Differential Reactivity of Two Homogeneous LDL-Cholesterol Methods to LDL and VLDL Subfractions, as Demonstrated by Ultracentrifugation and HPLC

SHINICHI USUI,1 HAJIME KAKUUCHI,2 MOTOI OKAMOTO,1 YUKI MIZUKAMI,3 and MITSUYO OKAZAKI4*

Background: The analytical and clinical performance of homogeneous LDL-cholesterol assays has been reported, but their reactions with subfractions of LDL and VLDL have not been described in detail.

Methods: We evaluated reaction selectivity of two homogeneous LDL-cholesterol assays, LDLk (Kyowa Medex) and LDLd (Daiichi Pure Chemical), with ultracentrifugally isolated VLDL and LDL subfractions to identify the lipoprotein particles from which the cholesterol recognized by these assays originates.

Results: The LDLd (y) and LDLk (x) methods correlated highly for whole serum samples: $y = 0.986x - 39.5$ mg/L ($r = 0.966; n = 34$). In isolated VLDL, the LDLk and the LDLd methods recovered 17.3% and 23.8% of cholesterol, respectively; but correlation analysis revealed differential reactivity to small and large VLDL particles. For the isolated LDL subfraction of density 1.019–1.040 kg/L, the LDLd method had significantly higher reactivity (95.6–98.7%) than the LDLk (88.4–92.0%). Both methods, however, demonstrated poor recovery (~50%) for the 1.050–1.063 kg/L fraction, indicating incomplete reactivity with small, dense LDL. Reactivity with lipoprotein(a) was better (71.2–90.8%) for both methods than with small LDL. For intermediate-density lipoprotein (IDL), there was no significant difference in recovery between the two methods (71.7% for LDLk and 68.9% for LDLd), but the LDLk method appeared to be more sensitive to LDL particle size.

Conclusions: The two homogeneous assays for LDL-cholesterol demonstrate only partial reactivity to small, dense LDL and nonspecific reactions to VLDL particles. Modification will be required in the homogeneous methods to obtain LDL-cholesterol values equivalent to those obtained by ultracentrifugation.

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The Adult Treatment Panel (ATP)5 guidelines of the National Cholesterol Education Program, including the most recent update (ATP III), consider LDL-cholesterol the major indicator for initial classification of coronary heart disease risk status and identify lowering of LDL-cholesterol as the primary goal of therapy (1). The association of LDL-cholesterol with increased risk of developing coronary heart disease, also recognized in the previous ATP II guidelines (2), has been well established by various types of studies (1–6). Therefore, accurate and precise determination of LDL-cholesterol is important in achieving reliable classification of patients and in monitoring therapies.

The most widely accepted reference method for LDL-cholesterol is a version of β-quantification (BQ) combining ultracentrifugation and heparin-Mn2+ precipitation, as performed at the CDC (7, 8). The LDL-cholesterol values determined by the BQ method, which are calculated by subtracting HDL-cholesterol from cholesterol measured in the 1.006 kg/L bottom fraction obtained by

1 Faculty of Health Sciences, Okayama University Medical School, 2-5-1 Shikata-cho, Okayama-shi, Okayama 700-8558, Japan.
2 Department of Chemistry, Chiba University, 1-33 Yayoi-cho Inage-ku, Chiba-shi, Chiba 263-8522, Japan.
3 Faculty of Human Life Sciences, Jissen Women’s University, 4-1-1 Ohsakae Hino-shi, Tokyo 191-8510, Japan.
4 Laboratory of Chemistry, College of Liberal Arts and Sciences, Tokyo Medical and Dental University, 2-8-30 Kohomodai, Ichikawa-shi, Chiba 272-0827, Japan.
*Author for correspondence. Fax 81-47-300-7100; e-mail okazaki.las@tmd.ac.jp.

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ultracentrifugation, include intermediate-density lipoproteins (IDL; density, 1.006–1.019 kg/L) and lipoprotein(a) [Lp(a)] as well as LDL particles with a density of 1.019–1.063 kg/L. IDL and Lp(a) are also generally considered to be atherogenic, and their proportional contribution to the LDL-cholesterol measurement would be expected to be greater in populations at risk for coronary heart disease. Therefore, LDL-cholesterol values by the BQ method can be considered to represent the cholesterol contained in several potentially atherogenic lipoproteins (8).

To provide more efficient measurement of LDL-cholesterol in clinical laboratories, new fully automated homogeneous methods have been developed and are commercially available (9–13). Homogeneous LDL-cholesterol methods allow convenient determination of LDL-cholesterol values in a small sample volume on an automated analyzer without any sample pretreatment. However, to replace the current commonly used Friedewald calculation (14) in routine clinical practice, the homogeneous methods should have the ability to measure LDL-cholesterol in samples from nonfasting patients as well as those with hypertriglyceridemia (>4000 mg/L), in which the Friedewald calculation is not valid (14, 15).

Most evaluations of the homogeneous methods for LDL-cholesterol have involved comparisons with the BQ or modified BQ methods (9–13, 15–20). Few reports have considered the reaction specificities of the reagents to the lipoprotein subclasses, i.e., IDL, Lp(a), and triglyceride-rich lipoproteins (20–23). In addition, the reaction specificities of homogeneous LDL-cholesterol methods to other VLDL and LDL subfractions, such as small, dense LDL, have not been established. Our aim was to characterize the types and characteristics of lipoprotein particles recognized as LDL by homogeneous methods. The main purpose of this study was to evaluate the reaction selectivity of two homogeneous assays, Determiner L LDL-C (Kyowa Medex) and Cholestest LDL (Daiichi Pure Chemical), to LDL and VLDL subfractions from healthy and hyperlipidemic individuals by use of ultracentrifugation and HPLC methods (24, 25).

**Materials and Methods**

**SERUM SAMPLES**

Blood samples were collected into glass tubes without anticoagulant from 38 volunteers, ages 23–99 years (mean age, 55 years), reporting for an annual medical examination. Of the 38 volunteers, 17 were normolipidemic (total cholesterol <2000 mg/L and total triglycerides <1500 mg/L) and 21 were hyperlipidemic (total cholesterol >2000 mg/L and/or total triglycerides >1500 mg/L).

The blood samples were allowed to clot at room temperature and were centrifuged at 2000g for 15 min to obtain serum samples. All serum samples were stored at 4 °C and analyzed within 10 days after blood collection. All volunteers gave informed consent to participate in this study.

**HOMOGENEOUS LDL-CHOLESTEROL METHODS**

Two homogeneous LDL-cholesterol assays were used: Determiner L LDL-C (LDLk; Kyowa Medex; lot nos. 203AAF and 203AAI) and Cholestest LDL (LDLd; Daiichi Pure Chemical; lot nos. 117RBY and 116RLX). In the LDLk method (9), α-cyclodextrin sulfate blocks the enzymatic reaction of cholesterol in chylomicrons and VLDL, and a nonionic surfactant (polyoxyethylene-polyoxypropylene block copolymer) selectively solubilizes LDL without solubilizing HDL. The combination of α-cyclodextrin and the nonionic surfactant facilitates direct LDL-cholesterol determination. The LDLd method, on the other hand, first reacts with the cholesterol in lipoproteins other than LDL, by use of a special surfactant, to form a colorless product and then reacts with another surfactant that exposes the cholesterol in LDL to enzymatic reactions, leading to a colored product (11).

All procedures were performed on a Hitachi 7170S automated analyzer (Hitachi High-Technologies Corporation) according to the manufacturers’ instructions.

**ULTRACENTRIFUGATION**

Six lipoprotein fractions [density (d) <1.006, 1.006–1.019, 1.019–1.030, 1.030–1.040, 1.040–1.050, and 1.050–1.063 kg/L] were prepared by sequential density ultracentrifugation (25–27). A 1.0-mL serum sample was placed in a polycarbonate centrifuge tube (cat. no. 343778; Beckman Instruments) and centrifuged on a Beckman Optima-TLX preparative ultracentrifuge with a fixed-angle TLA 120.2 rotor at 527 000g for 3 h at 16 °C, to obtain a top (d <1.006 kg/L) and a bottom (d >1.063 kg/L) fraction; fractions were recovered by a tube-slicing technique using a Beckman CentriTube Slicer. The density of the 1.006 kg/L bottom fraction obtained by ultracentrifugation was adjusted to 1.019 kg/L by addition of a concentrated NaBr solution, and the fraction was centrifuged by the same manner to obtain a 1.006–1.019 kg/L fraction. Other lipoprotein fractions were also obtained similarly from each ultracentrifugation bottom fraction after density adjustment. The recovery of lipoproteins was calculated as follows:

\[
\text{Recovery of cholesterol (\%)} = \frac{\text{Sum of cholesterol in each ultracentrifugation fraction}}{\text{Total cholesterol in whole serum}} \times 100
\]

\[
\text{Recovery of triglycerides (\%)} = \frac{\text{Sum of triglycerides in each ultracentrifugation fraction}}{\text{Total triglycerides in whole serum}} \times 100
\]

Four individuals (two normolipidemic and two hyperlipidemic) were excluded from statistical analysis because their recovery values were not within the mean recov-
ery ± 4 SD. The final analytical recoveries (mean ± SD; n = 34) were 96.5% ± 18.1% for cholesterol and 91.9% ± 10.5% for triglycerides.

**ANALYSIS OF LIPOPROTEINS IN ULTRACENTRIFUGATION FRACTIONS BY HPLC**

We analyzed each of the ultracentrifugation fractions by HPLC with a gel-permeation column to confirm the purity and provide more detailed characteristics of the fractions (24, 25, 28). The HPLC system consisted of an AS-8020 autoinjector, CCPS and CCPM-II pumps, and two UV-8020 detectors (Tosoh). Lipoproteins were separated on a single Superose 6HR column (300 × 10 mm; Pharmacia) with 0.05 mol/L phosphate-buffered saline (pH 7.4) containing 0.15 mol/L NaCl at a flow rate of 0.5 mL/min. The running buffer was filtered through a 0.22 µm filter (Millipore Corp.) before use and was continuously degassed with a SD-8022 online degasser (Tosoh) during analysis. A 10-µL sample was injected into the column by an AS-8020 autoinjector with a presuction of 25 µL. The column effluent was split equally into two lines by a P-460 MicroSplitter (Upchurch Scientific Inc.); one effluent portion was mixed with cholesterol reagent and the other with triglyceride reagent. The two enzymatic reagents were each pumped at a flow rate of 0.25 mL/min. The absorbance at 550 nm was continuously monitored after the enzymatic reaction at 37 °C in a reactor coil [Teflon; 15 m × 0.4 mm (i.d.)].

**MEASUREMENT OF LIPIDS AND Lp(a)**

Cholesterol and triglyceride values in whole sera and their ultracentrifugation fractions were determined enzymatically on the Hitachi 7170S automatic analyzer with Determiner L TC II and Determiner L TG II reagent sets (both from Kyowa Medex), respectively. The triglyceride assay was corrected for the presence of endogenous glycerol. Serum Lp(a) concentrations were determined on a JCA-BM12 biochemical analyzer (Japan Electron Optics Laboratory Co., Ltd.) by an immunoturbidimetric method from Daiichi Pure Chemical.

**STATISTICS**

Data are expressed as the mean ± SD unless stated otherwise. A paired Student t-test was used to test the significance of differences between the two homogeneous methods, and P < 0.05 was considered statistically significant.

**Results**

**COMPARISON OF TWO HOMOGENEOUS LDL-CHOLESTEROL METHODS TO TRIGLYCERIDE-RICH LIPOPROTEINS**

Shown in Table 2 are the means (SD) for total cholesterol and triglycerides and the cholesterol/triglyceride ratio (mg/mg) in the 1.006 kg/L top fractions separated by ultracentrifugation. The 1.006 kg/L top fractions seemed to contain mainly VLDL without chylomicrons because there was a significant correlation between cholesterol (y) and triglyceride (x) values in those fractions: y = 0.17x + 165 mg/L (r = 0.892). Therefore, the 1.006 kg/L top fraction obtained by ultracentrifugation hereafter is referred to as the “VLDL fraction”. The cholesterol/triglyceride ratio (mg/mg) in the VLDL fraction, however, ranged from 0.15 to 1.11 (mean, 0.47), indicating the presence of several types of VLDL particles. Two distinct peaks corresponding to large and small VLDL particles were also included in almost all of the VLDL fractions, as shown in Fig. 2, demonstrating the heterogeneity of the VLDL fractions obtained by ultracentrifugation. Six participants had only large VLDL, but the remainder (n = 28) had both large and small VLDL.

We analyzed those whole VLDL fractions with a density <1.006 kg/L in parallel by the two homogeneous methods. Mean cholesterol values obtained with the LDLk and the LDLd methods were 60 and 84 mg/L, respectively (Table 2), which corresponded to 17.3% and 23.8% of total cholesterol values in the VLDL fraction. As shown in Fig. 3, the correlations between total cholesterol in the VLDL fraction and the cholesterol determined by both the LDLk (r = 0.476; P < 0.005) and LDLd (r = 0.932; P < 0.001) methods were significant, but the LDLd method seemed to have stronger reactivity to triglyceride-rich lipoproteins, in a dose-dependent manner, relative to

**Table 1. Lipid and Lp(a) profiles of the participants in this study.**

<table>
<thead>
<tr>
<th>Lipid Profile</th>
<th>n</th>
<th>Minimum, mg/L</th>
<th>Maximum, mg/L</th>
<th>Mean ± SD, mg/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol</td>
<td>34</td>
<td>1447</td>
<td>3066</td>
<td>2150 ± 397</td>
</tr>
<tr>
<td>Total triglycerides</td>
<td>34</td>
<td>700</td>
<td>6609</td>
<td>1969 ± 1558</td>
</tr>
<tr>
<td>Lp(a)</td>
<td>34</td>
<td>26</td>
<td>1186</td>
<td>253 ± 323</td>
</tr>
<tr>
<td>Cholesterol by LDLk</td>
<td>34</td>
<td>659</td>
<td>2024</td>
<td>1308 ± 333</td>
</tr>
<tr>
<td>Cholesterol by LDLd</td>
<td>34</td>
<td>685</td>
<td>1925</td>
<td>1250 ± 340</td>
</tr>
</tbody>
</table>

mg/L by the LDLd, as shown in Table 1. The paired Student t-test revealed that the LDLd method significantly underestimated LDL-cholesterol values relative to the LDLk method (P < 0.001).

The homogeneous LDLd method (y) correlated highly with the LDLk method (x), as shown in Fig. 1A; the regression equation was: y = 0.986x − 39.5 mg/L (r = 0.966). The mean difference (LDLd – LDLk) was −58 mg/L, but the Bland–Altman plot (29) showed no relationship between the differences and LDL-cholesterol values as shown in Fig. 1B.

**COMPARISON OF REACTIVITY OF TWO HOMOGENEOUS LDL-CHOLESTEROL METHODS TO TRIGLYCERIDE-RICH LIPOPROTEINS**

...
the LDLk method. As shown in Fig. 4, the cholesterol measured in the VLDL fractions by the LDLd method also correlated highly and significantly with both the small VLDL-cholesterol ($r = 0.757; P < 0.001$) and large VLDL-cholesterol ($r = 0.856; P < 0.001$) concentrations that were calculated from chromatographic patterns, whereas the cholesterol measured by the LDLk method correlated significantly with the small VLDL-cholesterol ($r = 0.894; P < 0.001$) but weakly with the large VLDL-cholesterol ($r = 0.319; P = 0.065$) concentrations, suggesting different selectivities for the two homogeneous methods toward the VLDL subfractions.

Fig. 1. Relationship between LDL-cholesterol measured by the LDLk and the LDLd methods on whole-serum samples (A), and Bland–Altman plot (B).

(A), solid line represents a linear regression equation of: $y = 0.986x - 39.5$ ($r = 0.966; n = 34$). (B), differences (LDL-cholesterol by LDLd - LDL-cholesterol by LDLk) are plotted against the average values by the two methods.

Table 2. Characteristics of the VLDL fractions obtained by ultracentrifugation ($n = 34$).

<table>
<thead>
<tr>
<th></th>
<th>Minimum</th>
<th>Maximum</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol, mg/L</td>
<td>95</td>
<td>1152</td>
<td>388 ± 272</td>
</tr>
<tr>
<td>Triglycerides, mg/L</td>
<td>98</td>
<td>5564</td>
<td>1317 ± 1464</td>
</tr>
<tr>
<td>Cholesterol/triglyceride ratio, mg/mg</td>
<td>0.15</td>
<td>1.11</td>
<td>0.47 ± 0.31</td>
</tr>
<tr>
<td>Cholesterol by LDLk, mg/L</td>
<td>0</td>
<td>180</td>
<td>60 ± 42</td>
</tr>
<tr>
<td>Cholesterol by LDLd, mg/L</td>
<td>18</td>
<td>174</td>
<td>84 ± 44</td>
</tr>
</tbody>
</table>

Fig. 2. Representative chromatographic patterns of the 1.006 kg/L top fractions from two individuals with contrasting distributions of small and large VLDL particles.

One individual has large and small VLDL-cholesterol concentrations of 12.5 and 6.5 mg/L, respectively (A). The other individual has large and small VLDL-cholesterol concentrations of 3.7 and 14.6 mg/L, respectively (B). Solid and dashed lines indicate cholesterol and triglycerides, respectively.
COMPARISON OF REACTIVITY OF TWO HOMOGENEOUS LDL-CHOLESTEROL METHODS TO LDL SUBFRACTIONS

The characteristics of the LDL subfractions obtained by ultracentrifugation are shown in Table 3. LDL was mainly distributed in fractions with densities of 1.019–1.040 kg/L, which comprised ~64% of the LDL particles. One-way ANOVA indicated a significant difference (P < 0.05) in the cholesterol/triglyceride ratio (mg/mg) between LDL subfractions (d = 1.019–1.063 kg/L) even when the 1.006–1.019 kg/L fraction (IDL) was excluded from the statistical calculation. Elution times of the peaks observed in chromatographic patterns, which reflect the particle size of the lipoproteins contained in each LDL subfraction, increased noticeably as density increased, indicating that the LDL subfractions obtained by ultracentrifugation were of different particle sizes (Table 3).

The cholesterol values obtained with the two homogeneous methods were similar (Table 3) and highly correlated in all LDL subfractions (r = 0.942–0.998). The reactivities of the two homogeneous methods to each LDL subfraction ranged from 54.1% to 92.0% for the LDLk method and from 46.1% to 98.7% for the LDLd method, as shown in Fig. 5, demonstrating that the LDLd method provided slightly but significantly better recovery of LDL-cholesterol in major LDL fractions (d = 1.019–1.040 kg/L) than the LDLk method. Both homogeneous methods, however, were less reactive (46.1–54.1%) with lipoproteins in the 1.050–1.063 kg/L fraction, which contains small, dense LDL and/or Lp(a). In the case of the 1.050–1.063 kg/L fractions (n = 3) with a prominent Lp(a) peak detected in the chromatogram (Fig. 6), the LDLk and LDLd methods gave relatively good recoveries of 71.2–87.4% and 80.1–90.8%, respectively, suggesting that Lp(a)-cholesterol might be measured more effectively with the homogeneous methods than is small, dense LDL-cholesterol.

In the 1.006–1.019 kg/L fractions, there was no significant difference in the reaction sensitivities of the two homogeneous methods (recovery, 71.7% for LDLk and...
68.9% for LDLd), as shown in Fig. 5. However, the recovery obtained with the LDLk method correlated highly with the elution time of IDL detected by the HPLC method ($r = -0.496; P < 0.005$), as shown in Fig. 7, suggesting the existence of a differential reactivity between the two homogeneous methods with the IDL fraction. The LDLk method would be more sensitive to IDL particle size.

**Discussion**

LDL consists of heterogeneous lipoprotein particles that are different in hydrated density, particle size, and chemical composition. Traditionally the hydrated density range of 1.019–1.063 kg/L has been used to define LDL, which includes some Lp(a), usually occurring in the density range of 1.050–1.100 kg/L (30). On the other hand, LDL-cholesterol values determined by the reference method, which is widely known as the BQ method, include all cholesterol associated with apolipoprotein B-containing lipoproteins with densities >1.006 kg/L, thus including IDL with densities of 1.006–1.019 kg/L and all of the Lp(a). Newly developed LDL-cholesterol assays, therefore, should be evaluated not only in comparison with the reference method, but also in their selectivity and reactivity against IDL, Lp(a), and the triglyceride-rich lipoproteins with densities <1.006 kg/L. In this study, we determined the types and characteristics of lipoprotein particles that are recognized as LDL by two homogeneous methods, LDLk and LDLd.

The LDLk and the LDLd methods gave similar LDL-cholesterol values on whole-serum samples and VLDL and LDL subfractions obtained by ultracentrifugation, but the LDLk assay gave a significantly poorer recovery

![Fig. 5. Mean recovery of cholesterol associated with LDL subfractions by the LDLk (●) and the LDLd (○) methods. N.S., no significant difference between the two methods, as assessed by paired Student t-test. Bars, SD.](https://academic.oup.com/clinchem/article-abstract/48/11/1946/5642284)

![Fig. 6. Representative chromatographic pattern of the 1.050–1.063 kg/L fraction from an individual with a high Lp(a) concentration (1190 mg/L). Solid and dashed lines represent cholesterol and triglycerides, respectively.](https://academic.oup.com/clinchem/article-abstract/48/11/1946/5642284)

**Table 3. Characteristics of the LDL subfractions with densities of 1.006–1.063 kg/L.**

<table>
<thead>
<tr>
<th>LDL subfraction, kg/L</th>
<th>1.006–1.019 (n = 34)</th>
<th>1.019–1.030 (n = 34)</th>
<th>1.030–1.040 (n = 29)$^b$</th>
<th>1.040–1.050 (n = 30)$^b$</th>
<th>1.050–1.063 (n = 25)$^{b,c}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol, mg/L</td>
<td>129 ± 71</td>
<td>319 ± 203</td>
<td>381 ± 234</td>
<td>192 ± 154</td>
<td>64 ± 54</td>
</tr>
<tr>
<td>Triglycerides, mg/L</td>
<td>79 ± 49</td>
<td>117 ± 95</td>
<td>107 ± 93</td>
<td>42 ± 35</td>
<td>20 ± 14</td>
</tr>
<tr>
<td>Chol/Trig ratio,$^d$</td>
<td>1.74 ± 0.54</td>
<td>3.06 ± 1.33</td>
<td>4.60 ± 2.67</td>
<td>4.68 ± 2.78</td>
<td>3.52 ± 2.44</td>
</tr>
<tr>
<td>Elution time, min</td>
<td>25.8 ± 0.2</td>
<td>26.6 ± 0.2</td>
<td>27.2 ± 0.3</td>
<td>27.6 ± 0.4</td>
<td>28.0 ± 0.4</td>
</tr>
<tr>
<td>Cholesterol by LDLk, mg/L</td>
<td>97 ± 60</td>
<td>283 ± 170</td>
<td>361 ± 231</td>
<td>181 ± 158</td>
<td>40 ± 56</td>
</tr>
<tr>
<td>Cholesterol by LDLd, mg/L</td>
<td>89 ± 49</td>
<td>304 ± 187</td>
<td>380 ± 234</td>
<td>172 ± 154</td>
<td>29 ± 32</td>
</tr>
</tbody>
</table>

$^a$ Data are expressed as the mean ± SD.

$^b$ Fractions with apparently incomplete ultracentrifugal separation or no elution peak because low cholesterol concentration observed in their HPLC patterns were excluded.

$^c$ Fractions from volunteers with high Lp(a) concentrations (>1000 mg/L) were excluded.

$^d$ Ratio of cholesterol (Chol) to triglycerides (Trig).
(88.4–92.0%) for LDL subfractions with densities of 1.019–1.040 kg/L than did the LDLd assay (95.6–98.7%). Incomplete recovery of the 1.019–1.040 kg/L fractions by the LDLk method will possibly have an effect on LDL-cholesterol values determined in whole serum because these fractions represent the major subpopulation of whole LDL. Nauck and Rifai (16) previously documented the negative bias between the homogeneous assays and the modified BQ method, which indicates incomplete measurement of the cholesterol content of all of the “broadcut” LDL fraction, especially by the LDLk method. Other researchers have also shown a systemic negative bias of the LDLk and LDLd methods compared with the reference method (13, 15, 17–19). Our results can partially explain and support these previous data.

In other LDL subfractions, the recoveries by the two homogeneous methods were similar and the differences did not reach statistical significance. The lowest recovery, ~50% for the 1.050–1.063 kg/L fraction, by both homogeneous methods, however, should be noted, although the low recovery for this dense LDL fraction may not likely make a big difference in the results for LDL-cholesterol determined in whole serum in most cases. Small, dense LDL, which is fractionated mainly into a layer with a density 1.050–1.063 kg/L by ultracentrifugation, has been established as one of the more atherogenic lipoproteins and often increases with hypertriglyceridemia (31, 32). The poor recovery of cholesterol in the 1.050–1.063 kg/L fraction will be a problem in hypertriglyceridemic individuals with increased concentrations of small, dense LDL. A portion of Lp(a) is also included in the same dense LDL subfraction and has generally been accepted as one of the atherogenic lipoproteins as well. Therefore, ideally these two types of atherogenic lipoproteins should be recognized as LDL by the homogeneous methods, similar to the BQ method.

As for Lp(a)-cholesterol measurement, relatively higher recoveries of 71.2–90.8% were obtained for the 1.050–1.063 kg/L fractions that apparently had a major chromatographic peak corresponding to Lp(a), indicating that Lp(a)-cholesterol might be measured more effectively with both homogeneous methods than cholesterol associated with small, dense LDL. Consistent with our results, two previous studies (9, 20) indicated that Lp(a)-cholesterol would be included in homogeneous LDL-cholesterol values because, as Lp(a) concentrations increased, there was no increase in the bias for LDL-cholesterol values determined by the homogeneous methods compared with the BQ method. We can speculate that the ability of the homogeneous assays to distinguish Lp(a) from small, dense LDL particles may be attributable to the Lp(a) particle size, which is similar to that of IDL or large buoyant LDL but not of small, dense LDL.

As for IDL-cholesterol measurements, both homogeneous methods were similarly reactive with ~70% of cholesterol in the 1.006–1.019 kg/L fraction. Sakaue et al. (21) previously reported the differential recoveries of IDL-cholesterol by the LDLk (64%) and the LDLd (47%) methods from ultracentrifugally pure IDL fractions. Fei et al. (22) also reported that the LDLk and LDLd methods measured IDL-cholesterol at 52.4% and 31.2%, respectively, in IDL isolated from hypertriglyceridemic samples. Our study results were not consistent with these previous data, but indicated that the reactivity of the LDLk method to IDL might depend on particle size, as shown in Fig. 7. The LDLk and LDLd methods apparently do not recognize the same IDL particles, although we found no significant difference in the IDL recoveries by the two homogeneous methods.

Another problem associated with hypertriglyceridemic samples was the nonspecific reactivity of the homogeneous methods to the VLDL fractions, which produced a 17.3% recovery for the LDLk method and 23.8% for the LDLd method, nearly consistent with the previous report (21). Additionally, our study indicated that the LDLd method was similarly reactive with both large and small VLDL-cholesterol in a dose-dependent manner, whereas the LDLk showed no correlation with large VLDL-cholesterol and a significantly strong correlation with small VLDL-cholesterol. Considering that small VLDL is characterized as cholesterol-rich VLDL (25), the two homogeneous methods might include part of the remnant lipoproteins. Esteban-Salán et al. (18) recently indicated that the triglyceride bias might be dependent on the nature and/or composition of the triglyceride-rich lipoproteins used in the interference studies. They demonstrated that the LDL-cholesterol bias of the LDLk method with the BQ method correlated significantly and propor-

![Fig. 7. Relationship between elution time of the IDL fraction and recovery (%) of the IDL fraction by the LDLk (•) and the LDLd (○) methods. Solid line, LDLk (r = −0.496; P < 0.005); dotted line, LDLd, (r = −0.026; P = 0.980).](https://academic.oup.com/clinchem/article-abstract/48/11/1946/5642284)
tionally with the VLDL-cholesterol/serum triglyceride ratio and suggested that cholesterol-rich VLDL would be recognized as LDL by the LDLk method. We did not observe in the VLDL fraction a significant correlation between lipid composition (cholesterol/triglyceride ratio) and cholesterol values determined by the two homogeneous methods, but VLDL particle size might be associated with reaction specificity, especially in the LDLk method. We do not think that part of the VLDL fraction should be included in LDL-cholesterol value, although the recovery of the VLDL fraction by the homogeneous methods may be attributable to remnant lipoproteins, which are generally accepted as atherogenic particles, because (a) the BQ method values do not include remnants in the 1.006 kg/L top fraction and (b) nonspecific reactivity to remnants will lead to misclassification of type III hyperlipidemia as type IIb hyperlipidemia, leading to unsuitable treatment by an inappropriate selection of lipid-lowering drugs. Additionally, the method for the measurement of remnant-like particle-cholesterol has already been established by Nakajima et al. (33) and is commercially available and highly sensitive (34). To clarify whether remnant-like particles are recognized by the homogeneous LDL-cholesterol methods, further studies will be required.

We should remark on whether the observed performance of the homogeneous methods for ultracentrifugation fractions exactly reflected that of native sera. This problem is important but may be unavoidable. In our present study, very fast ultracentrifugation was conducted to obtain lipoprotein subfractions (3-h run for each fraction) to avoid alteration of the native properties of lipoprotein particles as much as possible. Pietzsch et al. (27) reported that a very short run time had a minimal effect on apolipoproteins B-100, A-I, E, and C-I to C-III and that the procedure was suitable for the assessment of lipid and protein constituents in lipoproteins. Therefore, our study design for ultracentrifugation was considered suitable, and in fact, HPLC analysis of the isolated ultracentrifugation fractions produced reasonable results with respect to their lipid composition and particle size.

In conclusion, we have demonstrated that two homogeneous LDL-cholesterol assays have reduced reactivity to small, dense LDL and nonspecific reactivity to VLDL. Both homogeneous methods had opposite reactivities to VLDL and small LDL, differences likely to compensate in hypertriacylglyceremidic individuals. Our results suggest that to obtain LDL-cholesterol values by the homogeneous methods that agree with the BQ reference method, some modifications will be required in the present homogeneous formulations.

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