $(HDL_{2a})_{gge}$ designate HDL subpopulations within the HDL_2 (d 1.063–1.125 g/ml) ultracentrifugal fraction. Particle size intervals defining these subpopulations are $(HDL_{2b})_{gge}$, 12.9–9.7 nm; $(HDL_{2a})_{gge}$ 9.7–8.8 nm; $(HDL_{3a})_{gge}$ 8.8–8.2 nm; $(HDL_{3b})_{gge}$, 8.2–7.8 nm; and $(HDL_{3c})_{gge}$, 7.8–7.2 nm (25). Unlike adult plasma HDL, CB HDL contained two distinct peaks within the $(HDL_{2a})_{gge}$ interval and often within the $(HDL_{3c})_{gge}$ region as well. The double peaks in the $(HDL_{2a})_{gge}$ and $(HDL_{3c})_{gge}$ were designated $(HDL_{2a1})_{gge}$, $(HDL_{2a2})_{gge}$, $(HDL_{3c1})_{gge}$, and $(HDL_{3c2})_{gge}$, respectively. CB contains a valley in the $(HDL_{3a})_{gge}$ region (4) where normally there is a major peak in adult plasma (25).

PEAK	R_f	DIAM (nm)	O.D. (cm)	relative O.D. (%)
2b	0.514	11.2	1.8	13
$2a_1$	0.645	9.4	3.6	25
$2a_2$	0.696	8.9	3.7	26
3b	0.818	7.9	3.2	23
3c	0.911	7.4	1.8	13

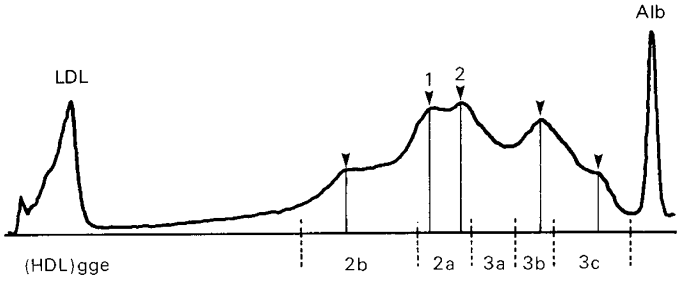

Fig. 1. A representative gradient polyacrylamide gel electrophoresis scan pattern of the plasma d < 1.21 g/ml fraction of a normal term infant (TG, 49.1 mg/dl; TC, 63.8 mg/dl) on a 4–30% gel demonstrating how the scans were quantitatively evaluated. The HDL peaks and shoulders are indicated by *arrows*; the two peaks under $(HDL_{2a})_{gge}$ are designated *1* and *2*. The designations of $(HDL)_{gge}$ are described in "Materials and Methods" and in References 11 and 25. The optical density (*O.D.*) is measured as peak height in cm (*vertical lines*) and then converted to relative optical density (%) for each peak. R_f values and corresponding particle size (diameter in nm), absolute and relative optical density for the five peaks are shown in tabular form. The relative optical density values are used to compare particle size distribution among individual cord blood samples. The low-density lipoprotein (*LDL*) region, as indicated, forms a sharp peak in the *left side* of the scan while albumin (*alb*) forms a sharp peak at the *right side*.

Statistical analysis. Mean differences in relative peak height between infants with different gradient gel electrophoresis patterns in each HDL region were assessed using analysis of variance (26). For multiple comparisons, significance values were adjusted using the Bonferioni inequality (26). TC, TG, and HDL-C means by group and gradient gel electrophoresis patterns were compared using the same method. Because the distributions of these variables were skewed, natural log transformations were performed for the statistical tests. Reported *p* values are based on these transformed values, however, the means and SDs reported are not transformed, so that they are clinically meaningful. Frequency distributions of gradient gel electrophoresis patterns were also compared using a χ^2 test (27).

RESULTS

The mean TG and TC values for the 880 CB plasmas were 41.5 ± 15.5 and 68.2 ± 17.5 mg/dl, respectively. The range for both measurements was large: 11–192 mg/dl for TG and 25–150 mg/dl for cholesterol. Of these samples, 5% had TC values above 100 mg/dl while 1% had TG values above 100 mg/dl. Only 0.5% of the samples had both elevated cholesterol and TG. These cholesterol and TG distributions are similar to those previously reported (5, 7, 10, 28–34).

The 154 samples for whom gge was performed were grouped according to the following criteria: *Group I*: infants with TG and TC levels below 100 mg/dl, Apgar scores greater than 8, gestational age greater than 37 wk, appropriate weight for gestational age, and no pre- or perinatal complications. This group was considered the control group. *Group II*: term infants with gestational age greater than 37 wk, pre- or perinatal complications and/or TG and/or TC values above 100 mg/dl. *Group III*: premature infants with gestational age < 37 wk, regardless of plasma lipid levels.

Gradient polyacrylamide gel electrophoresis of the 154 cord plasmas revealed that at least three distinct patterns are recognizable; representative scans of these patterns are shown in Figure 2. The first pattern (Fig. 2*A*) is distinguished by a prominent double peak in the $(HDL_{2a})_{gge}$ region and a pronounced peak in the $(HDL_{3b})_{gge}$ region. Minor peaks or shoulders are seen in the $(HDL_{2b})_{gge}$ and $(HDL_{3c})_{gge}$ regions and a valley is present in the $(HDL_{3a})_{gge}$ region. Although not shown in Figure 2, the shoulder in the $(HDL_{3c})_{gge}$ region frequently resolves into two small components. We have termed this pattern the normal (gge) pattern and it is similar to that previously described for pooled normal CB samples (4). The second pattern is shown in Figure 2*B* and contains a major peak in the $(HDL_{2b})_{gge}$ region rather than the shoulder seen in the normal pattern. The double peak is still visible in $(HDL_{2a})_{gge}$ but this region and the $(HDL_{3b})_{gge}$ and $(HDL_{3c})_{gge}$ regions are less pronounced than in the normal (gge) pattern. We have termed this the 2b(gge) pattern. The third pattern, as demonstrated by the two scans (*solid and broken line*) in Figure 2*C*, is characterized by a paucity of material in the $(HDL_{2b})_{gge}$ region and a single peak in the (HDL_{2a}) region, the (HDL_{2a2}) peak. The most prominent feature of this pattern is the relative increase in the $(HDL_{3b})_{gge}$ region (*solid line*) or the simultaneous increase in both $(HDL_{3b})_{gge}$ and $(HDL_{3c})_{gge}$, indicated by the *broken line*, as compared with the normal pattern.

The pattern represented by both these scans is termed the 3b(gge) pattern.

Table 1 summarizes the mean values of the relative optical density for the three $(HDL)_{gge}$ patterns from 152 of the 154 cord plasmas. One gradient gel pattern did not fit into any of the categories and one had a poor baseline; both were excluded from the analysis. Of the total scans, 64% had the normal (gge) pattern while 26 and 10% had 2b(gge) and 3b(gge) patterns, respectively. The mean relative optical density distribution for each of the patterns reflects the particle distribution seen in the representative scans shown in Figure 2. In the normal (gge) pattern, approximately 50% of the HDL material is in the $(HDL_{2a})_{gge}$ region as a double peak. About 20–25% of the HDL is in the $(HDL_{3b})_{gge}$ region and the remaining 25% is distributed between very large and very small particles. A major difference in the 2b(gge) pattern is a shift of HDL into the larger-sized $(HDL_{2b})_{gge}$ region at the expense of the smaller sized $(HDL_{3c})_{gge}$ components as compared to the normal (gge) pattern. The peak in the $(HDL_{2b})_{gge}$ region is double the average intensity of that of the normal pattern (see Table 1). Within the $(HDL_{2a})_{gge}$ region, the $(HDL_{2a1})_{gge}$ component, which has a mean particle diameter of 9.3 ± 0.1 nm, has slightly more material; coincidentally, the $(HDL_{2a2})_{gge}$ component with a mean particle diameter of 8.8 ± 0.1 nm is decreased. These changes are small but statistically significant as seen in Table 1. In the 3b(gge) pattern, the HDL are shifted toward the smaller particle sizes. There is almost a complete absence of larger, less dense particles in the $(HDL_{2b})_{gge}$ region and in the $(HDL_{2a1})_{gge}$ peak (Table 1). Although the $(HDL_{2a2})_{gge}$ peak is still apparent, its intensity is lower in comparison with the normal (gge) pattern but statistically not different from the 2b(gge) pattern (Table 1). The 3b(gge) pattern has detectable material in the $(HDL_{3a})_{gge}$ region, probably reflecting some spill over material from the extremely prominent $(HDL_{3b})_{gge}$ region. The average intensity of the $(HDL_{3b})_{gge}$ peak is double that of the normal (gge) pattern. The $(HDL_{3c1})_{gge}$ peak, which has a mean particle diameter of 7.6 ± 0.1 nm, is slightly increased but is not statistically significantly different from the normal (gge) pattern. The $(HDL_{3c2})_{gge}$ peak (mean particle diameter of 7.4 ± 0.1 nm), however, is significantly increased, reflecting the presence of the sharp peak in certain patterns as shown in the *broken line* in Figure 2*C*. These data support the premise that three distinct HDL subclasses exist in CB.

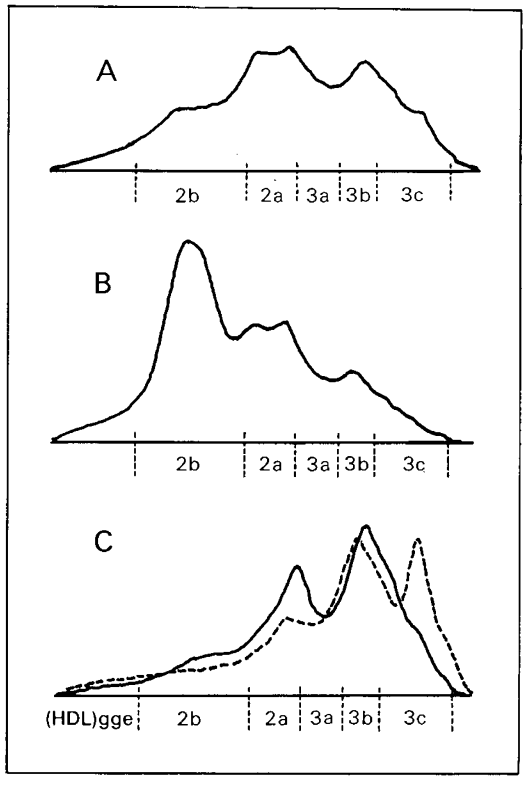

Fig. 2. Representative scans of the three types of gradient gel patterns noted in cord blood. *A*, normal (gge) pattern. *B*, 2b(gge) pattern. This is characterized by a pronounced increase in the $(HDL_{2b})_{gge}$ region and an elevation of cord blood cholesterol. *C*, 3b(gge) pattern. Two different forms of the pattern are noted: the *solid line* with a major peak in $(HDL_{3b})_{gge}$ and a shoulder in $(HDL_{3c})_{gge}$; and the *broken line* with prominent peaks in both the $(HDL_{3b})_{gge}$ and $(HDL_{3c})_{gge}$ regions. This pattern is associated with elevated TG levels.

Table 2 shows the frequency distribution of the gradient gel patterns in the group I, II, and III cord blood samples. The normal (gge) pattern is clearly the predominant pattern (89%) in group I (control) infants and accounts for 50 and 43% of the patterns in group II and III infants, respectively. The 2b(gge) pattern appears most frequently (49%) in the group III (premature) infants and also accounts for a considerable number (30%) of group II infants. The 3b(gge) pattern is observed less frequently (10% of all patterns) but is most frequent among the group II infants. The difference in pattern distribution between group I infants and the other two groups is statistically significant ($\chi^2 = 33.03$, $p \leq 0.0001$).

Table 3 shows the relationships between gradient gel patterns and plasma lipid values of the three infant groups. The mean concentrations for TG, TC, and HDL-C in infants with the normal (gge) pattern were within the limits of normal lipid values for newborn infants and averages ranged from 30–43, 62–85,

Table 2. *Frequency distribution of gradient gel electrophoretic patterns in group I, II, and III infants*

Gradient gel pattern	Group I *n* (%)	Group II *n* (%)	Group III *n* (%)	Total *n* (%)
Normal(gge) pattern	55 (89)	28 (50)	15 (43)	98 (64)
2b(gge) pattern	5 (8)	17 (30)	17 (49)	39 (26)
3b(gge) pattern	2 (3)	11 (20)	3 (9)	16 (10)
Total	62	56	35	153

Table 1. *Mean (± SD) relative optical density of HDL subpopulations according to gradient gel pattern and peak location within (HDL)gge distribution*

GGE pattern	*n*	Location of peak in HDL scan: 2b	2a: Peak 1	2a: Peak 2	3a	3b	3c: Peak 1	3c: Peak 2
Normal pattern	98 (64%)	16 ± 5	20 ± 8	25 ± 5	0	22 ± 6	9 ± 9	9 ± 7
2b(gge) pattern	39 (26%)	30 ± 6*	23 ± 5†	20 ± 7*	2 ± 6	15 ± 4*	6 ± 6	5 ± 6†
3b(gge) pattern	15 (10%)	4 ± 5*,§	0*,§	19 ± 11*	5 ± 12*,‖	44 ± 18*,§	13 ± 15‖	16 ± 16*,§

* $p < 0.01$; † $p < 0.05$ for comparison of means with the normal pattern samples.
§ $p < 0.01$; ‖ $p < 0.05$ for comparison of means with the 2b(gge) pattern samples.

Table 3. *Mean values of TG, TC, and HDL-C (± SD) by gradient gel pattern and infant group*

GGE pattern	TG*			TC†			HDL-C†		
	Group I	Group II	Group III	Group I	Group II	Group III	Group I	Group II	Group III
Normal	34.2 ± 11.9	43.4 ± 15.5	30.1 ± 11.3	62.5 ± 11.6	80.1 ± 20.8	85.4 ± 26.5	33.6 ± 7.5	40.8 ± 12.6	37.3 ± 10.9
2b(gge)	27.9 ± 1.9	39.7 ± 13.2	32.4 ± 8.2	84.1 ± 9.1	101.8 ± 19.6	97.0 ± 24.3	51.5 ± 6.0	56.1 ± 13.9	49.1 ± 11.1
3b(gge)	87.8 ± 5.7	86.5 ± 42.7	78.3 ± 5.5	72.5 ± 4.1	56.8 ± 24.2	75.3 ± 27.0	30.0 ± 8.1	19.5 ± 6.8	22.8 ± 19.7

* $p < 0.01$, for comparison of means between 3b(gge) pattern with normal and 2b(gge) patterns based on natural log transformed variables.
† $p < 0.01$, for comparison of means between all three patterns based on natural log transformed variables.

and 34–41 mg/dl, respectively, in the three groups. Compared with the normal (gge) pattern, infants with the 2b(gge) pattern have significantly higher TC (mean 84–102 mg/dl) and HDL-C (mean 49–56 mg/dl) but normal TG levels. The TC levels of the infants with the 3b(gge) pattern were somewhat lower than the control group, but TG levels (mean 78–88 mg/dl) were significantly elevated. Infants with this pattern had statistically significantly lower HDL-C (mean 20–30 mg/dl) levels.

These data strongly suggest that elevated CB cholesterol is associated with the appearance of the 2b(gge) pattern, while elevated TG is associated with the 3b(gge) pattern, irrespective of the infant group.

DISCUSSION

Several investigators have shown that prenatal and perinatal complications can influence CB lipid levels (12–20). It is well known that prematurity (gestational age less than 37 wk) is associated with elevated TC and low TG (12, 14, 17–19, 21) and that TC is elevated in infants of diabetic mothers (19, 35, 36). In addition, placental insufficiency, low Apgar, nuchal cord, and meconium staining are associated with elevated CB TG (13, 22). It has not been reported whether the change in lipid levels is also accompanied by a change of the subclass distribution of neonatal HDL, and this question was examined in the present report.

Davis *et al.* (4), using nondenaturing gradient gel electrophoresis, demonstrated that pooled and individual CB samples from full-term normal infants had a very characteristic size distribution profile which was different from that of adults. They observed that cord blood HDL possessed larger-sized particles in the $(HDL_{2b})_{gge}$ region, a double peak in the $(HDL_{2a})_{gge}$ region, and smaller HDL in the $(HDL_{3c})_{gge}$ region; additionally, there was little or no HDL in the $(HDL_{3a})_{gge}$ region. Our present results with infants having the "normal" (gge) pattern show three prominent peaks of almost equal intensity: two within the $(HDL_{2a})_{gge}$ region with estimated particle diameters of 9.3 ± 1.3 and 8.8 ± 1.2 nm and one within the $(HDL_{3b})_{gge}$ region with a particle diameter of 7.9 ± 0.9 nm. This pattern agrees well with that previously described by Davis *et al.* (4). Of our "control" group 89%, *i.e.* normal term infants with TG and TC concentrations below 100 mg/dl, had the normal (gge) pattern. Of the 880 original cord blood samples, the TG and TC distributions of 131 consecutive births of full-term infants with normal Apgar scores and no pre- or perinatal complications were analyzed retrospectively. In this sample, the 95th percentiles were 65 mg/dl for TG and 85 mg/dl for TC. If these cutoff points are used, the percentage of group I infants with a normal (gge) pattern increases to 96% since seven samples would be excluded from the original control group (group I) of 62 infants. This high degree of uniformity suggests that healthy full-term infants with normal lipid levels have a predictable HDL subclass distribution. This consistency of size distribution probably reflects the constancy of the milieu of the fetus *in utero* where diet and environmental factors have little impact.

The 2b(gge) pattern with its increased $(HDL_{2b})_{gge}$ and $(HDL_{2a1})_{gge}$ peaks is associated with CB cholesterol levels greater than 85 mg/dl. This pattern is also associated with increased HDL-C levels. Not surprisingly, the 2b(gge) pattern is seen most frequently in group III infants (approximately 50% of the group), since prematurity has been described by several investigators to be associated with elevated cholesterol levels (12, 14, 17–19, 21). The parallel increase in the $(HDL_{2b})_{gge}$ and $(HDL_{2a1})_{gge}$ peaks in this pattern, together with a decrease in intensity of peaks which correspond to smaller, more dense particles, suggests a metabolic transformation from smaller to larger particles. Although the metabolic origin of HDL subclasses is not fully understood, it has been postulated that lipases, lecithin:cholesterol acyltransferase, and lipid transfer protein have functional roles in determining the overall HDL pattern in adults. Lipoprotein lipase, by hydrolysis of TG-rich particles, generates surface remnant components (including phospholipid and cholesterol) which are taken up by HDL thus forming larger particles (37–39). Phospholipid and free cholesterol are substrates for lecithin: cholesterol acyltransferase, which generates cholesteryl ester that forms the core of larger, less dense HDL (40). It is thought that cholesteryl ester in the large HDL is exchanged for TG in very low-density lipoproteins through the action of the lipid transfer protein (41–43). The HDL TG is subsequently hydrolyzed by hepatic lipase which in effect transforms the large HDL particle to a smaller, more dense one (44, 45). One would expect that if all these factors are functional in CB, the $HDL_{2b})_{gge}$ peak would be reduced in intensity. Since this peak in infants with the 2b(gge) pattern is approximately twice that of the normal (gge) pattern, an impairment in the transformation of large HDL to small HDL is suggested. CB lecithin:cholesterol acyltransferase activity, although low (46–48), functions normally since the cholesteryl ester to free cholesterol ratio is similar to adults (49). Lipoprotein lipase activity in the newborn is also similar to that of adults while hepatic lipase activity is 2- to 3-fold higher in newborn infants (50). Although speculative, the unusual elevation of $(HDL_{2b})_{gge}$ may be the result of very low-density lipoprotein levels in cord blood. Deficiency of very low-density lipoprotein, the preferred donor of TG and acceptor of cholesteryl ester in the TG-for-cholesteryl-ester exhange process, could limit interconversion of large particles to small ones. The preceding hypothesis assumes that the level of lipid transfer protein in cord plasma is normal; however, at present such data are not available.

In the present study, we found that elevation of CB TG (> 65 mg/dl) was associated with a distinct 3b(gge) pattern which shows a paucity of larger, less dense HDL particles. This pattern is also associated with a significant decrease in HDL-C levels. The 3b(gge) pattern is most frequently encountered in term infants with pre- and perinatal complications, conditions associated with elevated CB TG levels. Recent reports on adult subjects with hypertriglyceridemia have shown, upon gradient gel electrophoresis, that elevated TG is associated with a pronounced increase in the $(HDL_{3b})_{gge}$ region (25, 51). The study of Chang *et al.* (51) provides evidence that the shift from larger particles to smaller ones in adults is progressive as plasma TG levels increase. At plasma TG levels > 330 mg/dl they noted that the predominant peak has a diameter of 7.8 nm, corresponding to the $(HDL_{3b})_{gge}$ peak in CB. Although the elevation of TG in CB is far less in absolute concentrations than that of adult hypertriglyceridemic plasma, the response of HDL size distribution is similar: CB plasma with TG levels above 65 mg/dl is associated with a decrease in larger, less dense HDL, and an increase in smaller, more dense HDL. The metabolic mechanism for depletion of HDL_2 particles and coincident increase in smaller HDL_3 particles in hypertriglyceridemia are not fully understood. It is interesting, however, that in adults an inverse relationship has been noted between hepatic lipase activity and levels of HDL_2 (52, 53).

Simultaneous elevation of CB TG and cholesterol occurs very infrequently. In the present series, two infants were in this category. The first had TG and cholesterol levels of 139 and 93 mg/dl, respectively, while the second had values of 72 and 106 mg/dl, respectively. The former infant had low HDL-C (28 mg/dl) while the latter had slightly elevated HDL-C (46 mg/dl). In both cases, the HDL exhibited the 3b(gge) pattern. Apparently, high cholesterol levels and even higher HDL-C levels do not allow the metabolic formation of large, less dense HDL particles if TG levels are concurrently elevated. Thus, where both TG and cholesterol are elevated, it is suggested that TG is the major determinant of fetal HDL subclass distribution.

Our study demonstrates that cord blood lipid levels are the most important factors influencing HDL subclass distribution. High TC is related to an increase of particles in the $(HDL_{2b})_{gge}$ region. High TG levels are associated with a decrease in the amount of particles in the $(HDL_{2b+2a})_{gge}$ region and with a concomitant increase of particles in the $(HDL_{3b})_{gge}$ region.

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