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Levels and correlates of LDL and VLDL particle sizes among children: the Bogalusa heart study

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Abstract

Levels of lipids and lipoproteins among children vary by sex and race/ethnicity, and are correlated with age, obesity, and other characteristics. There is, however, little information on the distribution and correlates of low-density lipoprotein (LDL) and very-low-density lipoprotein (VLDL) subclasses in early life. We used nuclear magnetic resonance (NMR) spectroscopy to determine mean LDL and VLDL particle sizes among 10- to 17-year-olds ($n = 918$) who participated in the 1992–94 examination of the Bogalusa heart study. As compared with girls, boys had a smaller (0.1 nm) mean LDL particle size and a larger (0.9 nm) mean VLDL size; furthermore, the average size of VLDL particles increased with age among white boys but not among other children. Although there were also black/white differences in particle sizes, with black children having larger LDL and smaller VLDL particles, these racial contrasts could be attributed to differences in lipid levels. Levels of triglycerides, insulin, and relative weight were associated with the size of VLDL (positive) and LDL (negative) particles. These results suggest that the analysis of lipoprotein subclasses may provide a better understanding of the role of various risk factors in the development of coronary heart disease

Keywords: Nuclear magnetic resonance spectroscopy; Lipids; Lipoproteins; VLDL; LDL; Lipoprotein subfractions; Children; Blacks

1. Introduction

The initial stages of atherosclerosis among children and young adults vary by race, sex and age, and are associated with lipid levels [1,2]. Of the various lipoprotein classes, levels of high density lipoprotein (HDL) cholesterol among adolescents show the most striking changes with age, but there are also differences in levels of triglyceride (TG) and low-density lipoprotein (LDL) cholesterol. TG levels are substantially higher among white children than among black children, and tend to increase with age among whites [3,4]. Although the mean LDL cholesterol level is higher among girls than boys, this difference narrows during sexual maturation [4].

Despite the importance of these lipids and lipoproteins in the development of atherosclerosis, the traditional risk factors are only moderately predictive of coronary heart disease (CHD). This misclassification may, in part, arise because the standard, density-based classification of lipoproteins (very-low, low, and high) results in heterogeneous categories that contain particles differing in diameter, composition, and possibly, atherogenicity [5,6]. Although LDL subclasses have received the most attention [7–9], subclasses of very-low-density lipoproteins (VLDL) may also differ in atherogenicity [10,11]. In addition, large VLDL particles may reflect delayed chylomicron clearance [12], a metabolic condition that has been related to disease severity [13,14].

Despite the potential importance of lipoprotein subclasses, few studies [15–17] have examined these characteristics in early life. The current analyses, based on

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the analysis of frozen, archived plasma samples, describe the distribution of LDL and VLDL subclasses and particle sizes by race, sex, age, insulin levels, and relative weight.

2. Methods

2.1. Sample

The 918 children and adolescents in the current analyses participated in the 1992–94 examination of the Bogalusa heart study, an epidemiologic study of cardiovascular disease risk factors in early life [18]. The surrounding community, Ward Four of Washington Parish (Louisiana), is fairly typical of semi-rural towns in the South, with an economy that is dominated by a lumber mill; the 1990 population of 43 000 was $\approx 1/3$ black. Cross-sectional examinations of 5–17-year-olds have been conducted in Bogalusa schools every 3–5 years since 1973.

Subjects in the current analyses were selected from 10- to 17-year-olds who were examined in the 1992–94 cross-sectional examination. Subjects were excluded if they were not fasting ($n = 261$), did not have an insulin or glucose determination ($n = 72$), were missing data for height, weight, or skinfolds ($n = 6$), or had a reported race/ethnicity other than white or black ($n = 3$). Of the 1643 eligible children, 918 were randomly selected for the determination of lipoprotein subclasses. The reproducibility of the NMR measurements was assessed for an additional 92 (blind) duplicate specimens.

2.2. Anthropometry

Height was measured to the nearest 0.1 cm with a manual height board, and weight to the nearest 0.1 kg using a balance beam scale; a gown, underpants, and socks were worn during the examination. Because age was moderately associated ($r = 0.28$) with Quetelet (kg/m^2) Index but not with Rohrer (kg/m^3) Index ($r = 0.02$), the latter is used as a measure of relative weight in the current analyses.

2.3. Chemical analyses of lipids and lipoproteins

All chemical analyses were performed on fresh blood samples in the Bogalusa Heart Study Core Laboratory. Serum concentrations of cholesterol and TG were determined using enzymatic procedures (Abbott VP; North Chicago, IL). Following the heparin-calcium precipitation of β- and pre-β- lipoproteins, the concentration of LDL cholesterol was determined from the densitometric (electrophoretic) ratio and cholesterol contents of the two lipoproteins [19]. Plasma insulin determinations were made using a commercial radioimmunoassay procedure (Phaadebas Insulin Kit, Pharmacia Diagnostics).

The laboratory met the performance requirements of the CDC Lipid Standardization Program. Laboratory reproducibility was also assessed daily using duplicate aliquots of blood (drawn into an additional tube) from an $\approx 10\%$ random sample of children. In the current analyses, levels of TG, LDL cholesterol, and HDL cholesterol always refers to these chemical, rather than NMR, determinations.

2.4. NMR spectroscopy

Plasma samples that had been stored at -70°C for 4–6 years were sent to LipoMed., (Raleigh, NC) for analysis of lipoprotein subclasses. Freezing under these conditions does not discernibly alter lipoprotein subclass levels of normotryglyceridemic (< 400 mg/dl) plasma, and in the current study, the median TG level was 76 mg/dl, with a 95th percentile of 174 mg/dl. Proton NMR spectra of freshly-thawed aliquots (0.25 ml) were acquired in duplicate at 47°C using a dedicated 400 MHz NMR analyzer

Lipoprotein subclasses of different size broadcast distinguishable lipid methyl group NMR signals, the intensities of which are proportional to the lipid mass of the particles [20]. Since the signals overlap substantially, a computer deconvolution is performed to derive their intensities. After converting these intensities to more familiar concentration units (mg/dl triglyceride for VLDL and mg/dl cholesterol for LDL and HDL), the concentrations of 15 (6 VLDL, 1 IDL, 3 LDL, 5 HDL) lipoprotein subclasses are obtained [21].

Larger subclass numbers denote larger particles, and the approximate diameter ranges (in nm) of the VLDL subclasses used in the computations are: V6 (150 ± 70), V5 (70 ± 10), V4 (50 ± 10), V3 (38 ± 3), V2 (33 ± 2), and V1 (29 ± 2). Approximate diameter ranges (in nm) of the three LDL subclasses are: L3 (22 ± 0.7), L2 (20.5 ± 0.7), and L1 (19 ± 0.7). The diameter ranges were determined by calibration with purified VLDL and LDL subfractions that were isolated by agarose gel filtration chromatography and analyzed by electron microscopy for particle size distribution. The LDL subclass diameters, which are ~ 5 nm smaller than those estimated by gradient gel electrophoresis are consistent with calculations based on detailed lipid compositional data [22] and independent electron microscopy analyses [23].

Average particle sizes for VLDL and LDL were determined by weighting the relative mass percentage of each subclass by its diameter. Because measurement reproducibility of the individual LDL subclasses is lower than that of average LDL size, only the latter is considered in this report. Grouping the 6 VLDL sub-

classes as small (V1 + V2), intermediate (V3 + V4), and large (V5 + V6) also improved the reproducibility.

Subjects with an average LDL particle diameter of ≤ 20.5 nm were classified as LDL pattern B (predominantly small LDL). A similar NMR classification system of adults in the Framingham offspring study found that 36% of men and 13% of women were pattern B (Otvos JD and Schaefer EJ, unpublished data). These prevalences are similar to those based on the use of gradient gel electrophoresis and a cutpoint of 25.5 nm to classify pattern B [24].

2.5. Statistical methods

The repeatability of the NMR determinations was assessed, with the laboratory blinded, in 92 pairs of samples. The calculated statistics for the replicate samples include

1. the median, absolute (intra-pair) difference,
2. the coefficient of variation (CV), and
3. the intra-class correlation coefficient (ICC).

The CV expresses the within-subject variability, defined as $(\Sigma\Delta_i^2/2N)^{1/2}$ in which the squared intra-pair differences are summed over all N pairs, as a percentage of the overall mean. In contrast, the ICC compares the within-subject variability to the variability across subjects [25]. Ideally, a laboratory determination would have a low CV and a high ICC.

Average particle sizes and subclass levels were compared across the four race–sex groups, and associations with age were examined using Spearman correlation coefficients and lowess (locally weighted scatterplot smoother) curves. Lowess is a smoothing technique [26] that relies on nearby data points to determine the functional form of the relation; the current analyses used a neighborhood width of 50%. Similar statistical techniques were used to examine the relation of subclass levels to levels of lipids, lipoproteins, insulin, and relative weight. Regression analyses were used to assess if the observed race and sex differences were independent of lipid and lipoprotein levels.

3. Results

The reproducibility of the examined characteristics is shown in Table 1. Among the 92 pairs of replicate NMR determinations, intra-class correlation coefficients (ICC) ranged from 0.69 (LDL size) to 0.97 (levels of intermediate VLDL); these values can be compared with ICCs above 0.95 for the chemically determined lipid levels. The modest ICC (0.69) for LDL size, along with its very low CV (1%), indicates that the small intra-pair differences were relatively large when compared to the variability across subjects. The median absolute difference of 0.2 nm for LDL size, for example, was almost half of the standard deviation (0.5 nm). In contrast, the relatively large measurement error for large VLDL (CV = 41%) was much smaller than the overall variability, resulting in an ICC of 0.96. The reproducibility the VLDL subclass determinations was fairly comparable to that for insulin levels, which had a CV of 18% and an ICC of 0.93.

Table 1
Repeatability statistics for the LDL and VLDL subclasses

	Number of pairs[a]	Mean ± SD levels		Median absolute difference	CV[b]%	Intra-class correlation
		Orignal	Duplicate			
NMR determinations						
LDL size (nm)	92	21.2 ± 0.5	21.3 ± 0.5	0.2	1	0.69
VLDL size (nm)	92	42.2 ± 7.9	41.6 ± 6.9	2.0	9	0.76
V1 + V2 (small), (mg/dl)	92	10 ± 5	10 ± 5	1.8	21	0.91
V3 + V4 (intermediate), (mg/dl)	92	21 ± 20	21 ± 19	2.8	17	0.97
V5 + V6 (large), (mg/dl)	92	8 ± 17	8 ± 18	1.2	41	0.96
Chemical determinations						
TC (mg/dl)	313	167 ± 28	167 ± 28	3.0	2	0.99
LDLC (mg/dl)	313	101 ± 25	102 ± 24	2.8	3	0.98
TG (mg/dl)	313	83 ± 45	83 ± 46	2.0	3	0.99
HDLC (mg/dl)	313	53 ± 12	53 ± 12	2.0	4	0.96
Insulin (μU/mL)	218	12 ± 9	12 ± 8	0.9	18	0.93

[a] Number of pairs of replicates for the analysis of blind duplicates. A 10% random sample of children selected on each screening day to have a second tube of blood drawn to assess laboratory reproducibility. The 92 pairs are the numbers of blind duplicate pairs among the 918 samples selected for lipoprotein subclasses analysis; the reproducibility of the chemical determinations is based on all blind duplicate samples from the 1992–94 cross-sectional examination.

[b] The CV was calculated as $100 \times [(\Sigma\Delta_i^2/2N)^{1/2}/\text{mean}]$. Δ_i^2 is the squared differences within pair i; the difference is summed over the i pairs.

Table 2
Mean levels and percentiles for NMR determinations, by race and sex

		Concentrations				Differences[a]	
	Percentile (*P*) or mean	White boys ($n=285$)	White girls ($n=266$)	Black boys ($n=182$)	Black girls ($n=185$)	Black–white	Female–male
NMR determinations							
LDL size (nm)	5*P*	20.2	20.4	20.5	20.6	–	–
	25*P*	20.8	21.0	21.0	21.1	–	–
	50*P*	21.2	21.3	21.4	21.5	–	–
	75*P*	21.5	21.7	21.7	21.8	–	–
	95*P*	21.9	22.0	22.0	22.0	–	–
	Mean ± SD	21.1 ± 0.5	21.3 ± 0.5	21.3 ± 0.5	21.4 ± 0.5	0.2**	0.1**
VLDL size (nm)	5*P*	34.5	35.1	32.2	32.7	–	–
	25*P*	38.3	38.0	35.7	35.6	–	–
	50*P*	41.8	41.0	38.8	37.9	–	–
	75*P*	46.5	45.4	42.9	41.0	–	–
	95*P*	60.6	57.4	52.0	49.5	–	–
	Mean ± SD	43.5 ± 7.6	42.7 ± 6.8	40.1 ± 6.7	39.0 ± 5.6	−3.6**	−0.9*
Chemical determinations							
Total cholesterol (mg/dl)	Mean ± SD	165 ± 29	166 ± 27	167 ± 31	169 ± 31	3.0	0.6
Triglycerides (mg/dl)	Mean ± SD	96 ± 51	101 ± 7	71 ± 30	72 ± 27	−26.8**	3.2
LDL cholesterol (mg/dl)	Mean ± SD	101 ± 25	101 ± 23	99 ± 27	101 ± 27	−0.3	0.1
HDL cholesterol (mg/dl)	Mean ± SD	48 ± 10	49 ± 11	57 ± 14	57 ± 13	8.1**	0.5

[a] As assessed in regression models that included race, sex, age, age 2, and age 3 as predictor variables. A − indicates that the adjusted mean level is higher among whites or boys. Values in parentheses have also been adjusted for levels of HDLC and TG.

* $P<0.05$.

** $P<0.001$.

Race and sex differences in particle size are shown in Table 2. The mean LDL size was 0.2 nm larger among blacks than among whites, and 0.1 nm larger among girls than among boys. These differences were most evident at the lower part of the LDL size distribution: the fifth percentile differed by 0.4 nm across race–sex groups, while the 95th percentile varied by only 0.1 nm. In contrast, the average VLDL size was larger among whites than among blacks, and boys tended ($P=0.04$) to have slightly larger particles than girls. There were also differences in mean lipid and lipoprotein levels, with black children having higher (+ 8 mg/dl) levels of HDL cholesterol and lower (− 27 mg/dl) levels of TG than white children. Sex differences in lipid levels were much smaller, and none were statistically significant.

Smoothed levels of lipoprotein particle sizes by age are shown in Fig. 1 for each race–sex group, and median levels are presented in Table 3. The LDL size showed no consistent trend with age, but race and sex differences appeared to narrow with increasing age. The prevalence of pattern B (average LDL size ≤ 20.5 nm) ranged from 5% (blacks) to 13% (white boys). Among white children, the prevalence of LDL pattern B decreased from 14 to 3% across the four age groups, but there was only a small (0.1 nm) age-related increase in the median size of LDL particles. These somewhat contrasting findings arose, in part, because 11 (of the 25) 10–11-year-old white children with pattern B had an average LDL particle diameter at the upper cutpoint (20.5 nm) of this classification. In contrast, the two 16–17-year-old white children with pattern B had mean particle sizes of 20.1 and 20.2 nm.

Of the three VLDL subclasses, intermediate VLDL had the highest concentration with a median level (24 mg/dl) that was about 2.5 times higher than that for other VLDL subclasses (Table 3). (The distributions of intermediate and large VLDL subclasses were skewed towards higher values.) Furthermore, racial differences were particularly marked for intermediate VLDL, with white children having an almost three-fold higher median level did black children (33 vs. 13 mg/dl). The sex difference was less striking, but after age 14, boys

tended to have higher levels of intermediate VLDL than did girls. The largest age-related changes in levels of VLDL subclasses were seen among white boys (Fig. 2). Between the ages of 10 and 17 years, smoothed levels of intermediate VLDL increased by about 70%, while levels of small VLDL decreased by about 20%. Although levels of large VLDL were not significantly correlated with age, levels of this subclass increased rapidly after age 15.

There were strong, non-linear associations with TG levels (Fig. 3). For example, neither the size of LDL (top panel) or VLDL (middle) particles varied greatly at TG levels below the median (76 mg/dl), whereas at higher TG levels, the mean size of both LDL ($r = -0.37$) and VLDL ($r = 0.73$) particles were associated with the TG level. Associations between levels of TG and VLDL subclasses were also non-linear (bottom). At TG levels below 55 mg/dl ($\sim$ 20th percentile), levels of small VLDL were highest (mean, 7 mg/dl), while at higher TG levels, intermediate VLDL tended to predominate. Levels of large VLDL increased rapidly at TG levels >100 mg/dl, but median levels of this subclass were close to 0 at TG levels below the median.

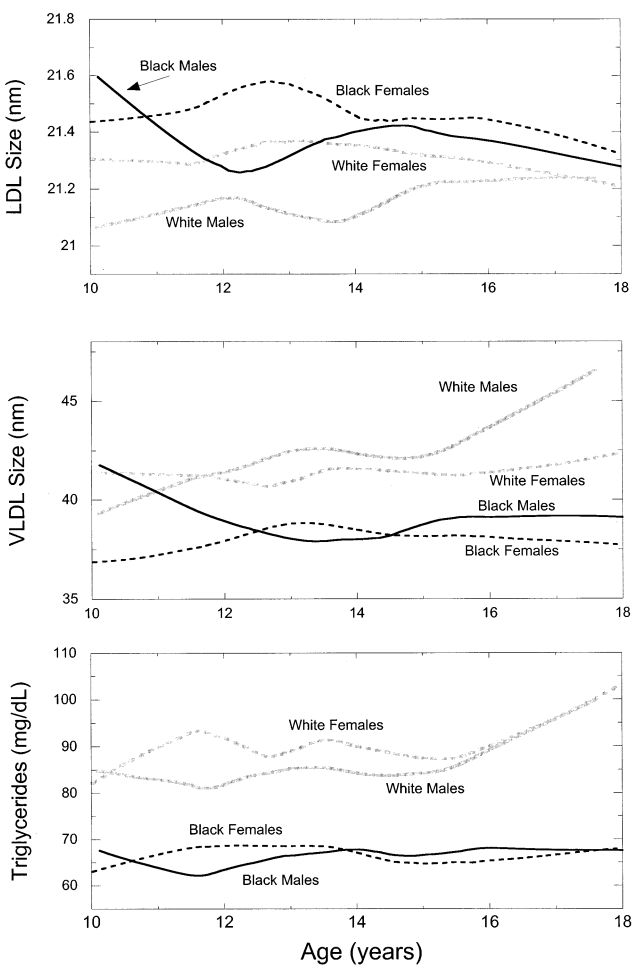

Fig. 1. Smoothed levels of average LDL size (top), average VLDL size (middle), and TG levels (bottom) by age; these smoothed levels roughly correspond to median levels. Within each race–sex group, lowess curves were constructed using a robust linear regression fitting procedure and a neighborhood width of 50%. Black lines represent black children, while lighter lines represent white children; solid lines are boys, while dashed lines are girls.

Race-, sex-, and age-adjusted associations with various characteristics are summarized in Table 4. (Correlation coefficients in parentheses have also been adjusted for TG levels, and values >0.11 are statistically significant at the 0.001 level.) In addition to its association with TG levels, the average LDL particle size was related positively to levels of LDL and HDL cholesterol ($r = 0.17$–0.35) and inversely to levels of insulin and relative weight ($r = -0.22$ to 0.30). In contrast, the average size of VLDL particles was associated inversely with HDL cholesterol, and positively with insulin and relative weight, reflecting contrasting associations with subclass levels. For example, the LDL cholesterol level was related more strongly ($r = 0.51$) to levels of small VLDL than with levels of the larger VLDL subclasses. In contrast, insulin and relative weight showed little association with small VLDL. Although adjustment for TG levels reduced the associations with VLDL size, associations with LDL size remained statistically significant.

We then examined whether the observed race and sex differences in particle size could be attributed to differences in lipids and lipoproteins. Regression analyses that included levels of TG and HDL cholesterol as covariates, indicated that at equivalent levels of these characteristics, girls had larger LDL ($+0.1$ nm) particles and smaller VLDL (-1.2 nm) particles than did boys; $P < 0.001$ for each difference. In contrast, the observed racial differences in LDL and VLDL particle sizes were almost entirely attributable to differences in lipid levels, and neither remained statistically significant after adjustment.

4. Discussion

Most studies of lipoprotein subclasses have focused on LDL, with high levels of small, dense particles having been found to be predictive of CHD [5,7–9,27]. Although there are several mechanisms by which small LDL particles could promote atherosclerosis [27,28], many (but not all [9]) investigators have found that adjustment for lipid levels greatly reduces the magnitude of the association with CHD risk [7,8]. It is also likely that VLDL subclasses differ in atherogenicity, but whereas some investigators have suggested that smaller VLDL particles may be the most atherogenic [10,29], others have found larger particles to be most strongly related to occlusive disease [11] and obesity [30,31]. It is possible that large VLDL particles may be selectively retained in the intima of the arterial wall [32]

Table 3
Median levels of lipoprotein subclasses, by race, sex, and age

Race–sex	Overall ($n=918$)	Age (years)				Correlation with age[a]
		10–11 ($n=279$)	12–13 ($n=285$)	14–15 ($n=217$)	16–17 ($n=137$)	
LDL size (nm)						
White boys	21.2	21.1	21.1	21.2	21.2	0.07
White girls	21.3	21.2	21.1	21.3	21.3	0
Black boys	21.4	21.3	21.4	21.4	21.3	0
Black girls	21.5	21.4	21.6	21.5	21.3	−0.06
LDL pattern B[b] *(%)*						
White boys	13%	16%	10%	15%	3%	–
White girls	8%	13%	7%	4%	2%	–
Black boys	5%	6%	5%	7%	4%	–
Black girls	5%	2%	12%	2%	3%	–
VLDL Size (nm)						
White boys	41.8	40.7	41.4	42.1	44.4	0.19**
White girls	41.0	41.8	40.6	41.1	41.7	0.01
Black boys	38.8	40.4	38.3	38.7	38.8	−0.04
Black girls	37.9	36.6	39.2	37.5	38.9	0.05
Small VLDL (mg/dl)[c]						
White boys	10.8	12.4	11.5	9.4	10.1	−0.21**
White girls	11.0	11.4	10.8	9.9	11.7	−0.11
Black boys	9.2	8.2	9.2	9.7	10.7	0.12
Black girls	9.7	11.5	10.2	8.7	9.0	−0.17*
Intermediate VLDL (mg/dl)[c]						
White boys	34.0	28.1	37.5	37.9	47.2	0.15*
White girls	32.0	36.7	31.5	28.2	34.6	−0.01
Black boys	13.6	11.2	11.8	16.1	19.4	0.11
Black girls	12.2	10.8	16.4	9.6	12.2	0
Large VLDL (mg/dl)[c]						
White boys	2.0	2.1	1.8	1.8	4.0	0.08
White girls	2.3	3.0	1.4	2.4	2.7	−0.03
Black boys	1.1	2.3	0.7	1.0	1.1	−0.13
Black girls	0.9	0.7	1.2	1.0	0.9	−0.01

[a] Spearman (rank) correlation coefficient.
[b] Pattern B was defined as an average LDL size 20.5 nm.
[c] Small VLDL was define as the sum of V1 and V2 (27–35 nm), intermediate VLDL as the sum of V3 and V4 (35–60 nm), and large VLDL as the sum of V5 and V6 (60–220 nm).
* $P<0.05$
** $P<0.001$.

or may be a marker for delayed chylomicron clearance [12], a metabolic condition associated with occlusive disease [13,14].

In parallel with the sex difference in CHD incidence, the mean LDL particle size is smaller among men than among women [17,31,33,34]. We found that the mean LDL size to be 0.1 nm smaller among boys than among girls, a difference that persisted even after adjustment for lipid and lipoprotein levels. Although others have reported that boys and girls have a similar mean LDL size [17] or a similar prevalence of small LDL particles [15], these studies may have lacked sufficient statistical power; furthermore, findings among Japanese children [15] may not be applicable to the US. There have been fewer studies of VLDL subclasses, but as compared with women, men appear to have higher levels of large VLDL and lower levels of small VLDL [35]. We found that after ages 12–14, boys generally have larger VLDL particles than do girls. Although the sex differences in lipoprotein particle sizes were relatively small, these contrasts were seen in the absence of sex differences in levels of lipids and lipoproteins.

Differences in the size of lipoprotein particles across racial/ethnic groups have not been extensively investigated. The LDL cholesterol ÷ apoB ratio, which is moderately associated ($r=0.60$) with LDL size [36], is higher among black children than among white children [37], and Mexican-American adults appear to have

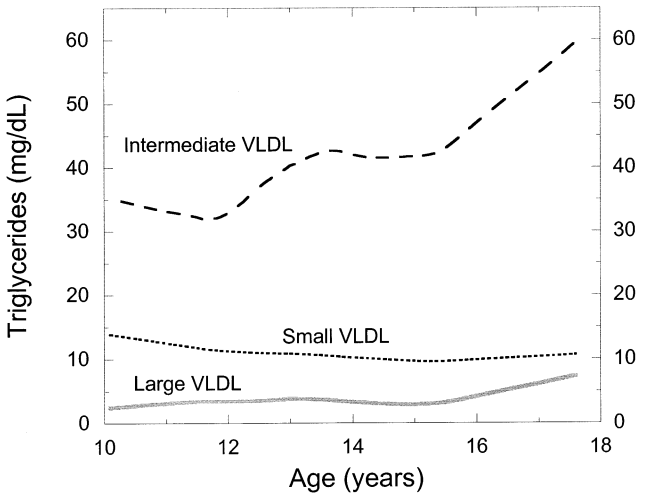

Fig. 2. Smoothed levels of VLDL subclasses by age among white boys. Lowess curves were constructed using a robust linear regression fitting procedure and a neighborhood width of 50%.

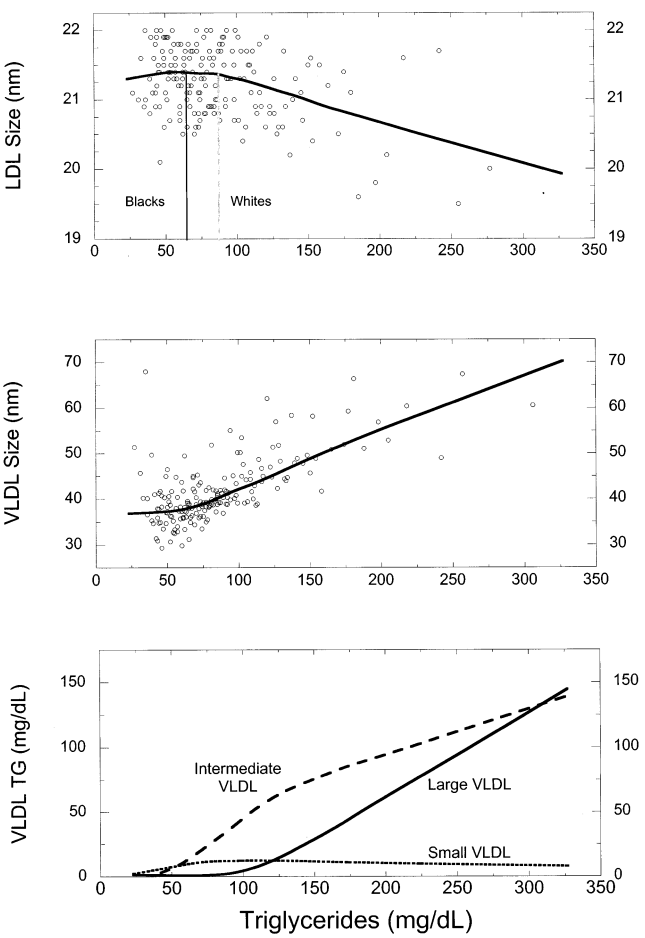

Fig. 3. Smoothed levels of average LDL size (top), VLDL size (middle), and VLDL subclasses (bottom) by levels of TG. Lowess curves were constructed using a robust linear regression fitting procedure and a neighborhood width of 50%, and a random 10% sample of subjects are shown for LDL size and VLDL size. The median TG levels among whites and blacks are represented by vertical lines in the top panel.

relatively small LDL particles [38]. We are not aware of previous studies that assessed if particle size differences are independent of other racial/ethnic differences [3,18], and we found that differences in particle sizes were entirely attributable to levels of TG and HDL cholesterol. The importance of TG levels likely reflects the exchange of triglycerides and cholesterol esters between VLDL and LDL particles, with the subsequent hydrolysis of triglycerides [28,36].

Although a decrease in LDL particle size with age has been reported among adults [39], others have observed only a weak [38] or a positive [33] association. In agreement with the lack of association in the current study, other investigators have reported

1. only a small difference in the mean size of LDL particles between children and adults [17], and
2. no trend in the LDL cholesterol/apoB with age among 8- to 17-year-olds [37].

Although we found that the prevalence of LDL pattern B among white children decreased with age, this was due to the large number (11 of 25) of 10- to 11-year-olds who had an average LDL size at the upper end (20.5 nm) of the pattern B range. As the association between LDL particle size and susceptibility to oxidation varies over a range of particle sizes [28], a simple dichotomous classification may obscure important differences. The observed increase in mean VLDL size among older boys parallels the decrease in HDL cholesterol levels [4] that occurs during maturation.

The TG level is strongly related to the size of VLDL particles and to the relative amount of each VLDL subclass. Although intermediate VLDL generally showed the highest concentration, levels of small VLDL were highest at relatively low TG levels, and the relative proportion of large VLDL increased rapidly at higher TG levels. As TG levels increase among adults, the relative amount of large VLDL increases [35], and hypertriglyceridemics have an accumulation of large VLDL particles. However, it is possible to have a low TG level but a large mean VLDL size, and among the 138 children in the current study whose TG level was < 50 mg/dl, the mean VLDL size ranged from 29 to 79 nm. This discordance suggests that information on VLDL subclasses may provide information not available with a simple TG determination.

The observed correlation between TG levels and LDL particle size ($r = -0.21$) was weaker than those ($r = -0.4$ to -0.7) that have been reported among adults [36,38,40]. This may be the result of the non-linear association between TG levels and LDL size [41], along with age-related increases in TG levels with age. In the current study there was little association between TG levels and the mean LDL particle size among children with a TG level below the median (76 mg/dl); this TG value is approximately the 25th percentile among adult men [3]. Studies of adults [9,35,36,42] have

Table 4
Relation of lipoprotein sizes and subclasses to levels of lipids, insulin, and relative weight

	Triglycerides	LDL cholesterol	HDLC	Insulin	Relative weight[a]
LDL size	−0.21[b]	0.17 (0.23)	0.35 (0.30)	−0.22 (−0.15)	−0.30 (−0.25)
VLDL size	0.59	−0.06 (−0.26)	−0.24 (−0.06)	0.30 (0.08)	0.25 (0.07)
Small VLDL (V1+V2)	0.40	0.51 (0.46)	−0.16 (−0.02)	0.11 (−0.02)	0.11 (−0.06)
Intermediate VLDL (V3+V4)	0.85	0.16 (−0.11)	−0.31 (−0.04)	0.30 (−0.06)	0.26 (−0.10)
Large VLDL (V5+V6)	0.47	0.11 (−0.01)	−0.26 (−0.12)	0.32 (0.16)	0.27 (0.14)

[a] Weight/height^3

[b] Values are Spearman correlation coefficients. With a sample size of 918, a correlation coefficient of 0.11 would be statistically significant at the 0.001 level. Values in parentheses have been adjusted for levels of TG in addition to race, sex, and age.

also found that the association between TG levels and LDL particle size is weak at TG levels below 100–125 mg/dl. In the current study, the Pearson correlation coefficient between TG and LDL size was −0.56 among children whose TG level was >100 mg/dl.

In agreement with our findings for relative weight, studies in adults have found the strongest associations with levels of the larger VLDL subclasses [30,31,43] and smaller LDL subclasses [17,30,31,34,36,43]. Comparable associations with small LDL have also been observed among university students [42], and children [15]. These findings may reflect an increased amount of cholesterol ester-triglyceride exchange among persons with relatively high TG levels. Insulin resistance and hyperinsulinemia also influence VLDL metabolism, leading to high levels of large VLDL [44] and smaller LDL particles [36,40,45].

Several limitations of the current study should be considered. Our results are based on the use of samples that had been frozen for 4–6 years at −70°C, but freezing under these conditions does not discernibly alter lipoprotein subclass levels if the TG level is <400 mg/dl. (The 95th percentile of TG in the current study was 174 mg/dl.) Indirect support for the validity of our NMR determinations of lipoprotein subclasses in frozen samples is provided by the similarity many of our findings to those of other investigations, particularly the relation of particle sizes to levels of TG, relative weight and insulin

The observed reproducibility of LDL size measured by NMR was good (CV = 1%), comparing favorably with CVs of 2–3% that have been reported for split samples using gradient gel electrophoresis [27,36,38]. The observed ICC of 0.69, however, was only moderate, and it is likely that this non-differential measurement error decreased the magnitudes of the observed associations. Although the measurement error CVs for the VLDL subclasses were larger, they were relatively small compared with the inter-person variability, and the resulting ICCs were >0.90.

We have previously shown that NMR-determined levels of small LDL and large VLDL are associated with the extent of occlusive disease [11]. Our current findings suggest that the measurement of lipoprotein subclasses, in addition to levels of LDL cholesterol and TG, may provide a better understanding of the role of sex, obesity, and TG levels in the development of coronary heart disease. The relation of other characteristics, including cigarette smoking and alcohol consumption, to lipoprotein subclasses should be examined.

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