

# A Large High-Density Lipoprotein Enriched in Apolipoprotein C-I

## A Novel Biochemical Marker in Infants of Lower Birth Weight and Younger Gestational Age

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LOW BIRTH WEIGHT IS ASSOCIATED with cardiovascular risk factors and death in adulthood.<sup>1</sup> Differences in the size, molecular weight, composition, and quantity of lipoprotein subclasses are associated with coronary artery disease (CAD).<sup>2</sup> In our initial study of lipoprotein heterogeneity in cord blood, infants born small for gestational age (SGA) had higher levels of triglyceride-rich very low-density lipoprotein (VLDL) and intermediate low-density lipoprotein (IDL) than infants born appropriate for gestational age.<sup>3</sup> This finding extended the observations of others in SGA infants<sup>4-7</sup> and suggested a link between higher triglyceride-rich VLDL subclasses in SGA infants and future CAD.

We assessed a large high-density lipoprotein (HDL) subclass in cord blood<sup>8-15</sup> enriched in apolipoprotein C-I (apo C-I). Apolipoprotein C-I is a 6.6-

**Context** Low birth weight is associated with increased cardiovascular disease in adulthood, and differences in the molecular weight, composition, and quantity of lipoprotein subclasses are associated with coronary artery disease.

**Objective** To determine if there are novel patterns of lipoprotein heterogeneity in low-birth-weight infants.

**Design, Setting, and Participants** Prospective study at a US medical center of a representative sample of infants (n=163; 70 white and 93 black) born at 28 or more weeks of gestational age between January 3, 2000, and September 27, 2000. This sample constituted 20% of all infants born during the study period at this site.

**Main Outcome Measures** Plasma levels and particle sizes of lipoprotein subclasses and plasma concentrations of lipids, lipoproteins (high-density lipoprotein [HDL] and low-density lipoprotein [LDL]), and apolipoproteins.

**Results** An elevated lipoprotein peak of a particle with density between 1.062 and 1.072 g/mL was identified using physical-chemical methods. This subclass of large HDL was enriched in apolipoprotein C-I (apo C-I). Based on the amount of the apo C-I-enriched HDL peak, 156 infants were assigned to 1 of 4 groups: 0 (none detected), 17%; 1 (possibly present), 41%; 2 (probably present), 22%; 3 (elevated), 19%. Infants in group 3, compared with those in the other 3 groups, had significantly ( $P<.001$ ) lower mean birth weight (2683.7 vs 3307.1 g) and younger mean gestational age (36.2 vs 39.3 wk). After correction for age, infants in group 3 had significantly higher levels of total and large HDL cholesterol and of total and large LDL cholesterol and LDL particle number. However, infants in group 3 had lower levels of small HDL, very low-density lipoproteins, and triglycerides than infants in the other 3 groups. This lipoprotein profile differed from that in infants born small for gestational age, who had significantly higher triglyceride ( $P<.001$ ) and apo B ( $P=.04$ ) levels, but lower levels of total and large HDL cholesterol ( $P<.001$ ) and apo A-I ( $P<.001$ ).

**Conclusions** Because apo C-I-enriched HDL, and purified apo C-I alone, promotes apoptosis in vitro, increased amounts of this particle may have physiological significance and identify a novel group of low-birth-weight infants apparently distinct from traditionally classified small-for-gestational-age infants.

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kDa apolipoprotein, and in adults it is a component of VLDL, IDL, and HDL.<sup>16</sup> Apolipoprotein C-I displaces apo E

from VLDL and IDL, thereby decreasing their clearance from plasma.<sup>17</sup> Apolipoprotein C-I decreases the binding

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of  $\beta$ -VLDL to a remnant receptor, the LDL receptor-related protein,<sup>18,19</sup> and apo E-mediated binding of VLDL and IDL to the LDL receptor.<sup>20,21</sup> Such inhibitory effects of apo C-I on VLDL and IDL removal from blood may promote hypertriglyceridemia and atherosclerosis.<sup>16</sup> In that regard, Bjorkegren and coworkers<sup>22,23</sup> reported a significant enrichment of apo C-I in postprandial chylomicron remnants and VLDL remnants in normolipidemic patients with CAD<sup>22</sup> or early asymptomatic atherosclerosis.<sup>23</sup>

Apolipoprotein C-I also decreases the transfer of cholesteryl esters from HDL to VLDL by inhibiting cholesterol ester transfer protein (CETP).<sup>24</sup> Apolipoprotein C-I stimulates lecithin cholesterol acyl transferase,<sup>25</sup> an enzyme that esterifies cholesterol and produces the formation of the mature, spherical HDL from nascent HDL. The effects of apo C-I on both CETP and lecithin cholesterol acyl transferase may therefore promote increased amounts of a large HDL, which may be antiatherogenic, unless the presence of apo C-I on large HDL renders it dysfunctional. For example, we found that both apo C-I-enriched HDL and purified apo C-I promote apoptosis of cultured human arterial smooth muscle cells through the induction of neutral sphingomyelinase and the subsequent steps involved in apoptosis.<sup>26</sup> If such an effect occurs in vivo, this might promote the rupture of an unstable plaque, leading to myocardial infarction.

## METHODS

### Patients

We studied a group of 163 infants (31 white males, 39 white females; 47 black males and 46 black females), who were previously characterized.<sup>3</sup> There were 23 SGA infants, defined as a birth weight for gestational age of 10% or less. The infants were studied anonymously, using cord blood that was routinely obtained after birth. The Joint Committee on Clinical Investigations at Johns Hopkins determined that the study met the requirements for exempt research. Therefore, informed consent was not obtained.

### Lipid, Lipoprotein, and Apolipoprotein Measurements

Plasma from cord blood was analyzed for levels of cholesterol, triglycerides, LDL and HDL cholesterol, lipoprotein (a), and apo A-I, A-II, B, C-I, C-III, and E.<sup>3,26</sup> Fifteen lipoprotein subclasses, the number of LDL particles, and the average sizes of VLDL, LDL, and HDL were determined by nuclear magnetic resonance spectroscopy.<sup>3,27</sup> Lipoprotein density profiles for VLDL, LDL, and HDL were obtained after sucrose density gradient ultracentrifugation (DGU).<sup>28,29</sup>

### Preparation of Lipoprotein Fractions

Fractions from the lipoprotein density profile were thawed and a portion subjected to delipidation.<sup>28,29</sup> The samples were analyzed in duplicate by capillary electrophoresis using the P/ACE 5510 instrument (Beckman Coulter Inc, Fullerton, Calif) at 17 kV for 30 minutes.

The lipoprotein fractions were thawed, centrifuged to pellet particulate matter, and subjected to solid-phase extraction delipidation.<sup>28,29</sup> The apolipoproteins were eluted and concentrated, and an aliquot was taken for matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry analysis, using a Voyager Elite XL DE mass spectrometer (PerSeptive Biosystems, Framingham, Mass). The remaining samples were evaporated to dryness, reconstituted in 250  $\mu$ L of 8.0-M urea containing 2% CHAPS (3-[(3-cholamidopropyl)dimethylamino]-1-propane-sulfonate), sonicated, and degassed. Electrophoresis was performed using the IPGphor unit (Amersham Pharmacia, Uppsala, Sweden) as described.<sup>28,29</sup>

### Statistical Analysis

The relationships among lipids, lipoprotein cholesterols, apolipoproteins, lipoprotein subclasses, and lipoprotein sizes in 4 groups of infants, sorted by the amount of the apo C-I-enriched HDL peak, were evaluated, first using analysis of variance on data that were not adjusted for age, and then linear regression to correct for influ-

ence of gestational age. *P* values were also estimated using the Kruskal-Wallis test due to the small size of 2 of the groups ( $n=5$  each in group 0 and 3). To evaluate differences in these lipid-related variables between white and black infants and male and female infants, a  $\chi^2$  test was performed. All *P* values  $<.05$  were considered significant. All analyses were conducted using STATA software version 7.0 (STATA Corp, College Station, Tex).

## RESULTS

### Definitions of 4 Groups of Infants

Using lipoprotein density profiles (FIGURE 1), 156 infants for whom we had adequate plasma samples were classified into 4 groups, based on the gray intensity scale in the area between LDL and HDL. Group 0 infants ( $n=27$ ) had no inflection above baseline (no detectable apo C-I-enriched HDL); group 1 infants ( $n=64$ ) had a small inflection (blip) above baseline (1 to 5 on scale) (possible apo C-I-enriched HDL); group 2 infants ( $n=35$ ) had a peak above baseline ( $>5$  and  $<50$  on scale) (probable apo C-I-enriched HDL); and group 3 infants ( $n=30$ ) had a large peak above baseline ( $\geq 50$  on scale) (elevated apo C-I-enriched HDL).

### Lipoprotein Density Profiling

A prominent characteristic of the lipoprotein density profiles was the presence or absence of a distinct peak of a particle with density between 1.062 and 1.072 g/mL between the major peaks for LDL and HDL (Figure 1). The peak density of lipoprotein(a) in adult plasma is close to 1.055 g/mL, and thus potentially occurring within the density range between 1.062 and 1.072 g/mL. Lipoprotein(a) levels in cord blood, however, are very low.<sup>3</sup> The mean lipoprotein(a) level in group 3 infants was 1.2 (SD, 1.3) mg/dL and in group 0 infants was 0.6 (SD, 0.9) mg/dL; these minimal values while statistically different ( $P<.05$ ) were not quantitatively significant.

One infant each from group 3 and group 0 were first selected for detailed analyses of this lipoprotein peak. The

lipoprotein(a) levels in these 2 infants were low (3 mg/dL in one and undetectable in the other). Three lipoproteins, namely, LDL, the lipoprotein with a peak of density 1.062 to 1.072 g/mL, and HDL, were isolated by sucrose DGU, delipidated, and prepared for the following analyses.

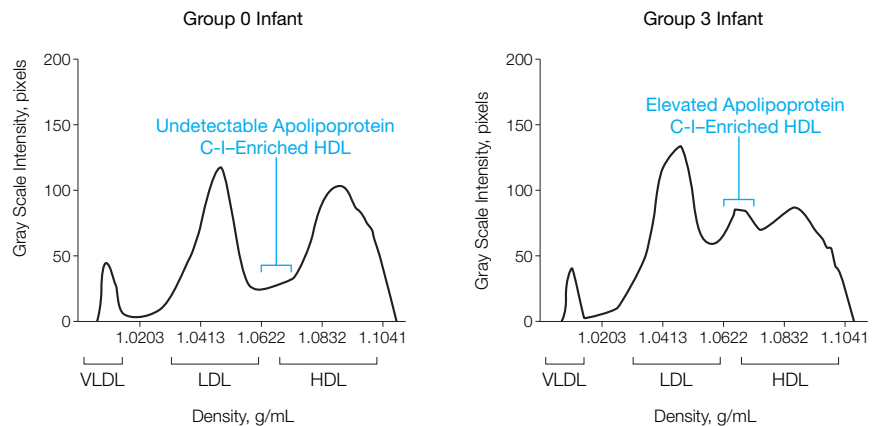
**Capillary Electrophoresis and Isoelectric Focusing.** After capillary electrophoresis was performed, apo A-I (47.7%) was the major apolipoprotein in the lipoprotein of density 1.062 to 1.072 g/mL from the group 3 infant, and apo C-I, ordinarily a minor component of HDL, was the second most prevalent apolipoprotein (37.6%). Negligible amounts of apolipoproteins were detected in the same lipoprotein density segment from the group 0 infant. These results were confirmed by isoelectric focusing, showing clearly apo A-I (isoelectric point [pI], 5.43) and apo C-I (pI 6.70) bands in the elevated lipoprotein peak from the group 3 infant but not the group 0 infant.

**MALDI-TOF Mass Spectrometry Analyses.** The apolipoproteins in the lipoprotein peak of density 1.062 to 1.072 g/mL from the group 0 infant were barely detectable (FIGURE 2A). In the elevated lipoprotein peak of density 1.062 to 1.072 g/mL (Figure 2B) from the group 3 infant, the intensity of apo C-I, relative to the intensity of apo A-I, was notably greater than in HDL (Figure 2D). There was little difference in the spectra for HDL of usual density between the group 3 (Figure 2D) and the group 0 infants (Figure 2C). These observations were confirmed in another group 3 infant and in a healthy control. There was no difference detected in LDL spectra.

#### Gradient Gel Electrophoresis of HDL

Plasma from 4 infants in group 3, 1 in group 2, and 3 in group 0 were ultracentrifuged at a density of less than 1.21 g/mL and gradient gel electrophoresis was performed.<sup>13,15</sup> As shown in representative densitometric scans of the gels (FIGURE 3), group 3 infants differed from group 0 infants. The largest HDL

**Figure 1.** Lipoprotein Profiles From Cord Blood After Sucrose Density Gradient Ultracentrifugation



Lipoprotein profiles from cord blood were obtained after sucrose density gradient ultracentrifugation. The density scale on the x-axis is not a linear scale because it is determined through mathematical transformation of locations along the vertical axis of the centrifuge tube (0-35 mm) based on sampled measured densities after completion of the spin. The density range used to define VLDL, LDL, and HDL by this method is given by the width of the braces under the x-axes. HDL indicates high-density lipoprotein; LDL, low-density lipoprotein; and VLDL, very low-density lipoprotein.

subclass in group 3 infants had a mean diameter of 11.6 (range, 11.5-11.8) nm compared with 9.4 (range, 8.8-10.8) nm in group 0 infants and 10.8 nm in 1 group 2 infant.

Apolipoprotein C-I was found in each of different HDL subclasses (Figure 3), as judged by immunoblots of the gradient gel electrophoresis gels using an anti-apo C-I antibody. These results are consistent with those from MALDI-TOS mass spectrometry and indicate that all HDL subclasses contained apo C-I.

#### Immunochemical Characterization of Apolipoproteins in Infants With Elevated vs Undetectable Amounts of the Lipoprotein of Density 1.062 to 1.072 g/mL

In a larger group of infants, the mean (SD) plasma level of apo C-I of 7.7 (3.2) mg/dL in 17 group 3 infants was significantly higher ( $P=.04$ ) than that of 5.1 (3.1) mg/dL in 13 infants from group 0. More extensive apolipoprotein analyses were performed in a smaller group of 10 infants (TABLE 1).

The distribution of apo A-I, A-II, B, C-I, and C-III between apo B-containing lipoproteins (VLDL, IDL, LDL, and lipoprotein[a]) and non-apo B-contain-

ing lipoproteins (HDL) was also determined without prior ultracentrifugation in infants from group 3 ( $n=5$ ) and group 0 ( $n=5$ ). The apo B-containing lipoproteins in 1 mL of plasma were precipitated with heparin-manganese chloride, and the apolipoprotein levels were measured in plasma, heparin-manganese supernatants (non-apo B-containing lipoproteins) and resolubilized precipitates (apo B-containing lipoproteins) using rocket immunoelectrophoresis.<sup>26,30</sup>

**Apolipoprotein B.** The plasma levels of total apo B were higher in group 3 than in group 0 infants but did not reach statistical significance (Table 1). All of the apo B was in the heparin manganese precipitates and none was detected in the supernatants.

**Apolipoprotein C-I.** The mean levels of apo C-I in both whole plasma and the heparin-manganese supernatants were about 2-fold higher in group 3 than in group 0 infants (Table 1). All of the apo C-I was in the supernatants and none was detected in the precipitates, distinctly different than later in life when a significant portion of apo C-I is associated with the apo B-containing lipoproteins.<sup>30</sup> These immunochemical results

further indicate that the apo C-I-enriched lipoprotein peak is an HDL subclass rather than a LDL subclass.

**Apolipoprotein C-III.** Infants in group 3 had significantly more apo C-III associated with non-apo B-containing lipoproteins, while infants in group 0 had significantly more apo C-III associated with apo B-containing lipoproteins (Table 1).

**Apolipoproteins A-I and A-II.** The apo A-I levels were higher in both the supernatants and precipitates in the infants in group 3 than in group 0. The mean apo A-II levels between groups 3 and 0 were very similar for whole plasma, supernatants, and precipitates (Table 1).

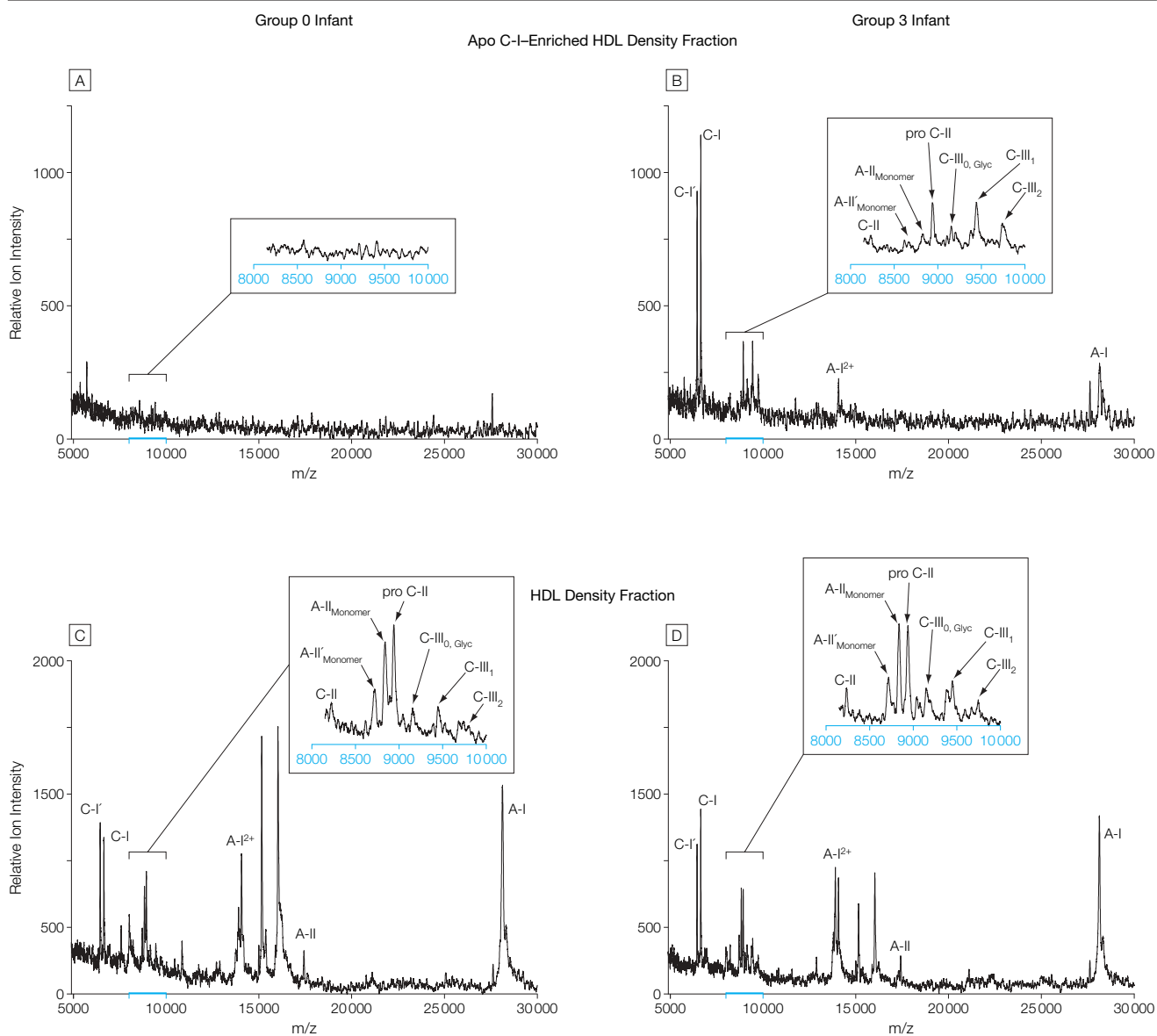
**Apolipoprotein E.** In a separate experiment, the mean concentration of apo E in group 3 infants was higher than

in group 0 infants in pooled whole plasma (12.9 v 5.8 mg/dL) and in heparin-manganese supernatants (7.9 v 4.8 mg/dL).

**Lipid, Lipoprotein, Apolipoprotein, and Lipoprotein Subclasses and Lipoprotein Sizes**

Lipoprotein density profiling was performed in 156 of the 163 infants

**Figure 2.** MALDI-TOF Mass Spectrometry of Apo C-I-Enriched HDL and Normal HDL



Apo indicates apolipoprotein; MALDI-TOF, matrix-assisted laser desorption/ionization time-of-flight; and HDL, high-density lipoprotein. Apo C-I-enriched HDL (A and B) and normal HDL (C and D) were isolated from plasma of a group 0 infant (A and C) and a group 3 infant (B and D) and prepared for MALDI-TOF mass spectrometry (see "Methods" section).

(95.7%) previously reported<sup>3</sup> to determine the frequency of appearance and degree of enrichment of the lipoprotein density 1.062 to 1.072 g/mL peak.

The levels of the lipid-related variables were determined in the 4 groups of infants (TABLE 2). Because of the influence of gestational age on the apo B- and apo A-I-containing lipoproteins in this population,<sup>3</sup> the *P* values were determined using data both nonadjusted and adjusted for gestational age. Before age adjustment, all the variables except apo B and small VLDL were significantly different. After age adjustment, the only LDL variables that remained significantly higher in group 3 were LDL subclasses L3 and L1 and LDL size (Table 2). In contrast, all the HDL and VLDL related variables remained significantly different after correction for gestational age. Large HDL levels were higher while small HDL and the VLDL related variables were lower in group 3. We also examined age-corrected means, which were very similar to the measured mean levels shown in Table 2 for the HDL- and VLDL-related variables. Differences between large LDL and large HDL among all groups were independent of triglycerides and VLDL. Despite the fact that the gestational ages were very similar in groups 0, 1, and 2, there were also impressive dose-response relationships for the levels of all 6 of the HDL-related variables from group 0 through groups 1, 2, and 3 infants (Table 2). These analyses indicate further that the differences for the HDL subclasses shown in Table 2 were independent of age.

**Distribution of Gestational Age**

The mean (SD) gestational ages of the infants were 39.7 (1.8) weeks in group 0; 39.3 (1.3) weeks in group 1; 38.8 (1.7) weeks in group 2; and 36.2 (4.2) weeks in group 3 and differed significantly (*P*<.001). The mean gestational age in group 3 infants was not only younger but had a distribution that was clearly broader than that in groups 0, 1, and 2 (FIGURE 4).

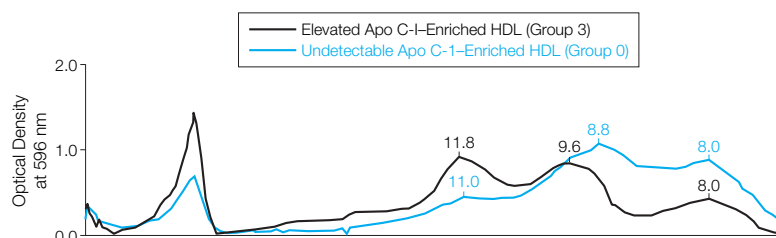
The mean (SD) birth weights of the infants were 3268.6 (631.9) g in group

0; 3412.2 (548.3) g in group 1; 3240.6 (609.2) g in group 2; and 2683.7 (783.3) g in group 3 and differed significantly (*P*<.001), being particularly low in group 3. After correcting for gestational age, the birth weights were no longer significant (*P*=.15).

There were no significant differences in the numbers of male and female (*P*=.38), white and black (*P*=.88), or SGA and appropriately sized (*P*=.34) infants among the 4 groups.

Multivariable linear regression models showed that after correcting for ges-

**Figure 3.** Gradient Gel Electrophoresis of HDL



Plasma lipoproteins were isolated from 4 group 3 infants, 1 group 2 infant, and 3 group 0 infants by ultracentrifugation at density greater than 1.21 g/mL and prepared for electrophoresis. Following electrophoresis, gels were stained for protein and densitometric scans performed at 596 nm. The relative amount of the high-density lipoprotein (HDL) subclasses, using an internal standard, is depicted on the y-axis. Numbers on the curves indicate HDL particle diameter in nanometers.

**Table 1.** Apolipoprotein Content of Plasma, Heparin-Manganese Supernatant, and Heparin-Manganese Precipitate in Infants With Undetectable (Group 0) or Elevated (Group 3) Apolipoprotein C-I-Enriched HDL

Apolipoproteins	Mean (SD), mg/dL		P Value
	Group 0 (n = 5)	Group 3 (n = 5)	
Apolipoprotein B			
Plasma	21.4 (2.3)	27.4 (12.2)	.53
Heparin-Mn <sup>+2</sup> Supernatant	0	0	>.99
Precipitate	16.3 (1.5)	23.1 (12.6)	.46
Apolipoprotein C-I			
Plasma	4.8 (2.4)	8.7 (1.9)	.08
Heparin-Mn <sup>+2</sup> Supernatant	3.3 (1.8)	6.9 (3.1)	.03
Precipitate	0	0	>.99
Apolipoprotein C-III			
Plasma	4.3 (1.1)	5.4 (1.1)	.25
Heparin-Mn <sup>+2</sup> Supernatant	3.1 (0.6)	4.7 (0.9)	.01
Precipitate	1.3 (0.6)	0.6 (0.3)	.05
Apolipoprotein A-I			
Plasma	99.6 (17.1)	119.7 (38.8)	.46
Heparin-Mn <sup>+2</sup> Supernatant	83.0 (11.6)	101.6 (30.7)	.12
Precipitate	3.1 (0.8)	4.9 (0.9)	.01
Apolipoprotein A-II			
Plasma	21.1 (1.6)	21.4 (2.1)	.60
Heparin-Mn <sup>+2</sup> Supernatant	19.8 (0.8)	19.8 (2.2)	.46
Precipitate	0.9 (0.2)	0.9 (0.2)	.91

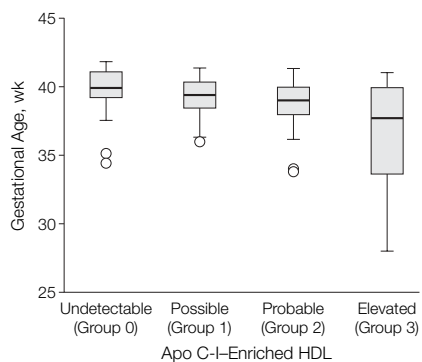
Abbreviations: HDL, high-density lipoprotein; Mn, manganese. SI conversion factors: To convert from mg/dL to μmol/L divide by the following conversion factors: apo B, 51.272; apo C-I, 0.663; apo C-III, 0.880; apo A-I, 2.902; and apo A-II, 1.741.

**Table 2.** Plasma Levels of Lipids, Lipoproteins, Apolipoproteins, Lipoprotein Subclasses, and Lipoprotein Sizes in Infants (N = 156), by Amounts of Apolipoprotein C-I-Enriched HDL\*

Variable	Mean (1 SD), by Group				P Value	
	0 (n = 27)	1 (n = 64)	2 (n = 35)	3 (n = 30)	Unadjusted†	Adjusted for Gestational Age‡
Total cholesterol, mg/dL	52.3 (11.1)	60.6 (18.3)	64.6 (11.1)	81.0 (23.3)	<.001	<.001
<b>LDL</b>						
Total particles, nmol/L	398.4 (150.9)	400.2 (188.8)	354.7 (146.6)	510.2 (339.8)	.03	.39
Total levels, mg/dL	22.11 (7.39)	27.89 (14.4)	27.0 (7.6)	37.7 (19.4)	<.001	.12
Size, nm	20.17 (0.64)	20.47 (0.84)	21.05 (0.68)	20.89 (0.8)	<.001	<.001
Small subclass (L1), mg/dL	16.88 (12.02)	14.74 (12.35)	6.80 (10.62)	16.78 (20.49)	.009	.009
Large subclass (L3), mg/dL	8.10 (6.95)	12.29 (10.61)	17.19 (13.1)	23.67 (17.42)	<.001	.02
Apolipoprotein B, mg/dL	18.51 (3.76)	18.31 (8.01)	16.45 (4.45)	17.58 (4.46)	.47	.41
<b>HDL, mg/dL</b>						
Total levels	20.0 (5.7)	25.2 (7.6)	30.7 (6.2)	36.7 (10.1)	<.001	<.001
Small subclasses (H1, H2)	10.09 (2.12)	9.18 (1.72)	8.49 (1.88)	6.17 (3.08)	<.001	<.001
Large subclasses (H3, H4, H5)	8.14 (3.20)	14.23 (7.25)	21.02 (6.60)	26.44 (7.29)	<.001	<.001
Largest subclass (H5)	0.75 (1.33)	4.7 (5.03)	9.53 (4.85)	16.66 (7.81)	<.001	<.001
Apolipoprotein A-I, mg/dL	68.67 (11.3)	73.95 (13.5)	81.75 (12.37)	85.23 (17.34)	<.001	<.001
<b>VLDL, mg/dL</b>						
VLDL triglycerides	19.27 (18.41)	7.5 (8.8)	7.04 (8.25)	6.69 (7.84)	<.001	<.001
Small subclasses (V1, V2)	2.88 (3.68)	2.47 (4.37)	1.90 (2.67)	3.15 (5.26)	.64	.74
Intermediate subclasses (V3, V4)	14.1 (13.09)	4.85 (6.15)	5.02 (6.24)	3.35 (4.09)	<.001	<.001
Total triglycerides, mg/dL	50.85 (19.33)	30.74 (12.95)	35.0 (14.16)	33.2 (8.73)	<.001	<.001

Abbreviations: HDL, high-density lipoprotein; LDL, low-density lipoprotein; VLDL, very low-density lipoprotein.  
 \*See the "Results" section for definitions of the infant groups 0-3. Lipoprotein subclasses and lipoprotein sizes were determined by nuclear magnetic resonance spectroscopy. For some variables the SD goes beyond zero because none of that variable was detected in the sample. Levels of LDL and HDL and their subclasses are expressed in terms of their cholesterol content. Levels of VLDL and its subclasses are expressed in terms of their triglyceride content.  
 †Determined using analysis of variance and not corrected for gestational age.  
 ‡Corrected for the influence of gestational age by linear regression.

**Figure 4.** Gestational Age in 156 Study Infants



The median and 25th and 75th percentiles (box) and 5th and 95th percentiles (whiskers) for gestational age are shown. The circles represent outliers.

tational age and race, levels of total and large HDL and apo A-I were significantly higher in the group 3 infants than in the SGA infants without elevated apo C-I-enriched HDL (TABLE 3). Conversely, the total triglycerides and apolipoprotein B levels, indicative of higher

triglyceride-rich lipoproteins, were significantly higher in the SGA infants than in the group 3 infants (Table 3). There were no significant differences in LDL particle number, LDL levels, and LDL subclasses between the 2 groups. We further directly compared the group 3 infants who had elevated apo C-I-enriched HDL (n = 24) with infants who were SGA (n = 13); 6 infants who were both in group 3 and the SGA group were excluded from the analyses. Results similar to those in Table 3 were found.

We next plotted the levels of large LDL (L3) and largest HDL (H5) against gestational age for the group 3 and group 0 infants (FIGURE 5). Group 3 infants had higher values of L3 than group 0 infants, but these L3 levels decreased dramatically with increasing gestational age. When all 30 infants in group 3 were included, the slope of the line for LDL3 vs age had a  $\beta$  of  $-2.55$  (95% CI,  $-1.32$  to  $-3.78$ );  $P < .001$  and correlation coefficient of  $r = 0.61$ . Af-

ter excluding the one outlier (a 28-week infant with L3 of 73 mg/dL), the slope of the line for L3 vs age had a  $\beta$  coefficient of  $-1.99$  (95% CI,  $-3.20$  to  $-0.78$ ;  $P = .003$ ) and correlation coefficient of  $r = 0.53$ . In distinct contrast, the higher H5 levels in group 3 did not decrease with gestational age, indicating strongly that the elevated amount of apo C-I-enriched HDL in group 3 persisted and was not simply a consequence of younger gestational age (Figure 5).

**COMMENT**

We report here the novel observation that the large HDL subclass in cord blood<sup>8-15</sup> is enriched in apo C-I. Infants with elevated apo C-I-enriched HDL (group 3) were further unique in that they had notably lower birth weights and younger gestational ages and significantly different plasma levels of lipids, lipoproteins, apolipoproteins, and lipoprotein subclasses and lipoprotein size than infants with un-

**Table 3.** Comparison of Variables in Infants With Elevated Apolipoprotein C-I-Enriched HDL (Group 3) vs Infants Born Small for Gestational Age (SGA)

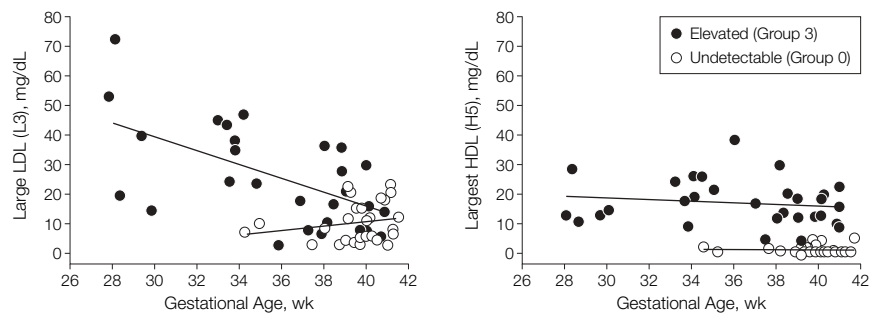
Variable	Mean (SD), mg/dL		Model 1*		Model 2*	
	Apo C-I-Enriched HDL (n = 24)	SGA (n = 13)	Mean Difference	P Value	Mean Difference	P Value
Total cholesterol	80.79 (17.58)	62.77 (18.43)	18.02	.02	13.39	.09
LDL						
Particles, No.†	507.09 (275.63)	412.21 (164.29)	94.88	.37	8.74	.93
Levels†	37.50 (17.19)	29.85 (16.43)	7.65	.24	1.82	.77
Small subclasses†	16.15 (17.11)	13.52 (9.52)	2.62	.68	-0.25	.97
Large subclasses†	24.59 (18.20)	12.42 (12.78)	12.17	.04	6.54	.21
Size	20.89 (0.83)	20.42 (0.78)	0.47	.10	0.44	.14
Apolipoprotein B†	17.64 (4.33)	24.72 (14.65)	-7.08	.03	-7.29	.03
HDL						
Small subclasses	6.29 (3.02)	8.93 (2.36)	-2.64	.01	-1.39	.08
Large subclasses	26.62 (7.15)	11.09 (5.43)	15.50	<.001	15.86	<.001
Levels	36.58 (9.44)	21.23 (6.14)	15.35	<.001	16.24	<.001
Apolipoprotein A-I	84.42 (18.14)	72.15 (14.17)	12.26	.04	18.23	<.001
VLDL						
Small subclasses†	3.11 (4.74)	3.57 (5.04)	-0.46	.80	-0.44	.82
Medium subclasses†	3.76 (4.46)	15.94 (14.51)	-12.18	<.001	-11.38	<.001
VLDL triglycerides†	7.11 (8.15)	21.95 (22.21)	-14.85	.003	-13.76	.01
Total triglycerides†	33.71 (9.57)	58.38 (23.75)	-24.68	<.001	-23.19	<.001
Lipoprotein(a)†	1.83 (1.90)	1.85 (2.61)	-0.013	.99	0.26	.75
Gestational age, wk	35.92 (4.37)	38.29 (2.13)	-2.37	.07	NA	NA
Birth weight, g	2754.04 (819.76)	2450.23 (549.77)	303.81	.23	712.95	<.001

Abbreviations: HDL, high-density lipoprotein; LDL, low-density lipoprotein; NA, not applicable; VLDL, very low-density lipoprotein. SI conversion factors: To convert from mg/dL to mmol/L divide by the following: cholesterol, 38.7; triglycerides, 88.5. \*Model 1 was unadjusted and model 2 was adjusted for gestational age and race. †Data that were skewed were log transformed for each model.

detectable (group 0), possible (group 1), or probable (group 2) apo C-I-enriched HDL.

An elevated lipoprotein peak in density range 1.062 to 1.072 g/mL, identified in about 1 in 5 infants in this well-characterized population,<sup>3</sup> was a large HDL particle enriched in apo C-I, expressed in all infants regardless of sex or race. The existence of an apo C-I-enriched HDL was supported by the congruent results of a number of physical-chemical and immunochemical methods. At birth all the plasma apo C-I was found in HDL, and group 3 infants had higher apo C-I levels than did group 0 infants. The distributions of the quantity of apo C-III and apo A-I in the apo B- and apo A-I-containing particles were also significantly different in the group 3 and group 0 infants, suggesting other differences between their HDL particles. Consistent with our prior work<sup>26</sup> and that of others,<sup>9,10</sup> the apo E content was higher in HDL from

**Figure 5.** Analysis of Gestational Age vs Large LDL and Largest HDL Levels in the Elevated and Undetectable Apo C-I-Enriched HDL Groups



Plots of gestational age vs large (L3) low-density lipoprotein (LDL) cholesterol and largest (H5) high-density lipoprotein (HDL) cholesterol in group 3 (elevated; n = 30) and group 0 (undetectable; n = 27) infants. Regression lines are depicted for each group. For L3 vs age the regression coefficient  $r = 0.61$  for group 3 infants includes the 1 outlier (28-week old infant with LDL3 of 73 mg/dL). If the outlier is excluded, the regression coefficient ( $r = 0.52$ ) is still significant. For group 0 infants the regression coefficient was  $r = 0.22$ . For H5 vs age, the regression coefficients were  $r = 0.16$  for group 3 infants and  $r = 0.01$  for group 0 infants. To convert from mg/dL to mmol/L, divide by 38.7.

group 3 infants. Using immunoaffinity chromatography, an apo C-I-enriched HDL and an apo C-I-poor HDL were isolated and characterized from pooled plasma of group 3 and of

group 0 infants, respectively.<sup>26</sup> Such apo C-I-enriched HDL, but not apo C-I-poor HDL, promoted apoptosis in human cultured arterial smooth muscle cells.<sup>26</sup> These observations support fur-

ther the enriched apo C-I content of HDL from group 3 infants, and indicate its potential physiologic and clinical significance.

Apolipoprotein C-I influences the activities of CETP, lecithin cholesterol acyl transferase, and hepatic lipase.<sup>16</sup> Apolipoprotein C-I in HDL inhibits CETP.<sup>24</sup> Adults deficient in CETP<sup>31</sup> have large HDL particles, but in a single study, the large HDL in cord blood did not appear to be due to a deficiency of CETP.<sup>5</sup> We assessed indirectly the activity of CETP. In a subset of 40 infants, there was no relationship between CETP activity and apo C-I levels (data not shown). The association of the D442G (aspartic acid→glycine at codon 442) mutation of the *CETP* gene with increased HDL cholesterol in adults was not seen in cord blood.<sup>32</sup> Although lecithin cholesterol acyl transferase activity is low in cord blood,<sup>33,34</sup> cholesterol esterification is normal and does not appear to account for the large HDL species in cord blood.<sup>5</sup> Additional studies in a larger number of group 3 and 0 infants are needed to determine the role of apo C-I in the production of the large, apo C-I-enriched HDL particle and its effect on CETP and lecithin cholesterol acyl transferase activities.

Our hypothesis is that infants with elevated apo C-I-enriched HDL at birth will have higher apo C-I levels in childhood. After the infant exits the intrauterine environment, where there is no ingestion of dietary fat, to the postprandial state, a transfer of apo C-I from HDL to chylomicrons occurs. A significant enrichment of apo C-I in chylomicrons is associated with exaggerated postprandial triglyceridemia,<sup>22</sup> delayed chylomicron remnant removal,<sup>22,23</sup> and enrichment of apo C-I in VLDL remnants in normolipidemic patients with CAD<sup>22</sup> or early asymptomatic atherosclerosis.<sup>23</sup> Thus, in children older than 2 years and in adults who have elevated apo C-I levels, a low-fat diet may be indicated to decrease the formation of chylomicrons and VLDL remnants. If apo C-I-enriched HDL were also present in children and adults with elevated apo C-I levels (as it is in

apo C-I-transgenic mice), both the delayed removal of triglyceride-rich remnants and the effect of an apparently dysfunctional HDL could promote atherosclerosis and CAD.

Conde-Knape et al<sup>35</sup> described an apo C-I-enriched HDL in a moderately expressing *APOC-I* transgenic mouse model on an *APOE* null background. Apolipoprotein C-I-enriched HDL (but not VLDL) inhibited hepatic lipase but not lipoprotein lipase. We found no relationship between a common *HpaI* restriction fragment-length polymorphism<sup>36</sup> in the promoter of *APOC-I* in all infants. A -514 C→T restriction fragment-length polymorphism in the promoter of the hepatic lipase gene (*LIPC*) explains about 30% of hepatic lipase activity,<sup>37</sup> but we found no relationship between this variant and apo C-I-enriched HDL.

We examined whether elevated apo C-I HDL might be a normal concomitant of the earlier gestational age observed in group 3 infants. Sixty percent of the group 3 infants were born older than 36 weeks' gestational age, and their large HDL levels were as elevated as those in the younger age groups, indicating that the expression of elevated apo C-I-enriched HDL was not simply a normal consequence of younger gestational age.

An unexpected finding was that group 3 infants had a birth weight that was 584.8 g (1.29 lb) lower than group 0 infants. The lipoprotein profile of group 3 infants was characterized by higher total and large HDL cholesterol but lower total and VLDL triglycerides, in distinct contrast to that of our relatively hypertriglyceridemic SGA infants.<sup>3</sup> Furthermore, of the 30 infants in group 3, only 6 were SGA, and their mean gestational age of 37.5 weeks was actually higher than that of 35.9 weeks in the 24 non-SGA group 3 infants. The low mean birth weight in group 3 infants was present in both the SGA (2402.1 g) and the non-SGA (2754.0 g) infants. Thus, elevated apo C-I-enriched HDL identified a new group of low-birth-weight infants, apparently distinct from SGA.

The relationship between low birth weight and adult cardiovascular disease is attributed to intrauterine effects on fetal tissue development<sup>1</sup> but might also be explained by genes that influence birth weight and cardiovascular risk in later years. There is substantial evidence that genes influence birth weight.<sup>38</sup> Infants born SGA are a heterogeneous group and may be constitutionally small or have either symmetrical or asymmetrical pathological intrauterine growth restriction.<sup>1</sup> The higher triglyceride-rich lipoproteins and apo B levels found in SGA infants may provide a link to future cardiovascular disease.<sup>3,7</sup> The distinct lipoprotein phenotype of elevated apo C-I-enriched HDL, if it persists throughout childhood into adulthood, may be a novel risk factor for cardiovascular disease. There are other possible effects of a dysfunctional HDL beyond apoptosis<sup>26</sup> that may promote atherosclerosis. Some patients with CAD and elevated HDL<sup>39</sup> have a proinflammatory HDL because it fails to prevent the formation of, or inactivation of, oxidized phospholipids.<sup>40</sup> A dysfunctional HDL might also be less efficient at mediating efflux of cholesterol from macrophages.

How might apo C-I influence the expression of low birth weight in infants with the elevated apo C-I-enriched HDL phenotype? Given our data on the promotion of apoptosis in vitro by apo C-I, we were intrigued by the observation that apoptosis, impoverished villous development, and fetoplacental angiogenesis were significantly higher in placentas from pregnancies with low birth weight due to intrauterine growth restriction.<sup>41-44</sup> Clearly, detailed genetic, clinical, and biochemical studies of the apo C-I-enriched HDL phenotype will be necessary to elucidate the role of apo C-I in the pathogenesis of CAD.

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**Study concept and design:** Kwiterovich.  
**Acquisition of data:** Kwiterovich, Cockrill, Virgil, Otvos, Knight-Gibson, Alaupovic, Forte, Farwig, Macfarlane.  
**Analysis and interpretation of data:** Kwiterovich, Cockrill, Garrett, Otvos, Knight-Gibson, Alaupovic, Forte, Zhang, Farwig, Macfarlane.

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*Statistical analysis:* Virgil, Garrett, Zhang.

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