Quantification of Lipoprotein Cholesterol in Serum from Children with Different Lipoprotein Profiles: Heparin–Calcium Precipitation and Ultracentrifugation Compared

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We compared the serum lipoprotein cholesterol concentrations in subgroups of children (n = 360), ages 5–17 years, as measured by the heparin–Ca\(^{2+}\) and preparative ultracentrifugation methods. Children were grouped from the total population on the basis of their previous results for serum \(\beta\)- and \(\pre-\beta\)-lipoprotein cholesterol (Group I: low \(\beta\)- and low \(\pre-\beta\)-; Group II: high \(\beta\)- and low \(\pre-\beta\)-; Group III: high \(\beta\)- and high \(\pre-\beta\)-; Group IV: low \(\beta\)- and high \(\pre-\beta\)-). The values for very-low-density (VLDL) cholesterol by ultracentrifugation method were 44, 53, 15, and 10 mg/L greater than the values for \(\pre-\beta\)-lipoprotein cholesterol by the heparin–Ca\(^{2+}\) method in Groups I, II, III, and IV, respectively; the differences were not significant in Group IV. The values of low-density (LDL) cholesterol were 64, 137, 144, and 73 mg/L less than the values for \(\beta\)-lipoprotein cholesterol in Groups I, II, III, and IV, respectively (\(p < 0.005\)). On the other hand, high-density (HDL) cholesterol concentrations in the respective four groups were 10, 37, 93, and 52 mg/L greater than \(\alpha\)-lipoprotein cholesterol concentrations; the differences were significant for Groups II, III, and IV (\(p < 0.005\)). Overall, the values for LDL-cholesterol correlated highly with \(\beta\)-lipoprotein cholesterol (\(r = 0.94\)), whereas correlations for VLDL- and HDL-cholesterol values with \(\pre-\beta\)-lipoprotein cholesterol (\(r = 0.76\)) and \(\alpha\)-lipoprotein cholesterol (\(r = 0.77\)) were somewhat lower. The differences between these two methods may result from their different operational definitions for measuring serum lipoproteins and the possibility that without appropriate corrections the values obtained by preparative ultracentrifugation do not serve as reference values.

Additional Keyphrases: pediatric chemistry · heart disease

Both clinical and epidemiologic studies have shown that susceptibility to coronary artery disease is related positively to low-density lipoprotein (LDL) and inversely to high-density lipoprotein (HDL) concentrations in serum (1). Because of this awareness, there is a demand for evaluating lipoprotein abnormalities in the general population, including children. In general, lipoproteins are measured in terms of cholesterol content by partitioning serum total cholesterol into very-low-density lipoproteins (VLDL), LDL, and HDL. Currently, polyvalent–divalent cation precipitation, either by itself or in combination with preparative ultracentrifugation, remains the most favored approach to measuring individual lipoprotein cholesterol (2–4).

Based on our observations of lipoprotein–glycosaminoglycan interactions, we have introduced a heparin–Ca\(^{2+}\) procedure that can selectively and quantitatively precipitate lipoproteins containing apoprotein-B (apoB) from serum (5, 6). When combined with measurement of serum total cholesterol and serum lipoprotein electrophoresis, this procedure allows quantification of \(\pre-\beta\)-, \(\beta\)-, and \(\alpha\)-lipoprotein cholesterol concentrations (7). Having tested its validity independently against analytical ultracentrifugation (7) and a method that involves a combination of ultracentrifugation and dextran sulfate precipitation (8), we have applied this procedure to measure serum lipoproteins in a large pediatric population (Bogalusa Heart Study) (9). However, detailed information is lacking on the comparability of results by the heparin–Ca\(^{2+}\) procedure with fractionation by preparative ultracentrifugation in children with different lipoprotein profiles.

As part of the Bogalusa Heart Study we have recently conducted a special lipid study designed to explore the biochemical determinants of serum lipoprotein concentrations in children whose concentrations of \(\beta\)-lipoprotein cholesterol and (or) \(\pre-\beta\)-lipoprotein cholesterol were at either extreme of the distribution of values. Here we compare the values for serum lipoprotein cholesterol concentrations as determined by the heparin–Ca\(^{2+}\) procedure and the ultracentrifugation method in these subgroups of children.

Materials and Methods
Population Sample
A detailed description of the sample selection is reported elsewhere (10). Briefly, all children who were examined and were fasting during both the 1973–1974 and 1976–1977 cross-sectional studies were entered into the sampling frame. The children were between ages 5 and 17 years during the second cross-sectional survey. The \(\beta\)- and \(\pre-\beta\)-lipoprotein cholesterol values (measured by the heparin–Ca\(^{2+}\) procedure) from these two cross-sectional studies were averaged and ranked separately. Four subgroups were selected on the basis of age-, race-, and sex-specific distributions. To increase the sample size of Groups II and IV, quartiles rather than quintiles were chosen, as follows: Group I, both \(\beta\)- and \(\pre-\beta\)-lipoprotein cholesterol concentrations were in the lowest quintile; Group II, \(\beta\)- and \(\pre-\beta\)-lipoprotein cholesterol concentrations were in the highest and lowest quartiles, respectively; Group III, both \(\beta\)- and \(\pre-\beta\)-lipoprotein cholesterol concentrations were in the highest and lowest quintiles; Group IV, \(\beta\)- and \(\pre-\beta\)-lipoprotein cholesterol concentrations were in the lowest and highest quartiles, respectively. Thus, phenotypically, the above Groups II, III, and IV are somewhat analogous to Types IIa, IIb, and IV, respectively, in the World Health Organization's classification system. Of the 447 children who were residing in Bogalusa in 1978, 388 were actually tested; of these, only fasting children with cholesterol values available on all the three lipoprotein fractions after ultracentrifugation are included in this report \((n = 360)\). Both electrophoretic and ultracentrifugal nomenclatures are used to describe respec-

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Nonstandard abbreviations: VLDL, LDL, and HDL, very-low-density, low-density, and high-density lipoproteins, respectively; apo, apoprotein; \([\text{Lp}(\alpha)]\), sinking \(\pre-\beta\)-lipoprotein.

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tive lipoprotein values obtained by the heparin–Ca$^{2+}$ procedure and the ultracentrifugation method.

Collection of Blood Specimens

Venous blood was collected in Vacutainer Tubes (Becton Dickinson, Rutherford, NJ 07070) and allowed to clot at room temperature for about 1.5 h. After centrifugation, sera were collected in tubes containing thimerosal (Aldrich Chemical Co., Milwaukee, WI 53233), packed in a box cooled with frozen packs, and sent to New Orleans Core Lipid Laboratory of the Specialized Center of Research—Arteriosclerosis. Specimens were handled and analyzed routinely the next day.

Serum Cholesterol and Triglycerides

Serum total cholesterol (or lipoprotein cholesterol) and triglycerides were determined with an AutoAnalyzer II (Technicon Instruments Corp., Tarrytown, NY 10591), according to the Laboratory Manual of the Lipid Research Clinics Program (3). An isopropanol extract of the sample (0.2 mL of whole serum or lipoprotein fraction) was used for the determination. A serum calibrator material provided by the Centers for Disease Control (CDC), Atlanta, GA 30333, was used to convert the cholesterol values obtained with the AutoAnalyzer II to be comparable with values obtained by the comparison method of Abell et al. (11). The laboratory has been designated as "standardized" by the CDC and is currently in the surveillance phase of its quality-control program.

Serum Lipoprotein Cholesterol

**Heparin–Ca$^{2+}$ method.** Serum β- + pre-β-lipoprotein cholesterol: These lipoproteins were selectively precipitated by the addition of heparin and Ca$^{2+}$ (5, 8, 9). Briefly, this method consists of mixing 0.2 mL of serum, 3.2 mL of distilled water, 0.1 mL of a 2.5 g/L solution of beef lung heparin (=140 USP units/mg; Upjohn Co., Kalamazoo, MI 49001), and 0.5 mL of CaCl$_2$ (0.5 mol/L), in that order. After letting the mixtures stand for 15 min, the precipitate is centrifuged (1500 × g, 30 min) and analyzed for the corresponding β- and pre-β-lipoprotein cholesterol content.

Electrophoretic ratio of β- and pre-β-lipoproteins: Serum (10–20 μL) was electrophoresed on agar–agarose gel plates (8.5 × 10 cm) with use of a barbital buffer (pH 8.6, 50 mmol/L) at 22 mA per plate (12). The lipoprotein bands, stained with Oil Red O, were scanned with a densitometer to measure the relative percentages of β- and pre-β-lipoprotein as % and y%, respectively, keeping x + y = 100. Although different lipoproteins have dissimilar lipid content and composition, the studies of Hulley et al. (13) indicated that the average dye (Oil Red O) uptake of the lipid portions of the three major lipoproteins was similar. We therefore corrected the densitometric ratios of the β- and pre-β-lipoproteins by taking into account that 1.0 mg of β-lipoprotein takes up the same amount of dye as 0.86 mg of pre-β-lipoprotein (14).

Estimation of β- and pre-β-lipoprotein cholesterol: The estimation of serum β- and pre-β-lipoprotein cholesterol concentrations was based on the corrected electrophoretic ratio of β- and pre-β-lipoprotein (x% and y%), β- + pre-β-lipoprotein cholesterol concentration (z, mg/L), and the reported fractional cholesterol content of β-lipoprotein (46.9%) and pre-β-lipoprotein (22.2%) molecules (7, 9). We used the following equations to calculate lipoprotein cholesterol, in milligrams per liter: β-lipoprotein cholesterol, 46.9x/z(46.9x + 22.2y); pre-β-lipoprotein cholesterol, 22.2y/z(46.9x + 22.2y). Changes in lipoprotein cholesterol estimations owing to variations in fractional cholesterol content of these molecules in normal individuals were considered to be small (7).

α-Lipoprotein cholesterol: The value for α-lipoprotein cholesterol was obtained by subtracting β- + pre-β-lipoprotein cholesterol from total cholesterol.

**Ultracentrifugation method.** VLDL-, LDL-, and HDL-cholesterol: Lipoprotein fractions were separated by ultracentrifugation by using a type-35 rotor fitted with seven-hole adaptors (8). Aliquots of serum (2.0 mL) were transferred to 11 × 79 mm polycarbonate centrifuge tubes without caps and diluted to 3.0 mL with a solution of density 1.066 kg/L. The contents were centrifuged in a Model L2-65B preparative ultracentrifuge (Beckman Instruments Inc., Palo Alto, CA 94304) at 33 000 rpm for 16 h at 17 °C to separate VLDL (the top 1.0 mL). The bottom fraction was then centrifuged at density 1.083 kg/L for 20 h. At the end of the run, the top 1.0-mL and bottom 2.0-mL fractions, LDL and HDL, respectively, were separated. All three ultracentrifugal fractions were analyzed for corresponding lipoprotein cholesterol content. Densities of the solutions were adjusted with NaCl–NaBr according to the procedure of Havel et al. (15). No attempt was made to ascertain the presence of β-VLDL (floating-β) and sinking pre-β-lipoprotein [Lp(a)] by electrophoresis or to re-analyze the samples to reduce methodologic error if there were disparities between the results of the two methods.

Measurement Error

Errors in measurements of serum total and individual lipoprotein cholesterol values were evaluated independently for separate venipuncture blood samples from a random sample constituting approximately 10% of the total sample (n = 33) (16). During the five-month period of this study, overall coefficients of variation (CVs) between the original and blind duplicate measurements for serum total, β-, pre-β-, and α-lipoprotein cholesterol measurements were 1.8, 2.6, 14.3, and 7.2%, respectively. The blind duplicate samples were indistinguishable from the other samples throughout the laboratory processing and the entire data processing. Because the objective of this study was to compare methods, we made special efforts to eliminate errors associated with data processing by editing values obtained for each sample by the computer. Owing to limitations of sample (serum) volume, we were unable to include similar blind duplicates for the ultracentrifugation method. The low cholesterol concentrations being technically more difficult to assay, we also performed replicate (n = 12) analyses of a low-concentration cholesterol (458 mg/L) pool; the CV we obtained was 3.3%.

Statistical Analysis

To compare the results from the ultracentrifuge and the heparin–Ca$^{2+}$ precipitation method, we used a two-factor (method and lipoprotein group) analysis of variance with a repeat measure on one factor (method) (17). This analysis is particularly sensitive to the main effect of methods and to the method/lipoprotein group interaction. If the interaction term was statistically significant, then simple effect comparisons between the methods were examined for each lipoprotein group. We found essentially the same results for untransformed and log-transformed data; hence, we present only the results from the untransformed data.

Results

Analytical Recovery by Ultracentrifugation

In view of the manipulative loss of lipoprotein fractions during ultracentrifugation, attempts have been made to
evaluate the analytical recovery of the combined lipoprotein fractions (VLDL + LDL + HDL). The frequency distribution of percentage recovery of lipoprotein cholesterol in all samples (n = 360) is given in Figure 1. Overall recovery of combined lipoprotein fractions by ultracentrifugation averaged 98.7% (2SD = 13.3%) of the serum total cholesterol, with recovery ranging from 90 to 106% in most (92%) of the samples. The mean ± SE analytical recovery for Groups I, II, III, and IV was 99.4 ± 0.7%, 97.8 ± 0.8%, 98.2 ± 0.5%, and 99.4 ± 0.9%, respectively, and the values did not differ significantly among the four lipoprotein groups.

Comparison of Methods

Serum concentrations of VLDL, LDL, and HDL-cholesterol obtained by the ultracentrifugation method in different lipoprotein subgroups were compared with corresponding values for β-, pre-β-, and α-lipoprotein cholesterol obtained by the heparin–Ca²⁺ method (Table 1). Proportionate differences in values by the two methods varied, depending on the lipoprotein fraction and lipoprotein subgroup. Values for VLDL-cholesterol were respectively 44, 53, 15, and 10 mg/L greater than the values for pre-β-lipoprotein cholesterol in Groups I, II, III, and IV; the differences were statistically significant in Groups I, II, and III (p < 0.005). We stress that, although concentrations of pre-β-lipoprotein cholesterol among the four lipoprotein subgroups followed the criteria of sample selection, concentrations of VLDL-cholesterol did not vary proportionately among the lipoprotein subgroups. For example, mean VLDL-cholesterol concentrations in Groups II and IV differed by only 18 mg/L, although the two groups were selected from low and high quartiles of distribution for pre-β-lipoprotein cholesterol, respectively. Values for LDL-cholesterol were 64, 137, 144, and 73 mg/L less than the values for β-lipoprotein cholesterol in Groups I, II, III, and IV, respectively (p < 0.005). Because the β- and pre-β-lipoprotein cholesterol concentrations were derived indirectly on the basis of electrophoretic ratios, we compared the results of direct determinations of combined β- + pre-β-lipoprotein cholesterol with the combined cholesterol values of the VLDL and LDL fractions. Values for β- + pre-β-lipoprotein cholesterol were 20, 83, 129, and 62 mg/L greater than values for VLDL- + LDL-cholesterol in Groups I, II, III, and IV, respectively (p < 0.005). This trend was also reflected in the differences between LDL-cholesterol and α-lipoprotein cholesterol. HDL-cholesterol concentrations were 10, 37, 93, and 52 mg/L greater than α-lipoprotein cholesterol concentrations, respectively, for Groups I, II, III, and IV; these differences were significant, however, only for Groups II, III, and IV (p < 0.005).

To determine whether manipulative loss of serum lipoproteins during fractionation by ultracentrifugation could account for some of the discrepancies, we compared the results by two methods in samples with 95–105% analytical recovery of serum total cholesterol (Table 2). The results for individual lipoprotein fractions by lipoprotein subgroups were essentially the same.

![Figure 1. Frequency distribution of the percentage of analytical recovery of total lipoprotein cholesterol by ultracentrifugation](image)

Overall correlation coefficients between LDL-cholesterol (y) and β-lipoprotein cholesterol (x) values (mg/L), shown in Figure 2, were high (r = 0.94; y = 1.05x + 57.0). On the other hand, correlations for VLDL-cholesterol with pre-β- and LDL-cholesterol (r = 0.76; y = 0.86x - 11.7) and for HDL-cholesterol with α-lipoprotein cholesterol (r = 0.77; y = 0.86x + 27.6) were somewhat lower. Combined cholesterol values of VLDL and LDL fractions correlated highly with direct determinations of β- + pre-β-lipoprotein cholesterol (r = 0.95).

**Discussion**

We measured serum lipoprotein cholesterol concentrations in four subgroups of pediatric population whose earlier concentrations of β- and (or) pre-β-lipoprotein cholesterol were in the extreme percentiles of the distribution; the measurements were made under standardized laboratory conditions, by the ultracentrifugation and the heparin–Ca²⁺ methods. The values for pre-β-lipoprotein cholesterol and α-lipoprotein cholesterol by the heparin–Ca²⁺ method were less than the values for VLDL-cholesterol and HDL-cholesterol by ultracentrifugation, whereas the β-lipoprotein cholesterol concentrations remained higher than the LDL-cholesterol concentrations. The proportionate differences in values by the two methods varied with the lipoprotein fraction and the group of children selected (reflecting various concentrations of serum lipoproteins).

The conditions specified in this report provide quantitative isolation of major lipoprotein fractions as defined by the two methods. Overall recovery of combined lipoprotein fractions by ultracentrifugation averaged 98.7% of the serum total cholesterol. Considering the highly technical nature of the ultracentrifugation method and the routine manner in which the samples were analyzed, lipoprotein fractionation should be considered highly quantitative. The heparin–Ca²⁺ method quantitatively and selectively precipitates from serum the equivalent of 2000 mg of β- + pre-β-lipoprotein cholesterol per liter with no detectable apoB-containing lipoprotein in the supernate (18). (We diluted the

---

**Table 1. Serum Lipoprotein Cholesterol Concentration by Lipoprotein Selection Group as Determined by Two Methods**

<table>
<thead>
<tr>
<th>Group</th>
<th>β</th>
<th>Pre-β</th>
<th>n</th>
<th>VLDL*</th>
<th>Pre-β*</th>
<th>LDL*</th>
<th>β*</th>
<th>VLDL + LDL*</th>
<th>β + pre-β*</th>
<th>HDL*</th>
<th>α*</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Low</td>
<td>Low</td>
<td>122</td>
<td>84 (4)c</td>
<td>40 (3)</td>
<td>676 (13)c</td>
<td>740 (13)</td>
<td>760 (14)c</td>
<td>780 (13)</td>
<td>575 (13)</td>
<td>565 (14)</td>
</tr>
<tr>
<td>II</td>
<td>High</td>
<td>Low</td>
<td>56</td>
<td>100 (6)c</td>
<td>47 (6)</td>
<td>1125 (29)c</td>
<td>1262 (31)</td>
<td>1225 (28)c</td>
<td>1308 (30)</td>
<td>618 (18)c</td>
<td>581 (17)</td>
</tr>
<tr>
<td>III</td>
<td>High</td>
<td>High</td>
<td>129</td>
<td>162 (8)c</td>
<td>148 (8)</td>
<td>1108 (21)c</td>
<td>1252 (22)</td>
<td>1271 (22)c</td>
<td>1400 (23)</td>
<td>535 (9)c</td>
<td>442 (10)</td>
</tr>
<tr>
<td>IV</td>
<td>Low</td>
<td>High</td>
<td>53</td>
<td>118 (10)</td>
<td>108 (9)</td>
<td>.713 (22)c</td>
<td>786 (20)</td>
<td>832 (23)c</td>
<td>894 (22)</td>
<td>565 (16)c</td>
<td>513 (16)</td>
</tr>
</tbody>
</table>

*Ultracentrifugation method. †Heparin–Ca²⁺ method. ‡Difference between methods significant, p < 0.005 (F-test).
Table 2. Serum Lipoprotein Cholesterol Concentration by Lipoprotein Selection Group as Determined by Two Methods

<table>
<thead>
<tr>
<th>Group</th>
<th>β Pre-β</th>
<th>Pre-β</th>
<th>LDL</th>
<th>β</th>
<th>HDL</th>
<th>α</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Low</td>
<td>Low</td>
<td>89</td>
<td>4</td>
<td>13</td>
<td>14</td>
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<tr>
<td>II</td>
<td>High</td>
<td>Low</td>
<td>36</td>
<td>7</td>
<td>32</td>
<td>35</td>
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<tr>
<td>III</td>
<td>High</td>
<td>High</td>
<td>80</td>
<td>16</td>
<td>27</td>
<td>29</td>
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<tr>
<td>IV</td>
<td>Low</td>
<td>High</td>
<td>34</td>
<td>12</td>
<td>10</td>
<td>10</td>
</tr>
</tbody>
</table>

Footnotes as in Table 1. Results shown are for the 239 samples for which analytical recovery of cholesterol by ultracentrifugation was 95–105%.

![Graph](image)

Fig. 2. Relationship between LDL-cholesterol (ultracentrifugation method) and β-lipoprotein cholesterol (precipitation method) concentrations in children grouped by this serum concentrations of lipoprotein

Letters represent multiple samples having the same values

serum appropriately in cases in which concentrations exceeded this limit.) As we have demonstrated elsewhere (18), the heparin-Ca²⁺ system does not have any affinity for apoE-containing HDL subfractions. However, the lipoprotein cholesterol values by the two methods showed statistically significant differences, even for the samples with 95–105% analytical recovery by ultracentrifugation (Table 3), suggesting intrinsic differences between the methods.

Certain possible reasons for these differences between methods become obvious on considering the nature of the two methods. In preparative ultracentrifugation the lipoproteins (VLDL, LDL, and HDL) have been operationally defined in terms of hydrated density. However, there is known to be an overlap of different lipoproteins within an operationally defined density range. For example, the occurrence of floating β-lipoproteins (β-VLDL), HDL, and sinking pre-β-lipoprotein (Lp(a)), respectively, in density ranges commonly used to separate VLDL, LDL, and HDL is well documented (19–22). Heparin–Ca²⁺ procedure, on the other hand, selectively precipitates all apoB-containing lipoproteins, irrespective of their hydrated density, leaving non-apoB-containing lipoproteins in the supernate. Therefore, it is reasonable to expect relatively greater values for β + pre-β-lipoprotein cholesterol and low values for α-lipoprotein cholesterol after heparin–Ca²⁺ precipitation than after ultracentrifugation (Table 2). In terms of concentrations of β- and pre-β-lipoprotein cholesterol individually, however, the presence of β-VLDL and (Lp(a)) would introduce bias because the measurements are based on electrophoretic distributions of β- and pre-β-lipoproteins.

To make the preparative ultracentrifugation method a standard method for α-lipoprotein cholesterol, one must correct for the occurrence of apoB-containing lipoproteins in the density range 1.063–1.21 kg/L. In fact, Warnick et al. (23) have successfully improved the accuracy of HDL-cholesterol values in the density range 1.063 to 1.063 fractions by correcting for manipulative loss and cholesterol derived from apoB-containing lipoproteins, including [Lp(a)]. They found substantial amounts of apoB-associated cholesterol (46–66 mg/L) in the density range 1.063 fractions in a limited number of children, women, and men with normal serum concentrations of lipids. In our study, the difference between HDL-cholesterol and α-lipoprotein cholesterol was 10, 37, 93, and 52 mg/L, respectively, for lipoprotein selection Groups I, II, III, and IV, which might represent apoB-associated cholesterol. Thus the amount of apoB-containing lipoproteins in density range 1.063 fractions may depend upon the lipoprotein profile of an individual or group. Although α-lipoprotein cholesterol represented 4.0 to 42.0% of the serum total cholesterol in different lipoprotein subgroups, the values by heparin–Ca²⁺ method did not exceed the values by ultracentrifugation in any of these subgroups.

Despite the close relationships of β + pre-β-lipoprotein cholesterol vs VLDL + LDL-cholesterol and β-lipoprotein cholesterol vs LDL-cholesterol by these two methods, there were considerable differences in values between pre-β-lipoprotein cholesterol and VLDL-cholesterol, especially in lipoprotein selection groups (I and II) who had low pre-β-lipoprotein values earlier. One possible reason for this may be that the heparin–Ca²⁺ method measures pre-β-lipoprotein cholesterol values indirectly based on the agarose gel electrophoretic ratio. Comparative studies by Lindgren et al. (24) indicated that measurements of pre-β-lipoprotein cholesterol by quantitative electrophoresis on agarose gel (standardized against analytical ultracentrifugation) were lower than the values obtained by other methods. However, the children with low pre-β-lipoprotein cholesterol (Groups I and II) had higher cholesterol/triglyceride ratios in VLDL, LDL, and HDL than children with high pre-β-lipoprotein cholesterol (Groups III and IV) (25); this compositional difference might affect slightly the degree to which lipoproteins take up Oil Red O (13). Finally, because the serum concentrations of triglycerides, pre-β-lipoprotein, and VLDL are very low in Groups I and II, even small absolute errors (or differences) in measurements by either method will yield large proportionate errors (26, 27).

Thus, ultracentrifugal, electrophoretic, and precipitation methods for measuring serum lipoproteins should be considered as independent methods that demonstrate different properties. Although no ideal method or operational definitions for measuring serum lipoproteins on a large scale are currently available, the heparin–Ca²⁺ method is simpler to perform and is more than adequate to describe the age-, race-, and sex-related differences in populations. The present studies, as well as the studies of Warnick et al. (23), emphasize that, without appropriate corrections, the lipoprotein concentrations obtained by preparative ultracentrifugation may not serve as reference values.
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