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Very low-density lipoprotein in the cord blood of preterm neonates

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Abstract

Human fetuses have markedly low levels of serum lipids and a unique lipoprotein profile with respect to quality, with low-density lipoprotein (LDL)–like particle as the dominant cholesterol carrier. However, little is known about triglyceride (TG) distribution. In addition, lipid metabolism is important in lung development, with indications that TG from very low-density lipoprotein (VLDL) is essential for surfactant synthesis. We investigated TG distribution in preterm neonate cord blood and the relationship of VLDL-TG levels with respiratory distress syndrome (RDS). The study included 103 appropriate-for-gestational-age neonates (61 males). We performed serum lipoprotein analyses in cord blood by high-performance liquid chromatography with gel permeation columns. Term neonates had low cord blood TG concentrations distributed equally to the LDL and VLDL fractions. However, preterm neonates had even lower TG concentrations, with VLDL as the dominant carrier. The LDL-TG and high-density lipoprotein–TG concentrations in cord blood increased gradually with gestational age, but cord blood VLDL-TG concentrations increased dramatically from 32 to 34 weeks of gestational age. Neonates with RDS exhibited no RDS-specific lipoprotein profile; however, most were born before the timing of the dramatic VLDL-TG increase. Our results suggest that 34 weeks of gestation is a critical period for TG metabolism, indicating the need for evaluation of the lipid nutritional state in preterm neonates.

1. Introduction

Human fetuses have markedly low levels of serum lipids, although pregnant women have hypercholesterolemia and hypertriglyceridemia [1]. In addition, human fetuses have a unique lipoprotein profile with respect to its quality. In cord blood, low-density lipoprotein (LDL) is rich in triglycerides (TG) compared with adult blood; and in the high-density lipoprotein (HDL) fraction, apolipoprotein E–rich large particles are dominant [2-4]. In term neonates, gestational age appears to markedly influence the LDL profile, but not the HDL profile, in cord blood [3,4]. Very low-density lipoprotein (VLDL) is a large, TG-rich particle synthesized in the liver. In cord blood, VLDL is reported to be TG poor compared with adult serum [2]. However, little is known about TG distribution in cord blood and its relationship with gestational age.

Lipid metabolism is important in fetal development, including lung maturation [5]. Cord lipid concentrations have been analyzed in human neonates with respiratory distress syndrome (RDS) [6,7]. Gunes et al [7] reported that total cholesterol (TC), HDL, and LDL cholesterol levels were lower in infants with RDS and in their mothers. Lane et al [6], on the other hand, demonstrated elevated TC and TG levels in larger RDS infants with birth weight of 2000 to 2499 g and impaired lipid transport across the placenta in smaller RDS infants. However, the VLDL profile in neonates with RDS has never been investigated.

Recently, high-performance liquid chromatography (HPLC) with gel permeation columns was established for classifying and quantifying lipoproteins on the basis of differences in particle size [8]. This method can provide

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detailed information on cholesterol and TG levels in lipoprotein fractions simultaneously. It may be useful in studies of preterm neonates because only a small serum sample (<10 μL) is necessary for analysis.

In the current study, this HPLC method was used to examine the distribution of TG in cord blood, especially the relationship of VLDL-TG levels with gestational age. The lipoprotein profile in preterm neonates in association with RDS was also examined.

2. Subjects and methods

2.1. Subjects

From September 2004 to March 2005, 103 appropriate-for-gestational-age neonates (61 males, 42 females) who were born by vaginal delivery or cesarean delivery in the maternity ward of Nihon University were included consecutively in the study; 67 were term infants and 36 were preterm infants. Of the preterm infants, 8 had RDS and 28 did not. Mothers with preeclampsia or maternal diabetes were excluded, and no mothers were administered steroids during gestation.

2.2. Methods

At birth, the umbilicus was double clamped; and cord blood was sampled from the umbilical vein. Total cholesterol and TG concentrations were measured by enzymatic methods. Serum lipoprotein analyses were performed using HPLC with gel permeation columns (LipoSEARCH; Skylight-Biotec, Akita, Japan), which measured the cholesterol and TG concentration in each lipoprotein fraction [8,9].

The diagnosis of RDS was made on the basis of the clinical, radiographic, and/or pathologic criteria, as described previously [10]. None of the neonates had been treated prophylactically with surfactant. Informed consent was obtained from all parents, and the study was approved by the University Ethics Committee (Nihon University, Itabashi Hospital).

2.3. Statistical analysis

All statistical analyses were conducted using STATVIEW (version 4.5; Abacus Concepts, Berkeley, CA). Data are reported as mean ± standard error. Differences in measured parameters between term neonates and preterm neonates and between preterm neonates with RDS and those without RDS were analyzed using Mann-Whitney *U* tests. *P* values less than .05 were considered significant.

3. Results

3.1. Characteristics of the subjects

Preterm neonates had higher LDL cholesterol and VLDL cholesterol concentrations than term neonates. On the other hand, the LDL-TG and VLDL-TG concentrations were significantly lower in preterm neonates than in term neonates. The HDL cholesterol and HDL-TG concentrations did not differ between term and preterm neonates (Table 1).

3.2. Relationship between gestational age and TG distribution

The LDL-TG ($r = 0.317$, $P = .0016$), HDL-TG ($r = 0.220$, $P = .0315$), and VLDL-TG ($r = 0.328$, $P = .0011$) concentrations exhibited positive linear relationships, respectively, with gestational age. Fig. 1 shows that LDL-TG and HDL-TG increased gradually with gestational age. On the other hand, cord blood VLDL-TG distribution exhibited a dramatic change from 32 to 34 weeks of gestational age. Neonates born before 33 weeks of gestational age had markedly low TG concentrations in cord blood (HDL-TG, 2.7 ± 0.3 mg/dL; LDL-TG, 6.2 ± 0.7 mg/dL; and VLDL-TG, 3.0 ± 0.3 mg/dL).

Table 1
Characteristics of the study population

	Term infants	Preterm infants	*P* value[a]	Preterm infant		*P* value[b]
	n = 67	n = 36		RDS n =8	Non-RDS n =28	
Gestational age (wk)	38.7 ± 0.2	33.1 ± 0.4		30.5 ± 0.9	33.8 ± 0.4	.0036
Birth weight (g)	2966.6 ± 60.4	1888.9 ± 64.3		1648.5 ± 157.1	1957.6 ± 65.4	.0834
Male-female	38:29	23:13		5:3	18:10	
TC (mg/dL)	64.9 ± 2.0	72.1 ± 3.5	.0511	77.0 ± 5.3	70.6 ± 4.3	.3042
HDL cholesterol (mg/dL)	36.6 ± 1.3	36.9 ± 1.9	.5943	39.2 ± 4.0	36.3 ± 2.2	.6480
LDL cholesterol (mg/dL)	20.7 ± 0.8	25.4 ± 1.7	.0143	29.0 ± 2.1	24.3 ± 2.1	.1378
VLDL cholesterol (mg/dL)	7.6 ± 0.5	9.7 ± 0.8	.0201	8.8 ± 1.6	10.0 ± 0.9	.5177
TG (mg/dL)	26.3 ± 2.1	23.5 ± 3.1	.0511	15.5 ± 3.5	25.8 ± 3.8	.0650
HDL-TG (mg/dL)	4.0 ± 0.3	4.0 ± 0.3	.7582	3.4 ± 0.7	4.2 ± 0.4	.4133
LDL-TG (mg/dL)	10.3 ± 0.5	4.3 ± 0.7	.0380	6.1 ± 0.8	9.5 ± 0.8	.0347
VLDL-TG (mg/dL)	11.9 ± 1.6	10.7 ± 2.4	.0227	6.0 ± 2.3	12.0 ± 3.0	.2536

Mean ± SE.

Mann-Whitney *U* test:

[a] Term vs preterm.

[b] RDS vs non-RDS.

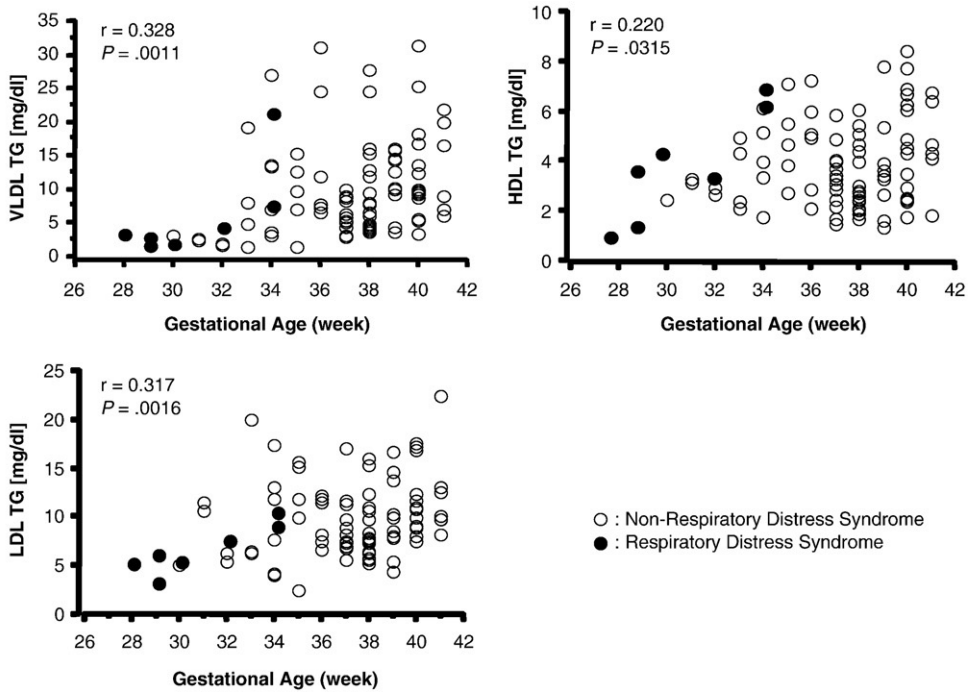

Fig. 1. Relationship between TG distribution and gestational age. Open circles represent the subjects without RDS, and closed circles represent the subjects with RDS. The VLDL-TG levels drastically increased at 32 to 34 gestational weeks, and the LDL-TG and HDL-TG levels gradually increased with gestational age. There seemed to be no difference in levels of TG in each lipoprotein fraction among subjects with or without RDS.

3.3. Lipoprotein profile in neonates with RDS

The lipoprotein profile was investigated in preterm neonates with or without RDS. We diagnosed 8 neonates as having RDS at gestational ages ranging from 28 to 34 weeks, and this group had lower LDL-TG concentrations. However, compared with gestational-age–matched preterm neonates without RDS (28-34 weeks), neonates with RDS exhibited no difference in lipoprotein profiles (data not shown).

4. Discussion

The present study demonstrated that term neonates had low cord blood TG concentrations, which were equivalently distributed to the LDL and VLDL fractions. In preterm neonates, the TG concentration was even lower, with VLDL as the dominant carrier. The LDL-TG and HDL-TG concentrations in cord blood increased gradually with gestational age. On the other hand, cord blood VLDL-TG concentrations exhibited a drastic change from 32 to 34 weeks of gestational age. Neonates with RDS demonstrated no RDS-specific lipoprotein profile; however, most of them were born before the timing of the dramatic increase in VLDL-TG.

The characteristic distribution of TG in term neonates, especially the relatively high LDL-TG concentration, may partly be explained by a reduction in hepatic lipase activity [4]. However, lipase activity, even including lipoprotein lipase (LPL), cannot fully explain the mechanism of TG distribution because TG concentrations were markedly low. Another possible mechanism is low lipid transfer across the placenta and low lipogenesis in the liver. Placental lipid transfer has been investigated in many studies. Maternal cholesterol can cross the placenta [11], and cholesterol concentrations in maternal serum affect concentrations in neonates [1]. However, this influence appears to be limited; fetuses can synthesize enough cholesterol when mothers have low circulating cholesterol [11]. On the other hand, essential fatty acids, which cannot be formed de novo by mammalian cells, must ultimately be derived from the mother by placental transfer [12]. Because TG is not transported intact, in the fetomaternal unit, free fatty acids originating in the maternal circulation are the major source of fatty acids for transport across the placenta [13]. Therefore, maternal LPL must be active to facilitate placental uptake of free fatty acids from circulating TG. Preterm infants have a significantly reduced essential fatty acid status compared with full-term neonates [14], suggesting that placental fatty acid transfer depends on gestational age.

Previous studies in human neonates have suggested an association between RDS and impairment of lipid metabolism. In addition, a study in rat fetal alveolar type II cells demonstrated that VLDL, together with LPL, stimulates surfactant synthesis [5]. Furthermore, fatty acid transferred via placenta is an important component of fetal surfactant because maternal loading of VLDL also stimulates surfactant synthesis [15]. In the present study, we found no difference

in lipoprotein profiles between neonates with RDS and preterm neonates (28-32 weeks) without RDS. However, none of the neonates born after 34 weeks of gestation developed RDS. Therefore, we speculate that the increase in VLDL-TG accelerates surfactant synthesis and protects against the development of RDS. In the lungs of rat fetuses, LPL activity was high (70%-80% of adult activity), contrary to levels in the heart (trace) in the late gestational period, indicating that the lung may be a major organ to remove VLDL-TG from circulation [16]. Lane et al [6] reported that cord blood lipid profile in infants with RDS depended on their birth weight. Larger infants with RDS (2000-2499 g) had higher cord blood TG and TC levels, whereas, in smaller infants with RDS (1000-1999 g), lipid transfer across the placenta is suggested to be impaired because of a reduction in cord serum arachidonic acid concentrations. Another study should be done to clarify the VLDL profile in neonates with RDS with respect to quality and serum concentration.

In conclusion, the cord blood VLDL profile depends on gestational age; and preterm neonates have markedly low VLDL-TG concentrations. When preterm neonates are not given enough fatty acid support after birth, their growth and development may be impaired. The growth of dendritic arbors and peak formation of synapses are reported to extend from about 34 weeks of gestation through 24 months after birth [17]. In the present study, we found that 34 weeks of gestation was a critical period for TG metabolism in neonates because cord blood VLDL-TG increased drastically from 32 to 34 weeks and the development of RDS seemed to be inhibited after this period. Our results indicate that further investigations of preterm neonates in this context would be useful.

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