Intermediate density lipoproteins (IDL) \((d = 1.006 \text{ to } 1.019 \text{ g/ml})\) and low density lipoproteins (LDL) \((d = 1.019 \text{ to } 1.063 \text{ g/ml})\) were isolated from human umbilical cord blood plasma by sequential ultracentrifugation. The concentration, chemical and apolipoprotein composition, size and size distribution of the neonatal IDL and LDL for both sexes were determined. The IDL and LDL from the neonates showed no sex-related differences in composition or concentration. The IDL and LDL were lower in concentration and differed in composition with regard to each other and with regard to the comparable adult fractions. The apolipoprotein (apo) composition showed only the high molecular weight form of apo B present in the IDL, while the LDL showed the presence of two lower molecular weight forms of apo B in addition to the high molecular weight form, along with appreciable amounts of apo E and apo A-I. The size distribution of the neonatal IDL and LDL showed a constant pattern, with peaks at approximately 300 Å for IDL and 257 and 244 Å for neonatal LDL. The alterations in composition, size and size distribution, as well as the lower concentrations present in the neonate, point to differences between the neonate and the adult in the metabolism of lipoproteins with a density of 1.006 to 1.063 g/ml.

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Lipoprotein and lipid levels of the human neonate are of scientific interest for a variety of reasons. Knowledge of what constitutes a normal lipoprotein pattern in a neonate may allow the identification of abnormalities in lipoprotein and lipid metabolism which in later life may give rise to coronary heart disease. Early diagnosis would allow for medical intervention at a point prior to overt symptoms of coronary heart disease. Additionally, studies of the neonatal lipid metabolism may provide further insights into the factors and processes that interact to give rise to the lipoprotein species, distributions, and concentrations in the adult.

Previous studies have shown that the lipid and lipoprotein levels\(1^\text{st} - 10^\text{th}\) and apolipoprotein concentrations\(11^\text{th}\) of neonatal umbilical cord blood are markedly different from those observed in the adult. Total plasma lipid levels — triglycerides (TG) and total cholesterol (TC) — are only one-third of those found in the adult population. The distribution of the various lipoproteins in the neonate is unlike that of the adult since the high density lipoproteins (HDL) represent the predominant lipoprotein species, and the low density lipoproteins (LDL) are reduced relative to the HDL. The very low density lipoproteins (VLDL) are present in only trace amounts.

While several investigators have provided information on the chemical composition and size of the neonatal fractions \(d = 1.006 \text{ to } 1.063 \text{ g/ml}\),\(^{2,5,6-10}\) no information is available on the

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physical and chemical properties of neonatal intermediate density lipoproteins (IDL), d = 1.006 to 1.019 g/ml, and the LDL, d = 1.019 to 1.063 g/ml. Furthermore, the studies reported give a wide range of values for composition and concentrations of d = 1.006 to 1.063 g/ml lipoproteins, which may reflect methodological differences or peculiarities of the populations studied. The aim of this report is to characterize the concentration, composition, and size distribution of the neonatal IDL and LDL.

Methods
Isolation of Lipoproteins

Human umbilical cord blood from normal full-term deliveries was collected from the umbilical cords into tubes containing EDTA (1 mg/ml). The red blood cells were removed by centrifugation at 4000 × g for 10 minutes at 4°C. The plasma was then collected and analyzed individually for TG and TC levels with the enzymatic assay kits (TG with kits from Worthington or Gilford Diagnostics; Worthington Diagnostics, Freehold, New Jersey; Gilford Diagnostics, Cleveland, Ohio); TC with kits from Worthington or BMC (Biodynamics/BMC, Indianapolis, Indiana) using a Gilford 3500 analyzer. Cord plasma having levels of either TG or TC exceeding 100 mg/100 ml were excluded, and the remaining cord plasmas were either pooled according to sex or used individually in an effort to examine only “normal” lipoprotein species. Cord blood samples having either TG or TC levels above 100 mg/dl were excluded, as this exceeded the average obtained from the literature by more than two standard deviations.

The lipoproteins were isolated from the pooled or individual plasma by ultracentrifugation according to Lindgren, except that the separations were all performed at 4°C; all solutions contained 0.1% EDTA and 0.02% sodium azide to minimize degradation. The VLDL were removed by centrifugation at 100,000 × g for 24 hours at d = 1.006 g/ml. The density of the subnatant was then adjusted to d = 1.063 g/ml and centrifuged for 24 hours at 100,000 × g to isolate the total LDL fraction. The total LDL (d = 1.006 to 1.063 g/ml) was separated into IDL and LDL by adjusting the density to 1.019 by dialysis followed by centrifugation at 100,000 × g for 24 hours. The IDL, the upper 3 ml, and the LDL, the lower 3 ml, were then pipetted into screw-cap vials with Teflon caps and stored at 4°C until used for analysis.

Analytical Procedures

The protein content of the lipoprotein fractions was determined by the modified Lowry protein determination of Markwell et al using bovine serum albumin (BSA) (Miles, Elkhart, Indiana) as a protein standard. Phospholipid, as inorganic phosphate, was determined by ashing, followed by a phosphate analysis as detailed by Ames. Free and total cholesterol were determined by the enzymatic method of Allain et al, and the esterified cholesterol level was obtained as a difference between the total and free cholesterol. Triglyceride was determined using either the chemical method of Biggs et al or the enzymatic method using the triglyceride reagent kit from Worthington. Values for triglyceride, cholesterol, esterified cholesterol, and phospholipid were calculated using 850, 387, 650, and 775 as the respective molecular weights. Mean values and standard error (SEM) were calculated by standard techniques. Data comparisons were analyzed for statistical significance by the Student’s t test.

Sodium dodecylsulfate (SDS) gel electrophoresis on 10% polyacrylamide gels was performed by the method of Weber and Osborn using mercaptoethanol as the reductant. SDS gel electrophoresis using 3% polyacrylamide gels was performed as described by Kane et al except that ovalbumin was added as an internal standard and the Rf was measured relative to the ovalbumin band. Gradient gel electrophoresis (GGE) on 2% to 16% gels (Pharmacia, Piscataway, New Jersey) was performed as described by the manufacturer. The gels were fixed and stained as detailed by Blanche et al. Stained gels were scanned with an RFT densitometer (Transidyne Corporation, Ann Arbor, Michigan) at a wavelength of 630 nm. The molecular weight and diameter standards employed were thyroglobulin (Schwartz/Mann, Orange, New Jersey), cross-linked BSA (Sigma, St. Louis, Missouri), the high molecular weight standard kit (Pharmacia, Piscataway, New Jersey), and carboxylated latex beads (Dow Diagnostics, Indianapolis, Indiana). For electron microscopy, the samples were dialyzed against 1% ammonium acetate, pH 7.4, and then negatively stained with 2% sodium phosphotungstate, as previously described. The size and size distribution were determined using the computerized particle analysis system described previously.

Results

The cord blood plasma TG and TC levels were determined on 273 female and 309 male neonates. The TG values were 41.9 ± 17.6 SEM mg/100 ml and 40.4 ± 16.7 mg/100 ml for females and males, respectively, with no statistically significant difference between the two values. The TC level for female neonates was 67.5 ± 16.3 mg/100 ml, and for male neonates, 62.3 ± 14.6 mg/
100 ml, again with no statistically significant difference between the two values. Approximately 3% of the cord blood plasma analyzed had levels of either TG or TC exceeding the 100 mg/100 ml cutoff point, and thus were excluded.

The composition and concentration of the neonatal IDL and LDL of both sexes are presented in table 1. It is immediately apparent that the diminished lipid levels of the neonate cord blood are mirrored in the greatly reduced concentrations of the IDL (6.57 ± 0.96 mg/100 ml in females; 6.82 ± 0.72 mg/100 ml in males) and the LDL (76.86 ± 5.99 mg/100 ml in females; 74.41 ± 6.78 mg/100 ml in males).

The distribution of lipid and protein moieties in both IDL and LDL is shown in table 2. The IDL of both male and female neonates was characterized by an enrichment of the TC and phospholipid. The LDL of the neonate showed a TG elevation; approximately 12% of the mass is TG compared to approximately 5% in the adult LDL (d = 1.019 to 1.063 g/ml). Cholesteryl ester, however, is the major core lipid of LDL particles, as indicated by the EC/TG ratio of 3.75 seen in table 3. IDL, in contrast, has a relatively larger portion of the core composed of TG, as shown by the EC/TG ratio of 1.24. The molar ratio of free cholesterol to phospholipid (FC/PL) is constant for both fractions, although the esterified cholesterol to free cholesterol (EC/FC) is decreased in both the IDL and LDL fractions (1.45 and 2.09, respectively) compared to the adult values (2.04 and 2.38 for adult IDL and LDL, respectively).

The size distributions of the neonatal IDL and LDL, as determined by densitometric scanning of the 2% to 16% gradient gels after staining for protein, are presented in figure 1 along with the size distribution for the total LDL fraction (d = 1.006 to 1.063 g/ml). The IDL, when analyzed by GGE, showed a single somewhat broad peak with a maximum corresponding to a calculated diameter of 300 Å, which is in good agreement
Figure 1. Representative densitometric scans of Coomassie blue R-250 stained 2% to 16% polyacrylamide gradient gel. A. Total low density lipoprotein levels (LDL) from cord blood plasma (d = 1.006 to 1.063 g/ml). B. LDL (d = 1.019 to 1.063 g/ml). C. Intermediate density lipoprotein levels (IDL) (d = 1.006 to 1.019 g/ml).

with the 310 Å obtained by electron microscopy (data not shown). The neonatal LDL on GGE had a large major peak at 257 Å and a minor peak component at 240 Å; by comparison, the EM value for LDL was 257 Å. The GGE patterns of particle size were similar for both sexes and for individual and pooled IDL and LDL fractions. LDL peak positions were remarkably reproducible, with peak sizes of 255 ± 3 Å and 242 ± 3 Å for individual samples analyzed (n = 15), and 256 ± 4 Å and 244 ± 6 Å for the pooled samples (n = 6).

The apoprotein content of the IDL and LDL fractions from neonates are shown in figures 2 and 3. Figure 2 presents the results of SDS gel electrophoresis of LDL on 10% polyacrylamide; apo B, which remained near the top of the gel, was the major apolipoprotein, but in addition, small quantities of apo A-I and E were also present. The identification of apo A-I and apo E was confirmed by Ouchterlony immunodiffusion kindly provided by Dr. P.N. Herbert (personal communication). Again, there was no difference between male and female neonates with regard to the presence of apo A-I and apo E. Figure 3 shows the analysis of the neonatal IDL and LDL by SDS gel electrophoresis on 3% polyacrylamide gels. The IDL displayed only one high molecular weight form of apo B, with a calculated molecular weight of 540,000 daltons. The LDL from the neonate contained, in addition to the high molecular apo B, two smaller molecular weight forms of apo B, with calculated molecular weights of 480,000 and 180,000 daltons, respectively. The neonate IDL and LDL showed a consistent pattern for both pooled and individual samples of high molecular weight apo B in both IDL and LDL, while LDL fractions had, in addition, the low molecular weight forms of apo B. Adult LDL treated in the same way (data not shown) consistently revealed the presence of the high molecular weight apo B, but the presence of the lower molecular weight forms was variable, being dependent on the individual sample.

Discussion

The TG and TC levels for neonatal cord blood plasma examined here are comparable to those reported by earlier workers.1-6 The values show a bias toward the higher values, similar to those reported by Hardell.1 However, the level of total cholesterol in the female vs male neonate, while somewhat higher, is not significantly different, in
Intermediate density lipoprotein (IDL) and low density lipoprotein (LDL) from pooled male and female cord blood plasma, reduced (+ ME, mercaptoethanol), and unreduced, upon sodium dodecyl sulfate (SDS) polyacrylamide electrophoresis on 3% gels. The LDL gels were loaded with approximately 50 μg of protein, and the IDL gels with approximately 25 μg of protein.

Figure 3.
maintaining the size of the core. In the case of the neonatal LDL, this component appears to be TG since the particles are enriched with TG compared to that of the adult particle (12% vs 5% for adult LDL). The IDL, however, do not show such a pattern of TG enrichment; rather they are enriched in total cholesterol (35%) and phospholipid (24%) compared to the adult IDL cholesterol (29%) and phospholipid (17%). Enrichment of TG in the LDL may reflect the fact that the fetal energy needs are apparently met mostly by carbohydrate. Such reliance on carbohydrate may result in an alteration of the lipoprotein composition similar to that observed in studies of hepatic VLDL during high carbohydrate regimens.

The apolipoprotein compositions of the neonatal IDL and LDL are different from those of the comparable adult fractions. Cord blood IDL is unusual in that apo B is the sole apolipoprotein present; in contrast, adult IDL contain, in addition to apo B, the smaller molecular weight apo E and C. The absence of apo C from the IDL is consistent with the finding of McConathy and Lane that lipoproteins containing apo B, in combination with either apo C or apo E or both, are markedly diminished or absent in cord blood relative to adult levels. Cord blood LDL contain, in addition to apo B, appreciable amounts of apo E and apo A-I. The presence of apo A-I and apo E does not appear to be due to any contamination of the LDL fraction, as the fraction did not show any VLDL- or HDL-like particles by either GGE or electron microscopy. Although the presence of apo A-I and apo E is established, it is unclear whether apo A-I and/or apo E exist on the same particle along with apo B, or whether they constitute a separate population of particles. The relatively high cord blood plasma apo C levels reported by McConathy and Lane combined with our finding of only trace amounts of apo C in the LDL fractions of neonates suggest that other lipoprotein density classes must be enriched in apo C.

Apo B of IDL and LDL was examined for the polymorphism reported for adult LDL by Kane et al. In cord blood IDL, apo B consisted of a single, high molecular weight band with a calculated molecular weight of 540,000 daltons. The LDL from the neonate had, in addition to the high molecular weight band, two other bands of lower molecular weight, one with a calculated molecular weight of 480,000 daltons and the other, a molecular weight of 180,000 daltons. Under the nomenclature proposed by Kane et al. to allow comparisons between laboratories, the 540,000 dalton band is designated B100, while the 480,000 dalton band is B83, and the 180,000 dalton band is B31. The numbers obtained differ slightly from those published by Kane et al. for adult LDL, but the differences are not likely to be significant as the molecular weight calculations are sensitive to the measurement errors introduced by the easy deformability of the 3% gels.

The size determined for neonatal IDL by GGE is comparable to that found for adult IDL (300 Å; T. Forte, unpublished observation) but show some variability between pooled and individual samples. Neonatal LDL size distributions, as determined by GGE, are noteworthy for their constancy when both pooled samples and individuals are analyzed. The LDL (d = 1.019 to 1.063 g/ml) give well-defined, reproducible peaks at approximately 255 Å (major peak) and 240 Å (minor peak). Although the size of the cord blood LDL particles falls within the range of the particle sizes found in the adult (230 to 280 Å), the size and distribution of the sizes are much more homogeneous compared to those evident in the adult LDL. That is, while the LDL from cord blood consistently shows the presence of two peaks with diameters of 255 and 240 Å, the adult LDL shows no consistent pattern, with a variety of particles appearing in a wide range of sizes. Examination of several adult LDL fractions by GGE reveals the presence of several distinct bands of varying size within an individual's LDL. This size heterogeneity occurs in addition to the density heterogeneity reported by Shen et al. LDL fractions obtained by the method of Shen et al. when run on gradient gels reveal the presence of several different size particles within the same density region.

The origin of the observed size homogeneity of neonatal LDL is obscure. One hypothesis is that the turnover of the neonatal LDL is so rapid that the particles do not undergo the exchanges and alterations of the components that probably produce the heterogeneity observed in the adult. That the LDL of the neonate does turn over rapidly has been inferred by Parker et al. who have sought to link the fetal adrenal steroidogenic activity with the level of LDL in the fetus. They have proposed and presented data to show that the levels of LDL and DS (dihydroepiandrosterone sulfate), an indicator of fetal adrenal steroidogenesis, are inversely related. The fetal adrenal gland has been shown to preferentially use LDL as a source of cholesterol for DS biosynthesis and, to that end, has been shown to possess the greatest concentration of LDL receptors of any organ tested. If we assume that only 50% of the cholesterol needs of the fetal adrenal gland are met by LDL cholesterol, which is a conservative estimate (see Carr et al.), this would result in the turnover of neonatal LDL exceeding 200% of the plasma pool per day, far in excess of the turnover rate of adult LDL, approximately 44%. In total, the evidence points to a significant, if not determining, role for the fetal adrenal gland in the level and turnover of fetal LDL, and this may in turn determine the relative homogeneity of the LDL species observed. It is of interest to note that after birth, while the lipoprotein pattern is
evolving toward that of the adult, the neonatal adrenal gland undergoes a profound change in morphology with the loss of a fetal zone of tissue found only in the developing adrenal gland.14

In summary, the IDL and LDL of neonates are significantly different in composition, size distribution, concentration, and, most probably, metabolism when compared to adult IDL and LDL. The significance of these differences is unclear but may point to differences in the metabolism of IDL and LDL which, when combined with the data derived from the study of adult IDL and LDL, may allow a better insight into the metabolism of lipoproteins and its control.

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Neonatal umbilical cord blood lipoproteins. Isolation and characterization of intermediate density and low density lipoproteins.

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