Lipoprotein Heterogeneity at Birth: Influence of Gestational Age and Race on Lipoprotein Subclasses and Lp (a) Lipoprotein

Peter O. Kwiterovich Jr, MD; Donna G. Virgil, MS; Elizabeth S. Garrett, PhD; James Otvos, PhD; Rita Driggers, MD; Karin Blakemore, MD; Steven L. Cockrill, PhD; Ronald D. Macfarlane, PhD

Objective: To determine the influence of gestational age, gender, and race, on lipoprotein heterogeneity at birth.

Design: Prospective study of representative sample of infants.

Setting: The Johns Hopkins Hospital.

Participants: 163 infants (70 White and 93 Black) >28 weeks gestational age.

Intervention: None.

Main Outcome Measures: Lipids, lipoprotein subclasses, apolipoproteins, Lp (a) lipoprotein.

Results: The number of low-density lipoprotein (LDL) particles, large LDL subclass, and LDL cholesterol level, were all significantly higher in the younger infants. The large high-density lipoprotein (HDL) subclass was significantly higher, while the small HDL subclass was significantly lower in the younger infants. Female infants had a greater HDL size than did males (P=.03). There were no differences between the age groups for HDL cholesterol, very low-density lipoprotein subclasses, or levels of triglycerides, or apolipoproteins B and A-I. White infants had a notably higher mean (SD) level (nmol/L) of total LDL particles (476 [251]), compared to the Black infants (372 [177]) (P=.009). The Black infants had a significantly (P=.02) higher mean (SD) Lp (a) lipoprotein level (mg/dL), compared to the White infants, 2.8 (3.2) vs 1.7 (2.4). Black small-for-gestational age infants had significantly higher levels of very low and intermediate density lipoproteins and apolipoprotein B, compared to appropriate-for-gestational age infants.

Conclusions: Gestational age has a significant effect on both LDL and HDL subclasses. Differences in LDL particle number and Lp (a) between White and Black infants mirror those seen later in life. (*Ethn Dis*. 2004;14:351–359.)

Key Words: Apolipoproteins, HDL Subclasses, LDL Subclasses, Lp (a) Lipoprotein, Small-for-gestational Age, VLDL Subclasses

The Johns Hopkins Medication Institutions, Baltimore, Maryland (POK, DGV, ESG, RD, KB); LipoScience, Inc., Raleigh, North Carolina (JO); The Laboratory for Cardiovascular Chemistry at Texas A & M, College Station, Texas (SLC, RD).

Address correspondence and reprint requests to Peter O. Kwiterovich; 550 North Broadway; Suite 308; Baltimore, MD 21205; 410-614-0972; 410-955-1276 (fax); pkwitero@jhmi.edu

Introduction

The plasma levels of the major lipoproteins, very low density lipoproteins (VLDL), low density lipoproteins (LDL), and high density lipoproteins (HDL), are often expressed in terms of their lipid content. This has clinical utility, since elevated levels of LDL cholesterol and of VLDL triglycerides, and low levels of HDL cholesterol, predict the development of coronary artery disease (CAD).[1] Very low density lipoproteins (VLDL), LDL, and HDL, however, are each a heterogeneous group of molecules that vary in size, density, content of their core cholesterol esters and triglycerides, and in the apolipoproteins on their surfaces.[2–4] Methods that identify and measure these lipoprotein subclasses include analytical ultracentrifugation,[2] density gradient ultracentrifugation (DGU),[2] gradient gel electrophoresis (GGE),[3,4] and nuclear magnetic resonance (NMR) spectroscopy.[4] As a result, greater insight has been obtained about the relationship of these subclasses with CAD. For example, larger VLDL, intermediate density lipoprotein (IDL), and smaller LDL, predict an increased risk of CAD, while larger HDL is related to a decreased risk for CAD.[1]

The concentrations of plasma lipids and lipoproteins at birth are considerably lower than in children and adults.[5] Nevertheless, certain metabolic inherited disorders of lipoprotein metabolism, such as familial hypercholesterolemia (FH),[5,6] hyperalphalipoproteinemia,[7,8] and hypobetalipoproteinemia,[9] are expressed in infants from families affected by these conditions. Familial hypercholesterolemia (FH) is strongly associated with premature CAD, while the other 2 disorders appear to be related to a decreased risk of CAD. In the general population, the ability of birth levels of LDL and HDL cholesterol to predict levels later in childhood, or in adulthood, has not been thoroughly determined.[8,10–13]

The heterogeneity of plasma lipoproteins at birth has also been examined, employing primarily DGU and GGE.[14–22] These studies have been labor intensive, and generally have investigated groups of infants too small to permit an examination of the influence of gestational age, race, and gender on the concentration and size of lipoprotein subclasses. Here we examine, in detail, lipoprotein heterogeneity at birth in a group of 163 White and Black infants using NMR spectroscopy, which provides simultaneous assessment of a number of VLDL, LDL, and HDL subclasses on a small amount of plasma. Small-for-gestational-age (SGA) infants were also included in our study, because SGA infants have been linked to adults who develop CAD associated with elevated LDL, low HDL, higher triglycerides, hypertension, insulin resistance, and diabetes.[23]

Methods

Patient Population

We studied 163 White and Black infants born at the Johns Hopkins Hos-

Here we examine, in detail, lipoprotein heterogeneity at birth in a group of 163 White and Black infants using NMR spectroscopy, which provides simultaneous assessment of a number of VLDL, LDL, and HDL subclasses on a small amount of plasma.

pital between January 3, 2000 and September 27, 2000. These infants constituted a representative sample of about 20% of all infants born during this period. Inclusion criteria for the study included the availability of gestational age (determined by date of last menstrual period, corrected by ultrasound, if necessary) and birth weight. Apgar scores (index of condition of infants at birth) and placental weight were also recorded. Exclusions included emergent births and infants less than 28 weeks gestation. The infants were studied anonymously, using cord blood that was routinely obtained after birth. Therefore, maternal informed consent was not obtained. The Joint Committee on Clinical Investigation at Johns Hopkins approved the study.

Definition of Small-for-Gestational Age (SGA) and Appropriate-for-Gestational Age (AGA) Infants

A SGA infant was defined as one whose weight for gestational age was = or < the 10th percentile, using the fetal growth curves from the Johns Hopkins Obstetrical Service, which are population-based estimates of normal fetal size across gestational age, specific to White and Black infants. An AGA infant was defined as one whose weight for gestational age was > the 10th percentile, using the same fetal growth curves. A separate growth curve was used for the twins (total infants = 9) in the data set, to assess whether their weight for gestational age was below the 10th percentile.[24]

Blood Sampling

Umbilical cord blood (10 mL) was collected in a tube containing liquid EDTA. The blood was refrigerated until the plasma was separated from the red blood cells.

Plasma Lipid, Lipoprotein Cholesterol, and Apolipoprotein Measurement

An aliquot of fresh plasma was used for the measurement of levels of total cholesterol, total triglycerides, and direct HDL cholesterol, as described elsewhere.[25] The CV for cholesterol measurement was <3%, and <5% for triglycerides, during the course of the study. Low density lipoprotein (LDL) cholesterol was estimated using the Friedewald formula, which has been verified for use with cord blood by preparative ultracentrifugation.[5,26,27] Apolipoprotein A-1 was determined using immunoturbidometric methods[28] with a CV of 5%. Apolipoprotein B was measured using an immunoturbidometric method that was modified from the manufacturers' (Sigma) instructions to provide a more sensitive measurement of apoB using a control pool with a low apoB value (42 mg/dL). Lp (a) lipoprotein levels were determined using an ELISA method, as previously described,[29] and had a CV of 8%.

Nuclear Magnetic Resonance Spectroscopy

The concentrations of 6 VLDL, 1 IDL, 3 LDL, and 5 HDL subclasses were determined by NMR spectroscopy, in duplicate, on fresh plasma samples (0.25 mL) at LipoScience, Inc. (Raleigh, NC), as previously described in detail.[4,30] The total LDL particle concentration (nmol/L) is the sum of the concentrations of LDL subclasses (including IDL). Very low density lipoprotein (VLDL) subclass concentrations are expressed in mg/dL of triglycerides, and those of IDL, LDL, and HDL subclasses are in mg/dL cholesterol. The NMR method also gave the average size (nm) of VLDL, LDL and HDL particles.[4,30]

Since the NMR signals of lipoprotein subclasses from cord blood are not noticeably different from those of adults, analyses of cord blood samples could be conducted successfully using the standard adult analysis model, modified only by use of a background protein signal derived from the d>1.21 g/mL bottom fraction of cord blood, rather than adult plasma. To account for the presence in some cord blood samples of a larger-than-usual large HDL subclass (H5), the reference signal of the H5 subclass was digitally shifted to represent what would be expected for a particle with a diameter approximately 1 nm larger than usual. Using this modified NMR analysis model, good agreement was observed with LDL and HDL subclass distributions measured by gradient gel electrophoresis.

To simplify analysis, the 15 measured subclasses were grouped into the following 9 subclass categories: large VLDL (V6V5) (60 nm–200 nm), intermediate VLDL (V4V3) (35 nm–60 nm), small VLDL (V2V1) (27 nm–35 nm), IDL (23 nm–27 nm), large LDL (L3) (21.3 nm–23 nm), intermediate LDL (L2) (19.8 nm–21.2 nm), small LDL (L1) (18.3 nm–19.7 nm), large HDL (H5H4H3) (8.2 nm–14 nm), and small HDL (H2H1) (7.3 nm–8.2 nm). Levels of the largest HDL subclass (H5, 10 nm–14 nm) were also examined. Average VLDL, LDL, and HDL particle sizes (nm) were determined by weighting the relative NMR signal contribution from each subclass by its mean diameter.

Statistical Methods

The relationships between lipids, lipoprotein cholesterols, apolipoproteins,

Table 1. Characteristics of the study population

	White	Black
Males	31	47
Females	39	46
Gestational age (weeks)	38.0 (3.2)	38.9 (2.3)
AGA*	38.3 (2.9)	39.0 (2.2)
SGA†	36.0 (4.5)	38.0 (2.5)
Birth weight (grams)	3184 (890)	3135 (545)
AGA*	3356 (762)	3245 (493)
SGA†	2024 (862)	2478 (430)

Values given are Mean (SD). AGA=appropriate for gestational age; SGA=small for gestational age.
* AGA, total 140 (White *N*=61, Black *N*=79).
† SGA, total 23 (White *N*=9, Black *N*=14).

lipoprotein subclasses and lipoprotein sizes, and gestational age were evaluated, treating gestational age as a categorical variable with 3 categories (<32 weeks, 32 weeks to 35.5 weeks, and 36 weeks and greater). *P* values comparing gestational age groups were estimated, using the Kruskal-Wallis test, due to the small size of 2 of the groups (*N*=7 in the <32 weeks group, and *N*=12 in the 32 weeks to <36 weeks group), and the skewness of the data. There was no difference between the results of the analyses using log transformed or untransformed data; therefore, the results are presented using untransformed data. ANOVA and linear regression (treating gestational age as continuous, and transforming outcome variables to adhere to assumptions of linear regression) were also performed, and the resulting *P* values were very similar to those found using the Kruskal-Wallis test. To evaluate differences in these lipid-related variables between the White and Black, and male and female, infants, linear regression was used, adjusting for categorical gestational age. Statistical significance was based on 2-sided Type I error (alpha) of 0.05 level.

Table 2. Lipids, lipoproteins, apolipoproteins, and lipoprotein subclasses in three groups of infants with different gestational ages

	Gestational Age (Weeks)			
Variable	<32 (*N*=7)	32 to 35.9 (*N*=12)	36 (*N*=144)	*P* Value
Total C*	91	70	60	.001
LDL particle number†	570.5	485.1	369.2	.007
LDL C*	51	27	26	.001
Large LDL C*	38.9	28.25	10.45	<.001
Small LDL C*	15.9	8.55	10.10	.77
LDL size‡	20.8	21.3	20.6	.045
ApoB*	18.3	17.80	16.96	.61
Large HDL C*	19.6	26.25	14.55	.01
Small HDL C*	4.5	5.3	8.9	.001
HDL C*	30	29	26	.33
ApoA-I*	68	74.5	76	.28
Medium VLDL TG*	3.6	2.95	3.05	.94
Small VLDL TG*	1.3	4.35	0.45	.15
VLDL TG*	6.1	8.25	5.15	.42
Total TG*	30.0	34.5	34.0	.52
Lp (a)*	1.0	1.5	1.0	.52

Note: The relationships between dependent variables and gestational age were evaluated treating gestational age as a categorical variable with 3 categories (<32 weeks, 32 weeks to 35.9 weeks, and 36 weeks and greater). *P* values comparing across gestational age groups were estimated using the Kruskal-Wallis test, due to the small size of 2 of the groups and the skewness of the data (see Methods). Total C=total cholesterol; LDL C=low density lipoprotein cholesterol; apoB=apolipoprotein B; HDL C=high density lipoprotein cholesterol; apoA-I=apolipoprotein A-I; VLDL=very low density lipoproteins; TG=triglycerides; Lp (a)=lipoprotein (a).
* Median (mg/dL).
† Median nmol/L.
‡ Median nm.

RESULTS

There were 70 White infants and 93 Black infants; 31 White males, 39 White females; 47 Black males and 46 Black females (Table 1). There were 23 SGA infants: 9 Whites (3 males, 6 females), and 14 Blacks (6 males, 8 females) (Table 1). No significant differences for gestational age were observed between the White and Black infants, or the male and female infants. The relationship between gestational age and birth weight for the entire study population (r=0.71), and for the SGA infants (r=0.93), appeared to be approximately linear. Similar relationships were found for the 4 sex and race groups.

Gestational Age and Birth Weight and Lipid and Lipoprotein Levels

Total cholesterol level, the total number of LDL particles, and LDL cholesterol level, were all significantly higher in the younger infants, and decreased with gestational age (Table 2). The size of LDL changed significantly with gestational age, manifesting a pattern of increased size, followed by a decrease in size after 36 weeks. The apoB levels decreased slightly with age, but this change was not statistically significant. These changes in LDL primarily resided in the large LDL subclass, which decreased significantly and notably with gestational age. There was no significant change in the levels of the small, dense LDL subclass (Table 2).

The HDL cholesterol and apoA-I

Table 3. Plasma levels of lipids, lipoprotein cholesterols and apolipoprotein levels in cord blood

Groups	TC	TG	HDL-C	LDL-C	ApoB	ApoA-I	Lp (a)
Total	64.6 (19.3)	38.3 (14.2)	27.8 (9.2)	29.2 (14.2)	17.8 (6.1)	77.1 (14.5)	2.4 (3.1)
White	67.6 (20.4)	39.3 (15.1)	27.6 (8.2)	32.2 (15.8)	17.2 (4.1)	75.2 (11.9)	1.7 (2.4)§
AGA	66.8 19.2)	38.6 (13.5)	27.5 (7.4)	31.6 (15.6)†	17.1 (4.0)	75.0 (10.7)	1.8 (2.5)‖
SGA	73.4 (28.1)	44.1 (23.5)	28.9 (13.1)	35.9 (17.7)	18.4 (5.4)	77.0 (18.8)	1.0 (0.9)
Black	62.5 (18.1)	38.2 (15.1)	27.9 (9.9)	27.0 (12.6)	18.3 (7.3)	78.6 (16.1)	2.8 (3.2)§
AGA	61.5 (16.5)	35.3 (10.5)*	28.3 (10.0)	26.2 (10.4)†	17.2 (4.3)‡	78.5 (16.8)	2.9 (3.3)‖
SGA	67.1 (25.9)	50.1 (21.3)*	25.4 (9.3)	31.6 (21.1)	24.3 (14.9)‡	79.2 (13.1)	2.9 (3.3)

Values are given as mean (SD) in mg/dL. TC=total cholesterol; TG=total triglycerides; HDL-C=high density lipoprotein cholesterol; LDL-C=low density lipoprotein cholesterol; ApoB=apolipoprotein B; ApoA-I=apolipoprotein A-I; Lp (a)=lipoprotein (a); AGA=appropriate-for-gestational age; SGA=small-for-gestational age.

* $P<.001$; † $P=.04$; ‡ $P=.001$; § $P=.02$; ‖ $P=.05$. All other comparisons were not statistically significant, after correcting for gestational age. Linear regression was used to evaluate differences in these variables between the White and Black infants, adjusting for categorical gestational age (see Methods).

levels did not change significantly with gestational age (Table 2). However, there was a significant re-distribution of the cholesterol within the HDL subclass levels. The levels of the large HDL subclass initially increased, then fell significantly, so that the older infants had the lowest levels of large HDL. In contrast, there was a step-wise and significant increase in the levels of the small HDL subclass (Table 2).

The plasma levels of total triglycerides, VLDL triglycerides, intermediate VLDL subclass, and small VLDL subclass, did not fall significantly with gestational age (Table 2). The large VLDL subclass was undetectable in more than 50% of the infants. The strongest correlation between the total triglycerides level and VLDL subclasses occurred with intermediate VLDL ($r=0.72$, $P<.0001$), although large VLDL ($r=0.29$, $P<.0001$) and small VLDL ($r=0.33$, $P<.0001$) were also significant.

The inverse relationships between gestational age and the plasma levels of total and LDL cholesterol, total number of LDL particles, large LDL, and large HDL described above for the entire study population were generally true for the White and Black, and male and female, infants, although there were some differences. The Black infants did not manifest as much of a change in the total number of LDL particles with age as did the White infants. The female infants had similar inverse relationships for all these parameters, while the change in the males was primarily manifested in the number of LDL particles.

Comparison of Plasma Levels of Lipids, Lipoproteins, Apolipoproteins and Lipoprotein Subclasses in the White Infants vs the Black Infants, in the Males Versus the Females, and in the SGA Versus the AGA Infants

White Infants vs the Black Infants

The White infants had a significantly ($P=.03$) higher mean (SD) level (mg/dL) of LDL-C (32 [16]) than did the Black infants (27 [13]), but this difference did not persist after age adjustment ($P=.10$) (Table 3). However, the White infants had a significantly ($P=.002$) higher mean (SD) level (nmol/L) of total LDL particles (476 [251]) than did the Black infants (372 [177]) (Table 4), a difference that did persist after age correction ($P=.009$). Both White and Black infants in the youngest gestational age group had the highest levels of total number of LDL particles (Figure 1). The mean White-Black difference was also reflected in notably higher values for the 95th percentiles for total number of LDL particles (nmol/L) in the White males (780) and White females (743), compared to the Black males (553) and Black females (652), respectively (Figure 1). Correction for gestational age was, therefore, critical, and all subsequent *P* values presented are corrected for age. We did not detect any significant differences ($P=.27$) in the average size of the LDL particles between the 2 groups (Table 4), indicating that the increased number of LDL particles in the Whites had a similar size and composition as those in the Blacks. There was no significant difference between the 2 groups for total apoB levels ($P=.31$) (Table 3). Therefore, measurement of the total number of LDL particles by NMR provided more information on White-Black differences at birth than did measurement of either LDL-C or apoB.

There was no significant mean (1 SD) difference ($P=.74$) for the HDL cholesterol levels between the White and Black infants (Table 3). The mean (1 SD) levels for apoA-I (mg/dL) was higher in the Black infants (78.6 [16.2]), compared to the White infants (75.0 [11.9]), but this did not reach statistical significance ($P=.24$) (Table 3). The average size of the HDL particles was larger in the Whites (9.54) than in the Blacks (9.32), but this difference did not reach statistical significance ($P=.24$) (Table 4). The Whites did not have higher levels of the larger HDL particles (H5H4H3), compared to the Blacks (Table 4).

There were no significant differences between White and Black infants for total and VLDL triglycerides, VLDL subfractions, or IDL cholesterol (Table 5).

The Black infants had a significantly higher mean (1 SD) Lp (a) lipoprotein

Table 4. Plasma levels and particle sizes of LDL subclasses and HDL subclasses in umbilical cord blood

	LDL Subclasses					HDL Subclasses		
	Cholesterol, mg/dL			LDL Size (nm)	LDL Particle Number (nmol/L)	Cholesterol, mg/dL		HDL Size (nm)
	Large	Intermediate	Small			Large	Small	
Total	15.6 (13.7)	4.4 (6.2)	13.7 (14.4)	20.7 (0.8)	416.2 (217.2)	17.29 (8.91)	8.56 (2.45)	9.41 (0.75)
White	17.2 (14.5)	5.0 (6.8)	16.3 (16.1)	20.6 (0.8)	475.8 (251.0)*	17.60 (8.02)	8.10 (2.75)	9.54 (0.78)
AGA	16.1 (13.7)	4.9 (6.7)	16.2 (14.2)	20.5 (0.7)	465.9 (217.1)†	17.1 (7.4)	8.32 (2.57)	7.48 (0.72)
SGA	24.2 (17.9)	5.2 (8.3)	16.5 (26.8)	21.0 (0.9)	542.5 (429.3)	21.1 (11.4)	6.6 (3.6)	10.0 (1.1)
Black	14.4 (13.1)	4.0 (5.7)	11.9 (12.9)	20.7 (0.9)	372.9 (176.8)*	17.14 (9.61)	8.88 (2.18)	9.31 (0.72)
AGA	14.2 (13.0)	4.1 (5.7)	11.9 (13.4)	20.7 (0.9)	370.5 (178.9)†	17.39 (9.83)	8.97 (2.15)	9.32 (0.71)
SGA	15.7 (14.3)	3.6 (5.7)	10.9 (9.9)	20.7 (0.9)	376.8 (174.1)	15.71 (8.69)	8.40 (2.37)	9.30 (0.77)

Values are given as mean (SD). LDL=low-density lipoproteins; HDL=high-density lipoproteins; AGA=appropriate-for-gestational age; SGA=small-for-gestational age.
* P=.009; † P=.012. All other comparisons were not statistically significant, after correcting for gestational age. Linear regression was used to evaluate differences in these variables between the White and Black infants, adjusting for categorical gestational age (see Methods).

level (mg/dL), compared to the White infants (2.8 [3.2] vs 1.7 [2.4], respectively) (adjusted for age, P=.02) (Table 3).

Male vs Female Infants

The only significant difference between all the male and female infants was that the females had a greater average HDL size (nm), compared to the males (age adjusted P=.034).

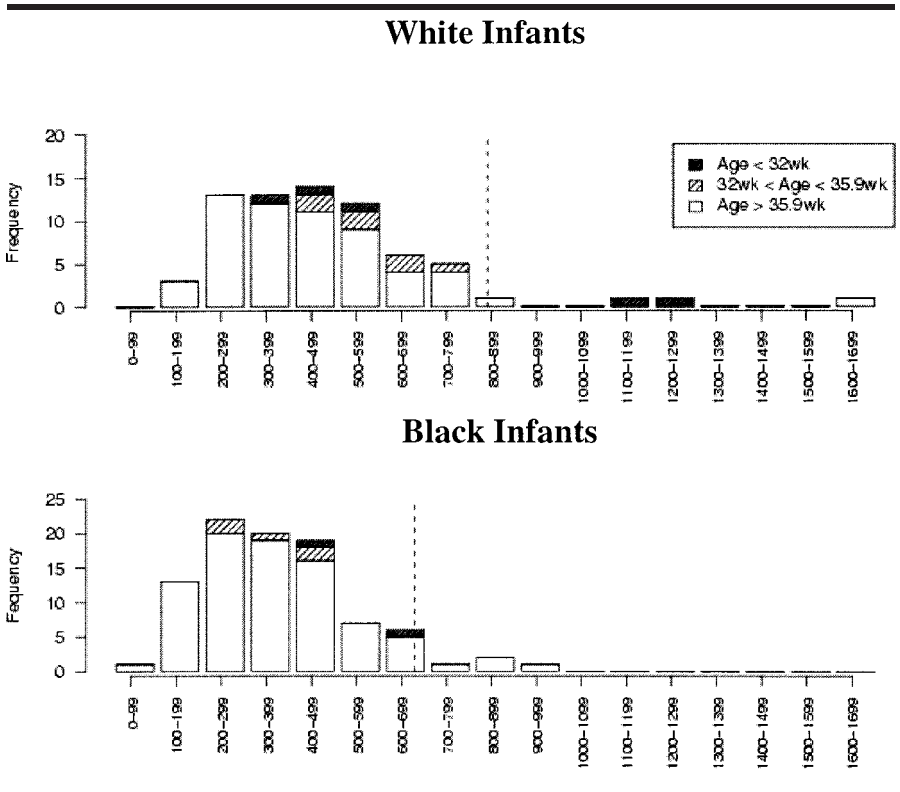

Fig 1. Frequency distributions of the LDL particle number (nmol/L) in the White infants (top panel) and the Black infants (bottom panel). The frequency of infants in each category of LDL particle number was divided by the sample size (N=70 [White infants] and 93 [Black infants]) and multiplied by 100. The dotted vertical line indicates the 95th percentiles for both groups. The infants were also subdivided into 3 age categories indicated in the key provided in the top panel

AGA vs SGA Infants

Appropriate-for-gestational age (AGA) White infants also had significantly higher levels of the total number of LDL particles, but lower Lp (a) levels, compared to the AGA Blacks (Tables 3, 4). Such differences were also apparent when the SGA White infants were compared to the Black SGA infants (Tables 3, 4), but did not reach statistical significance, most likely related to the relatively small number of SGA infants in this study.

The Black SGA infants had about 2-fold higher mean plasma levels of total VLDL (P=.006) and intermediate VLDL (.003) triglycerides, and IDL (.04) cholesterol compared to Black AGA infants (Table 5). Such differences in the triglyceride-rich lipoproteins resulted in a significantly higher mean (1 SD) total level of triglycerides (mg/dL) in the SGA Black infants (50.1 [21.3]), compared to the AGA Black infants (35.2 [10.5]) (P=.02), as well as significantly higher (.001) apoB levels (Table 3). Similar trends for higher total VLDL and intermediate VLDL triglyceride levels, and larger average VLDL size were observed when the SGA White infants were compared to the AGA White infants, but these were not statistically sig-

Table 5. Plasma levels and particle sizes of VLDL subclasses and IDL in umbilical cord blood

	VLDL Subclasses					
	TG, mg/dL					
	Large	Intermediate	Small	Total VLDL (TG, mg/dL)	VLDL Size (nm)	IDL (Cholesterol, mg/dL)
Total	0.38 (2.1)	6.1 (8.1)	2.7 (4.3)	9.2 (11.0)	35.7 (13.3)	1.6 (2.6)
White	0.5 (3.1)	6.7 (8.5)	2.9 (4.5)	10.1 (12.3)	37.2 (11.1)	1.6 (2.3)
AGA	0.2 (0.5)*	6.4 (7.6)	2.7 (4.3)	9.3 (9.1)	36.9 (11.7)	1.7 (2.3)
SGA	2.8 (8.5)*	8.3 (14.0)	4.6 (5.8)	15.8 (25.1)	39.9 (5.7)	1.4 (2.0)
Black	0.3 (0.8)	5.7 (7.7)	2.6 (4.1)	8.6 (10)	34.5 (14.7)	1.6 (2.8)
AGA	0.3 (0.7)	4.6 (6.2)†	2.4 (3.6)	7.3 (8.5)‡	33.2 (15.5)	1.4 (2.2)§
SGA	0.4 (1.1)	11.4 (12.2)†	3.8 (6.4)	15.6 (14.4)‡	41.5 (4.1)	3.0 (5.0)§

Values given are Mean (SD). VLDL=very low-density lipoproteins; IDL=intermediate low-density lipoproteins; TG=triglycerides; AGA=appropriate-for-gestational age; SGA=small-for-gestational age.

* P=.007; † P=.003; ‡ P=.006; § P=.04. All other comparisons were not statistically significant, after correcting for gestational age. Linear regression was used to evaluate differences in these variables between the White and Black infants, adjusting for categorical gestational age (see Methods).

We have expanded these observations to demonstrate that the total number of LDL particles and the average size of LDL both decrease with gestational age, and that such changes were characteristic of the entire study population.

nificant (Table 5). However, White SGA infants did have significantly higher large VLDL triglyceride levels, compared to White SGA infants (P=.007) (Table 5).

DISCUSSION

We report here a study of lipoprotein heterogeneity in a relatively large group of infants, 28 weeks of gestational age and older. Previous studies of lipoprotein heterogeneity in umbilical cord blood were performed in much smaller groups of infants, and usually addressed one major lipoprotein class at a time.[14–21] The development of NMR spectroscopy has provided a more facile method for assessing these characteristics of lipoproteins in a small amount of frozen plasma,[4,30] thus providing us with the opportunity to address more completely the influence of gestational age on lipoprotein heterogeneity, and to examine differences between White and Black, and male and female, infants. Since there is a linear relationship between the size of a lipoprotein particle and its hydrated density, there is a good correspondence between the size of lipoprotein subclasses by NMR, or GGE, and the density of the particle determined by DGU.[4,14–22] The data were collected from one hospital population, and, therefore, may not be representative of all infant populations. Further, the data are cross-sectional; however, a longitudinal assessment of the effects of gestational age on lipoprotein heterogeneity requires repeated intrauterine sampling, and is not feasible. Finally, we have not examined the effects of known modifiers of lipoprotein subclasses in newborns, including maternal factors, such as gestational diabetes, pre-eclampsia, or smoking.[23,31] A future prospective study on the influence of maternal, environmental, and genetic factors on lipoprotein heterogeneity at birth is planned.

Our finding that plasma levels of total cholesterol and LDL cholesterol declined significantly with gestational age is in agreement with most,[32–35] but not all,[36] other studies. We have expanded these observations to demonstrate that the total number of LDL particles and the average size of LDL both decrease with gestational age, and that such changes were characteristic of the entire study population. As suggested by others,[15] we found little evidence for increased LDL heterogeneity in human umbilical cord blood. The decrease in LDL levels with gestational age in the human fetus is directly related to an increase in hepatic LDL receptor activity.[34] The fetal adrenal gland, which produces large amounts of steroids, has the highest concentration of LDL receptors of any tissue studied.[37] The fetal plasma level of dehydroepiandrosterone sulfate, the major secretory product of the fetal adrenals, rises significantly between 33 weeks and 42 weeks of gestation.[33] Since LDL cholesterol is utilized as substrate for fetal adrenal steroidogenesis, Parker et al[33] suggested that the increasing rate of growth and steroid production by the fetal adrenals near term is causally related to the significant decline in the concentration of LDL cholesterol.

We also examined differences in the plasma levels of lipids, lipoprotein cholesterols, apolipoproteins, and lipoprotein heterogeneity between the White and Black infants. We found that the White infants had a notably higher total number of LDL particles, compared to the Black infants. This difference in LDL particle number was also found when comparing White and Black AGA infants, and White and Black SGA infants. However, no significant differences in LDL size were observed between these 2 groups at birth. Therefore, the smaller, dense LDL particles observed in White adolescents in Bogalusa[38] must emerge after the intrauterine period. The mean LDL cholesterol was also higher in our White infants than in our Black infants, but the difference was not

significant after adjusting for gestational age. This may explain why some,[26,39] but not all,[40] studies have reported that White infants have higher LDL cholesterol levels than Black infants. The difference between the Black/White groups for total number of LDL particles of 104 nmol/L should be reflected in a mean difference of about 5–6 mg/dL in apoB levels. The simplest explanation for the fact that we did not observe such a mean difference in apoB levels may reside in the difficulties of measuring such low levels of apoB in cord blood using an immunoturbimetric assay, while the NMR signals from lipoproteins in cord blood vs that from adults are very similar. Differences in LDL levels between White and Black infants do not appear to be explained by socioeconomic factors,[39] but are more likely due to genetic influences.[26]

In contrast to LDL, we found that Lp (a) lipoprotein was significantly higher in the Black infants than in the White infants, a difference that is also seen later in adolescents[41] and adults.[42] Rifai et al[43] were unable to detect differences in Lp (a) levels between White and Black infants at birth, perhaps related to the very low levels of Lp (a) at birth.[43–48] Lp (a) levels do not appear to be associated with birth weight, gestational age, gender or health status,[44–48] but correlate strongly with the sum of parental and fathers' Lp (a) levels, indicating that genetic factors are involved in determining infants' Lp (a) levels.[46] Vella and Calmarza[45] reported that children with a positive family history of cardiovascular heart disease had a higher mean Lp (a) level, and a higher prevalence of Lp (a) values, exceeding 5 mg/dL, compared to children with a negative family history. The ranking of Lp (a) levels at birth, highest in Indians, followed by Malays, and then Chinese, was concordant with the relative coronary mortality rates for the respective adult populations of Singapore.[47] Determining whether Lp (a) levels, particularly in Blacks, are independent predictors of CAD, is controversial.[42]

We also report here significant changes in the concentrations of the large and small HDL subclasses with gestational age; the plasma level of the large HDL particles decreased, while that of the small HDL particles increased. One possible explanation for the overall decrease in the large HDL particles with gestational age might reside in increased activity of either cholesterol ester transfer protein (CETP) with gestational age, leading to enhanced transfer of cholesterol esters from HDL to VLDL, or possibly hepatic lipase (HL), enhancing the conversion of large HDL to small HDL. Conversely, we found no significant change in this population in the plasma levels of HDL cholesterol or apoA-I with gestational age, consistent with the findings of others.[32,49] High density lipoprotein (HDL) metabolism may be different in SGA infants, but we were not able to demonstrate any differences in HDL cholesterol or HDL particles between our SGA and AGA infants. Others[50,51] have reported lower levels of HDL cholesterol in SGA infants; one study reported[50] that the large HDL particles were reduced, and CETP activity increased, while in another study,[51] both the large and small HDL particles were decreased, along with reduced activity of lecithin cholesterol acyl transferase (LCAT).

We found no significant change in total, intermediate, or small VLDL triglycerides, or in total triglycerides, with gestational age. In most of the infants, the large VLDL particles were usually undetected in the NMR spectra. In the Black group, we found that the total triglycerides and apoB levels were significantly higher in the SGA infants, compared to AGA infants, reflecting higher levels of total VLDL and intermediate VLDL triglycerides and IDL cholesterol. Higher levels of triglycerides have been reported in SGA infants.[35,50,51] Radunovic and co-workers[52] recently reported elevated apoB levels in 18 growth-restricted fetuses, compared to 23 normally grown fetuses. However, they did not measure the levels of triglycerides. Clearly, a more comprehensive study of lipids, apolipoproteins, and lipoproteins, in a larger number of SGA infants, is needed. Such infants should also be followed after birth, since Anderson et al[53] found that SGA Danish infants had raised LDL cholesterol levels in the first 4 years of life.

In summary, we found significant effects of gestational age on both LDL and HDL subclasses. The decrease in the large HDL particles, and the increase in the small HDL particles, with gestational age, occurred without a significant corresponding change in HDL cholesterol or apoA-I. White infants exhibited a significantly greater number of total LDL particles, compared to Black infants, a difference that is greater than expected, based on the differences in LDL cholesterol or apoB levels. To our knowledge, this is the first study to demonstrate that Black infants have significantly higher Lp (a) levels than do White infants. Future studies of a larger number of SGA infants should incorporate an assessment of lipoprotein heterogeneity, and how this is influenced by maternal factors.

ACKNOWLEDGMENTS

This work was supported by grants from the Thomas Wilson Foundation, Baltimore, Maryland, from the National Institutes of Health for a General Clinical Research Center, and by HL 068794.

REFERENCES

1. Kwiterovich PO Jr. The metabolic pathways of HDL, LDL, and triglycerides. A current review. *Am J Cardiol.* 2000;86(suppl 1):5–10.
2. Shen M, Krauss R, Lindgren F, Forte TM. Heterogeneity of serum low-density lipoproteins in normal human subjects. *J Lipid Res.* 1981;22:236–244.
3. Krauss R, Burke D. Identification of multiple subclasses of plasma low-density lipoproteins in normal humans. *J Lipid Res.* 1982;23:97–104.
4. Otvos J, Jeyarajah E, Bennett D, Krauss R. Development of a proton nuclear magnetic resonance spectroscopic method for determining plasma lipoprotein concentrations

and subspecies distributions from a single, rapid measurement. *Clin Chem.* 1992;38: 1632–1638.
5. Kwiterovich PO Jr, Levy RI, Fredrickson DS. Neonatal diagnosis of familial type II hyperlipoproteinemia. *Lancet.* 1973;1:118–122.
6. Vuorio AF, Turtola H, Kontula K. Neonatal diagnosis of familial hypercholesterolemia in newborns born to a parent with a molecularly defined heterozygous familial hypercholesterolemia. *Arterioscler Thromb Vasc Biol.* 1997; 17:3332–3337.
7. Glueck CJ, Gartside PM, Tsang RC, Mellies MJ, Steiner PM. Neonatal familial hyperalphalipoproteinemia. *Metabolism.* 1977;26: 469–472.
8. Tsang R, Neely K, Glueck C. Hyperalphalipoproteinemia, birth to age two years. *Pediatr Res.* 1981;15:66–69.
9. Andersen GE, Brokhattingen K, Lous P. Familial hypobetalipoproteinaemia in 9 children diagnosed as the result of cord blood screening for hypolipoproteinaemia in 10,000 Danish newborns. *Arch Dis Child.* 1979;54:691–694.
10. Andersen GE, Lous P, Friis-Hansen B. Screening for hyperlipoproteinemia in 10,000 Danish newborns. Follow-up studies in 522 children with elevated cord serum VLDL-LDL-cholesterol. *Acta Paediatr Scand.* 1979; 68:541–545.
11. Andersen G, Lous P, Friis-Hansen B. Hyperlipoproteinemia in newborn infants: a study of 1025 families. *Acta Paediatr Scand.* 1979; 68:683–690.
12. Fonnebo V, Dahl LB, Moe PJ, Ingebretsen OC. Does VLDL-LDL-cholesterol in cord serum predict future level of lipoproteins? *Acta Paediatr Scand.* 1991;80:780–785.
13. Miller N, Nestel P, Boulton T, Dwyer T, Leitch D. Cord blood high density lipoprotein concentration in 1797 births: relationship to family history of coronary disease. *J Chron Dis.* 1981;34:119–125.
14. Davis P, Forte T, Kane J, Hardman D, Krauss R, Blum C. Apolipoprotein and size heterogeneity in human umbilical cord blood low density lipoproteins. *Biochem Biophys Acta.* 1983;753:186–194.
15. Blum C, Davis P, Forte T. Elevated levels of apolipoprotein E in the high-density lipoproteins of human cord blood plasma. *J Lipid Res.* 1985;26:755–760.
16. Davis P, Forte T, Nichols A, Blum C. Umbilical cord blood lipoproteins isolation and characterization of high density lipoproteins. *Arteriosclerosis.* 1983;3:357–365.
17. Rosseneu M, Van Bievliet J, Bury J, Vinaimont N. Isolation and characterization of lipoprotein profiles in newborns by density gradient ultracentrifugation. *Pediatr Res.* 1983;17:788–794.
18. Genzel-Borovickzeny, Forte T, Austin M. High-density lipoprotein subclass distribution and human cord blood lipid levels. *Pediatr Res.* 1986;20:487–491.
19. Nichols A, Blanche P, Genzel-Boroviczeny O, Forte T, Gong E. Apolipoprotein-specific populations in high-density lipoproteins of human cord blood. *Biochem Biophys Acta.* 1991;1085:306–314.
20. Kherkeulidze P, Johansson J, Carlson L. High-density lipoprotein particle size distribution in cord blood. *Acta Paediatr Scand.* 1991;80:770–779.
21. Genzel-Boroviczeny O, D'Harlingue A, Kao L, Scott C, Forte TM. High-density lipoprotein subclass distribution in premature newborns before and after the onset of enteral feeding. *Pediatr Res.* 1988;23:543–547.
22. Kwiterovich Jr PO, Cockrill SL, Virgil DG, et al. A prominent large high-density lipoprotein at birth is enriched in apolipoprotein C-I and identifies a group of infants of lower birth weight and younger gestational age. *Circulation.* In press.
23. Godfrey KM, Barker DJP. Fetal nutrition and adult disease. *Am J Clin Nutr.* 2000; 71(suppl):1344S–1352S.
24. Min SJ, Luke B, Gillespi B, et al. Birthweight references for twins. *Am J Obstet Gyn.* 2000;182:1250–1257.
25. Shih WJ, Bachorik PS, Haga JA, Myers GL, Stein EA. Estimating the long-term effects of storage at −70 degrees C on cholesterol, triglyceride, and HDL-cholesterol measurements in stored sera. *Clin Chem.* 2000;46: 351–364.
26. Hardell LI, Carlson LA. Concentration and composition of human serum lipoproteins at birth. *Clin Chem Acta.* 1978;90:285–294.
27. Loughrey CM, Rimm E, Heiss G, Rifai N. Race and gender differences in cord blood lipoproteins. *Atherosclerosis.* 2000;148:57–65.
28. Bachorik PS, Lovejoy KL, Carroll MD, Johnson CL. Apolipoprotein B and A-I distributions in the United States, 1988–1991: results of the National Health and Nutritional Examination Survey III (NHANESIII). *Clin Chem.* 1997;43:2364–2378.
29. Weiss SR, Bachorik PS, Becker LC, Moy TF, Becker DM. Lipoprotein(a) and coronary heart disease risk factors in a racially mixed population: The Johns Hopkins Sibling Study. *Ethn Dis.* 1998;8:60–72.
30. Otvos JD. Measurement of lipoprotein subclass profiles by nuclear magnetic resonance spectroscopy. In: Rifai N, Warnick GR, Dominiczak MH, eds. *Handbook of Lipoprotein Testing.* Washington, DC: AACC Press; 2000:609–623.
31. Palinski W, Napoli C. Pathophysiological events during pregnancy influence the development of atherosclerosis in humans. *Trends Cardiovasc Med.* 1999;9:205–214.
32. Andersen GE, Friis-Hansen B. Cord serum lipid and lipoprotein-cholesterol values in normal and betamethasone-treated newborns of varying gestational age. *Acta Paediatr Scand.* 1977;66:355–360.
33. Parker CR Jr, Carr BR, Simpson ER, MacDonald PC. Decline in the concentration of low-density lipoprotein-cholesterol in human fetal plasma near term. *Metabolism.* 1983;32: 919–923.
34. Cai HJ, Xie CL, Chen Q, Chen XY, Chen YH. The relationship between hepatic low-density lipoprotein receptor activity and serum cholesterol level in the human fetus. *Hepatology.* 1991;13:852–857.
35. Diaz M, Leal C, Ramon y Cajal J, et al. Cord blood lipoprotein-cholesterol: relationship birth weight and gestational age of newborns. *Metabolism.* 1989;38:435–458.
36. Skinner ER, Klopper AI, Wilson GR, Toop KM. The composition and concentration of umbilical cord plasma lipoproteins; their relationship to the birth weight and other clinical factors of the newborn. *Clin Chim Acta.* 1983;135:219–228.
37. Brown MS, Kovanen PT, Goldstein JL. Receptor-mediated uptake of lipoprotein-cholesterol and its utilization for steroid synthesis in the adrenal cortex. *Recent Prog Horm Res.* 1979;35:215–257.
38. Freedman DS, Bowman BA, Otvos JD, Srinivasan SR, Berenson GS. Levels and correlates of LDL and VLDL particle sizes among children. The Bogalusa Heart Study. *Atherosclerosis.* 2000;152:441–449.
39. Frerichs RR, Srinivasan SR, Webber LS, Rieth MC, Berenson GS. Serum lipids and lipoproteins at birth in a biracial population: the Bogalusa heart study. *Pediatr Res.* 1978; 12:858–863.
40. Glueck CJ, Gartside PS, Tsang RC, Mellies M, Steiner PM. Black-White similarities in cord blood lipids and lipoproteins. *Metabolism.* 1977;26:347–350.
41. Srinivasen SR, Dahlen GH, Jarpa RA, Webber CS, Berenson GS. Racial (Black-White) differences in serum lipoprotein(a) distribution and its relation to parental myocardial infarction in children. Bogalusa Heart Study. *Circulation.* 1991;84:160–167.
42. Hobbs HH, White AL. Lipoprotein(a): intrigues and insights. *Curr Opin Lipidol.* 1999; 10:225–236.
43. Rifai N, Heiss G, Doetsch K. Lipoprotein (a) at birth, in Blacks and Whites. *Atherosclerosis.* 1992;92:123–129.
44. Abe A, Maeda S, Makino K, et al. Enzyme-linked immunosorbent assay of lipoprotein(a) in serum and cord blood. *Clin Chim Acta.* 1988;177:31–40.
45. Vella JC, Calmarza P. Lipoprotein(a) and other lipid parameters in cord blood: a study of 528 cases. *Ann Biol Clin (Paris).* 1998;56: 457–461.
46. Aasvee K, Jauhiainen M, Kurvinen E, Jordania R, Sundvall J, Ehnholm C. Lipoprotein (a), apolipoprotein A-I and B serum levels in young families from Tallin, Estonia. Relation-

ships with other cardiovascular risk factors and nationality. *Scand J Clin Lab Invest.* 1999;59:179–190.
47. Low PS, Heng CK, Saha N, Tay JSH. Racial variation of cord plasma lipoprotein (a) levels in relation to coronary risk level: a study in three ethnic groups in Singapore. *Pediatr Res.* 1996;40:718–722.
48. Wang XL, Wilcken DEL, Dudman NPB. Neonatal apoA-I, apoB, and apo(a) levels in dried blood spots in an Australian population. *Pediatr Res.* 1990;28:496–501.
49. Parker CR Jr, Fortunato SJ, Carr BR, Owen J, Hankins GD, Hauth JC. Apolipoprotein A-1 in umbilical cord blood of newborn infants: relation to gestational age and high-density lipoprotein cholesterol. *Pediatr Res.* 1988;23:348–351.
50. Kaser S, Ebenbichler CF, Wolf HJ, et al. Lipoprotein profile and cholesteryl ester transfer protein in neonates. *Metabolism.* 2001;50: 723–728.
51. Merzouk H, Lamri MY, Meghelli-Bouchenak M, Korso N, Prost J, Belleville J. Serum lecithin: cholesterol Acyltransferase activity and HDL2 and HDL3 composition in small for gestational age newborns. *Acta Paediatr.* 1997; 86:528–532.
52. Radunovic N, Kuczynski E, Rosen T, Dukanac J, Petkovic S, Lockwood CJ. Plasma apolipoprotein A-I and B concentrations in growth related fetuses. A link between low birth weight and adult atherosclerosis. *J Clin Endocrinol Metab.* 2000;85:85–88.
53. Anderson GT, Lifshitz C, Frus-Hanson B. Dietary habits and serum lipids during the first 4 years of life. A study of 95 Danish children. *Acta Paediatr Scand.* 1979;68:165–170.

AUTHOR CONTRIBUTIONS

Design and concept of study: Kwiterovich, Blakemore
Acquisition of data: Kwiterovich, Virgil, Otvos, Driggers, Blakemore, Cockrill, Macfarlane
Data analysis and interpretation: Kwiterovich, Virgil, Garrett, Otvos, Driggers, Blakemore, Cockrill, Macfarlane
Manuscript draft: Kwiterovich, Garrett
Statistical expertise: Kwiterovich, Virgil, Garrett
Acquisition of funding: Kwiterovich
Administrative, technical, or material assistance: Kwiterovich, Virgil, Otvos, Driggers, Cockrill, Macfarlane
Supervision: Kwiterovich, Macfarlane