Comparability of Results by Simplified Methods for Measuring Serum Lipoprotein Cholesterol in a Pediatric Population

Sathanur R. Srinivasan,¹ Theda A. Foster,² and Gerald S. Berenson¹

We compared serum lipoprotein cholesterol concentrations in a randomly selected subsample (n = 406) population of children (ages 5 to 17 years), as measured by the heparin-Ca²⁺ and the heparin-Mn²⁺ precipitation methods. Statistical analysis showed significant differences between results by these methods, especially in values for VLDL- and HDL-cholesterol (mean difference: VLDL-cholesterol, −81 mg/L; HDL-cholesterol, +108 mg/L). Lipoprotein electrophoretic data indicated that estimating VLDL-cholesterol from serum triglyceride concentrations invariably resulted in higher values for children with an extremely faint pre-β-lipoprotein band. Among the HDL-subfractions, 14 to 20% of HDL₂ was precipitated by heparin–Mn²⁺ despite the presence of 80 g of albumin per liter. Heparin affinity chromatography of HDL₂ in the presence of Ca²⁺ and Mn²⁺ showed an interaction of apolipoprotein E-containing HDL₂ subfraction only in conditions involving Mn²⁺. The heparin–Ca²⁺ procedure precipitated the equivalent of 2 g of β- plus pre-β-lipoprotein cholesterol per liter from serum, with no detectable apolipoprotein B in the supernatant. We conclude that these precipitations are affected by cation specificity and by differences in lipoprotein makeup of a given individual or population group.

Additional Keyphrases: lipoprotein quantitation • polyanionic precipitation method • pediatric chemistry • screening • electrophoresis • heparin affinity chromatography • apolipoproteins • interindividual differences

There is now general agreement that low-density lipoproteins (LDL) are atherogenic, but high-density lipoproteins (HDL) are an important independent negative risk factor for coronary heart disease (1). As a result, attention is being focused on methods for partitioning serum total cholesterol into the major classes of lipoproteins: very-low-density lipoproteins (VLDL), LDL, and HDL. Currently, a combination of ultracentrifugation and heparin–Mn²⁺ precipitation remains the most favored approach to measuring individual lipoprotein cholesterol (2). Because this procedure requires a large volume of serum and involves ultracentrifugation, it has limited applicability for population studies. Friedewald et al. (3) proposed a simplified procedure that eliminates the ultracentrifugation step by estimating VLDL-cholesterol from serum triglyceride concentrations.

Recent studies from this laboratory indicate that the characteristics and concentration of glycosaminoglycans, of serum lipoproteins, and of the divalent metal ions (e.g., Ca²⁺, Mg²⁺, or Mn²⁺) are all critical for quantitative and selective precipitation of the lipoproteins (4, 5). We developed a simplified procedure based on these observations for measuring serum lipoproteins. It is a combination of heparin–Ca²⁺ precipitation and electrophoresis (6, 7). Having tested its validity against the reference procedure, analytical ultracentrifugation (8), and by another independent method (9), we recently applied this procedure for measuring serum lipoproteins to a large pediatric population [the Bogalusa Heart Study (10)]. The HDL-cholesterol values for our pediatric population were about 16% higher than values for other pediatric population studies in which the heparin-Mn²⁺ precipitation method was used (e.g., white boys, 6–17 years: 610 mg/L vs 530 mg/L), although the LDL-cholesterol values remained comparable (11). However, the heparin-Ca²⁺ precipitation method gave similar values for adults (mostly men) as results obtained with the heparin-Mn²⁺ method (12).

In this study, we compared values for serum lipoprotein cholesterol concentrations in a randomly selected subsample of Bogalusa children by our method (LSU method) with the simplified heparin-Mn²⁺ precipitation method (Friedewald method). In addition, we studied the effect of Ca²⁺ and Mn²⁺ on HDL subfraction precipitation and the quantification of apolipoprotein B-containing lipoproteins by the heparin-Ca²⁺ procedure.

Materials and Methods

Samples

Some 4070 children—representing 88% of the children of ages 5 to 17 years living in Bogalusa, Louisiana—participated during the 1976–1977 second cross-sectional study of risk-factor variables in cardiovascular disease. Children were instructed to fast for 12 to 14 h before venipuncture. According to the reported fasting compliance, determined by interview on the morning of the examination, 11.9% of these children were nonfasting and were not included in the present study. Venous blood was collected (blood samples could not be obtained from 0.5% of the children) and was allowed to clot. After centrifugation, sera was collected in tubes containing thimerosal as the antibacterial agent, placed in a shipping box containing frozen packs, and sent by bus to the New Orleans Core Lipid Laboratory of the Specialized Center of Research—Arteriosclerosis. On every screening day approximately 10% of the sera were randomly assigned for lipoprotein cholesterol measurement by both the LSU and the Friedewald (3) method.

In addition, errors in measurement of serum lipids were evaluated independently from the 10% random subsample of blind duplicate determinations (10). Coefficients of variation of 2.7 and 7.7% were obtained for serum cholesterol and triglycerides, respectively. These values represent the composite...
errors associated with venipuncture, processing, and analysis of the samples, including transcription, editing, and computer processing of the final data.

Serum Cholesterol and Triglycerides

Serum total cholesterol (or lipoprotein cholesterol) and triglycerides were determined simultaneously with an AutoAnalyzer II (Technicon Instruments Corp., Tarrytown, NY 10591) according to the Lipid Research Clinics Laboratory method (2). An isopropanol extract of the sample (0.2 mL of whole serum or lipoprotein fraction) was used for the determination. A serum calibration material (provided by the Center for Disease Control, Atlanta, GA) was used to convert the cholesterol value obtained with the AutoAnalyzer II to values that would have been obtained with the method of Abell et al. (13). The Core Lipid Laboratory, which has been designated as "standardized" by the Center for Disease Control, is currently in the surveillance phase of its quality-control program.

Serum Lipoprotein Cholesterol

LSU Method

Determination of α-lipoprotein cholesterol: The serum α-lipoprotein (HDL) cholesterol values were determined after selective precipitation of serum β- (LDL) and pre-β-lipoprotein (VLDL) with heparin and CaCl2 (6, 9, 10). Briefly, this method consists of mixing serum (0.2 mL), distilled water (3.2 mL), beef-lung heparin (0.1 mL of a 2.5 g/L solution, ≈ 140 USP units/mL; The Upjohn Co., Kalamazoo, MI), and CaCl2 (0.5 mol/L, 0.5 mL), in that order. After the mixtures have stood for 15 min, the precipitate is centrifuged (1500 × g, 30 min) and analyzed for the corresponding β- and pre-β-lipoprotein cholesterol content by dissolving it in 0.2 mL of a 0.15 mol/L solution of NaCl. The value for α-lipoprotein cholesterol was obtained by subtracting β- plus pre-β-lipoprotein cholesterol from total cholesterol.

Quantification of apolipoprotein B-containing lipoproteins in this system was tested by measuring the cholesterol content (β- plus pre-β-lipoprotein) of the precipitates and the apolipoprotein B content of supernatants obtained from increasing amounts of serum (0.1 to 0.7 mL, pooled specimen). The volume of distilled water was adjusted to make the final volume 4 mL in all cases (6, 9).

Electrophoretic ratio of β- and pre-β-lipoproteins: Serum (10-20 μL) was electrophoresed on agar-agarose gel plates (8.3 × 10 cm), with use of barbital buffer (pH 8.6, 0.05 mol/L) at 22 mA per plate (6, 14). The lipoprotein bands, stained with Oil Red O, were scanned in a densitometer to assess the relative proportion of β- and pre-β-lipoprotein. We corrected the densitometric ratios of the β- and pre-β-lipoproteins as described previously, assuming that 1.0 mg of β-lipoprotein takes up the same amount of dye as does 0.86 mg of pre-β-lipoprotein (15).

Estimation of β- and pre-β-lipoprotein cholesterol: Our estimation of serum β- and pre-β-lipoprotein concentrations was based on the ratio of β- to pre-β-lipoprotein, β- plus pre-β-lipoprotein cholesterol concentration, and the reported mean values for cholesterol content of β-lipoprotein (469 g/kg) and pre-β-lipoprotein (222 g/kg) molecules. Any changes in lipoprotein estimations owing to variations in cholesterol content of these molecules in normal individuals, including children, were considered negligible (12). The β- and pre-β-lipoprotein cholesterol concentrations were estimated as follows: β-lipoprotein cholesterol = mg β-lipoprotein × 0.469; pre-β-lipoprotein cholesterol = mg pre-β-lipoprotein × 0.222.

Friedewald Method (3)

Determination of HDL-cholesterol: We determined serum HDL-cholesterol values after precipitating LDL and VLDL from the serum with heparin and MnCl2 (3). Briefly, this method consists of mixing serum (0.5 mL), heparin (20 μL of a 50 g/L solution), and MnCl2 (25 μL of a 1.0 mol/L solution), in that order. After the mixtures have stood for 30 min, 0.2 mL of the supernate obtained after centrifugation (1500 × g, 30 min) is assayed for HDL-cholesterol. The resulting values are multiplied by a factor of 1.09 to account for the dilution.

Estimation of VLDL- and LDL-cholesterol: We estimated the serum VLDL-cholesterol by dividing serum triglyceride concentration by 5. Values for LDL-cholesterol were calculated as follows: serum total cholesterol − (VLDL-cholesterol + HDL-cholesterol).

Preparation of Lipoprotein Fractions

We used a Beckman L2-65B preparative ultracentrifuge with a type 40.3 rotor, operated at 11 400 × g with a chamber temperature of 17 °C for 20 and 40 h, to separate VLDL + LDL and HDL subfractions, respectively (16). The densities of solutions to be centrifuged were adjusted with NaCl–NaBr solution (17). HDL subfractions were isolated from pooled serum samples after removing the VLDL and LDL by centrifugation at solvent density 1.063 g/mL. The infranatant serum was ultracentrifuged at solvent densities of 1.12 and 1.21 g/mL to obtain HDL2 and HDL3, respectively. Another HDL fraction was isolated as d 1.09–1.21 g/mL, to eliminate apolipoprotein B-containing lipoproteins, including lipoprotein (a). The isolated lipoprotein fractions were recentrifuged at their respective densities and dialyzed against 0.15 mol/L NaCl containing 1 mmol of EDTA per liter.

Heparin Affinity Chromatography

Samples of HDL2 (equivalent to 7 mg of cholesterol) were applied on a 0.9 × 30 cm column of heparinated agarose gel (Pierce Chemical Co., Rockford, IL) 0.4–0.5 mg of heparin per milliliter of gel) and eluted stepwise with Tris·HCl buffer, (5 mmol/L, pH 7.4) containing 50, 96, and 290 mmol of NaCl per liter (18). In addition to 50 mmol of NaCl per liter, the first buffer contained 25 mmol of MnCl2 or CaCl2 per liter. We collected 3.5-mL fractions and monitored the elution profile by measuring the absorbance at 280 nm.

Immunochemical Analysis

We examined the immunological properties of isolated lipoprotein fractions or heparin–MnCl2 precipitates of HDL subfractions by the Ouchterlony double-diffusion technique, using antibodies to human LDL (Hyland Laboratories, Inc., Los Angeles, CA), apolipoprotein Al, and apolipoprotein E (both generously provided by Dr. Paul S. Roheim, Dept. of Physiology, LSU School of Medicine, New Orleans, LA). The samples were allowed to diffuse for three days at room temperature.

The concentration of apolipoprotein B in the pooled serum (diluted 20-fold) and heparin–CaCl2 supernates (without further dilution) obtained from increasing amounts of pooled serum (0.1–0.7 mL) were quantitated by the electroimmunonasay procedure of Laurell (19). An antibody to human LDL (d 1.03–1.05 g/mL), raised in a goat, was used at a concentration of 7 mL/L in agarose gel (12 g/L). The electroimmunonasay was performed with use of a 25 mmol/L Tris–Tricine ("Tricine" is N-[tris(hydroxymethyl)methyl]glycine) buffer, pH 8.6, at 6 V/cm for 5 h.

Results

Characteristics of the Pediatric Population Subsample

To determine if the randomly selected subsample (n = 406) was representative of the total population examined, we
Table 1. Serum Lipoprotein Cholesterol Concentration (mg/L) as Determined by Two Different Methods

<table>
<thead>
<tr>
<th>n = 390</th>
<th>VLDL-cholesterol</th>
<th>LDL-cholesterol</th>
<th>HDL-cholesterol</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LSU</td>
<td>Friedewald</td>
<td>LSU</td>
</tr>
<tr>
<td>Mean</td>
<td>66</td>
<td>127</td>
<td>832</td>
</tr>
<tr>
<td>SD</td>
<td>48</td>
<td>53</td>
<td>211</td>
</tr>
<tr>
<td>SE</td>
<td>2.4</td>
<td>2.7</td>
<td>10.7</td>
</tr>
<tr>
<td>Range</td>
<td>3-306</td>
<td>52-396</td>
<td>274-1610</td>
</tr>
<tr>
<td>SD of difference*</td>
<td>46</td>
<td></td>
<td>110</td>
</tr>
<tr>
<td>t-value for paired data</td>
<td>-26.4b</td>
<td></td>
<td>-8.4b</td>
</tr>
</tbody>
</table>

* LSU value - Friedewald value.  b p < 0.01.

compared the mean values for certain anthropometric measurements and serum lipid and lipoprotein cholesterol concentrations (LSU method) for the two groups. Because these means did not differ significantly from each other (p > 0.05), we considered the subsample representative of the population of children examined.

Comparison of Methods

From the subsample, a total of 390 serum samples were analyzed for VLDL-, LDL-, and HDL-cholesterol by both the LSU and the Friedewald method (Table 1). It is apparent that while the LSU method gave a mean value 108 mg/L higher for HDL-cholesterol than did the Friedewald method (x), the latter method gave 61 mg/L and 47 mg/L higher values for VLDL- and LDL-cholesterol, respectively, than did the LSU method (y). Statistical analysis by paired t-test showed significant differences between these two methods, although the proportionate difference in LDL-cholesterol values by the two methods was far less than for the VLDL- and HDL-cholesterol values. The correlation coefficients for the two methods were high for LDL-cholesterol (r = 0.88; y = 0.98x + 6.22), while the correlations for VLDL-cholesterol (r = 0.59; y = 0.66x + 8.37) and for HDL-cholesterol (r = 0.57, y = 0.45x + 23.47) were lower.

Because samples from children had relatively low pre-β-lipoprotein bands (as seen from agar–agarose gel electrophoresis) and low serum triglycerides as compared to adults, we computed the serum triglyceride values for children with extremely faint electrophoretic pre-β-lipoprotein band (1–5%). Forty-seven children (12.1%) had a pre-β-lipoprotein band that comprised less than 5% of the densitometric total. Serum triglyceride values for children who had pre-β-lipoprotein bands amounting to 5% were similar to the values for children with trace amounts (1%) of pre-β-lipoprotein band [450 (SD 69) vs 502 (SD 178) mg/L].

Precipitation of HDL Subfractions with Heparin-Mn2+

Earlier studies from our laboratories indicated that heparin-Mn2+ partly precipitated ultracentrifugally isolated HDL subfractions (5). The presence of other serum proteins conceivably could prevent such partial precipitation. Although it was previously shown (30) that serum albumin alone cannot prevent HDL precipitation, a concentration-dependent effect, we have performed the precipitation procedure in the presence of excess albumin and whole serum. (A non-lipoprotein preparation of serum was not used for this purpose because it requires dialysis.) HDL-subfractions enriched with 8.0 g of albumin per liter were added in different amounts to the same serum pool (serum/HDL ratio by volume 9:1, 8:2, or 7:3) in the presence of heparin and Mn2+ (Table 2). While HDL3 precipitation by heparin-Mn2+ was insignificant (3–5%) and well within the measurement error of cholesterol, HDL (d 1.09–1.21 g/mL) and HDL2 were precipitated to a great extent as 10–14 and 14–20%, respectively. Our earlier studies have clearly demonstrated that heparin-Ca2+ systems do not form either soluble or insoluble complexes with apolipoprotein AI-containing HDL subfractions (5) but precipitate only apolipoprotein B-containing lipoproteins (4). Therefore we did not do the above experiments with heparin-Ca2+ for comparison. The heparin-Mn2+-precipitated proteins showed the presence of apolipoprotein AI by immunodiffusion (Figure 1).

Heparin Affinity Chromatography of HDL2

Heparin-Mn2+ precipitates the arginine-rich protein (apolipoprotein E)-containing HDL subfraction (21), so we subjected HDL2 to heparin affinity chromatography in the presence of Mn2+ and Ca2+, to test the affinity of the heparin-Ca2+ system for apolipoprotein E-containing HDL2 sub-

Table 2. Precipitation of HDL-Cholesterol from Serum Samples by Heparin-Mn2+ Treatment

<table>
<thead>
<tr>
<th>HDL fraction</th>
<th>Sample</th>
<th>Recovered in supernate</th>
<th>Percent precipitated</th>
</tr>
</thead>
<tbody>
<tr>
<td>HDL2 (d 1.063–1.12)</td>
<td>860</td>
<td>740</td>
<td>14</td>
</tr>
<tr>
<td>1250</td>
<td>990</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>1640</td>
<td>1310</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>HDL3 (d 1.12–1.21)</td>
<td>600</td>
<td>580</td>
<td>3</td>
</tr>
<tr>
<td>740</td>
<td>700</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>880</td>
<td>850</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>HDL (d 1.09–1.21)</td>
<td>760</td>
<td>680</td>
<td>10</td>
</tr>
<tr>
<td>1080</td>
<td>930</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>1390</td>
<td>1190</td>
<td>14</td>
<td></td>
</tr>
</tbody>
</table>

* Enriched with albumin, 80 g/L.

** Sample contained serum and enriched HDL in various proportions.

Fig. 1. Immunodiffusion pattern of apolipoprotein AI precipitated from serum and HDL by heparin-Mn2+ treatment

1–5, heparin-Mn2+ precipitates of serum containing increasing amounts of HDL-cholesterol: 0.76, 1.08, 1.39, and 1.71 g/L; 6, HDL (d 1.09–1.21); Ab; antibody to apolipoprotein AI.
Fig. 2. Elution profiles for heparin affinity chromatography of human HDL$_2$ (d 1.063–1.12) in the presence of Ca$^{2+}$ and Mn$^{2+}$

---, 50 mmol/L NaCl with 25 mmol of Mn$^{2+}$ per liter; - - - , 50 mmol/L NaCl with 25 mmol of Ca$^{2+}$ per liter

fractions (Figure 2). In the presence of Mn$^{2+}$, stepwise elution with 50, 95, and 290 mmol/L NaCl gave, respectively, three distinct fractions, as described by Weisgraber et al. (18). Immunodiffusion studies of the three fractions by use of antibodies against apolipoprotein AI, apolipoprotein E, and apolipoprotein LDL indicated the presence of apolipoprotein AI in peak I, apolipoprotein AI and apolipoprotein E in peak II, and apolipoprotein B in peak III, confirming the earlier findings (18). In contrast, in the presence of Ca$^{2+}$ all the HDL$_2$ subfractions were eluted with 50 mmol/L NaCl, indicating that the heparin-Ca$^{2+}$ system does not interact with apolipoprotein E-containing HDL$_2$. Both Mn$^{2+}$ and Ca$^{2+}$ interacted strongly with apolipoprotein B-containing lipoproteins appearing in the density range 1.063–1.12 g/mL, which could be either LDL or lipoprotein (a).

Precipitation of Apolipoprotein B-Containing Lipoproteins with Heparin-Ca$^{2+}$

The quantification of apolipoprotein-containing lipoproteins from increasing aliquots of serum by heparin-Ca$^{2+}$ was assessed by analyzing the corresponding precipitates for cholesterol and the supernates for apolipoprotein B (Figure 3). The amount of cholesterol found in the precipitate was linearly related to serum volume up to 0.5 mL, and no apolipoprotein B was detected in the supernates below this volume. [There was no trace of antigen(apolipoprotein B)–antibody interactions found in the electroimmunoassay at this range.] The serum alone, when diluted 20-fold as in the heparin-Ca$^{2+}$ procedure, gave a peak height of 12.4 mm. A contamination of apolipoprotein B at the amount of 5% in the heparin-Ca$^{2+}$ supernate at the corresponding dilutions will produce a peak height of 0.62 mm, which can be easily detected. The percentage of apolipoprotein B found in the supernates increased gradually (6.4–21.1%) between 0.5- and 0.7-mL aliquots of serum which contained 836 mg of apolipoprotein B per liter. The saturation point in terms of precipitable β- plus pre-β-lipoprotein cholesterol from serum represented 2100 mg/L.

Discussion

We concurrently measured serum lipoprotein cholesterol concentrations in a randomly selected subsample of a pediatric population, under standardized laboratory conditions, by the heparin-Ca$^{2+}$ and the heparin-Mn$^{2+}$ precipitation methods. Analysis by the LSU method gave a mean value of 66 mg/L for VLDL-cholesterol and 624 mg/L for HDL-cholesterol, as compared to Friedewald's 127 mg/L and 516 mg/L, respectively. However, the LSU and Friedewald methods gave relatively close values for LDL-cholesterol (832 mg/L vs 878 mg/L).

Certain potential reasons for these differences between results by the two methods emerge from our study. While VLDL-cholesterol measurement by the LSU method is based on a densitometric ratio of electrophoretically separated β- and pre-β-lipoproteins, the Friedewald method is based on the concentration of serum triglycerides. Friedewald et al. (3) have pointed out that VLDL-cholesterol estimation is not valid in individuals with a substantial degree of hypertriglyceridemia. In view of the low pre-β-lipoprotein band and low serum triglycerides encountered in children, calculation of VLDL-cholesterol based on serum triglycerides also could result in an over-estimation. Under these circumstances, serum triglycerides in the range of 450 to 500 mg/L could be derived mainly from HDL and LDL, because these two lipoprotein classes contribute by as much as 8% of their weight to the total serum triglycerides. Comparative studies by Lindgren et al. (22) indicated that measurements of VLDL-cholesterol by quantitative electrophoresis on agarose gel, a technique that was standardized against analytical ultracentrifugation, were 50% lower than the values obtained by the Friedewald method.

It has been suggested that this bias between the two methods is ascribable to incomplete precipitation of apolipoprotein B-containing lipoproteins by heparin-Ca$^{2+}$ (23). The present observations that the heparin-Ca$^{2+}$ procedure can precipitate 2000 mg/L equivalent of β- plus pre-β-lipoprotein cholesterol from serum without any detectable amount of apolipoprotein B in the supernate, which is well within the working range of the precipitation procedure (6, 7), rule out this possibility. We reduce the serum sample volume appropriately in cases where the values exceed the above limit and the turbidity produced by heparin-Ca$^{2+}$ exceeds 0.5 absorbance unit at 600 nm, which is an indirect measure of serum β- and pre-β-lipoprotein concentrations (4, 6). Such instances are rare in pediatric populations, because the 95th percentile for serum total cholesterol is 2100 mg/L (10).

In view of the above considerations, the differences between the two methods in HDL-cholesterol values can be attributed.

Fig. 3. Precipitation of serum apolipoprotein B-containing lipoproteins by the heparin–Ca$^{2+}$ method

The relative concentration of apolipoprotein B in the heparin–Ca$^{2+}$ supernates (without further dilution) obtained from increasing amounts of pooled serum (0.1–0.7 mL) were quantitated by electroimmunoassay (shown in the insert). The corresponding precipitates were analyzed for β- plus pre-β-lipoprotein cholesterol. Pooled 20-fold diluted serum was subjected to electroimmunoassay, for comparison. Supernates 1–7 represent final serum concentrations of 25 to 175 mL/L.
Table 3. HDL-Cholesterol Survey by the Center for Disease Control

<table>
<thead>
<tr>
<th></th>
<th>Mean HDL-cholesterol (and SD), mg/dL</th>
<th>Low pool</th>
<th>Medium pool</th>
<th>High pool</th>
</tr>
</thead>
<tbody>
<tr>
<td>LSU SCOR-A</td>
<td></td>
<td>345 (74)</td>
<td>513 (87)</td>
<td>596 (91)</td>
</tr>
<tr>
<td>Participating</td>
<td></td>
<td>349 (14)</td>
<td>525 (23)</td>
<td>605 (20)</td>
</tr>
<tr>
<td>labs combined</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CDC</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

n = 12 for each pool.

remains qualitative rather than quantitative." The current methods, although imperfect, are providing biological insight into lipoprotein changes in large numbers of individuals and are giving us leads to further refinement of techniques.

This research was supported by funds from the National Heart and Lung Institute, USPHS, and the Specialized Center of Research—Artherosclerosis (supported by grant HL15103) at Louisiana State University Medical Center in New Orleans.

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