Serum Lipoprotein Profile in Children from a Biracial Community

The Bogalusa Heart Study

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SUMMARY Serum lipoprotein profiles in 3182 children, ages 5–14 years, were studied in a biracial community as part of the Bogalusa Heart Study to describe the early natural history of atherosclerosis. White and black children showed similar mean levels of β-lipoproteins. Pre-β-lipoprotein levels, however, were significantly higher in white children, while significantly higher levels of α-lipoprotein were found in black children. Girls had generally higher levels of β- and pre-β-lipoprotein and lower levels of α-lipoprotein than boys, although the differences were not significant at each age group. With age there was little change in α-lipoprotein levels, a significant increase in pre-β-lipoprotein levels and a slight but significant decrease between 11 and 14 years in β-lipoprotein levels. The correlation of α-lipoprotein was negative with β-lipoprotein and, to a greater extent, with pre-β-lipoprotein. The above inverse relationships were significantly greater in white children than in black children, suggesting differences in lipoprotein profiles in the two groups. Lipoprotein values from a total community study are now available for comparison with the currently recommended upper normal limits for lipoproteins. Since only a very small percentage of children could be considered as hyperlipoproteinemic by those specific levels in this community, we suggest that distributions and percentiles be used to evaluate children for hyperlipoproteinemia.

ELEVATED LEVELS of certain serum lipoprotein fractions have been clearly recognized as one of the important risk factors for coronary artery disease. It is also known that coronary atherosclerosis begins early in life;1–8 consequently, attention has recently been focused on risk factors in children.4,8 There has been a surge of interest in identifying problems associated with hyperlipoproteinemia in children and in comparing lipoprotein levels of parents and their children.7–11 Serum lipids in free-living children have been studied mostly in terms of cholesterol and triglycerides.15–16 The introduction of simple methods to determine lipoproteins for clinical purposes now allows studies to be performed on large numbers of children. Recently serum lipoprotein profiles have been reported in only a limited number of free-living children.17–19 It was apparent from these studies that the extreme individual variability in lipoprotein levels makes larger samples necessary to detect subtle differences possibly related to race, sex, and early age.

The Bogalusa Heart Study was initiated as part of a Specialized Center of Research—Atherosclerosis (SCOR-A) in a biracial community (Bogalusa, Louisiana) to study the early natural history of atherosclerosis in a large pediatric population. The serum cholesterol and triglyceride levels in 5 to 14-year-old children of this community have been reported elsewhere.20 The following study describes the serum lipoprotein profile in these children.

Materials and Methods

Population Sample

We examined 3524 children, representing 93% of all children ages 5–14, residing in Ward 4 (Bogalusa) of Washington Parish, Louisiana, for coronary artery disease risk factors. Of the 1840 boys and 1684 girls examined, 37% were black and 63% were white. Children of all ages were examined throughout the school year to account for seasonal influences on serum lipoprotein levels. Children were instructed to fast for 12–14 hours prior to the exam. Accord-
ing to the fasting compliance, which was determined by interview on the morning of the examination. 7.5% of the children were nonfasting and were not included in the present study. Blood samples could not be obtained in 2.2% of the children.

Collection of Blood Specimens

Venous blood was collected in vacutainer tubes and allowed to clot at room temperature for about 1½ hours. After centrifugation the sera were collected in tubes containing Thimerosal21 (Aldrich Chemical Co., Milwaukee) and sent by bus to New Orleans in a box cooled with frozen packs. The specimens arrived at the laboratory the same day and were immediately refrigerated at 4°C prior to analysis the following day.

Serum Total Cholesterol and Lipoprotein Cholesterol Determinations

All analyses were performed in the SCOR-A Core laboratory in New Orleans which has been designated as "Standardized" by the Center for Disease Control (CDC), Atlanta, Georgia and currently is in the surveillance phase of its quality control program. Serum cholesterol and triglyceride were determined simultaneously in a Technicon Auto-Analyzer II according to the protocol developed by Lipid Research Clinics in collaboration with the CDC.22 An isopropanol extract of the sample was used for the determinations. A serum calibrator, provided by the CDC, was used to convert the cholesterol value obtained by the Auto-Analyzer II to the reference standard method of Abell-Kendall.23

\[ \beta + \text{Pre-}\beta\text{-lipoprotein Cholesterol} \]

Serum \( \beta + \text{pre-}\beta\text{-lipoprotein cholesterol} \) were measured in two ways:

1) Direct method: The \( \beta \)- and pre-\( \beta \)-lipoproteins were selectively precipitated by the addition of heparin and Ca\(^{2+} \) following a procedure previously described.24-27 Briefly, this method consists of mixing serum (0.2 ml), distilled water (3.2 ml), heparin (0.25% w/v, 0.1 ml \( \approx \) 140 units/mg beef lung heparin by courtesy of Upjohn Co., Kalamazoo, Mich.), and CaCl\(_2\) (0.5M, 0.5 ml) in the order given, and centrifuging the precipitate after allowing it to stand for 15 minutes. The precipitate was analyzed for the corresponding \( \beta \)- and pre-\( \beta \)-lipoprotein cholesterol content using the Auto-Analyzer II.

2) Indirect method (turbidimetric method): The indirect turbidimetric method, which is suitable for screening on a population level, consists of mixing serum, distilled water, heparin, and Ca\(^{2+} \) in the proportion described above and measuring the turbidity (O.D.) after 15 minutes at 600 nm against a blank containing a similar mixture but omitting heparin. Since the turbidity obtained by the addition of Ca\(^{2+} \) and heparin was quantitatively related to the serum \( \beta \)- and pre-\( \beta \)-lipoprotein concentrations, it was considered as a \( \beta + \text{pre-}\beta\text{-lipoprotein index}.27 \) The cholesterol content of these two classes of lipoproteins in a given serum was indirectly obtained by multiplying this index by 500. This factor was established in our laboratory based on the observation of standard curves relating the \( \beta + \text{pre-}\beta\text{-lipoprotein cholesterol} \) and the \( \beta + \text{pre-}\beta\text{-lipoprotein index} \) over a period of five years. Good agreement between the indirect turbidimetric method and the direct method has been noted in normal adult sera.28

The serum \( \beta + \text{pre-}\beta\text{-lipoprotein cholesterol} \) was measured for all the children by the indirect method. To assess the validity of applying this indirect method in large scale screening of children on every screening day, approximately 10% of the serum samples were randomly assigned for both direct and indirect determinations of \( \beta + \text{pre-}\beta\text{-lipoprotein cholesterol} \).

\( \alpha \)-lipoprotein Cholesterol

Although the \( \alpha \)-lipoprotein cholesterol can be measured directly in the supernatant after precipitation of the \( \beta \)- and pre-\( \beta \)-lipoproteins by heparin and Ca\(^{2+} \), a twenty-fold dilution of the serum in the above precipitation method will require concentration of supernatant before cholesterol analysis. Therefore, the difference between the serum total cholesterol and \( \beta + \text{pre-}\beta\text{-lipoprotein cholesterol} \) was considered as \( \alpha \)-lipoprotein cholesterol.

The validity of the above lipoprotein cholesterol measurements was further tested by sending samples to Dr. Ralph Ellefson (Lipid Research Laboratory, Mayo Clinic, Rochester, Minn.) for independent analysis by ultra-centrifugation of pre-\( \beta \)-lipoprotein, dextran sulfate-Ca\(^{2+} \) precipitation of \( \beta \)-lipoprotein and dextran sulfate-Mn\(^{2+} \) precipitation of \( \alpha \)-lipoprotein. Analysis of sera of 32 children, including blind duplicate samples, gave the following values (mg/100 ml, mean \( \pm \) SD): \( \beta + \text{pre-}\beta\text{-lipoprotein cholesterol} \) \( \approx \) 99 \( \pm \) 16 by the present method compared to 98 \( \pm \) 18 by the Mayo method; \( \alpha \)-lipoprotein cholesterol \( \approx \) 64 \( \pm \) 10 and 65 \( \pm \) 11 by the respective methods. Analyses using the paired \( t \)-test revealed no significant differences between the two methods in determinations of either \( \beta + \text{pre-}\beta\text{-lipoprotein cholesterol} \) or \( \alpha \)-lipoprotein cholesterol.

Electrophoretic Ratios of \( \beta \)- and Pre-\( \beta \)-lipoproteins

Electrophoresis of serum was performed on agar-agarose gel according to the method of Noble28 with some modifications.29 The lipoprotein bands were scanned in a Quick Scan densitometer equipped with a digital computer printout to obtain the relative proportion of \( \beta \)- to pre-\( \beta \)-lipoprotein. Since the dye uptake (per unit weight of lipoprotein) is known to differ among the lipoprotein classes29 and because of the differences in their protein and lipid content, the densitometric ratio of \( \beta \)- to pre-\( \beta \)-lipoprotein was corrected as described previously.18

Estimation of \( \beta \)- and Pre-\( \beta \)-lipoprotein Concentrations

Serum \( \beta \)- and pre-\( \beta \)-lipoprotein concentrations were calculated as previously described.18,20,26 The calculations were based on the densitometric ratio of \( \beta \)- to pre-\( \beta \)-lipoprotein, \( \beta + \text{pre-}\beta\text{-lipoprotein cholesterol} \), and reported average values of cholesterol present in \( \beta \)-lipoprotein (46.9%) and pre-\( \beta \)-lipoprotein (22.2%).21 The validity of the lipoprotein measurements was also established by comparison of our results with those obtained by analytical ultracentrifugation. Both sets of results were in agreement,
TABLE 1.  Selected Statistics Regarding Measurement Error and the Intraclass Correlation Coefficient for Selected Serum Lipids and Lipoproteins

<table>
<thead>
<tr>
<th>Serum variables</th>
<th>Number of pairs of blind duplicates</th>
<th>Mean</th>
<th>Measurement error</th>
<th>Intraclass correlation coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>β + pre-α-lipoprotein index (optical density units)</td>
<td>432</td>
<td>0.20</td>
<td>0.01</td>
<td>5.2</td>
</tr>
<tr>
<td>Cholesterol (mg/100 ml)</td>
<td>431</td>
<td>166.45</td>
<td>9.14</td>
<td>5.5</td>
</tr>
<tr>
<td>Electrophoresis β-lipoprotein (percentage*)</td>
<td>431</td>
<td>81.80</td>
<td>3.03</td>
<td>3.7</td>
</tr>
<tr>
<td>Electrophoresis pre-β-lipoprotein (percentage*)</td>
<td>431</td>
<td>18.19</td>
<td>3.03</td>
<td>16.6</td>
</tr>
</tbody>
</table>

*Denisomeric ratio, β + pre-β-lipoprotein = 100%.

with somewhat better reproducibility using the method described above. These lipoprotein values can be easily converted into the corresponding lipoprotein cholesterol values as follows: β-lipoprotein cholesterol = mg β-lipoprotein × 0.469; pre-β-lipoprotein cholesterol = mg pre-β-lipoprotein × 0.222.

Estimation of α-lipoprotein

Serum concentrations of α-lipoprotein were obtained by multiplying α-lipoprotein cholesterol by a factor of 5.9, assuming that the cholesterol content of the α-lipoprotein molecule is 17%. Measurement Errors

Every screening day a second independent blood sample was collected in random order on approximately 12% of the children to assess the lipid and lipoprotein measurement errors. Both new names and new ID numbers were placed on the duplicate samples to ensure blind analyses. The measurement errors for the variables that were directly determined in the laboratory are given in table 1; in addition, the mean and the intraclass correlation coefficient (within blind duplicates) are presented. Since α-lipoprotein cholesterol, β-lipoprotein, pre-β-lipoprotein, and α-lipoprotein were obtained through calculation, measurement errors are not presented for these variables. Since two independent samples were collected for each of the 432 individuals, it should be mentioned that the values given in table 1 represent the errors associated with the collection, processing, and analyses of the blood samples as well as the processing of the data (unpublished observations).

Results

A comparison between the direct and the indirect methods for measuring serum β + pre-β-lipoprotein cholesterol was made in sera from a random sample of 349 children to test the validity of applying the indirect method for all of the children studied. Analysis of quantitative differences between the direct and indirect method was performed as described by Friedewald et al. and Glueck et al., and is presented in table 2. The direct determination of cholesterol in the β- and pre-β-lipoprotein fractions obtained by heparin-Ca++ precipitation is considered the standard. A mean difference of −0.73 mg/100 ml between the two methods indicates that the indirect method gave slightly higher β + pre-β-lipoprotein cholesterol values than the direct method. However, the difference was not statistically significant. The percent error, which is an estimate of the error of the indirectly estimated value as compared to the directly measured value is well within the range obtained by previous investigators for their indirect estimation of β-lipoprotein cholesterol using the Friedewald formula. The indirect method was applied, therefore, to measure the lipoprotein concentrations in all children.

The Distribution of Serum Lipoproteins

The distributions by race of serum β-, pre-β-, and α-lipoproteins in children 5–14 years old is presented in figure 1. Whereas the distribution of β-lipoproteins showed no racial difference (mean level of 190 mg/100 ml in black children compared to 189 mg/100 ml in white children), both pre-β and α-lipoprotein distributions indicated a consistent racial difference. White children tended to have higher pre-β-lipoprotein levels than black children (mean level of 40 mg/100 ml in white children vs 33 mg/100 ml in black children, P < 0.001). On the other hand, black children tended to have higher α-lipoprotein levels than white children (mean levels of 436 mg/100 ml and 384 mg/100 ml, P < 0.0001). In both racial groups the median values for β-lipoprotein (184 mg/100 ml for both races) and α-lipoprotein (431 mg/100 ml in white children and 389 mg/100 ml in black children) were only slightly different from the corresponding mean values. The median values for pre-β-lipoprotein, however, were 21.7% and 23.8% less than the mean values in black and white children, respectively. The distributions of β-, pre-β-, and α-lipoproteins were unimodal, but a chi-square goodness of fit test indicated that none were normally distributed.

Serum Lipoprotein Levels in Individual Race-Sex Groups

Serum β, pre-β-, and α-lipoprotein levels were compared among the four race-sex groups at every age. The results are
shown in tables 3–5. All statistical analyses were performed on the logarithmically transformed data although the arithmetic mean and standard deviation values obtained from the untransformed data are presented in the tables. The \( \beta \)-lipoprotein levels given in table 3 showed no significant race difference among children ages 5–14 except among 5-year-old girls. Although girls from both races tended to have a slightly higher level of \( \beta \)-lipoproteins than boys (black children \( P < 0.05 \); white children \( P < 0.01 \)), these differences were not statistically significant for each age interval. Furthermore, a decrease in \( \beta \)-lipoprotein concentration was noticeable after age 11 in all children irrespective of sex or race. White children of both sexes showed a higher pre-\( \beta \)-lipoprotein level than black children (table 4); however, a statistically significant racial difference was observed only in girls (\( P < 0.01 \)), especially in the 12–14 year age group. Interestingly, like \( \beta \)-lipoprotein, the pre-\( \beta \)-lipoprotein levels were generally higher in girls than in boys in both racial groups (black children \( P < 0.01 \); white children \( P < 0.001 \)), although the differences were not significant for each age group. \( \alpha \)-lipoprotein levels (table 5) in children differed markedly between races in both boys (\( P < 0.001 \)) and girls (\( P < 0.001 \)), with black children tending to be higher than white children. This trend was consistent throughout the ten-year age span (except in 5-year-old girls). Though the overall (ages 5–14) mean levels of \( \alpha \)-lipoproteins were slightly higher in boys than in girls, the differences were significant only among white children (\( P < 0.05 \)).

When the lipoprotein concentrations were expressed in terms of their corresponding lipoprotein cholesterol values, the \( \alpha \)-lipoprotein cholesterol (with mean levels of 74 mg/100 ml in black children and 65 mg/100 ml in white children) constituted as much as 44% and 40% of the total lipoprotein cholesterol (total serum cholesterol) in black and white children, respectively.

Statistical Normal Limits (Percentiles)

To estimate the normal limits for serum lipoproteins in this pediatric population, the 5th, 50th (median), and 95th percentile values by race and sex were calculated and are given in table 6. Since serum lipoprotein concentrations are generally being measured and reported in terms of corresponding lipoprotein cholesterol, the percentile values for the individual lipoprotein cholesterol were also included in the table. The 5th and 95th percentile levels for this group of children showed very little difference between boys and girls within each race. Although the percentile levels for \( \beta \)-lipoprotein data were calculated on the logarithmic scale, the values were listed as arithmetic means and standard deviations.

### Table 3. Serum \( \beta \)-Lipoprotein Levels (mg/100 ml, Mean ± sd) in Children by Age, Race, and Sex

<table>
<thead>
<tr>
<th>Age (yr)</th>
<th>Black children</th>
<th>White children</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>Boys</td>
</tr>
<tr>
<td>5</td>
<td>30</td>
<td>206 ± 54</td>
</tr>
<tr>
<td>6</td>
<td>44</td>
<td>192 ± 58</td>
</tr>
<tr>
<td>7</td>
<td>48</td>
<td>192 ± 48</td>
</tr>
<tr>
<td>8</td>
<td>61</td>
<td>188 ± 43</td>
</tr>
<tr>
<td>9</td>
<td>80</td>
<td>197 ± 60</td>
</tr>
<tr>
<td>10</td>
<td>71</td>
<td>179 ± 38</td>
</tr>
<tr>
<td>11</td>
<td>70</td>
<td>190 ± 55</td>
</tr>
<tr>
<td>12</td>
<td>81</td>
<td>185 ± 48</td>
</tr>
<tr>
<td>13</td>
<td>68</td>
<td>169 ± 46</td>
</tr>
<tr>
<td>14</td>
<td>55</td>
<td>184 ± 41</td>
</tr>
<tr>
<td>5-14</td>
<td>608</td>
<td>187 ± 30</td>
</tr>
</tbody>
</table>

†Race differences at \( P < 0.05 \); \( P < 0.01 \) (F test after conversion to log \( \beta \)-lipoproteins).
*Sex differences at \( P < 0.05 \); \( P < 0.01 \) (F test after conversion to log \( \beta \)-lipoproteins).
proteins were very similar in both the races, the upper 95th percentile level for pre-β-lipoprotein in black children was about 26% lower than in white children. For α-lipoprotein, the upper 95th percentile levels in white children were about 10% lower than in black children.

The above three sets of percentile values by sex and race are also presented for every age in figures 2-4. The median and selected percentile levels of β-lipoproteins (Fig. 2) indicated no consistent racial differences at each age interval in either boys or girls. Although no definite age-related trend could be ascertained from these percentile levels, the median and upper 95th percentile levels indicated a decrease in β-lipoprotein between ages 11 and 14. For pre-β-lipoproteins (Fig. 3) the median level for white children exceeded black children at every age interval between the ages of 9 and 14; this difference was more pronounced in girls than in boys due to a strong age-related increase in pre-β-lipoprotein levels in white girls. The difference between the races in pre-β-lipoprotein levels was even greater at the 95th percentile level except for 13 and 14-year-old boys and 6 and 8-year-old girls. Interestingly, black children exceeded white children at every age interval in their median α-lipoprotein level (Fig. 4).

**Effect of Age**

To assess the effect of age on serum lipoprotein levels in these children, regression analyses were made by race and sex. The summary of the results is given in Table 7. In view of the skewed distribution of the serum lipoproteins, regression analyses were made after logarithmic transformation of the data as in the mode of previous reports. Since β-lipoprotein levels showed a definite tendency to decrease after age 11 (Table 3), the regression analyses were made separately on children of age groups 5-10 and 11-14 years. Although β-lipoprotein levels decreased slightly with age in all children (except white girls between ages 5 and 10) the decreasing trend, as shown by the negative correlation, was statistically significant ($P < 0.05$) only between ages 11 and 14 (except in black boys). Interestingly, the β-lipoprotein levels in white girls between ages 5 and 10 increased slightly with age ($P < 0.05$). A highly significant ($P < 0.0001$) increase in pre-β-lipoprotein levels with age was observed in all children, and the rate of increase with age (coefficient of linear regression) was higher in white children than in black children. There was little correlation with age in the case of α-lipoprotein levels on all four race-sex groups studied.

**Interrelationship Among Serum Lipoproteins and Lipids**

The interrelationship among serum lipoproteins and lipids in black versus white children is presented in Table 8. In both the races, β- and pre-β-lipoproteins were positively correlated. A similar relationship was observed for serum total cholesterol and triglycerides, both of which were measured independently. In contrast, the α-lipoprotein was negatively correlated with β-lipoprotein and to a greater extent with pre-β-lipoprotein, suggesting an inverse relationship between these lipoprotein classes. It should be noted that the α-lipoprotein was negatively correlated with serum triglycerides as well. The serum total cholesterol correlated with β-lipoproteins and triglycerides with pre-β-lipoprotein.
TABLE 6. Selected Percentile Levels by Race and Sex for Serum Lipoproteins in Children 5–14 Years Old

<table>
<thead>
<tr>
<th>Serum lipoproteins</th>
<th>5th</th>
<th>50th</th>
<th>95th</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-lipoprotein</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Boys, black</td>
<td>119 (56)*</td>
<td>181 (83)</td>
<td>286 (134)</td>
</tr>
<tr>
<td>white</td>
<td>119 (56)</td>
<td>181 (83)</td>
<td>272 (127)</td>
</tr>
<tr>
<td>Girls, black</td>
<td>128 (60)</td>
<td>188 (88)</td>
<td>284 (133)</td>
</tr>
<tr>
<td>white</td>
<td>126 (59)</td>
<td>188 (88)</td>
<td>278 (131)</td>
</tr>
<tr>
<td>Pre-β-lipoprotein</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Boys, black</td>
<td>4 (1)</td>
<td>24 (5)</td>
<td>79 (18)</td>
</tr>
<tr>
<td>white</td>
<td>4 (1)</td>
<td>27 (6)</td>
<td>106 (24)</td>
</tr>
<tr>
<td>Girls, black</td>
<td>6 (1)</td>
<td>27 (6)</td>
<td>83 (18)</td>
</tr>
<tr>
<td>white</td>
<td>5 (1)</td>
<td>35 (8)</td>
<td>113 (25)</td>
</tr>
<tr>
<td>α-lipoprotein</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Boys, black</td>
<td>231 (39)</td>
<td>440 (75)</td>
<td>673 (114)</td>
</tr>
<tr>
<td>white</td>
<td>182 (31)</td>
<td>392 (67)</td>
<td>596 (101)</td>
</tr>
<tr>
<td>Girls, black</td>
<td>230 (39)</td>
<td>425 (72)</td>
<td>637 (108)</td>
</tr>
<tr>
<td>white</td>
<td>171 (29)</td>
<td>381 (65)</td>
<td>575 (98)</td>
</tr>
</tbody>
</table>

*The corresponding lipoprotein cholesterol values are given in parentheses.

proteins. The absolute values of the above correlations were generally greater in white children than in black children. On the other hand, the correlation coefficient between total cholesterol and α-lipoprotein was higher in black children than in white children.

Frequency of Hyperlipoproteinemia

Since the three forms of hyperlipoproteinemia common in our population consist of elevated β- and/or pre-β-lipoproteins, their frequency in this pediatric population was calculated based on the currently recommended upper normal limits. Table 9 shows that only 0.5% of black and white children exceeded the upper β-lipoprotein cholesterol level of 170 mg/100 ml and can be considered hyper-β-lipoproteinemic (Type IIa). On the other hand, 1.7% of black children and 4.7% of white children showed hyper-pre-β-lipoproteinemia (pre-β-lipoprotein cholesterol level > 25 mg/100 ml) without associated hyper-β-lipoproteinemia (Type IV). Combined hyperlipoproteinemia, with both β- and pre-β-lipoprotein cholesterol levels exceeding the above cut-off points, was seen in only 0.1% of black and 0.1% of white children. Although α-lipoprotein levels are not considered when classifying hyperlipoproteinemias, it should be mentioned that 62% of black children and 46% of white children exceeded the suggested upper limits for α-lipoprotein cholesterol (male: 65 mg/100 ml; female: 70 mg/100 ml).

Discussion

The present study describes the race, sex, and age-related patterns of serum lipoproteins in a well-defined biracial population of children. The introduction of simple methods to quantitate lipoproteins on a population level made such observations feasible. A striking racial difference was observed in the lipoprotein makeup of these children with the black children having relatively higher α-lipoprotein levels and lower pre-β-lipoprotein levels than the white children. Our report on the same children indicated that white children had lower serum cholesterol and higher triglyceride levels than black children. The differences in pre-β-lipoprotein levels in these two groups seem to be in agreement with their triglyceride levels, since in the fasting state serum triglyceride exists predominantly as a component of pre-β-lipoprotein. The higher concentration of cholesterol observed in the black children could be attributed to the differences in their α-lipoprotein levels because the β-lipoprotein levels were very similar in both groups. Although serum cholesterol is generally considered to reflect the β-lipoprotein concentrations, the present study as well as earlier studies clearly show the need for
quantitating individual lipoprotein classes rather than extrapolating them from serum cholesterol and triglyceride values. Recently, Tyroler et al. reported that black adults had more \( \alpha \)-lipoprotein cholesterol (determined by preparative ultracentrifuge method) than did a comparable group of white adults. These observations suggest that the racial differences in lipoprotein makeup are likely to persist from childhood through adulthood. It would be interesting to see whether this difference occurs even in a neonatal stage. Furthermore, it is important to know whether the differences in lipoprotein levels between the races are due to genetic or environmental factors.

The sex and age-related changes in this group of children indicated a common tendency for girls to have higher \( \beta \)- and pre-\( \beta \)-lipoprotein and lower \( \alpha \)-lipoprotein concentrations than boys. The pre-\( \beta \)-lipoprotein showed a progressive increase with age, \( \beta \)-lipoprotein showed a slight tendency to decrease with age, especially between ages 11 and 14, and \( \alpha \)-

**Figure 3.** Selected percentile values (5th, 50th and 95th) for serum pre-\( \beta \)-lipoprotein by age, race and sex in children.

**Figure 4.** Selected percentile values (5th, 50th and 95th) for serum \( \alpha \)-lipoprotein by age, race and sex in children.
lipoprotein remained relatively unchanged. These results are in agreement with our earlier observations on a limited number of children from a nearby community, even though the race, sex, and age-related differences were not as marked as in the present study. Kwiterovich et al. observed no age-related change in any of the lipoprotein fractions within sex, although β-lipoprotein cholesterol decreased slightly with age when the sexes were combined. The tendency for decrease in β-lipoprotein concentrations and pronounced increase in pre-β-lipoprotein concentrations around puberty could be due to the influence of gonadal hormones on lipid and lipoprotein metabolism. It should be mentioned that a clear-cut pattern of age- and sex-dependent changes in the serum lipoprotein concentrations around puberty might not be apparent from a cross-sectional study because of the differences in sexual maturity among individuals of a given age group. The studies of Lee indicate that a distinct pattern of change can be observed when the lipid levels of individual children are examined longitudinally.

It is apparent from this study that the age and sex-related differences in individual lipoprotein classes differ markedly from the adult pattern. In general, adult males (above 20 years of age) show higher levels of pre-β- and β-lipoproteins and lower levels of α-lipoprotein than females; the increase in pre-β-lipoprotein with age is more pronounced in males than in females. The transition from the infantile to the adult pattern for lipoproteins has been reported to occur between the ages of 16 and 20 in males and after the age of 20 in females; however, these observations were based on a limited number of persons from an undefined population. Further studies are needed to ascertain at what age the characteristic adult patterns of lipoprotein develop.

The level of α-lipoprotein (cholesterol) observed in children was consistently higher than that found in young adults 23–25 years of age (using the same methodology). Interestingly, Kornerup found significantly higher phospholipid levels in children ages 1–15 than in adults above the age of 20, even though there were no significant differences in serum cholesterol levels between these two groups.

### Table 7. Summary of Regression Analyses with Age of Serum Lipoproteins in Children by Race and Sex

<table>
<thead>
<tr>
<th>Serum lipoproteins</th>
<th>Correlation with age (r)</th>
<th>Coefficient of linear regression</th>
<th>Intercept (y)</th>
<th>Standard error of the slope</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>β-lipoproteins, ages 5–10</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Boys, Black, N = 334</td>
<td>-0.08</td>
<td>-0.0060</td>
<td>2.32</td>
<td>0.004</td>
</tr>
<tr>
<td>White, N = 582</td>
<td>-0.01</td>
<td>-0.0008</td>
<td>2.27</td>
<td>0.003</td>
</tr>
<tr>
<td>Girls, Black, N = 298</td>
<td>-0.08</td>
<td>-0.0051</td>
<td>2.33</td>
<td>0.004</td>
</tr>
<tr>
<td>White, N = 555</td>
<td>0.09*</td>
<td>0.0058</td>
<td>2.23</td>
<td>0.003</td>
</tr>
<tr>
<td><strong>α-lipoproteins, ages 5–14</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Boys, Black, N = 608</td>
<td>0.17**</td>
<td>0.0254</td>
<td>1.08</td>
<td>0.006</td>
</tr>
<tr>
<td>White, N = 1033</td>
<td>0.21**</td>
<td>0.0331</td>
<td>1.05</td>
<td>0.005</td>
</tr>
<tr>
<td>Girls, Black, N = 565</td>
<td>0.17**</td>
<td>0.0213</td>
<td>1.19</td>
<td>0.005</td>
</tr>
<tr>
<td>White, N = 975</td>
<td>0.32**</td>
<td>0.0476</td>
<td>0.99</td>
<td>0.004</td>
</tr>
<tr>
<td>Pre-β-lipoprotein, ages 5–14</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Boys, Black, N = 608</td>
<td>0.01</td>
<td>0.0007</td>
<td>2.01</td>
<td>0.003</td>
</tr>
<tr>
<td>White, N = 1033</td>
<td>-0.02</td>
<td>-0.0018</td>
<td>2.57</td>
<td>0.003</td>
</tr>
<tr>
<td>Girls, Black, N = 565</td>
<td>0.01</td>
<td>0.0005</td>
<td>2.60</td>
<td>0.003</td>
</tr>
<tr>
<td>White, N = 976</td>
<td>-0.02</td>
<td>-0.0017</td>
<td>2.55</td>
<td>0.003</td>
</tr>
</tbody>
</table>

*p < 0.05.
**p < 0.001.
††††Race differences at P < 0.01; P < 0.001; P < 0.0001.

### Table 8. Correlation Coefficients (r) for Serum Lipoprotein and Lipid Variables by Race in Children, Ages 5–14 Years

<table>
<thead>
<tr>
<th></th>
<th>β-lipoprotein</th>
<th>Pre-β-lipoprotein</th>
<th>α-lipoprotein</th>
<th>Total cholesterol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-β-lipoprotein</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Black, N = 1173</td>
<td>0.231**</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White, N = 2009</td>
<td>0.393**,†††</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>α-lipoprotein</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Black, N = 1173</td>
<td>-0.093*</td>
<td>-0.359**</td>
<td></td>
<td></td>
</tr>
<tr>
<td>White, N = 2009</td>
<td>-0.291**,**</td>
<td>-0.505**,†††</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total cholesterol</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Black, N = 1174</td>
<td>0.727**</td>
<td>0.097*</td>
<td>0.505**,†††</td>
<td></td>
</tr>
<tr>
<td>White, N = 2009</td>
<td>0.745**</td>
<td>0.206**,†</td>
<td>0.441**</td>
<td></td>
</tr>
<tr>
<td>Triglyceride</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Black, N = 1174</td>
<td>0.282**</td>
<td>0.667**</td>
<td>-0.286**</td>
<td>0.127**</td>
</tr>
<tr>
<td>White, N = 2009</td>
<td>0.442**,†††</td>
<td>0.798**,†††</td>
<td>-0.422**,†††</td>
<td>0.253**,†††</td>
</tr>
</tbody>
</table>
α-lipoproteins are rich in phospholipids, the high levels found in children could reflect this class of lipoproteins. However, these observations are at variance with other studies using a heparin-Mn⁺⁺ precipitation method for quantitating α-lipoprotein cholesterol. The use of Ca⁺⁺ or Mg⁺⁺ in the precipitation methods is more specific and quantitative than Mn⁺⁺, which is known to coprecipitate other serum proteins, including α-lipoproteins. More recent studies on serum lipid-heparin interactions indicated that, among the subclasses of high density lipoproteins (HDL), 40% of HDL₂ and 10% of HDL₃ could be precipitated in the presence of Mn⁺⁺. Therefore, the degree of difference between methods using Ca⁺⁺ and those using Mn⁺⁺ is likely to be determined by the proportion of each of the HDL subclasses (HDL₁, HDL₂, and HDL₃) present in the sera.

Hatch et al., who obtained an excellent correlation between the quantitative agarose gel electrophoresis method and the analytical ultracentrifuge method, observed that individuals having sinking pre-β-lipoprotein would be subject to a small error in the estimated relative amounts of β- and pre-β-lipoproteins. The method used in these studies does not allow an estimation of the presence of this fraction in our population studies. Although combined electrophoretic and ultracentrifugal methods recorded a frequency of 10 to 15% sinking pre-β-lipoproteins in normal individuals, this is a very minor component of the serum and is particularly difficult to classify. Relatively simple and inexpensive methods for measuring serum lipoproteins are greatly needed for clinical and epidemiologic studies of cardiovascular disease risk factors. Our results show that major serum lipoprotein fractions can be measured in large populations to describe race, sex, and age-related patterns.

The interrelationship among different classes of lipoproteins indicates that the α-lipoprotein level in serum is inversely proportional to both pre-β- and β-lipoprotein levels. This is in agreement with the findings in an adult population by Nichols and Miller and Miller. The observed interrelationship among different classes of lipoproteins and lipids was attributed to the functional associations among the major classes of lipoproteins which apparently are related to the status of triglyceride metabolism. Interestingly, the body (tissue) cholesterol pool increased with decreasing serum α-lipoprotein levels, and low levels of α-lipoproteins have been associated with the incidence of coronary artery disease. α-lipoprotein is known to activate lipoprotein lipase and lecithin: cholesterol acyl transferase enzymes. Whether the low levels of pre-β-lipoprotein (and triglycerides) and higher levels of α-lipoproteins in children compared to adults reflect their status of lipid metabolism is not known. The observed differences between the races in the correlation coefficients for any two given variables of serum lipoproteins and lipids suggest differences in the lipoprotein distributions between the two groups. (For example, relatively lower levels of pre-β-lipoprotein [or triglyceride] with little difference in β-lipoprotein levels in black children could give a low order correlation coefficient in the black children compared to white children.) If an increase in α-lipoprotein levels does reduce the risk of coronary heart disease, and racial differences we observed in children continue through adulthood, one would expect blacks to have a lower risk of heart disease associated with a given cholesterol level than whites. Data from the only other major prospective epidemiologic study with a sizable black and white population (Evans County) seem to support this assumption.

When the upper limits for lipoproteins were set at the currently recommended levels of 170 mg/100 ml β-lipoprotein cholesterol and 25 mg/100 ml pre-β-lipoprotein cholesterol for children 0–19 years of age, only a small percentage of children could be considered as hyperlipoproteinemic. It is apparent that the above cut-off points are relatively higher than the upper 95th percentile levels for lipoproteins obtained in this pediatric population.

Although the upper 95th percentile may be a statistically valid upper normal limit for a given population, it is arbitrary and does not necessarily represent the optimal lipoprotein levels. Therefore, the mean or percentile values presented here should be considered only as a guideline in determining the baseline or range for serum lipids and lipoproteins in children. Because lipid levels vary widely among populations, appropriate comparative data in pediatric populations living in different cultural and geographic environments within the country may be necessary for obtaining more accurate limits of normal.

These observations are intended to describe the distributions found in free-living, presumably normal children in a total community setting. Such observations will serve as baselines for comparison to other populations and perhaps guidelines for consideration of intervention. The 95th percentile has been previously suggested and presented accordingly in our study as the limit for consideration of "hyper" lipoproteinemia. However, the high risk of heart disease in this country, the evidence of the early onset of the disease, and the consideration of risk factors as a continuum for probability of clinical disease suggest that for a population, the median is a more realistic limit for the present.

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