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Original Article

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Comparison of Two Homogeneous LDL-Cholesterol Assays Using Fresh Hypertriglyceridemic Serum and Quantitative Ultracentrifugation Fractions

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Aim: The purpose of this study was to compare two homogeneous assays of low-density lipoprotein-cholesterol (LDL-C) with a modified beta quantification reference measurement for LDL-C (BQ-LDL), fractions of chylomicron (CM), very low-density lipoprotein (VLDL) and intermediate-density lipoprotein (IDL) by quantitative ultracentrifugation in patients with hypertriglyceridemia.

Methods: Two homogeneous LDL-C assays (LDL-C(K), Kyowa Medex and LDL-C(S), Sekisui Medical) were used to measure 198 samples of fresh anonymized leftover sera with hypertriglyceridemia (≥ 150 mg/dL). Of these, 32 samples with discrepant LDL-C levels or hypertriglyceridemia (≥ 400 mg/dL) were used for further analysis. Quantitative ultracentrifugation was used to separate samples.

Results: The two homogeneous LDL-C assays had a strong correlation with each other for the samples from 198 patients with hypertriglyceridemia. LDL-C(K) and LDL-C(S) in 32 selected samples were strongly correlated with BQ-LDL. In both homogeneous assays, cholesterol in the CM and VLDL fractions was measured as part of the LDL-C. A weak correlation was found between cholesterol in the VLDL fraction and LDL-C using the two homogeneous assays, but no correlation was found with cholesterol in the CM fraction. Cholesterol in the IDL fraction was also measured as part of the LDL-C in both assays.

Conclusion: Both homogeneous assays partially detected cholesterol in the chylomicron and VLDL fractions, but LDL-C measured by both homogeneous assays correlated with BQ-LDL.

Key words: LDL cholesterol, Homogeneous assay, Ultracentrifugation, Hypertriglyceridemia

Introduction

Low density lipoprotein (LDL) cholesterol has been strongly correlated with cardiovascular disease in many epidemiological studies[1, 2]. The Friedewald equation is widely used to estimate LDL cholesterol (LDL-C = total cholesterol (TC) − high-density lipoprotein cholesterol (HDL-C) − triglyceride (TG)/5), which requires the measurement of serum TC, HDL-C, and TG[3]. The reference method for LDL-C is termed the "beta quantification" (BQ) procedure, which requires the use of ultracentrifugation to remove chylomicron (CM) and very low-density lipoprotein (VLDL) cholesterol, followed by the measurement of cholesterol in the LDL and high-density lipoprotein (HDL) containing "bottom" fraction, selective precipitation of LDL, and measurement of HDL-C in the supernatant[4]. The BQ procedure involves ultracentrifugation and complicated manual procedures; therefore, it is not applicable for routine work. Thus, most laboratories use direct homogeneous methods or the Friedewald equation to determine LDL-C concen-

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trations. However, the Friedewald equation is not applicable for samples containing chylomicrons or samples from patients with type III hyperlipoproteinemia[5]. The Friedewald equation is based on the idea that the concentration of VLDL-C is low compared with LDL-C. Reliable calculation using the Friedewald equation requires a specimen obtained after at least a 12-hour fast.

By homogeneous methods, LDL-C is separated from other cholesterol fractions with the characteristics of surfactants, and LDL-C is directly measured using an automatic analyzer. Several different homogeneous LDL-C assays are used for routine clinical analysis, but differences between each reagent in normal and abnormal lipid samples have been reported[6-8]. The heterogeneity of homogeneous assays might be explained by the distinct determination principles and the different reactivity to lipoproteins. Because of these issues, some homogeneous LDL-C assays are no longer used. The remaining homogeneous assays have been developed and improved over time. Homogeneous LDL-C assays offer many advantages, such as good precision, complete automation, and no requirement for a fasting sample.

Because of differences in the measurements between the remaining homogeneous assays, we suspect that homogeneous assays might not be completely consistent with LDL cholesterol by the BQ procedure. The aim of this study was to compare how much LDL cholesterol values of two frequently used homogeneous assays with different measurement principles are affected by the presence of other lipoprotein fractions in daily high triglyceridemic patients. This study evaluated the extent to which two homogeneous assays for LDL-C correlated with LDL-C by modified BQ (BQ-LDL) in samples from patients with hypertriglyceridemia and to analyze the cause of any discrepancy. The two homogeneous assays for LDL-C were compared with BQ-LDL, and fractions of CM, VLDL, and intermediate density lipoproteins (IDL) were determined using ultracentrifugation.

Material and Methods

Subjects

This single-center study was conducted at Fukuoka University Hospital between April and September 2013. TC, TG, LDL-C, and HDL-C were measured in 198 samples of fresh anonymized leftover sera from patients with hypertriglyceridemia (TG ≥ 150 mg/dL (1.69 mmol/L)) in our routine clinical chemistry laboratory. Because leftover samples from clinical examination were used, all analyses were performed with a single measurement. Sera were immediately stored at 4℃ and all measurements were performed within 2 days. The dietary intake status of patients was unknown. We excluded dyslipidemic patients with extremely high TG (>1000 mg/dL; 11.29 mmol/L). In addition, we excluded patients with severe systemic infections, decompensated liver cirrhosis, or cholestatic liver disease. Of these, 32 samples with discrepant LDL-C levels measured by two homogeneous LDL-C assays, Friedewald's formula or hypertriglyceridemia (TG ≥ 400 mg/dL; 4.52 mmol/L) were used for quantitative ultracentrifugation analysis. Serum lipids and apoB levels in 32 samples of whole serum and lipid-lowering therapy are shown in **Table 1**. Of the 32 samples, 12 samples had >15 mg/dL discrepancy in LDL-C levels as measured by the two homogeneous LDL-C assays (No. 1, 4, 7, 9, 11, 15, 17, 23, 26, 28. 30, 32), 11 samples had >20 mg/dL discrepancy in LDL-C levels between the homogeneous LDL-C assays and Friedewald equation (No. 1, 3, 5, 12-14, 19, 21, 29, 31, 32), and 13 samples showed hypertriglyceridemia (≥ 400 mg/dL; 4.52 mmol/L; No. 2, 6, 8, 10, 11, 16, 18, 20, 22, 24, 25, 27, 28). The three groups chosen had similar numbers of samples. Thirteen of the 32 samples were from patients who received lipid lowering therapy (**Table 1**). This study was approved by the ethics committee of Fukuoka University Hospital (#14310).

Methods

Levels of TC, TG, HDL-C, LDL-C(K), and LDL-C(S) in 198 samples from patients with hypertriglyceridemia were measured using the JCA-BM6010 analyzer (JEOL Ltd., Tokyo, Japan). The two homogeneous assays for LDL-C were as follows: a liquid selective detergent method (LDL-C(S); Cholestest® LDL, Sekisui Medical, Tokyo, Japan) and a selective solubilization method (LDL-C(K); MetaboLead® LDL-C, Kyowa Medex, Tokyo, Japan). The liquid selective detergent method (Sekisui Medical) uses a detergent polymer mixture (reagent 1) to release cholesterol from non-LDL lipoproteins and it reacts with cholesterol esterase, cholesterol oxidase, peroxidase, and 4-aminoantipyrene to give a colorless product. Then a second detergent reagent (reagent 2) that releases cholesterol from LDL is added. Cholesterol reacts with components of reagent 1 plus N, N-bis (4-sulfobutyl)-m-toluidine disodium in reagent 2 to form colored products that are measured spectrophotometrically. The selective solubilization method (Kyowa Medex) uses cyclodextrin sulfate, dextran sulfate and a magnesium complex to stabilize VLDL and chylomicron molecules. Reagent 1 also contains peroxidase and N-(2-hydroxy-3-sulfopropyl)-3,5-dime-

Table 1. Serum lipids and apoB levels in whole serum of 32 samples and lipid-lowering therapy

No.	Age (yr)	Sex	TC	TG	BQ-LDL	LDL-C(K)	LDL-C(S)	LDL-C(F)	HDL-C(K)	HDL-C(S)	ApoB	Lipid lowering therapy
			(mg/dL)									
1	58	M	225	160	104	122	81	131	62	56	67	No
2	20	M	200	456	47	42	50	(69)*	40	32	77	No
3	31	F	193	321	94	101	102	70	59	55	78	No
4	70	M	315	250	207	227	206	215	50	48	174	No
5	21	F	227	267	117	127	128	103	71	69	92	No
6	57	M	308	437	191	183	188	(204)*	17	16	196	No
7	79	F	379	245	270	279	262	273	57	55	197	No
8	21	M	242	428	145	153	152	(114)*	43	42	121	No
9	62	M	198	187	117	123	107	116	45	44	108	Pitavastatin 2 mg
10	72	M	177	413	75	75	74	(45)*	50	48	88	Atorvastatin 5 mg, Ezetimibe 10 mg
11	57	M	341	510	214	216	193	(192)*	47	42	199	EPA 1800 mg
12	56	F	175	204	89	82	86	61	73	70	69	No
13	53	M	222	209	135	133	139	118	62	62	105	Bezafirate 400 mg, Ezetimibe 10 mg
14	44	M	221	274	123	128	132	109	57	57	102	Atorvastatin10 mg
15	26	F	182	173	137	117	134	121	27	27	116	No
16	25	M	226	437	130	130	128	(101)*	38	37	103	No
17	39	F	192	175	121	104	124	121	36	36	105	No
18	59	M	160	400	71	72	75	(49)*	31	30	73	No
19	48	M	213	290	124	128	134	104	51	54	107	No
20	33	F	232	429	112	126	126	(105)*	41	43	115	Rosuvastatin 2.5 mg
21	64	M	193	329	121	120	119	94	33	35	110	Rosuvastatin 2.5 mg
22	44	F	155	536	64	69	71	(11)*	37	38	73	Atorvastatin 10 mg
23	58	M	348	256	249	260	230	252	45	47	196	No
24	83	M	226	427	119	125	112	(102)*	39	39	117	No
25	88	F	193	491	97	94	85	(67)*	28	28	105	No
26	57	M	262	167	203	191	169	180	49	47	156	Atorvastatin 10 mg
27	76	M	165	434	54	45	43	(12)*	66	63	70	Atorvastatin 10 mg, Ezetimibe 10 mg
28	43	M	303	577	136	141	120	(156)*	31	31	127	Clinofibrate 400 mg, EPA 1800 mg
29	63	M	164	226	83	83	85	60	58	55	63	Rosuvastatin 5 mg
30	51	F	350	328	229	248	223	237	48	48	187	No
31	74	F	192	280	104	106	94	82	54	48	90	No
32	77	F	209	225	85	101	72	103	61	55	66	Atorvastatin 5 mg

*Because serum triglycerides were above 400 mg/dL, these are provisional values.
EPA, eicosapentaenoic acid; yr, year; F, female, M, male.

thoxyaniline sodium. The second reagent contains a surfactant to form stable micelles with HDL as well as stabilized VLDL and chylomicron, which inhibits their reaction with cholesterol esterase and oxidase. Upon addition of the second reagent, cholesterol from LDL reacts with the components of reagent 1 and cholesterol esterase, cholesterol oxidase, and 4-amino-nonanthopylene in the second reagent to produce a colored product.

The two homogeneous assays for HDL-C were as follows: a selective solubilization method (HDL-C(S); Cholestest N® HDL, Sekisui Medical) and a selective inhibition method (HDL-C(K); Metabo-Lead® HDL-C, Kyowa Medex, Tokyo, Japan). Serum levels of TC and TG were measured by enzymatic methods using reagents from Kyowa Medex, Tokyo, Japan, and apolipoprotein (apo) B was measured using a turbidimetric immunoassay method from Sekisui Medical, Tokyo, Japan.

Quantitative ultracentrifugation was performed

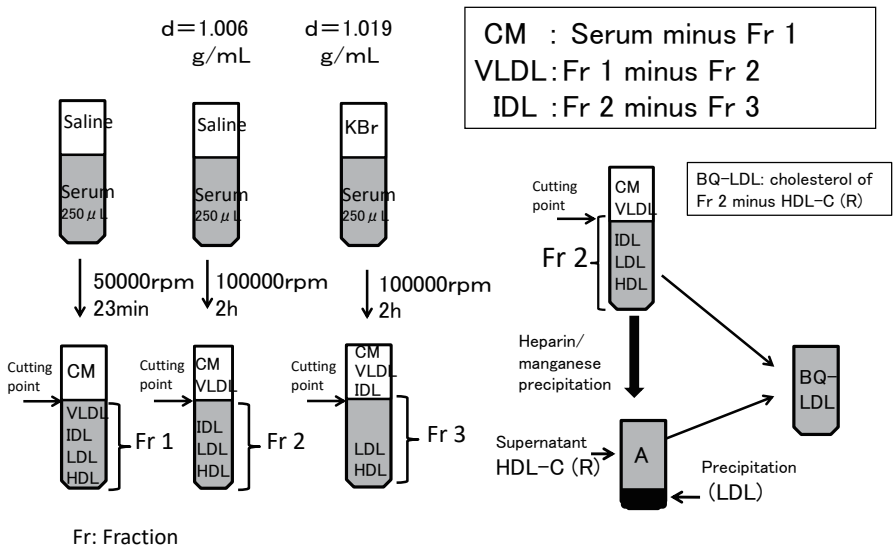

Fig. 1. Schematic presentation of fractionation by quantity ultracentrifugation and modified β-quantification reference measurements

For each sample, four ultracentrifugations, CM removal, CM and VLDL removal, CM, VLDL and IDL removal, and BQ-LDL were performed with 250 μL of serum. The serum Svedberg flotation rate (Sf) >400 fraction (fraction 1: serum after CM removal), serum d >1.006 g/mL fraction (fraction 2: serum after CM and VLDL removal) and serum d >1.019 g/mL fraction (fraction 3: serum after CM, VLDL and IDL removal) were separated using quantitative ultracentrifugation as described previously[10]. HDL-C(R) was separated from fraction 2 by the precipitation method. BQ-LDL was calculated as the difference between the total cholesterol of fraction 2 and HDL-C (R).

at 10℃ with a TLA 120.2 rotor in an Optima MAX-XP Benchtop Ultracentrifuge (Beckman Coulter, Fullerton, CA, USA). For quantitative ultracentrifugation, the serum is divided into small portions, layered with saline or a specific gravity liquid using KBr, ultracentrifuged by changing the centrifugation conditions, and the upper layer portion is removed, as described previously[9, 10]. For chylomicron separation, centrifugation was performed at 50,000 rpm for 23 minutes (Fig. 1). VLDL was depleted in the serum d >1.006 g/mL fraction, IDL was depleted in the serum d > 1.019 g/mL fraction, and LDL and HDL were retained in the >1.019 g/mL fraction (Fig. 1). The cholesterol level at the bottom of each fraction obtained by ultracentrifugation was measured to find the difference between each fraction. The difference between whole serum and fraction 1 was chylomicron, the difference between fraction 1 and 2 was VLDL, and the difference between fraction 2 and 3 was calculated as the IDL (Fig. 1). The LDL-C of the modified beta quantification procedure (BQ-LDL) was calculated from the difference in cholesterol value between fraction 2 and the supernatant separated by the heparin-Mn precipitation method (Fig. 1).

Statistical Analysis

Statistical analysis was performed at the University of Fukuoka using SAS software (Version 9.1, SAS Institute, Cary, NC, USA). Regression analysis was performed using the general linear model. Differences in the slopes of regression lines were examined by analysis of covariance (ANCOVA). LDL-C levels in hypertriglyceridemic patients measured by different methods were compared by analysis of variance (ANOVA). Data are presented as the mean ± SD, and the significance level was set at $p<0.05$ unless otherwise indicated.

Results

As shown in Fig. 2A, LDL-C(K) and LDL-C(S) were correlated with each other in 198 samples of fresh serum from patients with hypertriglyceridemia (TG ≥ 150 mg/dL; $r=0.980$, $p<0.001$). Fig. 2B shows that LDL-C(K) and LDL-C(S) also correlated well with the LDL-C value calculated by the Friedewald equation ($r=0.978$, $p<0.001$). Thirty-two samples of serum lipids, apoB, and lipid lowering therapy are shown in Table 1. No obvious characteristics were

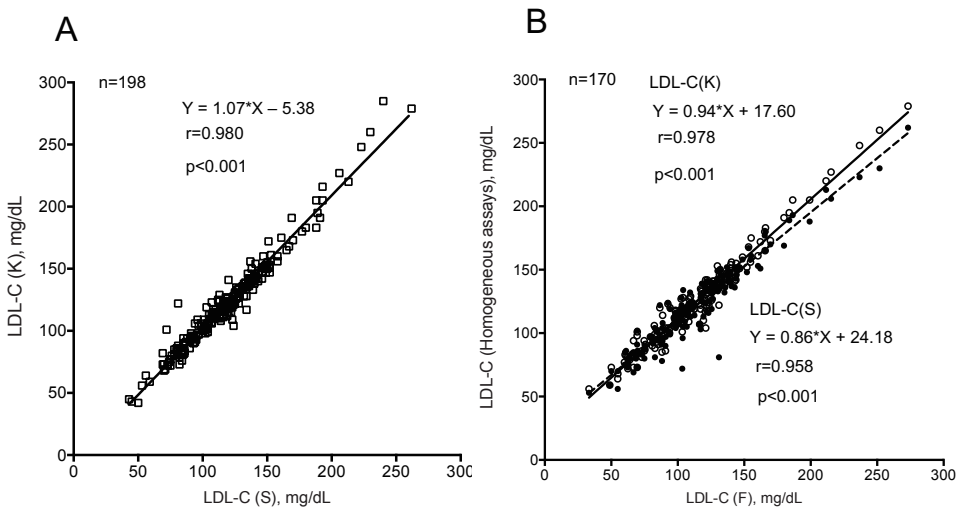

Fig. 2. Linear regression analysis plot of homogeneous LDL-C(S) vs homogeneous LDL-C(K) (A) and two homogeneous LDL-C assays vs LDL-C(F) for TG < 400 mg/dL (B)

For specimens containing less than 400 mg (4.52 mmol/L) triglyceride calculated by the Friedewald equation, LDL-C(K) and LDL-C(S) were compared with LDL-C(F). Open circles: LDL-C(K), closed circles: LDL-C(S).

Table 2. Whole serum data of the hypertriglyceridemic samples

	Total cholesterol	Triglyceride	LDL-C(K)	LDL-C(S)	HDL-C(K)	HDL-C(S)
	Mean ± SD (mg/dL)					
198 samples	204 ± 45	249 ± 140	116 ± 40	113 ± 37	51 ± 15	49 ± 13
32 samples	231 ± 62	329 ± 122	133 ± 60	126 ± 54	47 ± 14	46 ± 13

* $p < 0.01$.

observed regarding conflicts between the two homogeneous assays and the Friedewald equation or hypertriglyceridemia (TG ≥ 400 mg/dL). The means ± SD of TC, TG, LDL-C(K), LDL-C(S), HDL-C(K), and HDL-C(S) are shown for 198 samples of fresh serum from patients with hypertriglyceridemia (TG > 150 mg/dL), and for 32 samples of mismatched LDL-C levels or hypertriglyceridemia (TG ≥ 400 mg/dL; **Table 2**). There were no differences in the mean values of LDL-C(K) and LDL-C(S) in the 198 specimens, but in 32 selected samples, LDL-C(K) was significantly higher than LDL-C(S) (**Table 2**).

Linear regression analysis was performed for LDL-C(K) and LDL-C(S) vs BQ-LDL in the 32 selected samples. As shown in **Fig. 3A**, LDL-C(K) and LDL-C(S) had a good correlation with BQ-LDL ($r=0.987$, $p<0.001$ and $r=0.982$, $p<0.001$, respectively). ApoB 100 is an apolipoprotein that exists on a 1:1 basis on VLDL, IDL, LDL particles, and correlates with the number of particles, mostly LDL particles. ApoB occurs as two isoforms, apoB-100 and apoB-48, but most apoB in the serum samples was apoB-100. LDL-C(K) and LDL-C(S) in the 32 samples were also correlated with serum apoB ($r=0.922$, $p<0.001$ and $r=0.931$, $p<0.001$, respectively; **Fig. 3B**). The two homogeneous LDL-C assays of 32 samples were strongly correlated with each other ($y=1.07*x-5.38$, $r=0.980$), and LDL-C(K) and LDL-C (S) determined by the two homogeneous LDL assays were strongly correlated with LDL-C(F) ($y=0.94*x+17.60$, $r=0.978$ and $y=0.86*x+24.18$, $r=0.958$, respectively (data not shown)).

Quantitative ultracentrifugation was performed on the 32 selected samples as shown in **Fig. 1**. **Table 3** shows the mean values of cholesterol and LDL cholesterol in whole serum and each ultracentrifugal fraction. The means of LDL-C(K) and LDL-C(S) were not significantly different from the BQ-LDL-C. However, LDL-C(K) and LDL-C(S) in the CM-removed serum were lower than BQ-LDL-C. Both direct LDL-C values were significantly lower in the order CM-removed fraction, VLDL-removed fraction, and

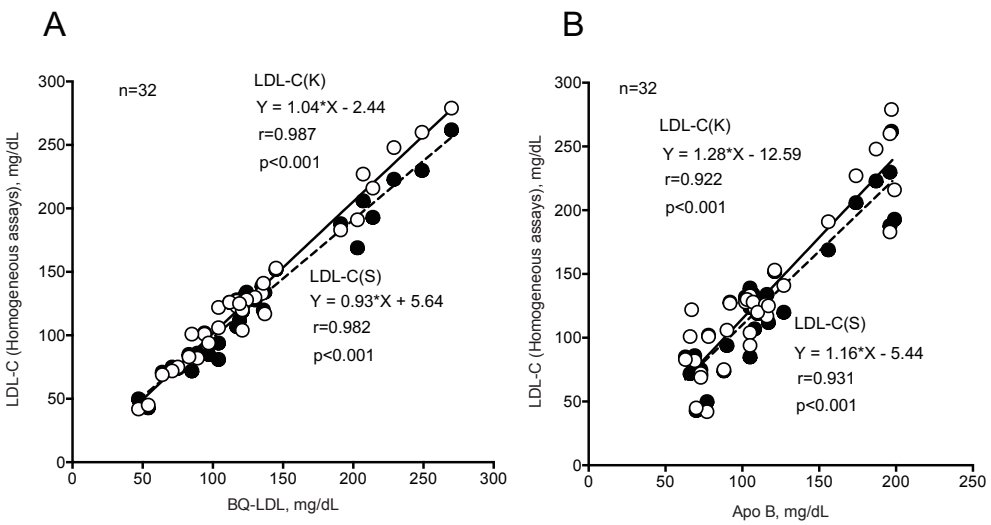

Fig. 3. Comparison of the correlation between two homogeneous LDL-C assays and β-quantification LDL (A) or serum apoB (B)

Open circles: LDL-C(K), closed circles: LDL-C(S). The solid lines represent regression lines for LDL-C(K) and the dashed lines represent regression lines for LDL-C(S).

Table 3. Comparison of cholesterol levels in whole serum and quantitative ultracentrifugation fractions

	Cholesterol	BQ-LDL	LDL-C(K)	LDL-C(S)
	Mean ± SD (mg/dL)			
Whole serum	231 ± 62	130 ± 57 (100%)†	**133 ± 60 (102%)†	**126 ± 54 (97.0%)†
CM removed serum	194 ± 60		*123 ± 56 (94.6%)†	**119 ± 52 (89.2%)†
VLDL removed serum	174 ± 57		**120 ± 54 (92.3%)†	**115 ± 52 (88.5%)†
IDL removed serum	157 ± 50		106 ± 48 (81.5%)†	104 ± 48 (80.0%)†
CM	36.7 ± 17.7		10.1 ± 5.0	7.5 ± 3.1
VLDL	20.5 ± 13.3		4.1 ± 4.7	4.4 ± 3.5
IDL	17.3 ± 13.0		13.1 ± 11.0	10.9 ± 7.7

$n = 32$.
*$p < 0.01$; **$p < 0.001$.
†Percentage of BQ-LDL.

IDL-removed fraction. In both direct assays, cholesterol in the CM, VLDL, and IDL fractions was measured as part of the LDL-C. As shown in **Table 3**, in the VLDL-removed fraction, LDL-C(K) and LDL-C(S) contained only about 92.3% and 88.5% of BQ-LDL, respectively.

In **Fig. 4A**, **B**, **C**, we compared the differences of cholesterol in the CM, VLDL, and IDL fractions, and differences in the values by subtracting BQ-LDL from the two homogeneous LDL-C values in whole serum, CM-removed and VLDL-removed fractions. There was no correlation between the cholesterol concentration in the CM and VLDL fractions or differences in values obtained by subtracting BQ-LDL from the two homogeneous LDL-C values in whole serum and the CM-removed fraction (**Fig. 4A**, **B**). These reactions did not depend on triglyceride in the CM and VLDL fractions (data not shown). However, a negative correlation was found between the cholesterol concentration in the IDL fraction and the difference obtained by subtracting BQ-LDL from the two homogeneous LDL-C values in the VLDL-removed fraction (**Fig. 4C**). Of note, a strong negative correlation was found between the cholesterol concentration of the IDL fraction and the difference obtained by subtracting BQ-LDL from LDL-C(S) in the VLDL-removed fraction.

Correlations of cholesterol and LDL-C measured by the two direct assays in the three fractions (CM, VLDL, IDL) are shown in **Fig. 5A**, **B**, **C**. No correla-

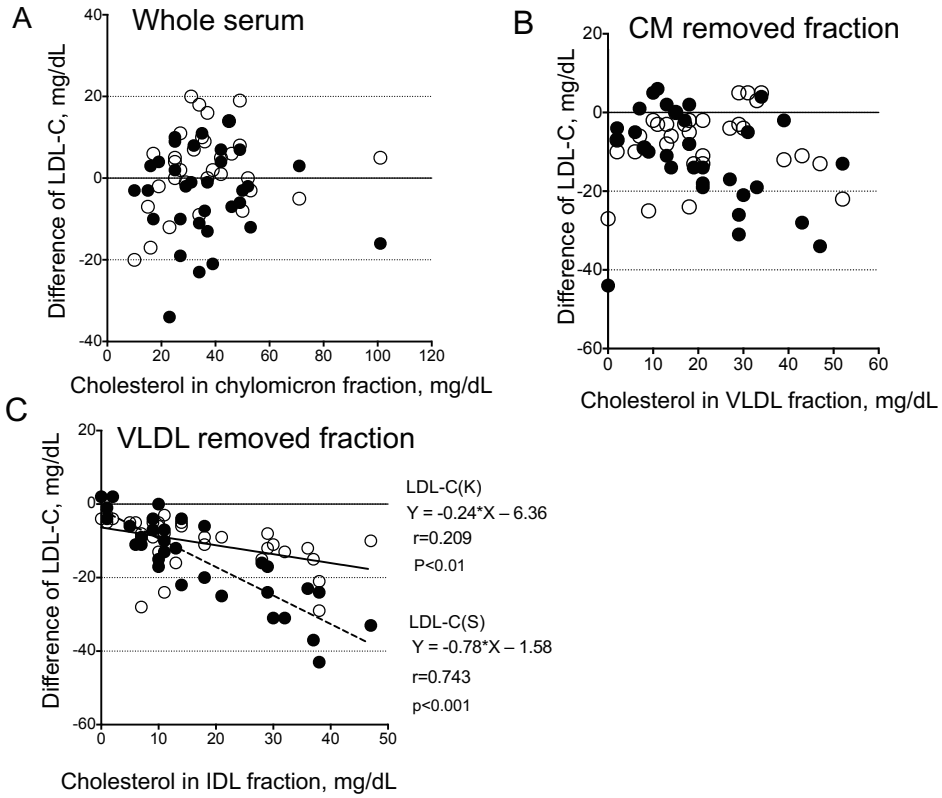

Fig. 4. Effect of increased cholesterol concentrations on the difference in LDL-C concentrations between the two homogeneous assays and modified β-quantification ($n=32$)

Plot of concentration difference vs the BQ-LDL for LDL-C (K) and LDL-C(S) in: whole serum vs cholesterol concentration in the chylomicron fraction (A), chylomicron-removed fraction vs cholesterol concentration in the VLDL fraction (B); and VLDL-removed fraction vs cholesterol concentration in the IDL fraction (C). Open circles: LDL-C(K), closed circles: LDL-C(S). The solid lines represent regression lines for LDL-C(K) and the dashed lines represent regression lines for LDL-C(S).

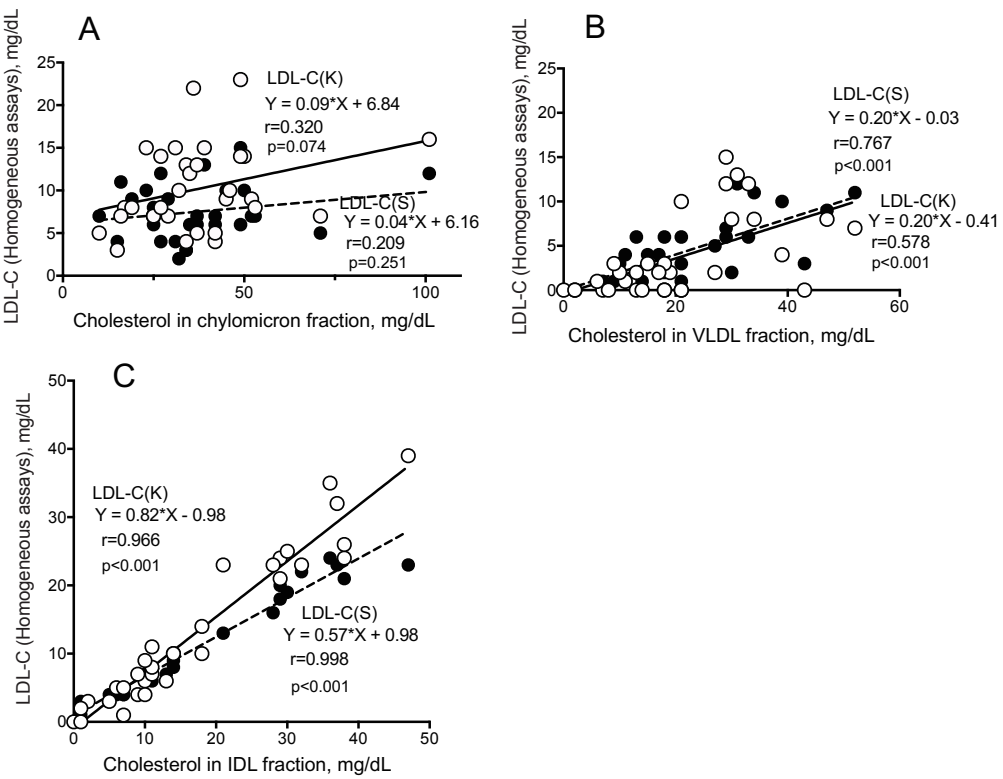

Fig. 5. Relationship between LDL-C levels measured by the two homogeneous assays and cholesterol levels in CM (A), VLDL (B), and IDL (C) fractions

Open circles: LDL-C(K), closed circles: LDL-C(S). The solid lines represent regression lines for LDL-C(K) and the dashed lines represent regression lines for LDL-C(S).

tion was found between cholesterol in the CM fraction and that measured by both direct methods (**Fig. 5A**). In the VLDL fraction, a weak correlation was also noted between cholesterol and that measured by the two direct methods (**Fig. 5B**). In theory, BQ-LDL contains cholesterol in the IDL fraction; therefore, it is natural that direct LDL includes cholesterol. There was a strong correlation between cholesterol in IDL and LDL-C in the IDL fraction as measured by both direct assays (**Fig. 5C**).

Discussion

Most homogeneous assays for LDL-C have satisfied all error targets of the National Cholesterol Education Program (NCEP) for non-diseased individuals in a fasting state compared with β-quantification[6, 9]. Previous studies reported no significant difference in LDL-C concentration as measured by homogeneous assay between fasting and non-fasting samples of the same individual and the postprandial change in LDL-C concentration determined by homogeneous assays and β-quantification was similar[11-13]. However, earlier studies revealed that some homogenous assays exhibited poor analytical performance in patients with common diseases, and even in disease-free subjects[6, 8, 14]. Other reports showed marked differences in measurements, particularly in samples with lipid abnormalities, possibly because homogeneous LDL-C methods from different manufacturers use different measurement principles[9]. A common problem was that when these reagents were used, hypertriglyceridemia often triggered a positive assay bias, especially for LDL-C measurements. After these issues were highlighted, some homogeneous LDL-C assays are no longer used. Previously, the Japanese Arteriosclerosis Society recommended the Friedewald equation as a method to measure the LDL-C level, on the basis of different measurements obtained between LDL-C homogeneous assays[15]. Recently, the Japan Atherosclerosis Society has accepted measurement by LDL-C homogeneous assay[16].

This study compared two homogeneous LDL-cholesterol assays, LDL-C(K) (a selective solubilization method) and LDL-C(S) (a liquid selective detergent method) using fresh hypertriglyceridemic serum and quantitative ultracentrifugation fractions. Because of the limited amount of sample, two widely used homogeneous assays with different measurement principles were chosen in this study.

We analyzed residual specimens that had been taken for routine examination. The two homogeneous LDL-C assays, LDL-C(K) and LDL-C(S), were strongly correlated with each other as well as with BQ-LDL.

CM and VLDL are the two major classes of TG-rich lipoprotein. We examined whether cholesterol in the CM, VLDL, and IDL fractions was contained in the LDL-C measured by direct methods. Both LDL-C(K) and LDL-C(S) were affected by the CM fraction. However, these reactions did not depend on cholesterol or triglyceride in the CM fraction. The two homogeneous LDL-C assays were also affected by the VLDL fraction. Part of these reactions depended on cholesterol in the VLDL fraction. The current study confirmed that both homogeneous LDL-C assays had a reaction in the CM fraction and a weak reaction in the VLDL fraction. A mean value of 10 to 15 mg/dL (7.7 to 11.5%) of BQ-LDL was missed in the homogeneous LDL-C assays (**Table 3**). In principle, the BQ-LDL method includes the IDL fraction as part of the LDL, so it is expected that homogeneous LDL-C assays will include some cholesterol in the IDL fraction. The major lipoproteins that can reach the subendothelia of vessels are LDL, IDL and HDL. Because of the large particle size, it is considered that CM and VLDL do not reach atherosclerotic lesions. Non-HDL cholesterol contains all cholesterol in CM and VLDL. As shown in the lower part of **Table 3**, 57.2 mg/dL cholesterol from CM and VLDL contained 14.2 mg/dL (25%) and 11.9 mg/dL (21%) of LDL-C(K) and LDL-C(S), respectively. Thus, the two direct LDL-C assays reduced the cholesterol effect in CM and VLDL to 21%–25%. Therefore, the direct LDL-C assay may be a better indicator than non-HDL cholesterol.

Taken together, the results of the two homogeneous LDL-C assays using hypertriglyceridemic serum were in good agreement, even though an appreciable misclassification percentage in lipoprotein categories must be considered (**Table 3**). The difference in the reactions with TG rich lipoprotein might influence the difference in the measurements of LDL-C by both homogeneous assays. According to the guidelines of the Japan Atherosclerosis Society, the treatment target for LDL-C is determined by the risk category[16]. It is necessary to consider that homogeneous assays are affected to some extent by CM and VLDL cholesterol.

Extreme hypertriglyceridemia of ≥ 11.29 mmol/L (1000 mg/dL) and special cases of dyslipidemia such as biliary tract diseases, were not included in the samples of this study. LDL cholesterol is a risk factor for arteriosclerosis. At a higher strata of triglyceride levels, larger particles such as chylomicrons and their remnants begin to predominate. In this important but much less commonly encountered subgroup, chylomicrons are too large to penetrate the arterial wall, and thus, atherosclerosis risk is relatively low in this situation. Another reason extreme hypertriglyceridemia

was not included in this study is that fractionation by ultracentrifugation cannot be performed accurately in the presence of excess chylomicron. We also excluded patients with biliary tract diseases. Lipoprotein-X (Lp-X), an abnormal lipoprotein found in the plasma at the same density range as LDL, is present in patients with biliary tract diseases and in patients with familial lecithin:cholesterol acyltransferase (LCAT) deficiency. ApoB, a major component of LDL and a key factor in the pathogenesis of atherosclerotic plaques, is missing from the structure of Lp-X, which affects the measurement of LDL cholesterol values by direct methods[6, 17].

Several authors have published studies regarding the handling and storage of samples for lipid determination by ultracentrifugation and precipitation methods. Zivkovic *et al*. reported that freezing prior to density-based fractionation introduced significant variability particularly in HDL and LDL cholesterol as well as LDL and VLDL triglyceride concentrations[18]. Sgouropoulou *et al*. reported that the size of LDL particles was affected by freezing isolated lipoproteins after sequential ultracentrifugation, and that the particles became smaller, which might influence their electrophoretic or chromatographic mobility[19]. To avoid any potential influence on lipid profile measurements after a freeze-thaw cycle, we used fresh samples in this study.

This study had some limitations. First, we could not examine multiple measurements because we used sera leftover from a routine clinical chemistry laboratory. Second, we did not consider the dietary condition of the patients. Third, it was a single center study and the sample size was small.

To the best of our knowledge, this is the first study to compare the ultracentrifugal fractions of fresh serum from patients with hypertriglyceridemia using homogenous LDL assays.

Conclusion

The results from this study and previous findings indicate that the two homogenous LDL-C assays tested exhibited important differences when using samples with hypertriglyceridemia. Part of the CM and VLDL was measured as the LDL-C using both direct methods. However, there was no correlation with the CM value. There was a difference in reactivity to CM and VLDL for each method. However, LDL-C measured by the two homogeneous assays correlated well with BQ-LDL. Therefore, the two homogenous LDL-C assays might be useful for evaluating patients with hypertriglyceridemia and unknown dietary status.

Conflict of Interest

All the authors have no conflicts of interest to report.

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