Distribution and Correlates of High-Density Lipoprotein Subclasses Among Children and Adolescents

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Levels of high-density lipoprotein (HDL) cholesterol among children vary by sex and race/ethnicity and are correlated with age, obesity, and other characteristics. Several studies of adults have indicated that atherogenicity of HDL particles may vary by size, but there is little information on the distribution and correlates of HDL subfractions in early life. We used nuclear magnetic resonance (NMR) spectroscopy to determine the mean HDL particle size and levels of 3 HDL subclasses among 10- to 17-year-olds (n = 918). We found the mean HDL particle size to be (1) inversely associated with age among boys, (2) larger among girls than boys, and (3) larger among black children than among white children. These associations with particle size reflected contrasting associations with various HDL subclasses; among boys, for example, levels of large HDL decreased with age, whereas levels of small HDL remained constant (black boys) or tended to increase (white boys). Furthermore, relative weight and levels of both triglycerides and low-density lipoprotein (LDL) cholesterol were associated inversely with levels of large HDL, but positively with levels of small HDL. These contrasting associations suggest that the role of HDLC in coronary heart disease (CHD) may be more complex than previously thought, and that the analysis of HDL subclasses may improve the accuracy of CHD prediction.

LEVELS OF HIGH-DENSITY lipoprotein cholesterol (HDLC) among children vary by sex, race/ethnicity, age, and other characteristics. Whereas mean HDLC levels are slightly higher among pre-adolescent boys than similarly aged girls, levels decrease markedly among boys during sexual maturation in most ethnic groups.[1-4] By the end of adolescence, the mean HDLC level is higher among girls than among boys, a difference that persists throughout adulthood.[5,6] Differences in HDLC levels by race/ethnic group are also well documented, with black males having a 5 to 10 mg/dL higher mean HDLC level than white males.[5,6] Several other characteristics, such as obesity, alcohol consumption, cigarette smoking, physical activity, and triglyceride levels are also associated with levels of HDLC.[7]

Despite the importance of HDLC levels in the development of coronary heart disease (CHD), lipoproteins and other risk factors are only moderately predictive of disease.[8] This may, in part, be because the standard lipid measures do not account for the chemical, physical, and atherogenic heterogeneity of particles within a lipoprotein class.[9,10] Although low-density lipoprotein (LDL) subclasses have received the most attention, the protective effect of HDLC has generally been strongest for the larger subclasses.[11-15] Furthermore, some evidence suggests that levels of the smaller HDL subclasses (HDL_{3b} and HDL_{3c}), as determined by gradient gel electrophoresis (GGE)[12-14] or nuclear magnetic resonance (NMR) spectroscopy,[15] may be atherogenic.

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Submitted July 1, 2000; accepted September 29, 2000.

Supported by National Institutes of Health (NIH) Grants No. HL 15103 and HL 32194.

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0026-0495/01/5003-0011$35.00/0

doi:10.1053/meta.2001.21027

Despite their potential importance, relatively few studies have examined HDL subclasses among children[16-18] and infants.[19,20] The current cross-sectional analyses of 918 10- to 17-year-olds describe the distribution and correlates of NMR-determined HDL subclasses; we have previously shown these determinations to be highly correlated (r = .88) with GGE-determined subclasses.[21,22] In particular, we assess whether HDL subclasses differ in their relation to race, sex, age, relative weight, and lipid levels. These findings could provide additional information on the determinants of CHD.

MATERIALS AND METHODS

Sample

The 918 children and adolescents in the current analyses participated in the 1992 to 1994 examination of the Bogalusa Heart Study, an epidemiologic study of cardiovascular disease risk factors in early life.[23] The surrounding community, Ward 4 of Washington Parish (Louisiana), had a 1990 population of 43,000. Bogalusa is fairly typical of semirural towns in the South, with a population that is about one third black and an economy that is dominated by a lumber mill. Seven cross-sectional examinations of school-aged children have been conducted since 1973.

Subjects in the current analyses were selected from 10- to 17-year-olds (n = 1,985) examined in the 1992 to 1994 cross-sectional examination. Informed consent was obtained for the examination, and all procedures were in accord with the ethical standards of the institutional review board of Tulane University. Subjects were considered ineligible for the current study if they were not fasting (n = 261), reported race/ethnicity as other than white or black (n = 3), or were missing information on levels of insulin or glucose (n = 72), or on height, weight, or skinfolds (n = 6). Of the 1,643 eligible children, 918 were randomly selected for the determination of lipoprotein subclasses. The reproducibility of the NMR measurements was assessed in 92 (blind) duplicate specimens.

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 metric scale; a gown, underpants, and socks were worn during the examination. The Rohrer Index (kg/m^3) is used as a measure of relative weight in the current analyses. Height is moderately associated (r = .35) with Quetelet Index (kg/m^2), but not with Rohrer Index (r = .01).

Chemical Analyses of Lipids, Lipoproteins, and Insulin

All chemical analyses, including levels of HDLC, were performed in the Bogalusa Heart Study Core Laboratory. Concentrations of serum cholesterol and triglycerides were determined using enzymatic procedures (Abbott VP; North Chicago, IL), and lipoprotein cholesterol determinations were performed using heparin-calcium precipitation and agar-agarose gel electrophoresis.[24] (In the current analyses, HDLC refers to the chemical, and not NMR, determination.) As previously described,[25] immunoprecipitation was used to characterize HDL particles that contain apolipoprotein A-I only (LpA-I) and those that contain both apolipoproteins A-I and A-II (Lp A-I:A-II); these measurements were available for 726 subjects. Plasma insulin determinations were made using a radioimmunoassay procedure (Phadebas Insulin Kit).

The laboratory met the performance requirements of the Lipid Standardization Program of the National Centers for Disease Control. Based on a 10% sample of children who were randomly selected each day, the intraclass correlation coefficients for the lipid and lipoprotein determinations between duplicate aliquots of bloods were ≥ 0.95, and for insulin levels, $r = .91$.

NMR Spectroscopy

Plasma samples, which had been stored at –70°C for 4 to 6 years, were sent to LipoMed, (Raleigh, NC) for analysis of lipoprotein subclass levels. Freezing under these conditions does not discernibly alter lipoprotein subclass levels of normotriglyceridemic (<400 mg/dL) plasma, and in the current study, the median (95th percentile) triglyceride level was 76 (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 (Varian, Palo Alto, CA). Spectral deconvolution was performed to determine the amplitudes of the lipid methyl group signals broadcast by particles of different size[21,26]; these amplitudes were used to quantify the lipoprotein subclass concentrations. Each subclass signal comes from the lipids (cholesterol, cholesterol esters, triglycerides, and phospholipids) in the particle and is unaffected by variations in lipid composition. For reporting purposes, the NMR signal amplitudes have been converted to mass concentration units (mg/dL) of cholesterol.[26] The current study focuses on HDL subclasses; another report[27] has described levels of LDL and very–low-density lipoprotein (VLDL) subclasses.

Based on these techniques, 3 HDL subclasses were quantified: large HDL (≈ 8.8 to 13.0 nm in diameter), intermediate HDL (8.2 to 8.8 nm), and small HDL (7.3 to 8.2 nm). Diameter ranges were determined by calibration with purified lipoprotein subfractions that were isolated by ultracentrifugation and agarose gel filtration chromatography. The correspondence between HDL subclasses determined by NMR and by GGE are: large HDL, approximately $HDL_{2b} + HDL_{2a}$; intermediate HDL, approximately HDL_{3a}; and small HDL, approximately $HDL_{3b} + HDL_{3c}$. The average HDL particle size, reflecting the relative concentration of each subclass, was determined by weighting the relative mass percentage of each subclass by its diameter. This estimate of HDL particle size, which is based on a continuous scale (8.2 to 10.3 nm in the current study) is highly correlated ($r = .88$) with GGE-determined HDL particle size.[21,22]

Statistical Methods

The repeatability of the subclass determinations was assessed with the laboratory blinded in 92 pairs of samples. The calculated statistics for the replicate samples include (1) the mean, absolute difference, (2) the coefficient of variation (CV), and (3) the intraclass correlation coefficient. The CV expresses the within-subject variability, defined as $(\Sigma\Delta_i^2/2N)^{1/2}$ in which the squared intrapair differences are summed over all N pairs as a percentage of the overall mean. In contrast, the intraclass correlation coefficient compares the within-subject to inter-subject variability.[28]

The average HDL size and subclass levels were compared across the 4 race-sex groups, and associations with age and other characteristics were examined using Spearman (rank) correlation coefficients (age was calculated as the number of days, divided by 365.25, between the examination and birth dates). Lowess (locally weighted scatterplot smoother) curves were also used to illustrate these associations; this robust smoothing technique relies on nearby data points to determine the shape of the relation,[29] and we used a neighborhood width of 50%. Stratification and regression analyses were also used to assess race and sex differences in HDL size and subclass levels and to determine whether the observed differences were independent of lipid and lipoprotein levels. Interaction (product) terms were included in several of these regression models to determine whether the observed race or sex differences varied significantly by age.

RESULTS

As determined from the analysis of blind duplicates (Table 1), the NMR determinations for the average HDL particle size (CV = 0.7%, $r = .97$) and the large HDL level (CV = 5.1%, $r = .99$) were very reproducible. Measurement errors were larger for levels of small and intermediate HDL, but intraclass correlation coefficients were ≥0.85. Subsequent analyses do not include levels of intermediate HDL, the subclass having the largest measurement error.

The average HDL particle size, as well as levels of the subclasses, varied substantially by race and sex (Table 2). As compared with whites, the mean HDL size was 0.3 nm larger among black children, a difference equal to approximately 75% of its standard deviation. Girls also had a larger (0.1 nm) average HDL size than did boys, and these race-sex differences

Table 1. Repeatability Statistics for Levels of HDLC and HDL Subclasses

	Mean ± SD Levels (mg/dL)*		Absolute Difference (mg/dL)*	Coefficient of Variation (%)	Intraclass Correlation
	Original	Duplicate			
HDLC	53.1 ± 11.8	52.9 ± 11.5	2.1 ± 2.5	4.3	0.96
NMR determinations					
HDL size	9.1 ± 0.4	9.1 ± 0.4	0.06 ± 0.07	0.7	0.98
Small HDL	15.0 ± 4.4	14.7 ± 4.5	1.7 ± 1.3	10.3	0.88
Intermediate HDL	8.2 ± 6.5	8.8 ± 7.0	2.8 ± 2.4	30.4	0.85
Large HDL	24.4 ± 12.9	24.6 ± 12.8	1.4 ± 1.1	5.1	0.99

NOTE. All statistics are based on the analysis of blind duplicates. There were 313 pairs of specimens for HDLC (which was determined chemically) and 92 pairs of specimens HDL size and HDL subclasses (which were determined using NMR).

*Except for HDL size, all characteristics are measured in units of cholesterol concentration (mg/dL). HDL size measurement is in nm.

Table 2. Mean Levels and Percentiles for HDLC and for the HDL Subclass Determinations by Race and Sex

	Percentile (P) or Mean ± SD	Concentrations				Differences in Means*	
		White Boys (n = 285)	White Girls (n = 266)	Black Boys (n = 182)	Black Girls (n = 185)	Black-White	Female-Male
HDLC (mg/dL)†	Mean ± SD	48 ± 10	49 ± 11	57 ± 14	57 ± 13	8.1‡ (0.8)	0.5 (0.8)
HDL size (nm)	Mean ± SD	8.8 ± 0.3	9.0 ± 0.3	9.2 ± 0.4	9.3 ± 0.4	0.3‡ (0.03)	0.1‡ (0.02)
	5 P	8.4	8.5	8.5	8.6		
	25 P	8.6	8.7	8.8	9.0		
	50 P	8.8	9.0	9.2	9.3		
	75 P	9.1	9.2	9.5	9.6		
	95 P	9.4	9.6	10.0	9.9		
Small HDL (mg/dL)	Mean ± SD	16 ± 4	14 ± 5	15 ± 4	15 ± 4	0.3 (0.3)	−1.3‡ (0.3)
Large HDL (mg/dL)	Mean ± SD	17 ± 10	20 ± 11	28 ± 14	30 ± 14	10.4‡ (0.8)	2.6‡ (0.8)

*The mean race and sex difference for each characteristic was assessed in regression models that included race, sex, age, age^2, and age^3 as predictor variables. A minus sign indicates that the mean level is higher among boys than among girls. Standard errors of the mean differences are shown in parentheses.

†Levels of HDLC were determined chemically: all other characteristics were measured using NMR.

‡$P < .001$.

were evident throughout the entire distribution. Of the HDL subclasses, the mean level of large HDL was substantially higher among black children (29 mg/dL) than among white children (18 mg/dL), a difference that paralleled the black/white difference in levels of HDLC; in contrast, mean levels of small HDL did not vary by race. (Whereas mean levels of large and small HDL were almost identical among white boys, the ratio of large to small HDL was about 2.0 among black children.) Sex differences were generally smaller, but as compared with boys, girls had higher levels of large HDL along with lower levels of small HDL.

Smoothed levels of HDLC and average HDL size by age are shown in Fig 1. HDLC levels were higher among blacks than among whites, and before approximately 12 years of age, boys had higher levels than did girls. However, HDLC levels among boys were inversely associated with age, and by 14 years of age, mean levels were higher among girls. Despite its strong association with the HDLC level ($r = .76$), the mean HDL particle size was larger among girls than boys at all ages (Fig 1B). As assessed in linear regression models, age significantly modified the male/female difference in HDLC levels ($P < .001$), but not the sex difference in mean HDL particle size. Furthermore, the sex difference in HDL particle size among white children reached a maximum between the ages of 12 and 13 years, an age at which boys and girls had very similar levels of HDLC.

Smoothed levels of HDL subclasses by age are shown in Fig 2. Levels of small HDL (Fig 2A) were significantly ($P < .001$) higher among boys than among girls at all ages, but with the possible exception of an increase with age among white boys ($r = .11$, $P = .08$), there was little difference in levels of small HDL by age or race. In contrast, mean levels of large HDL (Fig 2B) were (1) 8 to 14 mg/dL higher among blacks than whites at all ages, (2) generally higher among girls than among boys, and (3) inversely associated ($r = -.21$) with age among boys, but not girls. Similar to the results for HDLC levels, the sex difference in levels of large HDL varied significantly by age, with the female excess most evident among older adolescents. Among children over 16 years of age, white boys had the lowest levels of large HDL and the highest levels of small HDL, resulting in a mean ratio (large HDL ÷ small HDL) of 0.75. In contrast, mean levels of this ratio among 16- and 17-year-olds ranged from 1.5 to 2.0 in other race-sex groups.

The relationship of HDL size and subclass levels to lipids and other correlates is shown in Table 3 (all correlations have been adjusted for race, sex and age). The mean HDL size was strongly associated (inversely) with levels of triglycerides, insulin, and relative weight ($r = -.40$ to $-.54$); a child at the 75th percentile of each of these characteristics had, on average, a 0.3 nm smaller mean HDL particle size than did a child at the 25th percentile. Many of the inverse associations with HDL size were stronger than those with the HDLC level. Correlations with relative weight, for example, were −0.54 (HDL size) and −0.32 (HDLC), reflecting contrasting relations with levels of large ($r = -.50$) and small ($r = .15$) HDL subclasses. In addition, levels of LDL cholesterol were not related to HDLC ($r = -.06$), but they were associated positively ($r = .21$) with small HDL and inversely ($r = -.18$) with large HDL. Adjustment for HDLC levels reduced the magnitudes of most associations only slightly.

We then examined whether the observed race and sex differences in HDL size were independent of lipid and lipoprotein levels (Fig 3). Because HDLC levels and HDL particle size were strongly correlated ($r = .76$), it would be possible, for example, for the racial difference in HDL particle size to be entirely due to the higher HDLC level among black children. However, even at comparable levels of HDLC, there were still racial (Fig 3A) and sex (Fig 3B) differences in HDL particle size. Regression analyses (not shown) indicated that adjustment for levels of triglycerides, HDLC, and LDLC reduced the magnitude of the race difference in HDL size (from 0.3 to 0.1 nm), but did not alter the 0.1 nm difference between girls and boys ($P < .001$ for each difference).

DISCUSSION

HDL subclasses differ substantially in their relationship to many characteristics. As compared with white children, we found that black children had higher mean levels of large HDL, but similar levels of small HDL (Table 2). Furthermore, girls

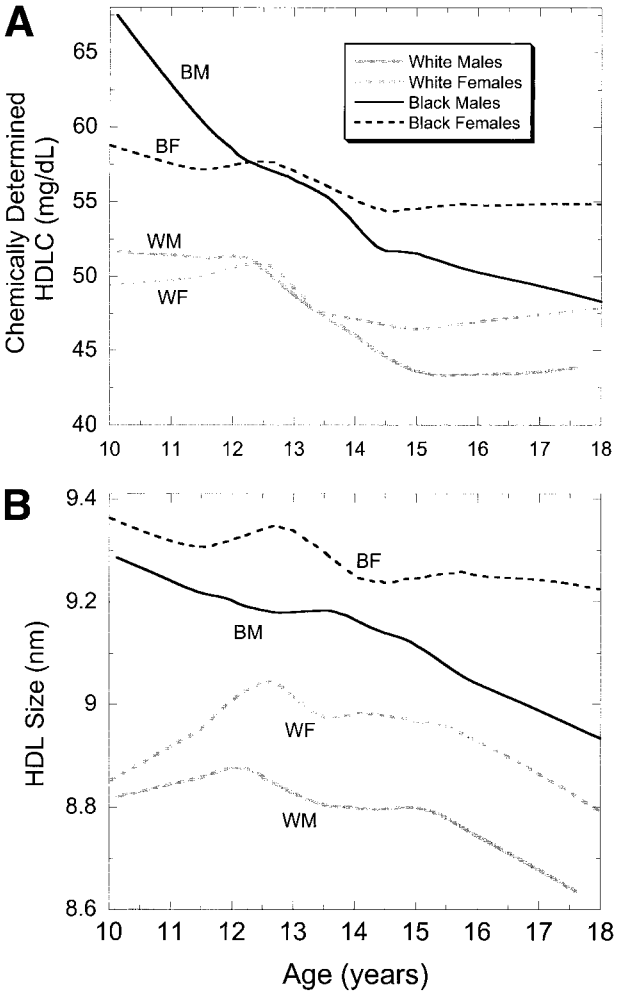

Fig 1. Smoothed levels of HDLC (A) and average HDL size (B) by age. Within each race-sex group, lowess curves were constructed using a linear regression fitting procedure with a neighborhood width of 50%; these smoothed levels roughly correspond to median values. Age was calculated as the number of days between the examination and birth dates divided by 365.25. BF, black females; BM, black males; WF, white females; WM, white males.

had a larger mean HDL particle size than did boys, and the decrease in HDLC levels during adolescence among boys was almost entirely due to large HDL (Fig 2). The inverse relationship of HDLC levels to triglycerides and relative weight were also limited to large HDL subclasses, and the lack of correlation with LDL cholesterol was due to the contrasting associations with small (positive) versus large (inverse) HDL (Table 3). Because the standard measurement of HDLC would obscure these associations, it has been suggested[10,30] that the analysis of HDL subclasses may help elucidate associations with CHD risk. Several results[12-15] suggest that the inverse relationship of HDLC levels to coronary artery disease is limited to the larger subclasses (HDL_{2a} and HDL_{2b}), whereas levels of small HDL are positively associated with disease severity. We have shown[21,22] that NMR and GGE determinations of HDL particle size are highly ($r = .88$) correlated.

It has been known for several decades that levels of HDL cholesterol decrease among boys during adolescence,[1-3] but we found this decrease to be limited to the large HDL subclass; levels of small HDL, in contrast, tended to increase with age among white boys (Fig 2). It is conceivable that the increased CHD risk among men (particularly, white men) is due to a low level of HDLC and a relatively high level of small HDL, a profile that develops during adolescence. Although other studies of GGE-determined HDL subclasses among children have typically included fewer than 100 subjects,[17,18] the sex and age differences that we observed in Table 2 and Fig 2 are in good agreement with previous findings.

Despite the high prevalence of hypertension among black men, their rates of CHD are no higher than those among white men, and it has been suggested that this may, in part, be due to differences in levels of HDLC.[31,32] Previous studies of black/white differences in HDL subclasses have typically examined only HDL_2 and HDL_3 (as determined by selective precipitation), and while blacks generally have higher levels of both subfractions,[16,32,33] it is likely that HDL_3 does not reflect levels of the smallest HDL subclasses.[14,30] As compared with whites, black adults have higher levels of apolipoprotein A-I, but

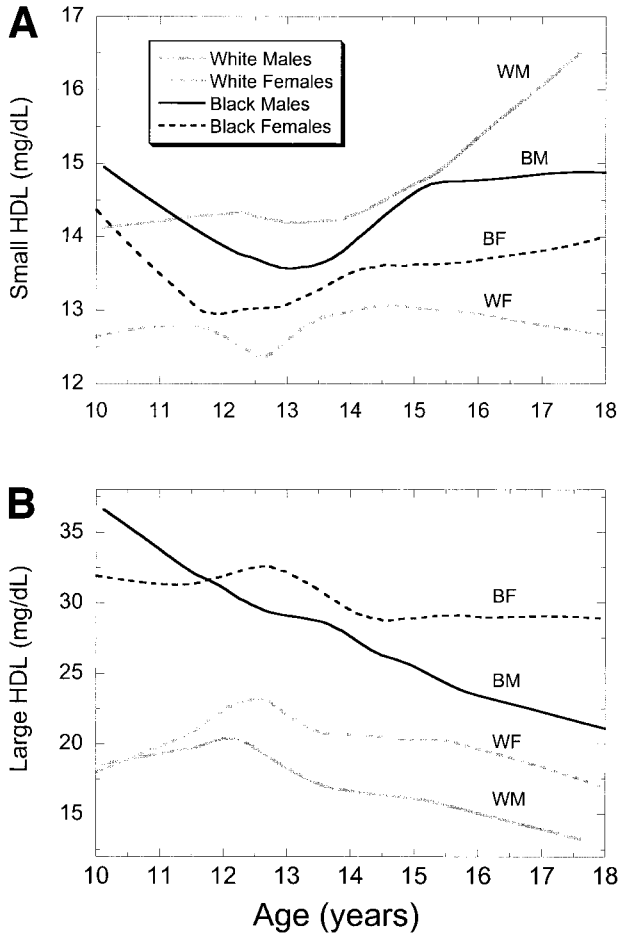

Fig 2. Smoothed levels of small HDL (A) and large HDL (B) by age. Within each race-sex group, lowess curves were constructed using a neighborhood width of 50%.

Table 3. Relation of HDL Size and HDL Subclasses to Levels of Lipids, Insulin, and Relative Weight

	Total Cholesterol	Triglycerides	LDL Cholesterol	HDLC*	LpA-I†	LpA-I: LpA-II†	Insulin	Relative Weight (W/H^3)
HDLC*	0.22‡	−0.34	−0.06	—	0.61	0.05	−0.29	−0.32
HDL Size	−0.05	−0.46	−0.25	0.76	0.40	−0.18	−0.40	−0.54
	(−0.35)§	(−0.34)	(−0.31)		(0.57)	(0.04)	(−0.28)	(−0.48)
Small HDL	0.19	0.15	0.21	−0.09	0.12	0.05	0.08	0.15
	(0.22)	(0.13)	(0.20)		(0.09)	(0.12)	(0.05)	(0.13)
Large HDL	0.05	−0.44	−0.18	0.86	0.49	−0.12	−0.38	−0.50
	(−0.28)	(−0.31)	(−0.23)		(0.45)	(−0.10)	(−0.27)	(−0.46)

*Levels of HDLC were determined chemically; HDL size, along with small, intermediate, and large HDL were measured using NMR.

†LpA-I and LpA-I:LpA-II determinations were available for 726 of the 918 subjects.

‡Values are Spearman correlation coefficients. With a sample size of 918, a correlation of 0.07 would be statistically significant at the 0.05 level, and a correlation of 0.11 would be statistically significant at the 0.001 level.

§Values in parentheses have been adjusted for levels of HDLC in addition to race, sex, and age.

similar levels of apolipoprotein A-II,[34] findings that agree with our observation (Table 2) that levels of large (but not small) HDL are elevated among black children.

As seen among adults[14,30,35,36] and newborns,[20] we also found that triglyceride levels were correlated inversely with levels of large HDL and positively with levels of small HDL (Table 3). These divergent associations were reflected in the inverse relationship of triglyceride levels to the mean HDL particle size[30,36] and may result from the bidirectional exchange of lipids between HDL and VLDL, with the subsequent hydrolysis of triglyceride.[35] Other investigators[17,36-38] have also found that relative weight and insulin levels are related inversely to levels of the larger HDL subclasses, but positively to levels of the smaller HDL subclasses. The differing relationships of LDL cholesterol to levels of small HDL (positive) and large HDL (inverse) likely accounts for the lack of association between levels of LDL cholesterol and HDLC (Table 3). Therefore, it may be important to control for levels of large and small HDL subclasses when assessing the relationship of LDL cholesterol to CHD; adjustment for (total) HDLC could result in overestimating the strength of the independent relationship of LDL cholesterol to disease risk.

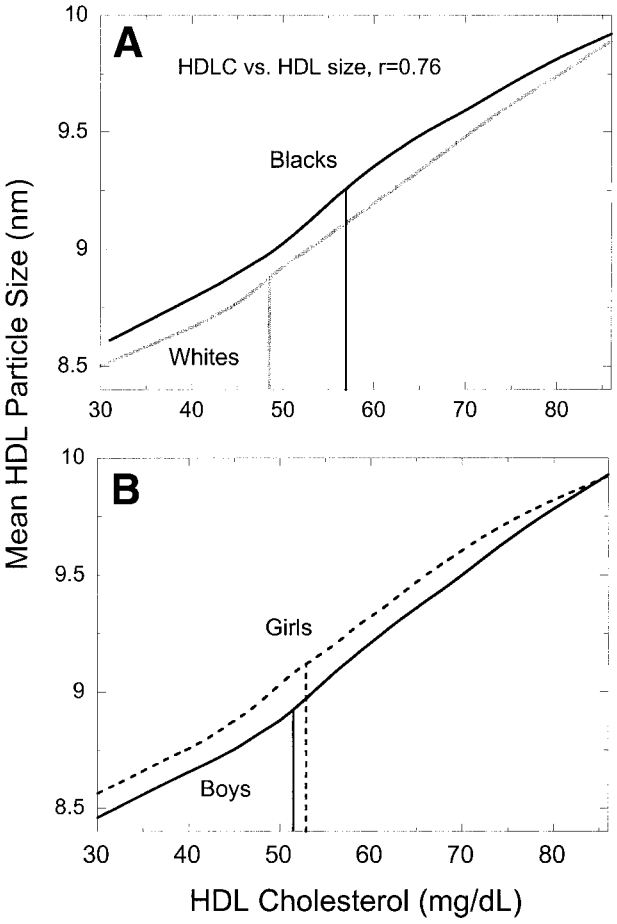

Fig 3. Smoothed levels of average HDL size by levels of HDLC. Lowess curves were calculated separately for white and black children (A) or for boys and girls (B). The vertical lines represent the median HDLC levels within race and sex groups.

Although we found that the HDL size and large HDL determinations were highly correlated ($r \geq .98$) among duplicate specimens, the possible influence of laboratory error on these cross-sectional analyses should be considered. NMR-determined levels of small HDL were less reproducible, but the measurement error (CV = 10%) was similar to the error obtained with other techniques.[14,17] Indirect support for the validity of the NMR determinations is provided by the similarity of our findings with those observed using GGE.[17,18,35-38] Of particular interest is the relationship ($r = .57$, Table 3) of HDL size to LpA-I, which is preferentially found in large HDL particles.

It should be realized that although the quantification of HDL_2 and HDL_3 by ultracentrifugation or selective precipitation has not improved the prediction of CHD,[39,40] it is likely that HDL_3 levels reflect intermediate-size HDL subclasses rather than the smallest subclasses.[14,30] For example, we have previously found that levels of both HDL_2 and HDL_3 are higher among black adolescents than among whites;[16] the different results in the current study (Table 2) for levels of small HDL are likely to be, at least in part, due to differences in laboratory methods. Although we have also found that levels of LpA-I: A-II are higher among white adolescents than among blacks,[25] the classification of HDL subclasses according to apolipoprotein composition differs substantially from one based on particle size.

The contrasting associations of small and large HDL subclasses to sex, race, lipids, and other characteristics suggest that the assessment of HDL-related CHD risk may be more complex than generally believed. These associations should be examined in additional studies, and it would be important to determine if levels of specific HDL subclasses or HDL size can predict CHD risk more accurately than levels of HDLC. The advantages of lipoprotein subclass analyses with NMR spectroscopy, particularly its rapid measurement, make it feasible to examine these associations in large studies.

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