Black-white differences in cholesterol levels of serum high-density lipoprotein subclasses among children: the Bogalusa Heart Study

S. R. Srinivasan, Ph.D., D. S. Freedman, Ph.D., L. S. Webber, Ph.D., and Gerald S. Berenson, M.D.

ABSTRACT Cholesterol levels of serum high-density lipoprotein (HDL) subclasses, HDL$_2$ and HDL$_3$, were examined in a random subsample (n = 561) of children (7 to 17 years of age) from a total biracial community. Overall, black children in younger (7 to 10 years) and older (11 to 17 years) age groups alike had significantly higher HDL$_2$ cholesterol (HDL$_2$-C) and HDL$_3$-C than their white counterparts. In addition, black children had a relatively higher frequency of joint occurrence of high levels of both HDL$_2$-C and HDL$_3$-C. A significant sex-related difference, with girls showing higher values than boys, was noted among younger age groups for HDL$_2$-C. A male-female crossover trend in HDL$_2$-C levels was apparent only among white children, with girls showing higher values after age 11. Both age and sexual maturation were inversely associated with HDL$_3$-C levels in white children, irrespective of sex (p < .001). Serum triglycerides were inversely related to both HDL$_2$-C and HDL$_3$-C only in white children (p < .001). A black-white difference in HDL$_2$-C persisted only among boys and girls in the older age group after adjusting for the covariates (sexual maturation, age, adiposity, oral contraceptive use, cigarette smoking, alcohol use, and serum triglycerides). With respect to HDL$_3$-C, the covariate-adjusted difference remained significant only among boys in the older age group. Metabolic variations between the races in response to both physiologic and environmental factors likely account for the divergence in antiatherogenic HDL pattern.

HDL₂ and HDL₃ in 5- to 17-year-old black and white children of both sexes.

Materials and methods

Population. The Bogalusa Heart Study is a long-term epidemiologic study of cardiovascular disease risk factors from birth through early adulthood in the biracial community (65% white, 35% black) of Bogalusa, LA. In 1984-1985, 2666 children in grades 3 to 12 were examined, representing 85% of all eligible individuals. Studies involving HDL₂-C and HDL₃-C were restricted to a 25% random subsample (561) of the 2327 fasting children. Results from six children were deleted from the data analyses because of missing data, leaving a total of 555 children. In this group there were 67% whites, 33% blacks, 52% boys, and 48% girls.

The Louisiana State University Medical Center Institutional Review Board reviews the experiments related to human subjects. Informed consent (written) was obtained from a parent or guardian of each child. Confidentiality was stressed throughout the study. For data handling purposes each subject was identified by a code number.

General examination. Sexual maturation was determined by visual assessment of secondary sex characteristics during a physical examination according to the method of Tanner. The ratings for sexual maturation ranged from 1 (no development) to 5 (complete development), according to the stages of female breast or male genitalia development. Subcapular skinfold thickness was measured, and is used as a measure of adiposity.

Information on lifestyle characteristics was obtained by questionnaires concerning smoking (grades 3 to 12), alcohol intake (grades 7 to 12), and oral contraceptive use (girls, grades 7 to 12).

Collection of blood specimens. Children were instructed to fast for 12 to 14 hours, and compliance was determined by interview on the morning of examination. Serum samples were obtained from antecubital venous blood and were sent in a cold-packed box by bus to the New Orleans Core Lipid Laboratory where it was kept at 4°C. Laboratory analyses were performed on the following day.

A random subsample (25%) of the fasting serum samples was selected for the analysis of HDL₂-C and HDL₃-C during each screening day. In addition, a second blood sample (blind duplicate) was collected on each screening day (an approximate 10% random subsample) to estimate measurement error.

Serum lipids. Serum total cholesterol and triglyceride levels were determined in a Technicon AutoAnalyzer II according to the laboratory manual of the Lipid Research Clinics Program. The laboratory has been designated as standardized by the Centers for Disease Control (CDC) in Atlanta.

Serum HDL₂-C and HDL₃-C. Cholesterol levels of the HDL subclasses were determined according to the method of Gideon et al. with some modifications. Total HDL cholesterol (HDL-C) and HDL₂-C in serum were measured directly by heparin microaffinity column chromatography (Isolab Inc., Akron, OH) and heparin-manganese-dextran sulfate precipitation procedures, respectively; HDL₃-C was calculated as the difference between these two values.

Cholesterol levels in the HDL fractions were determined with use of the enzymatic reagent according to the manufacturer's protocol (Boehringer/Mannheim Diagnostics, Houston). This enzymatic reagent was chosen because it has Tris rather than phosphate as the buffer; the manganese ion is known to interfere with enzymatic cholesterol assay systems involving phosphate buffer. Quality-control specimens from the CDC were used after diluting them to obtain cholesterol levels in the desired range for the analysis.

Measurement errors for HDL-C, HDL₂-C, and HDL₃-C are listed in table 1. The measurement error includes errors associated with collection, processing, and analysis of the samples, as well as with data processing. The relatively high coefficient of variation of HDL₂-C might be explained in part by its low concentration; furthermore, HDL₃-C is measured indirectly, and therefore the measurement is subject to errors in measurement for both HDL-C and HDL₃-C. It should be noted, however, that both HDL₂-C and HDL₃-C have the same intraclass correlation coefficient, reflecting the same order of reliability of measurement in both cases.

Statistical analyses. Race and sex differences in levels of HDL₂-C and HDL₃-C were examined by t tests. For this purpose data were grouped into prepubertal (7 to 10 years) and pubertal (11 to 17 years) groups based on sample size considerations and for physiologic reasons. Levels of HDL₂-C and HDL₃-C were divided into quintiles and these categories were then cross-tabulated to examine their joint distribution. Categories were constructed with the use of all children; the cross-tabulations are shown separately for each race.

The association of HDL₂-C and HDL₃-C levels with serum lipids and total HDL-C were examined with Spearman (rank-order) correlation coefficients. Within each race-sex group, polynomial regression models (including age, age², and age³ terms as predictor variables) were used to estimate the relation of levels of HDL₂-C and HDL₃-C with age. Since all HDL measures did not show a linear relation to age, a nonlinear model was considered appropriate. The relations of age and sexual maturation to HDL₂-C and HDL₃-C were also assessed with Spearman correlation coefficients. Analysis of covariance was used to determine if black-white differences in mean levels of HDL₂-C and HDL₃-C existed for younger (7 to 10 years) and older (11 to 17 years) age groups after appropriately controlling for sexual maturation (Tanner stage), age, age², age³, subcapular skinfold thickness, cigarette smoking (number/week), alcohol consumption (ml/week), and oral contraceptive use.

Results

Distributions and mean levels. Race-specific distributions of HDL₂-C and HDL₃-C are shown in figure 1; the mean values by race, sex and age group are given in table 2. The distributions of HDL₂-C and HDL₃-C were unimodal with median values very close to the means. However, the distributions were skewed to the right, particularly levels of HDL₂-C in black children. Overall, black children of younger (7 to 10 years) and

<table>
<thead>
<tr>
<th>Variable</th>
<th>No. of pairs (blind)</th>
<th>Mean (mg/dl)</th>
<th>Measurement error</th>
<th>Intraclass correlation coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>HDL₂-C²</td>
<td>76</td>
<td>58.7</td>
<td>58.7</td>
<td>3.0</td>
</tr>
<tr>
<td>HDL₃-C²</td>
<td>76</td>
<td>44.7</td>
<td>45.0</td>
<td>2.2</td>
</tr>
<tr>
<td>HDL₂-C³</td>
<td>76</td>
<td>14.0</td>
<td>13.7</td>
<td>3.4</td>
</tr>
</tbody>
</table>

CV = coefficient of variation

²Difference between duplicates not significant (paired t test).

²Measured directly.

³Measured indirectly (HDL₂-C = HDL-C - HDL₃-C).
older (11 to 17 years) age groups alike had significantly higher levels of HDL$_{2}$-C and HDL$_{3}$-C than their white counterparts. A significant male-female difference, with boys showing higher levels than girls, was noted among those in the younger age group for HDL$_{2}$-C.

Race-specific cross-tabulations of HDL$_{2}$-C and HDL$_{3}$-C (by quintiles) were then examined (table 3). At any given combination of HDL$_{2}$-C and HDL$_{3}$-C quintiles (e.g., quintiles 1-1, 5-1, 3-3, etc.) the percentage of children having that combination of lipoprotein cholesterol varied between the races. Changes in the percentage frequencies of lipoprotein cholesterol combinations from the upper left to the lower right in table 3 showed that, in general, a relatively greater number of black children had high levels of both HDL$_{2}$-C and HDL$_{3}$-C, whereas white children had a relatively higher frequency of joint occurrence of low levels of HDL$_{2}$-C and HDL$_{3}$-C. In addition, a lack of association between HDL$_{2}$-C and HDL$_{3}$-C was noted in children of both races.

**Relation to serum lipids and HDL-C.** The association of HDL$_{2}$-C and HDL$_{3}$-C levels to serum total cholesterol, triglycerides, and HDL-C in the four race-sex groups are shown in table 4. With respect to total cholesterol, HDL$_{2}$-C showed a significant positive correlation in white girls and black boys; a similar relationship was noted for HDL$_{3}$-C in white children of both sexes and in black boys. Both HDL$_{2}$-C and HDL$_{3}$-C were significantly inversely related to triglycerides only in white children of both sexes. Serum total HDL-C showed a strong direct relation to both HDL$_{2}$-C and HDL$_{3}$-C in

![Figure 1](http://circ.ahajournals.org/)

**FIGURE 1.** Distributions of HDL$_{2}$-C and HDL$_{3}$-C in 7- to 17-year-old children by race.

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### Table 2

| Serum levels (mean ± SD) of HDL$_{2}$-C and HDL$_{3}$-C in children by race, sex, and age group: the Bogalusa Heart Study |
|-----------------|-----------------|-----------------|-----------------|------------------|------------------|
| Age (yr)        | Boys            | Girls           | Race difference$^A$ | Sex difference$^A$ |
| HDL$_{2}$-C (mg/dl) | Black           | White           | Black           | White           | p<.001           | p<.05           |
| 7-10            | 18 ± 9          | 14 ± 8          | 15 ± 8          | 11 ± 9          | p<.001           | p<.05           |
| (n = 32)        | (n = 60)        | (n = 26)        | (n = 55)        |                  |                  |                  |
| 11-17           | 16 ± 9          | 11 ± 7          | 16 ± 8          | 13 ± 8          | p<.001           | NS              |
| (n = 67)        | (n = 132)       | (n = 61)        | (n = 128)       |                  |                  |                  |
| HDL$_{3}$-C (mg/dl) | 49 ± 9          | 47 ± 6          | 51 ± 7          | 47 ± 7          | p<.05            | NS              |
| 7-10            | (n = 32)        | (n = 60)        | (n = 26)        | (n = 55)        |                  |                  |
| 11-17           | 47 ± 7          | 42 ± 7          | 45 ± 6          | 43 ± 6          | p<.0001          | NS              |
| (n = 67)        | (n = 132)       | (n = 61)        | (n = 128)       |                  |                  |                  |

$^A$All children.
TABLE 3
Bivariate distributions of HDL₂-C and HDL₃-C in black (n = 184) and white (n = 371) children: the Bogalusa Heart Study

<table>
<thead>
<tr>
<th>HDL₃-C quintile</th>
<th>1 (Low)</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5 (High)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>HDL₂-C quintile</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Black</td>
<td>1.0</td>
<td>2.1</td>
<td>3.2</td>
<td>4.3</td>
<td>5.4</td>
<td>10.7</td>
</tr>
<tr>
<td>White</td>
<td>1.2</td>
<td>2.3</td>
<td>3.4</td>
<td>4.5</td>
<td>5.6</td>
<td>12.9</td>
</tr>
<tr>
<td>Total</td>
<td>2.2</td>
<td>4.4</td>
<td>6.6</td>
<td>8.8</td>
<td>11.1</td>
<td>25.4</td>
</tr>
</tbody>
</table>

Values are percent frequency.

all the race-sex groups. However, there was only very weak association between HDL₂-C and HDL₃-C, with correlation coefficients ranging from −0.04 to 0.10 in the four race-sex groups (data not shown in table).

Relation to age and sexual maturation. Levels of HDL₂-C and HDL₃-C were then related to age and sexual maturation (Tanner stage) in the four race-sex groups (table 5). Overall, HDL₂-C showed no association with either age or sexual maturation in any of the race-sex groups. In contrast, both age and sexual maturation were significantly inversely associated with HDL₃-C levels in white children of both sexes; among black children, age was significantly associated with HDL₃-C levels only in girls.

Race- and sex-specific levels of HDL₂-C and HDL₃-C between the ages of 7 and 17 years were estimated by a polynomial model regression to evaluate further the age-related trends. White children (figure 2, left), unlike black children (figure 2, right), showed a male-female crossover trend in HDL₂-C levels at age 11, indicating an interaction of age and sex. Before this age, higher levels were seen in boys, but girls had higher levels after this age. HDL₂-C levels tended to increase in girls between ages 7 and 11 years, whereas levels decreased in boys between the ages of 11 and 13 years, resulting in the male-female crossover. No such trends were seen for HDL₃-C in either white (figure 3, left) or black (figure 3, right) children.

Evaluation of black-white differences. Black-white differences (absolute and percent) in HDL₂-C and HDL₃-C levels were examined separately for each sex group in younger and older children, controlling for possible confounding covariates (sexual maturation, age, adiposity, cigarette smoking, alcohol use, and oral contraceptive use) (table 6). Because serum triglycerides (very low-density lipoproteins or VLDL) are inversely related to HDL₁,₁₇,₃₁ and black-white differences in triglycerides and VLDL (whites > blacks) are known,₁₇,₃₂ triglyceride was considered as an additional covariate. In the younger age group (7 to 10 years),

<table>
<thead>
<tr>
<th>HDL₂-C</th>
<th>Cholesterol</th>
<th>Triglyceride</th>
<th>HDL-C</th>
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</thead>
<tbody>
<tr>
<td>White boys</td>
<td>189</td>
<td>.18</td>
<td>−.30&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>White girls</td>
<td>181</td>
<td>.25&lt;sup&gt;b&lt;/sup&gt;</td>
<td>−.25&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Black boys</td>
<td>98</td>
<td>.32&lt;sup&gt;b&lt;/sup&gt;</td>
<td>−.09</td>
</tr>
<tr>
<td>Black girls</td>
<td>86</td>
<td>.21</td>
<td>.17</td>
</tr>
<tr>
<td>HDL₃-C</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White boys</td>
<td>189</td>
<td>.34&lt;sup&gt;b&lt;/sup&gt;</td>
<td>−.21&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>White girls</td>
<td>181</td>
<td>.26&lt;sup&gt;b&lt;/sup&gt;</td>
<td>−.22&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Black boys</td>
<td>98</td>
<td>.41&lt;sup&gt;b&lt;/sup&gt;</td>
<td>.03</td>
</tr>
<tr>
<td>Black girls</td>
<td>86</td>
<td>.23</td>
<td>−.05</td>
</tr>
</tbody>
</table>

<sup>a</sup>p < .01; <sup>b</sup>p < .001.

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TABLE 4
Relation of HDL₂-C and HDL₃-C to serum lipids and HDL-C in children by race and sex: the Bogalusa Heart Study

<table>
<thead>
<tr>
<th>Spearman rank correlation</th>
<th>n</th>
<th>Cholesterol</th>
<th>Triglyceride</th>
<th>HDL-C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White boys</td>
<td>190</td>
<td>−.11</td>
<td>−.43&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>White girls</td>
<td>181</td>
<td>.11</td>
<td>−.34&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Black boys</td>
<td>99</td>
<td>−.17</td>
<td>−.17</td>
<td></td>
</tr>
<tr>
<td>Black girls</td>
<td>86</td>
<td>.07</td>
<td>−.36&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Tanner stage</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White boys</td>
<td>189</td>
<td>−.13</td>
<td>−.42&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>White girls</td>
<td>178</td>
<td>.05</td>
<td>−.28&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Black boys</td>
<td>96</td>
<td>−.14</td>
<td>−.19</td>
<td></td>
</tr>
<tr>
<td>Black girls</td>
<td>86</td>
<td>—</td>
<td>−.16</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>p < .001.
the differences in HDL subclasses became statistically not significant after adjusting for the covariates (including triglycerides) in both sexes. On the other hand, in the older age group (11 to 17 years), the observed black-white differences in HDL subclasses were independent of differences in covariates (including triglycerides), with the possible exception of HDL_{2-C} differences among girls (table 6).

Discussion

The present report described the serum HDL_{2-C} and HDL_{3-C} profiles in a biracial sample of children from a community-based study. Earlier studies in children from the same community suggested the possibility of race-related differences in HDL subclasses based on the finding that the HDL-C/apolipoprotein (apo) A-I ratio was higher in blacks than in whites. Recently Tyrold et al., who found higher apo A-I/apo A-II ratios in adult blacks (especially men), hypothesized that levels of HDL_{2} may be relatively higher among blacks. These suggestions are based on the fact that the HDL subclasses differ in their composition, with HDL_{2} having relatively more cholesterol and less apo A-II molecules per mole of apo A-I. The results from
the present study show for the first time that the black-white difference in HDL-C (blacks > whites) is due to significant differences in both HDL₂-C and HDL₃-C subclasses.

The cross-sectional relation of HDL subclasses to both age and sexual maturation are discernible for HDL₂-C among white children, with values showing significant inverse associations. Of particular interest is the occurrence of a male-female crossover in HDL₂-C levels among white children, with girls showing lower values before age 11 and boys showing lower values after that age (figure 2). This pattern appears to continue through adulthood because white women are known to have higher HDL₃ than men. Although a similar crossover trend is not seen for HDL₃-C among black children, a recent report in young black adults indicates a sex differential (females > males) for both HDL₂-C and HDL₃-C. It is possible that in blacks HDL transitions, leading to characteristic adult pattern, occur after the completion of sexual maturation. Endogenous sex hormones, depending on race and sex, may in part influence the observed lipoprotein transitions in adolescence.

The finding that the relation of HDL₂-C and HDL₃-C is minimal suggests the occurrence of varying proportion of HDL subclasses among individuals. The bivariate distribution of HDL₂-C and HDL₃-C reflects this lack of relationship (table 3). The inverse relations of HDL₂-C and HDL₃-C to serum triglycerides among white children are in the expected directions, given the metabolic and functional associations between HDL and VLDL. The inverse association can be attributed to the finding that HDL particles are in part derived from the lipolysis of triglyceride-rich lipoproteins. Interestingly, inverse associations between HDL subclasses and triglycerides are not seen among black children, probably reflecting the low triglyceride (VLDL) levels in this group.

The present study shows that the black-white differences in levels of HDL subclasses are independent of confounding variables such as sexual maturation, age, adiposity, oral contraceptive use, cigarette smoking, and alcohol use in the older age group (11 to 17 years), with the possible exception of HDL₂-C difference among girls. The lack of a similar finding in the younger age group (7 to 10 years) may be either due to a smaller sample size or to an age-related phenomenon, with differences becoming stronger after prepubertal age. Earlier studies show that the black-white difference in HDL becomes clearly established at around 9 years of age and remains throughout adulthood. In the Bogalusa Heart Study, cross-sectional and longitudinal dietary studies have shown no significant differences between the two racial groups. Information is lacking on the amount of physical activity and its effect on HDL in the two racial groups. Although dietary intake and physical activity were not taken into account in the present study, it has been suggested that these variables could not account for the black-white difference in HDL-C. It is likely that metabolic responses to physiologic and environmental variables may be different between the two racial groups.

The observed black-white difference is also independent of serum triglycerides, which is relatively higher in whites than blacks, especially among the older age group. This implies that the black-white difference in cholesterol levels of HDL subclasses are not solely due to compositional changes related to bidirectional transfer of cholesteryl ester and triglycerides.
between HDL and VLDL.\textsuperscript{43-45} Instead, this black-white difference may reflect differences in lipoprotein particle number, since apo A-I, an indicator of HDL particle number, is significantly higher in blacks (especially men and boys) than in whites.\textsuperscript{20, 33} Because the catabolism of triglyceride-rich lipoproteins is a determinant of HDL concentrations (particle number), especially HDL\textsubscript{2},\textsuperscript{30, 46} it is tempting to speculate that blacks may inherently have an efficient lipid-clearing mechanism.

It should be noted that with respect to carbohydrate tolerance, a strong biologic determinant of lipoprotein metabolism,\textsuperscript{47, 48} black children showed significantly lower fasting plasma glucose and higher postglucose plasma insulin, insulin/glucose ratios, and insulin/free fatty acid ratios than white children, independent of obesity.\textsuperscript{49} These findings imply inherent metabolic differences between the two racial groups, resulting in divergent lipoprotein responses to similar physiologic and environmental impacts. However, as a caveat, certain limitations of the study with respect to statistical approaches to explain biologic variability should be taken into account before any generalization of the current findings is made. Comparative lipoprotein metabolic studies are obviously needed to illuminate the biologic basis for the racial differences in levels of HDL subclasses.

Among the HDL subclasses, HDL\textsubscript{2} is specifically inversely related to CHD.\textsuperscript{13, 14} It is of interest that HDL\textsubscript{2}-C levels are relatively higher among blacks, whereas the frequency of the joint occurrence of low levels of HDL-C and HDL\textsubscript{2}-C is relatively higher among whites. These results favor the idea that the low CHD risk among adult black men may be due in part to their antiatherogenic HDL pattern.\textsuperscript{8}

We are grateful to the children of Bogalusa and their parents, without whom this work would not have been possible. We thank the Bogalusa Heart Study Field Staff for data collection and the Core Laboratory Staff, especially Joanne Comeaux, for technical assistance.

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