Serum LDL- and HDL-cholesterol determined by ultracentrifugation and HPLC

Jun Dong,* Hanbang Guo,* Ruiyue Yang,* Hongxia Li,* Shu Wang,* Jiangtao Zhang,[†] and Wenxiang Chen[1],*,[†]

Institute of Geriatrics* and National Center for Clinical Laboratories,[†] Beijing Hospital, Beijing 100730, China

Abstract Simple and precise methods for LDL-cholesterol (LDL-C) and HDL-cholesterol (HDL-C) measurements are essential for assessment of cardiovascular disease (CVD) risks and for lipid and lipoprotein studies. We report here an ultracentrifugation (UC) and HPLC method that requires substantially less specimen volume and provides the necessary reliability and throughput required by large-volume, high-quality research and clinical studies. 2-Mercaptoethanol (ME) was used to dissociate serum lipoprotein [a] (Lp[a]) into apolipoprotein [a] and Lp[a] remnant (Lp[a−]) and eliminated the contamination of Lp[a] in HDL separated by UC. Serum aliquots were centrifuged at a density of 1.006 kg/l for the separation of HDL plus LDL, and in the presence of ME at a density of 1.063 kg/l for the separation of HDL. Cholesterol concentrations of the bottom fractions were analyzed by HPLC. LDL-C and HDL-C determined using this method were equivalent to those with β-quantification and the designated comparison method of the Centers for Disease Control. The total coefficient of variations for LDL-C and HDL-C were 0.65–1.12% and 0.96–2.07%, respectively. This method requires a small amount of specimen and is easy to operate.⬛⬛ This method may be used in research or in clinical laboratories where precise and specific lipoprotein cholesterol analysis is needed.—Dong, J., H. Guo, R. Yang, H. Li, S. Wang, J. Zhang, and W. Chen. **Serum LDL- and HDL-cholesterol determined by ultracentrifugation and HPLC.** *J. Lipid Res.* 2011. **52:** 383–388.

Supplementary key words low density lipoprotein-cholesterol • high density lipoprotein-cholesterol • high-performance liquid chromatography

It is well established that increased LDL-cholesterol (LDL-C) and decreased HDL-cholesterol (HDL-C) are major risk factors for cardiovascular disease (CVD). Reliable measurements of LDL-C and HDL-C are important for achieving a uniform interpretation of clinical data, which is essential for the prevention and management of CVD. Homogeneous methods have been widely used to directly measure LDL-C and HDL-C without pretreatment of specimens to separate the lipoproteins. Despite these improvements, large variations exist (1, 2). A difficult analytical challenge for these methods is to specifically measure LDL or HDL components in the presence of widely varying proportions of the different lipoprotein molecules encountered in clinical practice. In lipoprotein research and clinical studies requiring accurate HDL-C and LDL-C values, more-reliable methods for the measurement of lipoproteins are needed.

The most reliable method for LDL-C and HDL-C measurement is the CDC β-quantification method (3). The method combines removal of VLDL by ultracentrifugation (UC), isolation of HDL by precipitation of apolipoprotein B (apoB)-containing lipoproteins from the UC bottom fraction, and cholesterol analysis of the bottom fraction and the HDL supernatant by the CDC cholesterol reference method. The β-quantification method has been used to establish the concentrations of the major classes of lipoproteins in most epidemiologic and clinical trials that have become the basis of the guidelines for risk assessment of CVDs (4). The National Cholesterol Education Program (NCEP) has recommended this method as the reference method for LDL-C and HDL-C (5, 6). However, β-quantification may have limitations because it is technically demanding and time-consuming (especially the manual volumetric sampling and reconstitution of the bottom fractions), requires a large sample volume (5 ml), and has low throughput. To overcome the impracticalities, CDC has implemented a designated comparison

Abbreviations: apoB, apolipoprotein B; BFC, bottom fraction cholesterol; CRMLN, Cholesterol Reference Method Laboratory Network; CVD, cardiovascular disease; DCM, designated comparison method; HDL-C, HDL-cholesterol; LDL-C, LDL-cholesterol; Lpl[a], lipoprotein [a]; ME, 2-mercaptoethanol; NCEP, National Cholesterol Education Program; UC, ultracentrifugation.

[1] To whom correspondence should be addressed.
  e-mail: chenwenxiang@hotmail.com

method (DCM) (7) for HDL-C in the Cholesterol Reference Method Laboratory Network (CRMLN) standardization program. Modifications of the original β-quantification method have also been made to reduce the sample volume and to improve the throughput of the method (8, 9).

Lipoproteins were originally defined by their buoyant densities, and UC may analytically remain the most reliable separation technique for lipoproteins. One of the major obstacles for the use of UC separation has been the interference of lipoprotein [a] (Lp[a]) with the HDL fraction. Lp[a] consists of an LDL-like particle and an apolipoprotein [a] (apo[a]) molecule linked to each other through disulfide bonds and shows hydrated densities of 1.050–1.100 kg/l (10). It has been reported that Lp[a] can easily be dissociated to LDL-like particles by thiols (11, 12). This property of Lp[a] provides a potential approach for quantitative separation of HDL by UC.

In this study, we investigated the UC separation of LDL and HDL and, with an internal standard HPLC cholesterol analysis, developed a simple and reliable method for the measurement of LDL-C and HDL-C. The method requires a sample volume of 0.1 ml (0.05 ml × 2), allows automatic sampling, involves no bottom fraction reconstitution and precipitation, has a maximum throughput of 50 samples in one run, is highly precise, and gives results comparable to the CDC reference methods.

MATERIALS AND METHODS

Serum samples

Fresh serum samples were collected from the leftovers of patient samples in the Department of Laboratory Medicine of Beijing Hospital. Aliquots of about 1 ml of serum were taken from the leftovers and put in another series of vials. The specimens in the Department were bar coded, and the vials were labeled without relating to specimen identities. This study had been reviewed and approved by the Ethics Committee of Beijing Hospital. For method optimization and precision testing, serum aliquots were pooled and realiquoted and stored at $-70°C$ until analysis. For method comparison, individual serum samples were stored at 4°C and analyzed within 24 h.

LDL and HDL separation by UC

Separation principles. The UC separation of LDL and HDL involved spinning one aliquot of serum at a background density of 1.006 kg/l and another at a density of 1.063 kg/l in the presence of the Lp[a]-dissociating agent ME. HDL was represented by the 1.063 kg/l bottom fraction and LDL by the difference between the two bottom fractions. The UC separation was performed with serum samples (0.05 ml) diluted with density solutions (0.8 ml) to form specified background densities, which enabled automatic sampling and reduced sample volume.

Density solutions. For LDL plus HDL separation, a solution of 0.098 M NaBr was prepared by dissolving NaBr in deionized water. Its density was verified to be 1.006 kg/l using a density meter (DMA 4500M; Anton Paar, Austria) at 20°C. For HDL separation, a solution of 0.87 M NaBr containing 0.05 M ME was prepared. This solution showed a density of 1.0665 kg/l, and mixing of 0.8 ml of the solution with 0.05 ml of serum would form a back-

ground density of 1.063 kg/l, assuming the background density of serum is 1.006 kg/l and serum protein volume 6%.

UC separation. The UC separation was performed on a Beckman Coulter (Fullerton, CA) XL-90 ultracentrifuge with a Type 25 rotor (1 ml × 100 in three rows) and thick-wall polycarbonate UC tubes (1 ml, 8 × 51 mm). The sampling of serum was performed with a MicroLab 500 automatic dilutor (Hamilton; Reno, NV). The dilutor was first primed with the 1.006 kg/l density solution, and aliquots of 0.05 ml of serum samples were delivered with 0.8 ml of the solution to a series of UC tubes. The dilutor was then primed with the 1.0665 kg/l solution, and another set of serum aliquots was delivered. The UC tubes were loaded onto the rotor and spun at 23,000 rpm for 18.5 h at 20°C. The g forces for the outmost, middle, and innermost rows were 78,196, 68,843, and 59,431 g, respectively. After the centrifugation, the UC tubes were sliced at the middle of their contents with a CentriTube Slicer (Beckman Coulter), and bottom fractions were obtained for cholesterol analysis.

Cholesterol measurement

Measurement principle. An HPLC method (13) was used for the measurement of cholesterol in the bottom fractions. Cholesteryl esters were hydrolyzed with alcoholic potassium hydroxide and, in the presence of an internal standard (stigmasterol), extracted with hexane. The sterols were oxidized to 4-en-3,6-diones with chromic acid and analyzed by HPLC. The low detection limit of the method allowed measurement of cholesterol in dilute samples, and the internal standard calibration eliminated the need for volumetric reconstitution of the UC bottom fractions.

Calibrators and internal standard. A stock solution of 1,000 mg/dl was prepared by dissolving cholesterol reference material SRM 911b (National Institute of Standards and Technology, Gaithersburg, MD) in ethanol. Calibrators of 25, 50, 100, 200, and 300 mg/dl were prepared by diluting the stock solution with ethanol. An internal standard was prepared by dissolving stigmasterol in ethanol (200 mg/dl).

HPLC analysis and calculation. For cholesterol analysis by HPLC, the bottom fractions in the UC tubes were quantitatively transferred to 10 ml screw-capped glass tubes by first transferring the bottom fractions with a syringe and then washing the tubes with a mixture of ethanol and 8.9 M potassium hydroxide (80:20) (three times and total volume of 1 ml). With the same dilutor as for the UC sampling, aliquots of 0.05 ml of the calibrators were delivered with 0.8 ml of ethanol to 10 ml glass tubes. To the calibrator tubes, 0.2 ml of 8.9 M potassium hydroxide was added manually with a pipette. To all tubes (both bottom fractions and calibrators), also with the dilutor, 0.05 ml of the internal standard was added with 0.8 ml of ethanol. The tubes were incubated at 50°C for 2 h. After addition of 2 ml water and 4 ml hexane, the tubes were shaken on a mechanical shaker for 15 min. Aliquots of 0.5 ml of the hexane phases were dried down, oxidized with chromic acid, and analyzed by HPLC, as previously described (13).

Peak area ratios of cholesterol to stigmasterol for the calibrators were linearly regressed on the corresponding cholesterol concentrations, and the resulting equation was used to calculate cholesterol concentrations of centrifuged bottom fractions.

HDL-C DCM and modified β-quantification

The development and validation of the present method (UC/HPLC) involved several comparisons with the HDL-C DCM and a

modified β-quantification method. The DCM was performed as in the CDC CRMLN (7). The modified β-quantification was similar to the 1 ml procedure by Cole et al. (9), but the sampling of serum and reconstitution of bottom fractions were performed gravimetrically. Aliquots of 0.8 ml of serum samples were transferred into UC tubes with a pipette and weighed to 0.1 mg. The samples were spun under the conditions described above. After the UC, the tubes were marked at the middle of their contents and the top fractions were gently aspirated with a needle connected to a water pump. After wiping off the remnants from the tube wall, the tubes and their contents were reconstituted to their original weights with 0.9% NaCl. The reconstituted specimens were mixed gently using a piece of steel wire until the protein pellets in the bottom of the tubes were redissolved and the specimens well mixed. Cholesterol concentrations that represent bottom fraction cholesterol (BFC) (HDL-C and LDL-C) of serum samples were measured using the HPLC method (13).

RESULTS

Elimination of Lp[a] interference with UC HDL

It is generally recognized that 1.063 kg/l is the density cut point between HDL and LDL. The major issue in this study was to eliminate the interference of Lp[a] for the isolation of HDL by UC. It has been established that in Lp[a], apo[a] is covalently linked to apoB-100 and can easily be dissociated from it by subjecting chemically reduced Lp[a] to UC. The resulting lipoprotein remnant, Lp[a−], is similar in structure and density to those of autologous LDL (12). If Lp[a] can be effectively dissociated and the dissociation of Lp[a] does not affect the density properties of HDL, then HDL can be isolated directly by UC.

To test the dissociation of Lp[a] with chemical reduction, aliquots of a mixed serum with an Lp[a] concentration of about 50 mg/dl were centrifuged in the presence of various concentrations of ME (0, 0.01, 0.02, 0.04, and 0.08 M) at a density of 1.063 kg/l. BFC was determined by HPLC. The BFC values decreased with increased ME concentrations ranging from 0 to 0.02 M, but stayed unchanged when ME concentrations further increased (0.02–0.08 M) (**Fig. 1**). If the decrease of BFC results from the dissociation of Lp[a], then 0.02 M ME can effectively dissociate Lp[a]. For complete dissociation of Lp[a], the ME concentration used in this study was 0.05 M.

To test the effectiveness of Lp[a] dissociation and the stability of HDL in the presence of ME, serum samples from 49 individuals were ultracentrifuged in the presence and absence of ME at a density of 1.063, and BFC [$BFC_{1.063ME(+)}$ and $BFC_{1.063ME(−)}$] was determined by HPLC. Serum Lp[a] mass was measured by immunoturbidimetric assay, and HDL-C was measured by DCM (HDL-C_{DCM}). The results showed that the differences between $BFC_{1.063ME(−)}$ and $BFC_{1.063ME(+)}$ were highly correlated with Lp[a] mass (**Fig. 2**) ($r = 0.848$, $P < 0.001$), but no correlation was found between the differences and HDL-C levels determined by DCM ($r = −0.093$, $P > 0.05$). These results would indicate that the differences between $BFC_{1.063ME(−)}$ and $BFC_{1.063ME(+)}$ were caused mainly by the dissociation of Lp[a], and ME did not affect the density properties of HDL.
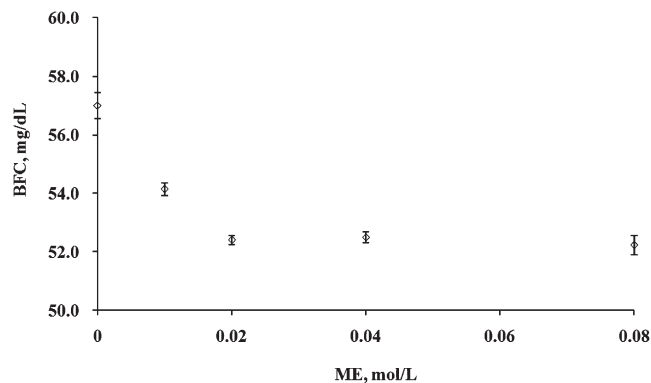

Fig. 1. Correlation between 2-mercaptoethanol (ME) concentrations versus d = 1.063 kg/l ultracentrifuged bottom fraction cholesterol (BFC) concentrations (n = 6). Aliquots of mixed serum with Lp[a] mass of about 50 mg/dl were centrifuged in the presence of 0, 0.01, 0.02, 0.04, and 0.08 M of ME at a density of 1.063 kg/l. Serum was spun three times in duplicate at 23,000 rpm for 18.5 h (78,196 g) at 20°C, and BFC concentrations were measured by HPLC.

To further test the Lp[a] dissociation, four individual serum samples with Lp[a] mass of 45.3, 50.5, 60.7, and 109.2 mg/dl were spun at a background density of 1.063 kg/l in the presence and absence of ME. Aliquots of 50 μl of the bottom fractions were electrophoresed on 6% SDS-PAGE gels and immunoblotted with a sheep anti-human apoB and HRP-conjugated rabbit anti-sheep IgG antibodies for detection of apoB. The results are shown in **Fig. 3**. As expected, in the absence of ME, a significant amount of apoB was detected, and in the presence of ME, most of the apoB (>90%) was removed. This result confirmed Lp[a] dissociation, and the dissociated Lp[a−] (containing one molecular apoB) had a density similar to that of LDL (<1.063) and had been removed from HDL.

Linearity of cholesterol measurement by HPLC

The linear correlation between cholesterol concentration (*x*) (the five calibrators, each in duplicate) and the
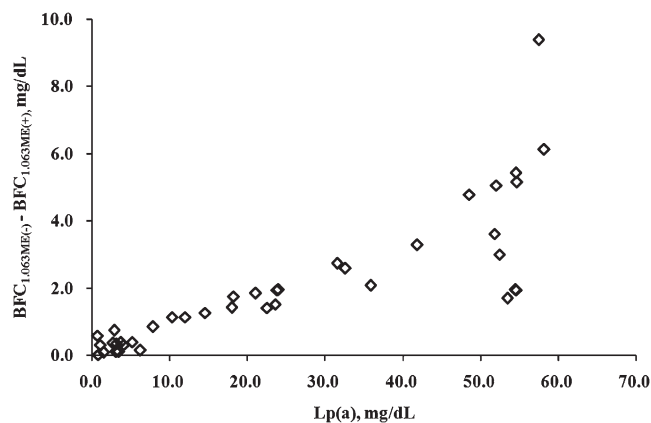
Fig. 2. Differences between $BFC_{1.063ME(−)}$ and $BFC_{1.063ME(+)}$ versus Lp[a] mass. Serum samples from 49 individuals were ultracentrifuged in the presence and absence of 2-mercaptoethanol (ME) at a density of 1.063 kg/l. Bottom fraction cholesterol [BFC, $BFC_{1.063ME(−)}$, and $BFC_{1.063ME(+)}$] was determined by HPLC. Serum Lp[a] mass was measured by immunoturbidimetric assay.

ME − + − + − + − +
Sample 1 Sample 2 Sample 3 Sample 4
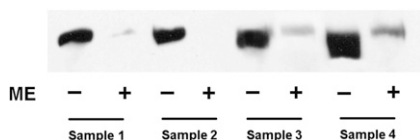
Fig. 3. Western blot of apolipoprotein B (apoB) in centrifuged bottom fractions. Individual serum samples were spun at a background density of 1.063 kg/l in the presence and absence of 2-mercaptoethanol (ME). Aliquots of 50 µl of each bottom fraction were fractionated by 6% SDS-PAGE and immunoblotted with a sheep anti-human apoB polyclonal antibody. Lp[a] concentrations of samples 1, 2, 3, and 4 were 45.3, 50.5, 60.7, and 109.2 mg/dl, respectively.

peak area ratio (y) of cholesterol to stigmasterol in 20 analytical runs was assessed by linear regression analysis. The correlation coefficients, slopes, intercepts, and standard errors of the y estimate, slopes, and intercepts are shown in **Table 1**.

Precision of the UC/HPLC method

To estimate the precision of the method, four frozen serum pools were repeatedly analyzed. Serum samples were centrifuged in duplicate, and BFC was analyzed in triplicate in four runs. LDL-C and HDL-C levels and the within-run and total CVs are presented in **Table 2**. The average within-run CV and the total CV were 0.45–0.78% and 0.65–1.12% for LDL-C, 0.60–1.21% and 0.96–2.07% for HDL-C, respectively.

Bottom fraction comparison with β-quantification method

In the CDC β-quantification method, whole serum was used for the separation of lipoproteins. In the present method, however, a diluted serum specimen was used for the UC separation. To evaluate whether serum dilutions would affect lipoprotein separation and to compare the BFC levels (d = 1.006 kg/l) under the two UC conditions, 67 serum samples (total cholesterol ranged from 102.3 to 266.6 mg/dl and triglycerides from 53.9 to 376.9 mg/dl) from individual patients were measured for BFC with both the UC/HPLC and the modified β-quantification methods. The mean BFC concentrations were 158.60 ± 37.04 and 158.59 ± 37.21 mg/dl for the two methods, respectively. Correlation and relative difference plots are shown in **Fig. 4**. Linear regression analysis showed a correlation of the UC/HPLC results (y) with the modified β-quantification results (x) of $y = 0.993x + 0.978$ (in mg/dl) with a correlation coefficient of 0.998 (Fig. 4A). The results from UC/HPLC showed an average absolute bias

TABLE 1. Linearity between cholesterol concentration and peak area ratio of cholesterol to the internal standard

	Mean	Range
Slope	0.00681	0.00646–0.00711
Intercept	0.00231	−0.00085–0.00579
Standard error of y estimate	0.00220	0.00091–0.00448
Standard error of slope	0.00001	0.00001–0.00002
Standard error of intercept	0.00116	0.00048–0.00235
R^2	0.99999	0.99996–0.99999

TABLE 2. Precision of ultracentrifugation/HPLC analysis of serum LDL-C and HDL-C

	Serum pools	Mean	CV Within-run	CV Total
		mg/dl	%	
LDL-C	Pool 1	111.8	0.48	1.12
	Pool 2	107.2	0.78	0.92
	Pool 3	160.1	0.45	0.65
	Pool 4	110.9	0.68	0.85
HDL-C	Pool 1	51.8	0.67	0.96
	Pool 2	85.0	0.60	1.01
	Pool 3	49.9	0.91	1.73
	Pool 4	34.0	1.21	2.07

LDL-C, LDL-cholesterol; HDL-C, HDL-cholesterol.

of 0.01 mg/dl (95% CI, −0.52–0.54 mg/dl) and an average relative bias of 0.05% (95% CI, −0.29–0.39%) (Fig. 4B). Paired t-test analysis showed that there was no difference between BFC levels measured using the two methods ($P > 0.05$). These comparison results demonstrate that
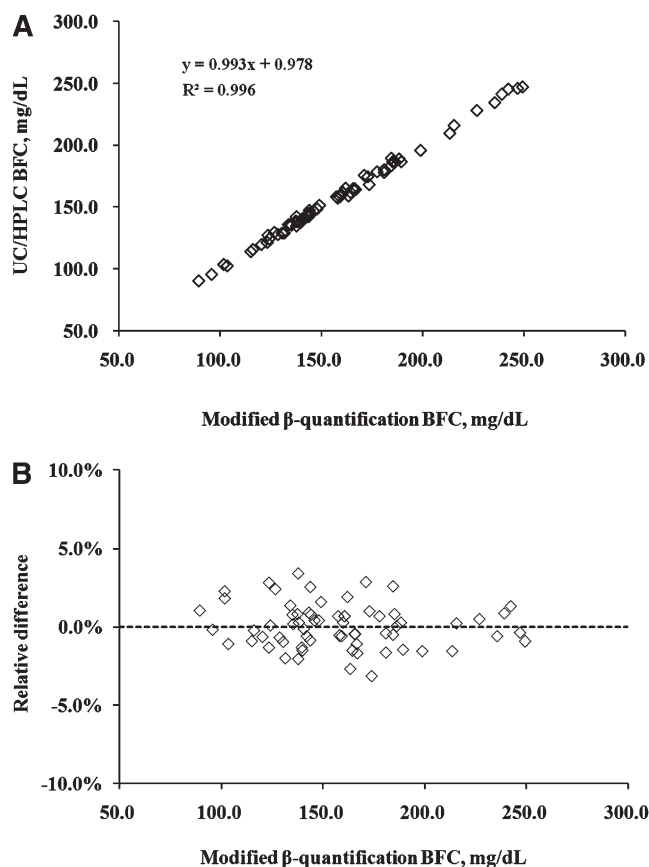
Fig. 4. Correlation (A) and relative difference (B) plots between ultracentrifugation/HPLC (UC/HPLC) and the modified β-quantification for the measurement of bottom fraction cholesterol (BFC) (LDL-C plus HDL-C) levels in 67 serum samples. For the UC/HPLC method, 50 µl serum samples were diluted with NaBr solutions and centrifuged at a density of 1.006. For the modified β-quantification, 0.8 ml serum samples were gravimetrically transferred into tubes and centrifuged. After UC, the bottom fractions were reconstituted gravimetrically. BFC was analyzed by HPLC. Relative difference is defined as the difference between UC/HPLC BFC and β-quantification BFC divided by β-quantification BFC.

serum dilution in the UC/HPLC method does not affect the UC separation of lipoproteins.

Comparison of HDL-C with DCM

The DCM used in the CRMLN has been proven to provide HDL-C results approximately equivalent to those of the CDC β-quantification reference method. As a CRMLN member, this laboratory performs the DCM method with <1 mg/dl SD and <1 mg/dl biases, as required by CDC. The cholesterol measurement is performed with the CDC Abell-Kendall cholesterol reference method in our CRMLN activities. The HPLC method measures less noncholesterol sterols and gives results approximately 1.1% lower than the Abell-Kendall method (13). To eliminate this difference in cholesterol measurement, the HPLC method was used in the comparison study.

To compare the UC/HPLC method for HDL-C (BFC$_{1.063ME}$) measurement with the DCM, 124 serum samples from individual patients (total cholesterol ranged from 73.0 to 319.3 mg/dl and triglycerides <200 mg/dl) were analyzed with the two methods. Correlation and relative difference plots are shown in **Fig. 5**. Regression analysis showed a correlation of the UC/HPLC results (y) with the DCM results (x) of $y = 0.933x + 1.792$ (in mg/dl) with a correlation coefficient of 0.995 (Fig. 5A). The UC/HPLC results showed an average absolute bias of -1.39 mg/dl (95% CI, $-1.70 - -1.16$ mg/dl) and an average relative bias of -2.68% (95% CI, $-3.21 - -2.15\%$). Biases at the medical decision points 35 and 60 mg/dl were -1.48% and -3.61%, respectively, as calculated from the linear regression equation. The total error was 4.36%, as calculated by the NCEP equation (bias + 1.96 CV) and $-8.80\% - 3.65\%$ as evaluated by nonparametric analysis (14). Despite the finding of a statistically significant difference by paired t-test between the two methods, the performance of the UC/HPLC method would be clinically acceptable because both bias and total errors were well within the NCEP specifications of $\leqslant 5\%$ and $\leqslant 13\%$, respectively (Fig. 5B).

DISCUSSION

For the precise measurement of serum LDL-C and HDL-C, effective separation of LDL and HDL is needed. However, lipoproteins, as heterogeneous mixtures of lipids and proteins, are highly complicated and are not rigidly defined. The most widely used nomenclature defines four main classes of lipoproteins based on their hydrated densities and UC (15): VLDL (d 0.95–1.006 kg/l), intermediate density lipoprotein (IDL, d 1.006–1.019 kg/l), LDL (d 1.019–1.063 kg/l), and HDL (d 1.063–1.21 kg/l). Lp[a] is a special class of lipoprotein (d 1.050–1.100 kg/l) in which apo[a] is covalently linked to apoB-100 of LDL-like particles. Although UC remains the most reliable lipoprotein separation technique, separation of LDL and HDL has not been achieved because the HDL fraction isolated by UC may contain a considerable amount of Lp[a]. Therefore, in the β-quantification and other methods (9), HDL is separated from LDL by chemical precipitation after the removal of VLDL by UC. With this approach, how-
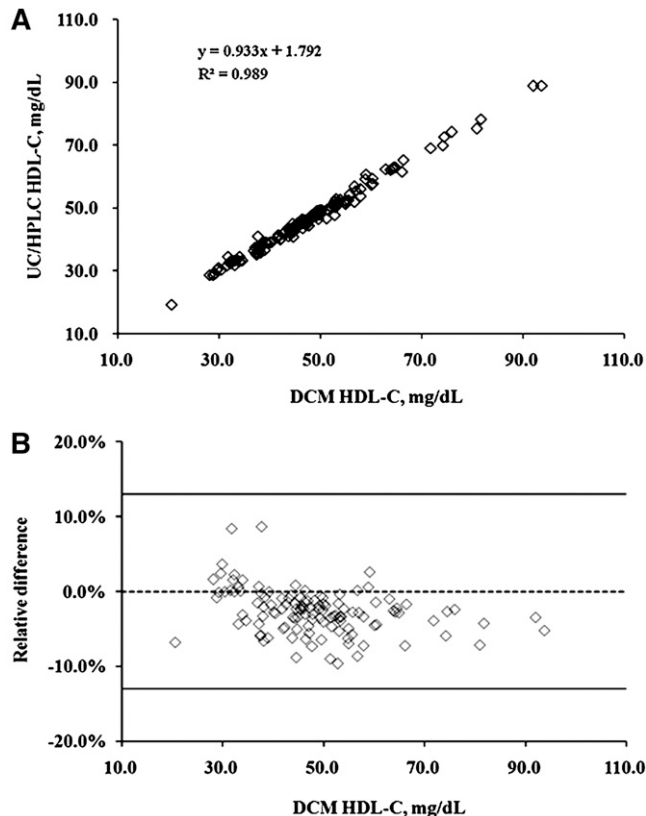
Fig. 5. Correlation (A) and relative difference (B) plots between ultracentrifugation/HPLC (UC/HPLC) and the designated comparison method (DCM) for the measurement of HDL-C in 124 serum samples. For the UC/HPLC method, serum solutions were centrifuged at a density of 1.063 kg/l in the presence of 0.05 M ME. For the DCM, the precipitation of apoB-containing lipoproteins was performed with dextran-sulfate. HDL-C concentrations were analyzed by the HPLC method. Relative difference is defined as the difference between UC/HPLC HDL-C and DCM HDL-C divided by DCM HDL-C. The total analytical error limits specified by the NCEP (<13%) are displayed (B).

ever, a large volume of serum samples is required for not only volumetric handling in UC, but also for the following preparation and determination of HDL. It is also difficult to use diluted serum samples for UC because the dilution may affect chemical precipitations.

The key aim of this study was to separate HDL by UC without Lp[a] interference. Lp[a] contains an LDL-like particle that is covalently coupled with apo[a]. It has been reported that Lp[a] can easily be dissociated by chemical reduction and UC and that the resulting Lp[a−] has a density similar to or slightly smaller than that of LDL (12). Therefore, isolation of HDL by UC can be achieved if Lp[a] is dissociated and if the dissociation does not affect the density properties of HDL. We tested this by ultracentrifuging serum specimens in the presence and absence of ME at a density of 1.063 kg/l and measuring the BFC. It was shown that BFC$_{1.063ME(-)}$ was generally higher than BFC$_{1.063ME(+)}$, and the difference was highly correlated with Lp[a] ($r = 0.848$) but not with HDL ($r = -0.093$). We also confirmed Lp[a] dissociation by detecting apoB in the bottom fraction of UC and found that most of the apoB

had been removed from HDL in the presence of ME. These results indicate that ME may eliminate the interference of Lp[a] on HDL isolation without changing HDL density properties.

Reliable measurement of LDL-C and HDL-C with a UC separation depends on accurate sampling of serum, quantitative transfer of bottom fractions, and precise measurement of cholesterol. The UC separation enabled the use of diluted serum samples and thus the use of a high-precision automated diluter for the introduction into the UC tubes of small-volume serum samples with density solutions. An internal standard HPLC method (13) was used for the measurement of cholesterol. The same diluter was used for the sampling of calibrators to ensure the same volume of specimen and calibrators. The HPLC method offered the required analytical sensitivity for the diluted samples, and the use of an internal standard eliminated the need for volumetric reconstitution of the bottom fractions.

Whole serum is generally used in UC separations of lipoproteins. To test whether the sample dilutions would cause any biases on the isolated lipoproteins, a modified reduced volume β-quantification method was established for comparison with the UC/HPLC method. Because of the small sample volume (0.8 ml) and the importance of volume accuracy, serum samples were transferred and their bottom fractions reconstituted gravimetrically. The density of the saline used for the reconstitution may not be exactly the same as that of the removed top fractions, but the volume biases caused would be negligible for the present purpose. Comparison of this method with the UC/HPLC method on 67 serum samples showed no significant difference in BFC, indicating the dilution would not influence the lipoprotein separation.

For HDL-C measurement, the UC/HPLC method was compared with the DCM on 124 individual patient samples. The UC/HPLC results correlated very well with the DCM results but showed an average bias of −2.68%. Our data demonstrated that the dissociation of Lp[a] with ME did not affect the density properties of HDL. The bias might be caused, at least in part, by the different separation principles. Such differences may be clinically acceptable and are well within the NCEP guidelines for acceptable bias and total error for HDL-C.

In this study, the separation of HDL and LDL by UC is based on the widely used lipoprotein definition. The measured LDL-C values include the contributions of IDL and Lp[a], which is in accordance with CDC reference method. Precision study showed that the total CVs for measurement of LDL-C and HDL-C by UC/HPLC were 0.65–1.12% and 0.96–2.07%, respectively.

In conclusion, a UC and HPLC method for measurement of LDL-C and HDL-C has been established. The method has several advantages: *a*) HDL and LDL are separated by UC, and HDL is not contaminated with Lp[a]; *b*) the sample volume required is 0.1 ml (2 × 0.05 ml) for LDL-C and HDL-C measurements; *c*) an automated diluter is used in place of a manual pipette for the precise introduction of both calibrators and specimens; *d*) an internal standard is used to eliminate the need for volumetric reconstitution of the bottom fractions and to ensure the precise measurement of cholesterol concentration in the bottom fractions; and *e*) a maximum of 50 samples can be analyzed in a single analytical run. The UC/HPLC method is highly precise and easy to operate and can be used in high-volume research or in clinical laboratories where precise and specific lipoprotein cholesterol analyses are needed.

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