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Non–HDL Cholesterol Shows Improved Accuracy for Cardiovascular Risk Score Classification Compared to Direct or Calculated LDL Cholesterol in a Dyslipidemic Population

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BACKGROUND: Our objective was to evaluate the accuracy of cardiovascular disease (CVD) risk score classification by direct LDL cholesterol (dLDL-C), calculated LDL cholesterol (cLDL-C), and non–HDL cholesterol (non–HDL-C) compared to classification by reference measurement procedures (RMPs) performed at the CDC.

METHODS: We examined 175 individuals, including 138 with CVD or conditions that may affect LDL-C measurement. dLDL-C measurements were performed using Denka, Kyowa, Sekisui, Serotec, Sysmex, UMA, and Wako reagents. cLDL-C was calculated by the Friedewald equation, using each manufacturer's direct HDL-C assay measurements, and total cholesterol and triglyceride measurements by Roche and Siemens (Advia) assays, respectively.

RESULTS: For participants with triglycerides <2.26 mmol/L (<200 mg/dL), the overall misclassification rate for the CVD risk score ranged from 5% to 17% for cLDL-C methods and 8% to 26% for dLDL-C methods when compared to the RMP. Only Wako dLDL-C had fewer misclassifications than its corresponding cLDL-C method (8% vs 17%; $P < 0.05$). Non–HDL-C assays misclassified fewer patients than dLDL-C for 4 of 8 methods ($P < 0.05$). For participants with triglycerides ≥2.26 mmol/L (≥200 mg/dL) and <4.52 mmol/L (<400 mg/dL), dLDL-C methods, in general, performed better than cLDL-C methods, and non–HDL-C methods showed better correspondence to the RMP for CVD risk score than either dLDL-C or cLDL-C methods.

CONCLUSIONS: Except for hypertriglyceridemic individuals, 7 of 8 dLDL-C methods failed to show improved CVD risk score classification over the corresponding cLDL-C methods. Non–HDL-C showed overall the best concordance with the RMP for CVD risk score classification of both normal and hypertriglyceridemic individuals.

LDL cholesterol (LDL-C),[9] a major risk factor for cardiovascular disease (CVD), is the primary target of lipid-lowering therapy, and is used to classify patients into various CVD risk categories *(1)*. Lipoproteins comprise a heterogeneous group of particles of varying size and lipid and protein composition *(2)*, making the development of specific methods for LDL-C challenging. The reference measurement procedures (RMPs) for LDL-C (rLDL-C) and HDL-C (rHDL-C) are based on ultracentrifugation to remove VLDL and chylomicrons, followed by heparin-manganese precipitation to remove LDL *(3)*. Although rLDL-C is impractical for routine use, it has been validated as a CVD biomarker in large clinical studies *(4, 5)* and is the standard to which all routine methods are compared *(6)*. Until recently, LDL-C was not usually directly measured but was instead estimated from total cholesterol (TC),

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[9] Nonstandard abbreviations: LDL-C, LDL cholesterol; CVD, cardiovascular disease; RMP, reference measurement procedure; rLDL-C, reference measurement procedures for LDL-C; rHDL-C, reference measurement procedures for HDL-C; TC, total cholesterol; HDL-C, HDL cholesterol; TG, triglyceride; cLDL-C, calculated LDL cholesterol; dLDL-C, direct measurement of LDL cholesterol; NCEP, National Cholesterol Education Program; dHDL-C, direct HDL cholesterol; apo, apolipoprotein.

HDL cholesterol (HDL-C), and triglyceride (TG) using the Friedewald equation *(7)*. It is known, however, that calculated LDL-C (cLDL-C) becomes progressively less accurate with increasing TGs, is not valid for type III hyperlipoproteinemia, and requires fasting samples *(7)*. In addition, bias and imprecision from the 3 separate measurements used in the calculation can adversely affect cLDL-C accuracy *(8)*.

To address these limitations, various homogeneous reagents for the direct measurement of LDL-C (dLDL-C) have been developed and are now widely adopted *(2)*. An advantage of these methods is that they do not depend on the measurement of TGs, and therefore are less influenced by nonfasting samples. Another advantage is that they are fully automated on various platforms and hence have relatively good precision *(9)*. Nevertheless, previous studies of dLDL-C methods have shown that they may not show complete specificity toward LDL-C and may not always offer a significant practical advantage over cLDL-C *(2, 8, 10, 11)*. These earlier studies, however, were sometimes limited, because they often examined only 1 direct method, and many did not test dyslipidemic populations or compare the results to rLDL-C *(2, 8, 10)*.

Recently, we completed a study comparing all the current dLDL-C methods on the market to rLDL-C *(9)*. We observed that dLDL-C methods frequently failed to meet National Cholesterol Education Program (NCEP) total error goals on dyslipidemic samples when compared to the β-quantification ultracentrifugation RMP for LDL-C. Using the same population, we examined in this study the concordance of CVD risk score classification by the various dLDL-C and cLDL-C methods, using the direct HDL-C (dHDL-C) method from each manufacturer in the calculation, to the CVD risk score obtained by rLDL-C. In addition, apolipoprotein (apo)-B and apoA-I, the main protein structural components of LDL and HDL, respectively, as well as non–HDL-C, were also assessed for CVD risk score classification.

Materials and Methods

PATIENT SAMPLES

Participants were recruited at the Virginia Commonwealth University Medical Center and NIH, with the approval of institutional review boards. Details of the population (n = 175), which included 37 healthy controls, with the majority of the remaining participants recruited from a lipid or CVD clinic, have been previously described *(9)*. A total of 104 participants fasted >12 h, 24 fasted 10–12 h, 11 fasted 8–10 h, and 36 fasted <8 h. Sera were stored at 4 °C, and all measurements were completed within 48 h of collection.

LIPID AND LIPOPROTEIN ANALYSIS

Results for rLDL-C, rHDL-C, dLDL-C, dHDL-C, TG, and TC from the previous study *(9)* were used. Ultracentrifugation reference measurement procedures for LDL-C and HDL-C were performed at the CDC. Direct LDL-C and HDL-C methods [Denka Seiken, Kyowa Medex, Sekisui Medical (formerly Daiichi), Serotec, Sysmex International Reagents, UMA, Wako Pure Chemical Industries, and Roche Diagnostics (distributor of Kyowa Medex reagents with Roche calibrator and controls)] were performed on a Hitachi 917 analyzer (Roche Diagnostics), using parameters recommended by each manufacturer. TC was measured by using Roche reagents adapted for a Siemens Advia 1650 analyzer. Total TG was measured, without glycerol blanking, using Siemens Advia reagents on an Advia 1650 analyzer. Method performance for TC and TG was verified by participation in the CDC Lipid Standardization Program *(12)*, and the mean biases compared to the CDC-RMPs were 0.2% (range −0.3% to 0.8%) for TC and −0.1% (range −3.0% to 2.5%) for TG.

LDL-C was calculated by the Friedewald equation: [cLDL-C (mmol/L) = TC (mmol/L) − HDL-C (mmol/L) − TG (mmol/L)/2.22] *(7)*, using dHDL-C from each manufacturer and TC and TG, as described above. Non–HDL-C was calculated by the following equation: (non–HDL-C = TC − HDL-C), using either dHDL-C from each manufacturer or rHDL-C and TC as described above. The reference values for VLDL cholesterol (rVLDL-C) were calculated by the following equation, using TC and RMPs for LDL-C and HDL-C: (rVLDL-C = TC − rLDL-C − rHDL-C. For dLDL-C values <0.08 mmol/L (3 mg/dL) or when cLDL-C was <0, a value of 0.05 mmol/L (2 mg/dL) was assigned.

apoA-I and apoB were measured on frozen samples stored at −70° C between 6 and 12 months and were performed in singleton in 1 analytical run, using a nephelometric method on the Dimension Vista® System (Siemens Healthcare Diagnostics). To verify traceability of results, apoB IFCC/WHO standard (SP3-08) and apoA-I IFCC/WHO standard (SP1-01) were measured in quadruplicate and yielded results close to their assigned values [SP3-08 apoB: 118 mg/dL vs mean (SD) 117 (2.2) mg/dL; SP1-01 apoA-1: 150 mg/dL vs 155 (3.7) mg/dL].

STATISTICAL ANALYSIS

JMP Statistical Software (SAS Institute) and Analyze-it for Microsoft Excel (Analyze-it Software) were used. Performance of dLDL-C and cLDL-C compared to rLDL-C was assessed by use of coefficients of determination and weighted Deming regression analysis. Performance of LDL-C methods

Table 1. dLDL-C and cLDL-C vs rLDL-C.

	Denka	Kyowa
dLDL-C vs rLDL-C [TGs < 2.26 mmol/L (200 mg/dL)] (n = 145)		
R^2	0.97	0.98
$S_{y\|x}$ mmol/L (mg/dL)	0.08 (3.09)	0.08 (3.09)
Slope (95% CI)	0.99 (0.89 to 1.10)	1.00 (0.88 to 1.12)
Intercept (95% CI), mmol/L, mg/dL	−0.02 (−0.25 to 0.22), −0.77 (−9.67 to 8.51)	−0.06 (−0.33 to 0.22), −2.32 (−12.76 to 8.51)
% Observed agreement (95% CI), κ	87% (80% to 92%), 0.83	90% (84% to 95%), 0.87
% In lower/higher risk category	6%/8%	8%/2%
% Exceeding total error goal	13%	8%
cLDL-C[a] vs rLDL-C [TGs < 2.26 mmol/L (200 mg/dL)] (n = 145)		
R^2	0.98	0.98
$S_{y\|x}$ mmol/L (mg/dL)	0.07 (2.71)	0.08 (3.09)
Slope (95% CI)	0.97 (0.86 to 1.08)	0.95 (0.78 to 1.13)
Intercept (95% CI), mmol/L, mg/dL	−0.02 (−0.26 to 0.23), −0.77 (−10.05 to 8.89)	−0.02 (−0.42 to 0.39), −0.77 (−16.24 to 15.08)
% Observed agreement (95% CI), κ	91% (85% to 95%), 0.88	88% (82% to 93%), 0.85
% In lower/higher risk category	7%/2%	10%/1%
% Exceeding total error goal	12%	14%
dLDL-C vs rLDL-C [TGs ≥ 2.26 mmol/L (200 mg/dL) and <4.52 mmol/L (400 mg/dL)] (n = 20)		
R^2	0.97	0.83
$S_{y\|x}$ mmol/L (mg/dL)	0.07 (2.71)	0.13 (5.03)
Slope (95% CI)	1.07 (0.98 to 1.15)	1.10 (0.92 to 1.28)
Intercept (95% CI), mmol/L, mg/dL	−0.12 (−0.31 to 0.07), −4.64 (−11.99 to 2.71)	−0.01 (−0.34 to 0.32), −0.39 (−13.15 to 12.37)
% Observed agreement (95% CI), κ	75% (51% to 91%), 0.69	60% (36% to 81%), 0.52
% In lower/higher risk category	10%/15%	5%/35%
% Exceeding total error goal	5%	30%
cLDL-C[a] vs rLDL-C [TGs ≥ 2.26 mmol/L (200 mg/dL) and <4.52 (400 mg/dL)] (n = 20)		
R^2	0.84	0.85
$S_{y\|x}$ mmol/L (mg/dL)	0.16 (6.19)	0.15 (5.80)
Slope (95% CI)	1.15 (0.94 to 1.36)	1.15 (0.96 to 1.33)
Intercept (95% CI), mmol/L, mg/dL	−0.53 (−1.00 to −0.06), −20.49 (−38.67 to −2.32)	−0.46 (−0.86 to −0.07), −53.75 (−33.26 to −2.71)
% Observed agreement (95% CI), κ	50% (27% to 73%), 0.38	55% (32% to 77%), 0.43
% In lower/higher risk category	35%/15%	35%/10%
% Exceeding total error goal	40%	40%

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[a] cLDL-C was calculated using direct HDL-C from each indicated manufacturer.

was evaluated in participants with TG concentrations <2.26 mmol/L (200 mg/dL) and between 2.26 and 4.52 mmol/L (200 and 400 mg/dL) and included both diseased and nondiseased individuals. Participants were classified into CVD risk categories on the basis of NCEP criteria *(1)* as described in the Data Supplement that accompanies the online version of this article at http://www.clinchem.org/content/vol57/

Table 1. dLDL-C and cLDL-C vs rLDL-C. *(Continued from page 492)*

Roche	Sekisui	Serotec	Sysmex
0.98	0.99	0.97	0.97
0.09 (3.48)	0.06 (2.32)	0.08 (3.09)	0.12 (4.64)
0.93 (0.77 to .09)	0.98 (0.92 to 1.05)	0.91 (0.81 to 1.01)	0.91 (0.63 to 1.20)
−0.02 (−0.38 to 0.35), −0.77 (−14.69 to 13.53)	−0.02 (−0.16 to 0.13), −0.77 (−6.19 to 5.03)	−0.01 (−0.25 to 0.22), −0.39 (−9.67 to 8.51)	0.11 (−0.65 to 0.65), 0.00 (−25.14 to 25.14)
80% (73% to 86%), 0.74	91% (85% to 95%), 0.88	74% (66% to 81%), 0.66	82% (75% to 88%), 0.76
19%/1%	7%/2%	265%/1%	17%/1%
19%	6%	35%	26%
0.98	0.98	0.98	0.98
0.07 (2.71)	0.08 (3.09)	0.07 (2.71)	0.08 (2.97)
1.00 (0.89 to 1.12)	0.99 (0.86 to 1.13)	0.99 (0.90 to 1.08)	1.00 (0.89 to 1.10)
−0.06 (−0.33 to 0.20), −2.32 (−12.76 to 7.73)	−0.02 (−0.34 to 0.29), −0.77 (−13.15 to 11.21)	−0.02 (−0.22 to 0.19), −0.77 (−8.51 to 7.35)	0.01 (−0.23 to 0.25), 0.49, (−8.80 to 9.74)
95% (89% to 98%), 0.93	92% (87% to 96%), 0.90	93% (88% to 97%), 0.91	90% (80% to 93%), 0.87
3%/3%	3%/5%	2%/5%	1%/9%
10%	9%	8%	10%
0.82	0.99	0.82	0.84
0.13 (5.03)	0.04 (1.55)	0.14 (5.41)	0.13 (5.03)
1.06 (0.88 to 1.24)	1.06 (1.00 to 1.11)	1.04 (0.83 to 1.24)	1.12 (0.93 to 1.31)
−0.04 (−0.37 to 0.29), −1.55 (−14.31 to 11.21)	−0.09 (−0.24 to 0.06), −3.48 (−9.28 to 2.32)	−0.30 (−0.75 to 0.16), −11.60 (−29.00 to 6.19)	−0.32 (−0.72 to 0.08), −12.37 (−27.84 to 3.09)
65% (41% to 85%), 0.57	90% (68% to 99%), 0.87	70% (46% to 88%), 0.62	80% (56% to 94%), 0.75
15%/20%	0%/10%	25%/5%	15%/5%
15%	0%	45%	10%
0.85	0.83	0.85	0.83
0.15 (5.80)	0.16 (6.19)	0.15 (5.80)	0.15 (5.80)
1.16 (0.97 to 1.35)	1.15 (0.95 to 1.35)	1.17 (0.99 to 1.35)	1.15 (0.96 to 1.34)
−0.44 (−0.85 to −0.03), −17.01 (−32.87 to −12.76)	−0.45 (−0.87 to −0.03), −17.40 (−33.64 to −1.16)	−0.47 (−0.85 to −0.09), −18.17 (−32.87 to −3.48)	−0.40 (−0.79 to −0.02), −15.47 (−30.55 to −0.77)
65% (41% to 85%), 0.57	45% (23% to 69%), 0.31	60% (36% to 81%), 0.50	55% (32% to 77%), 0.45
20%/15%	35%/20%	20%/20%	25%/20%
40%	45%	40%	35%

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issue3. The McNemar test was used to assess whether the rate of misclassification of participants with dLDL-C or cLDL-C differed significantly from the reference measurement procedure. The nominal data used for the McNemar analysis were misclassification rates for dLDL-C, cLDL-C, and non–HDL-C compared to their RMPs. For each method, misclassification rates were compared to their RMP as previously described *(13)*. For example, we considered a null hypothesis that any given dLDL-C method does not mis-

Table 1. dLDL-C and cLDL-C vs rLDL-C.
(Continued from page 493)

UMA	Wako
0.85	0.99
0.18 (6.96)	0.05 (1.93)
0.99 (0.90 to 1.07)	0.99 (0.98 to 1.01)
−0.04 (−0.23 to 0.15), −1.55 (−8.89 to 5.80)	0.05 (0.02 to 0.09), 1.93 (0.77 to 4.38)
86% (79% to 91%), 0.81	92% (86% to 96%), 0.89
9%/6%	3%/5%
15%	4%
0.96	0.97
0.12 (4.64)	0.05 (1.93)
0.93 (0.71 to 1.16)	0.93 (0.52 to 1.33)
0.04 (−0.48 to 0.55), 1.55 (−18.56 to 21.27)	−0.02 (−0.93 to 0.89), −0.77 (−35.96 to 34.42)
84% (77% to 90%), 0.79	83% (76% to 89%), 0.78
10%/6%	15%/1%
15%	25%
0.74	0.98
0.16 (6.19)	0.05 (1.93)
1.07 (0.85 to 1.35)	0.97 (0.91 to 1.04)
0.14 (−0.29 to 0.56), 5.41 (−11.21 to 21.66)	0.27 (0.12 to 0.43), 10.44 (4.64 to 16.63)
45% (23% to 69%), 0.33	70% (46% to 88%), 0.62
10%/45%	5%/25%
45%	30%
0.83	0.82
0.16 (6.19)	0.16 (6.19)
1.15 (0.96 to 1.35)	1.18 (0.96 to 1.41)
−0.41 (−0.82 to −0.00), −15.85 (−31.71 to 0.00)	−0.66 (−1.17 to −0.15), −25.52 (−45.24 to −5.80)
50% (27% to 73%), 0.38	50% (27% to 73%), 0.38
25%/25%	35%/15%
40%	50%

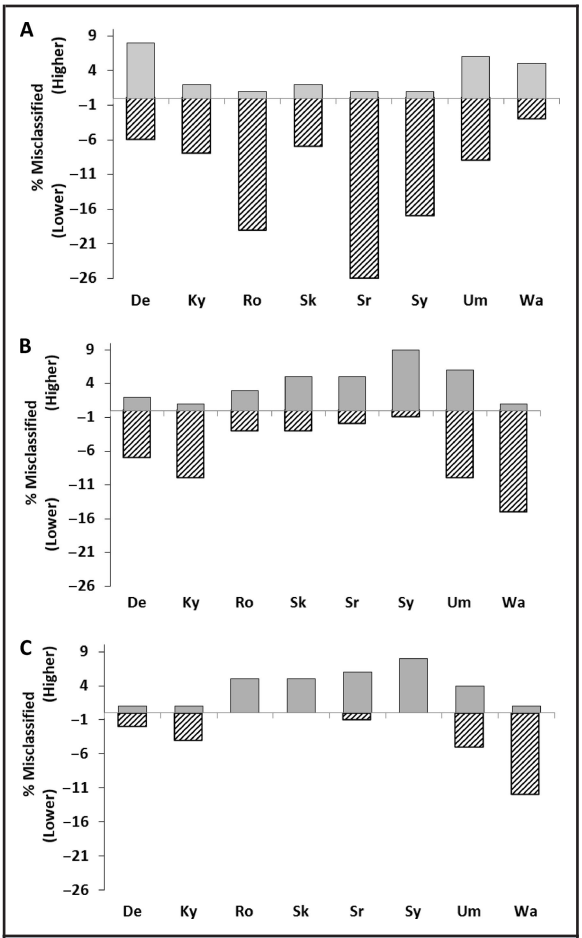

Fig. 1. Misclassification rate for CVD risk for those participants with TG levels <2.26 mmol/L (200 mg/dL), n = 145.

Percent of test results that were classified into either a higher (shaded bar) or lower (hatched bar) CVD risk category compared to RMPs are shown for dLDL-C (A), cLDL-C (B), or non–HDL-C (C). De, Denka; Ky, Kyowa; Ro, Roche; Sr, Serotec; Sk, Sekisui, Sy, Sysmex; Um, UMA; Wa, Wako.

classify patients either more or less frequently than its corresponding cLDL-C method. If both parts of this hypothesis are rejected, we assert equivalence in the rate of misclassification.

Results

COMPARISON OF DIRECT AND CALCULATED LDL-C METHODS FOR SAMPLES WITH TG <2.26 mmol/L (200 mg/dL)

Coefficients of determination (R^2) with rLDL-C ranged from 0.85 to 0.99 for dLDL-C assays and from 0.96 to 0.98 for cLDL-C assays (Table 1). All the assays also showed a relatively small proportional and fixed bias. The dLDL-C and cLDL-C results from each method were used to classify CVD risk score, according to NCEP risk categories and compared to the risk

scoreclassification obtained by using rLDL-C. The overall misclassification rate of CVD risk score classifications ranged between 5% and 17% for cLDL-C methods and was lower than that observed for 5 of the 8 corresponding dLDL-C methods, which had misclassification rates between 8% and 26%. Statistically, there were significantly ($P < 0.05$) more misclassifications with Roche and Serotec dLDL-C methods compared to their corresponding cLDL-C methods (Roche dLDL-C 20% vs cLDL-C 6%; Serotec dLDL-C 27% vs cLDL-C 7%). Only the Wako cLDL-C method showed significantly more misclassifications than its corresponding dLDL-C method (17% vs 8%) ($P < 0.05$).

The percentage of individuals classified by the dLDL-C methods into a lower risk category compared to rLDL-C ranged between 3% and 26%, whereas only 1%–8% of individuals were misclassified into a higher risk category (Fig. 1). Except for Denka and Wako dLDL-C methods, dLDL-C methods misclassified more patients into a lower rather than a higher risk category. Only in 2 cases, which both occurred with the UMA dLDL-C method, was any individual misclassified by more than 2 risk categories. In the case of cLDL-C methods, no consistent pattern was observed in terms of the direction of misclassifications (Fig. 1); 3 cLDL-C methods had a positive bias and 4 had a negative bias.

COMPARISON OF DIRECT AND CALCULATED LDL-C METHODS ON HYPERTRIGLYCERIDEMIC SAMPLES

This analysis (Table 1) was limited to 20 individuals with TG concentrations ≥2.26 mmol/L (200 mg/dL) and <4.52 mmol/L (400 mg/dL), because of the known limitation of the Friedewald equation for hypertriglyceridemic samples. In general, the dLDL-C methods performed better than their corresponding cLDL-C methods in this population when assessed by total error or the percent observed agreement with rLDL-C for cardiovascular risk score classification. The cLDL-C methods also appeared to show a bias for categorizing individuals into lower risk categories compared to dLDL-C methods (Table 1 and online Supplemental Fig. 1).

EXAMINATION OF FACTORS CONTRIBUTING TO ERROR IN cLDL-C

In Table 2, we present data for the contribution of errors from the dHDL-C assays in calculating LDL-C. For those patients with TG concentrations <2.26 mmol/L (200 mg/dL), residual SDs ($S_{y|x}$) for dHDL-C were relatively low [range 0.06–0.08 mmol/L (2.3–3.1 mg/dL)], except for the UMA assay (0.22 mmol/L, 8.5 mg/dL). Between 6% and 20% of values for dHDL-C methods exceeded total error goals in patients with TG concentrations <2.26 mmol/L (200 mg/dL). When compared to rHDL-C for CHD risk score classification, fewer misclassifications were observed for dHDL-C assays (Table 2) than were observed with dLDL-C assays (Table 1). All dHDL-C assays, however, except Sekisui, showed a substantial increase in the number of results that exceeded total error goals, in patients with TG concentrations ≥2.26 mmol/L (200 mg/dL).

The term TG (mmol/L)/2.22, used in the Friedewald equation, provides an estimate of VLDL cholesterol and is another source of error for cLDL-C. Part of the error is due to imprecision and bias from the TG measurement, including whether endogenous glycerol is subtracted. In addition, TG has a relatively large biologic variability of approximately 20% CV, which also contributes to errors in calculating LDL-C *(14)*. In our population, TG (mmol/L)/2.22 and VLDL cholesterol (n = 144, after exclusion of one outlier) showed a relatively weak relationship ($R^2 = 0.65$) and relatively large residual SD ($S_{y|x}$) [0.12 mmol/L (4.9 mg/dL)], even for those individuals with TG <2.26 mmol/L (<200 mg/dL) (online Supplemental Fig. 2), which is approximately twice the amount of error contributed from the dHDL-C methods.

NON–HDL-C FOR CVD RISK SCORE CLASSIFICATION

Non–HDL-C, which is a measure of cholesterol associated with all apoB-containing particles, was examined as an alternative for CVD risk score classification (Table 3 and online Supplemental Table 2). Non–HDL-C is unaffected by errors related to estimating VLDL cholesterol and is also unaffected by issues related to the lipoprotein specificity of dLDL-C methods toward the various apoB-containing lipoproteins. For patients with TG concentrations <2.26 mmol/L (200 mg/dL), non–HDL-C calculated by using dHDL-C methods showed a strong relationship ($R^2 \geq 0.97$) to non–HDL-C calculated with rHDL-C. The percent of individuals classified by the non–HDL-C methods into a lower risk category compared to the reference non–HDL-C method ranged between 0% and 11%, whereas 1%–8% were misclassified into a higher risk category. Except for the Wako dLDL-C, non–HDL-C methods showed overall less misclassifications than dLDL-C methods or cLDL-C methods (Fig. 1).

For patients with TG concentrations ≥2.26 mmol/L (200 mg/dL) and <4.52 mmol/L (400 mg/dL), the non–HDL-C methods, in general, showed a better correspondence to their RMP than did dLDL-C or cLDL-C methods (online Supplemental Fig. 1). The percent of individuals misclassified into a lower risk category ranged between 0% and 7%, whereas 0%–18% were misclassified into a higher risk category, which was better than that observed for either dLDL-C or cLDL-C methods.

Table 2. dHDL-C vs rHDL-C.

	Denka	Kyowa	Roche
dHDL-C vs rHDL-C [TGs < 2.26 mmol/L (200 mg/dL)] (n = 146)			
R^2	0.97	0.99	0.98
$S_{y\|x}$ mmol/L (mg/dL)	0.08 (3.09)	0.06 (2.32)	0.06 (2.32)
Slope (95% CI)	1.07 (1.02 to 1.12)	1.12 (1.08 to 1.15)	1.06 (1.02 to 1.09)
Intercept (95% CI), mmol/L, mg/dL	−0.08 (−0.13 to −0.02), −3.09 (−5.03 to −0.77)	−0.11 (−0.15 to −0.07), −4.25 (−5.80 to −2.71)	−0.10 (−0.14 to −0.61), −3.87 (−5.41 to −2.32)
% Observed agreement (95% CI), κ	93% (88% to 97%), 0.88	91% (85% to 95%), 0.86	93% (88% to 97%), 0.89
% In lower/higher risk category	5%/3%	1%/8%	5%/1%
% Exceeding total error goal	6%	8%	6%
dHDL-C vs rHDL-C [TGs ≥ 2.26 mmol/L (200 mg/dL)] (n = 28)			
R^2	0.93	0.97	0.96
$S_{y\|x}$ mmol/L (mg/dL)	0.10 (3.87)	0.11 (4.25)	0.11 (4.25)
Slope (95% CI)	1.10 (0.91 to 1.29)	0.93 (0.58 to 1.27)	0.85 (0.51 to 1.21)
Intercept (95% CI), mmol/L, mg/dL	−0.02 (−0.18 to 0.13), −0.77 (−6.96 to 5.03)	0.08 (−0.22 to 0.38), 3.09 (−8.51 to 14.69)	0.10 (−0.20 to 0.41), 3.87 (−7.73 to 15.85)
% Observed agreement (95% CI), κ	89% (72% to 98%), 0.76	89% (72% to 98%), 0.73	86% (67% to 96%), 0.63
% In lower/higher risk category	4%/7%	11%/0%	14%/0%
% Exceeding total error goal	25%	11%	14%

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apoB AND apoA-I FOR CVD RISK SCORE CLASSIFICATION

apoB correlated poorly with all dLDL-C methods, and coefficients of determination (R^2) ranged between 0.47 and 0.61 (online Supplemental Table 3). The coefficients of determination between apoB and rLDL-C were also relatively low ($R^2 = 0.56$). But the coefficient of determination between apoB and non–HDL-C was better and ranged between 0.83 and 0.84 (online Supplemental Table 4). When reference non–HDL-C was compared to apoB, the coefficient of determination was 0.86 (online Supplemental Fig. 3A).

The relationship between apoA-I and rHDL-C was fairly strong ($R^2 = 0.81$) (online Supplemental Fig. 3B). However, the relationships between apoA-I and the various dHDL-C methods were quite variable, with coefficients of determination ranging between 0.66 and 0.83 (online Supplemental Table 5).

Discussion

A major finding from this study is that dLDL-C methods, in general, did not offer an advantage over cLDL-C in classifying patients into NCEP risk score categories in a dyslipidemic population when compared to rLDL-C. In fact, for patients with TG concentrations <2.26 mmol/L (200 mg/dL), cLDL-C values based on Roche and Serotec dHDL-C methods more closely matched rLDL-C for CVD risk score classification than did their corresponding dLDL-C methods. dLDL-C methods did, however, appear to have an advantage over cLDL-C in CVD risk score classification for those patients with TG concentrations ≥2.26 mmol/L (200 mg/dL) (Table 1 and online Supplemental Table 1), because of the poorer performance of dHDL-C methods on hypertriglyceridemic samples (Table 2) and inaccuracies in VLDL cholesterol estimation (online Supplemental Fig. 2). These results suggest that from a practical and cost perspective, it may be better to use cLDL-C for risk score classification in the subset of patients with TG concentrations <2.26 mmol/L (200 mg/dL), because it does not involve doing the extra measurement for dLDL-C. dLDL-C methods may be best reserved for individuals with TG concentrations ≥2.26 mmol/L (200 mg/dL), in whom these methods usually showed an advantage for correctly classifying patients.

Table 2. dHDL-C vs rHDL-C. *(Continued from page 496)*

Sekisui	Serotec	Sysmex	UMA	Wako
0.98	0.97	0.97	0.85	0.98
0.07 (2.71)	0.08 (3.09)	0.08 (3.09)	0.22 (8.51)	0.07 (2.71)
1.01 (0.97 to 1.05)	1.09 (0.99 to 1.19)	1.01 (0.94 to 1.07)	1.29 (1.21 to 1.37)	0.98 (0.93 to 1.04)
−0.06 (−0.11 to −0.02), −2.32 (−4.25 to −0.77)	−0.16 (−0.28 to −0.03), −6.19 (−10.83 to −1.16)	−0.09 (−0.17 to −0.01), −3.48 (−6.57 to −0.39)	−0.33 (−0.41 to −0.26), −12.76 (−15.85 to −10.05)	0.10 (0.03 to 0.17), 3.87 (1.16 to 6.57)
93% (88% to 97%), 0.89	91% (85% to 95%), 0.86	88% (81% to 93%), 0.81	86% (80% to 91%), 0.79	87% (80% to 92%), 0.80
7%/0%	8%/1%	12%/0%	5%/8%	1%/12%
8%	12%	16%	17%	20%
0.98	0.78	0.9	0.62	0.8
0.07 (2.71)	0.26 (10.05)	0.38 (14.69)	0.21 (8.12)	0.18 (6.96)
1.07 (0.90 to 1.23)	0.58 (0.37 to 0.79)	1.07 (0.02 to 2.11)	0.91 (0.82 to 1.00)	0.62 (0.00 to 1.24)
−0.07 (−0.20 to 0.07), −2.71 (−7.73 to 2.71)	0.38 (0.19 to 0.57), 14.69 (−7.35 to 22.04)	−0.12 (−1.05 to 0.80), −4.64 (−40.60 to 30.94)	0.06 (0.02 to 0.09), 2.32 (−0.77 to 3.48)	0.45 (−0.12 to 1.03), 17.40 (−4.64 to 39.83)
86% (67% to 96%), 0.63	82% (63% to 94%), 0.55	79% (59% to 92%), 0.40	79% (59% to 92%), 0.56	75% (55% to 89%), 0.52
14%/0%	14%/4%	21%/0%	14%/7%	0%/25%
7%	25%	25%	29%	43%

Other factors to consider when evaluating dLDL-C and cLDL-C methods for CVD risk score classification is intraindividual biological variability and the requirement for fasting before sample collection. Although biological variability from all 3 variables, namely TC, TG, and HDL-C, affects cLDL-C, it has been shown that intraindividual variation for cLDL-C is similar to that for dLDL-C [7.3% (0.6%) for cLDL-C and 6.8% (0.5%) for dLDL-C] *(8)*. Accurate cLDL-C determination also requires that a patient fast before sample collection *(15, 16)*. A potential advantage, therefore, of dLDL-C methods is their use with nonfasting samples. A recent study of a dLDL-C method (Hitachi 917 analyzer, Roche Diagnostics), however, showed a lack of association of nonfasting dLDL-C with CVD risk, which raises questions about the clinical utility of at least this dLDL-C method in nonfasting patients *(17, 18)*. Another study evaluating a dLDL-C method (Sigma Diagnostics) also showed relatively poor performance in nonfasting patients *(19)*. Other studies have also revealed a physiological postprandial decrease in LDL-C values for some patients *(15, 20, 21)*.

The third Adult Treatment Panel of the NCEP currently recommends the use of non–HDL-C, which includes cholesterol from all apoB-containing lipoproteins, as a secondary target of lipid lowering for individuals with TG concentrations ≥2.26 mmol/L (200 mg/dL) *(1)*. In this study, non–HDL-C misclassified fewer cases irrespective of TGs than did either dLDL-C or cLDL-C when compared to their corresponding RMPs (Fig. 1). This reduced rate of misclassification was more pronounced for patients with TG concentrations ≥2.26 mmol/L (200 mg/dL), in whom both dLDL-C and cLDL-C methods showed poorer performance (online Supplemental Fig. 1). Non–HDL-C also requires the measurement of only 2 analytes, instead of the 3 used for cLDL-C, thus reducing costs.

Before non–HDL-C can be recommended as a primary screening test, it will be important to establish not only its superior correspondence to its own RMP, but also that non–HDL-C is at least equivalent to LDL-C for predicting CVD. In diabetic patients, with increased TGs, non–HDL-C has indeed been shown in several studies to be superior to LDL-C in predicting

Table 3. Comparison of results and classification based on direct non–HDL-C vs RMP non–HDL-C.

	Denka	Kyowa	Roche
Non–HDL-C vs RMP non–HDL-C [TGs < 2.26 mmol/L (200 mg/dL)] (n = 146)			
R^2	0.997	0.997	0.997
$S_{y/x}$ mmol/L (mg/dL)	0.04 (1.55)	0.03 (1.16)	0.03 (1.16)
Slope (95% CI)	1.00 (0.90 to 1.10)	1.00 (0.95 to 1.04)	1.01 (0.96 to 1.07)
Intercept (95% CI), mmol/L, mg/dL	−0.01 (−0.28 to 0.27), −0.39 (−10.83 to 10.44)	−0.02 (−0.13 to 0.10), −0.77 (−5.03 to 6.19)	0.00 (−0.15 to 0.16), 0.00 (−5.80 to 6.19)
% Observed agreement (95% CI), κ	97% (92% to 99%), 0.95	95% (90% to 98%), 0.93	95% (90% to 98%), 0.93
% In lower/higher risk category	2%/1%	4%/1%	0%/5%
% Exceeding total error goal	2%	1%	1%
Non–HDL-C vs RMP non–HDL-C [TGs ≥ 2.26 mmol/L (200 mg/dL)] (n = 28)			
R^2	0.998	0.998	0.996
$S_{y/x}$ mmol/L (mg/dL)	0.02 (0.77)	0.01 (0.39)	0.02 (0.77)
Slope (95% CI)	0.99 (0.97 to 1.01)	1.00 (0.97 to 1.02)	1.00 (0.97 to 1.02)
Intercept (95% CI), mmol/L, mg/dL	−0.02 (−0.10 to 0.06), −0.77 (−3.87 to 2.32)	0.00 (−0.08 to 0.09), 0.00 (−3.09 to 3.48)	0.04 (−0.06 to 0.14), 1.55 (−2.32 to 5.41)
% Observed agreement (95% CI), κ	100% (88% to 100%), 1.00	93% (77% to 99%), 0.91	89% (72% to 98%), 0.87
% In lower/higher risk category	0%/0%	0%/7%	0%/11%
% Exceeding total error goal	0%	0%	0%

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CVD risk *(22–24)*. This may be true because apoB-containing lipoproteins other than LDL, such as remnant lipoproteins, also significantly contribute to the pathogenesis of atherosclerosis in diabetic patients. Several large epidemiologic studies also have shown that non–HDL-C in the general population is at least equivalent to or better than LDL-C and apoB in predicting CVD risk *(25–28)*. In the Framingham Heart study, non–HDL-C was found to be superior to LDL-C in individuals who had TGs that were either increased or within the reference interval *(29, 30)*. Furthermore, non–HDL-C was still predictive of CVD in nonfasting individuals *(29, 30)*. A recent metaanalysis of 68 studies that included more than 300 000 individuals found that hazard ratios for CVD were at least equivalent for non–HDL-C and LDL-C, whether LDL-C was calculated or directly measured by several different methods *(31)*.

Recent guidelines from the American Diabetes Association and American College of Cardiology suggest that apoB may be superior to LDL-C as a target for cholesterol therapy *(32)*. apoA-I has also been shown in some studies to be equivalent to or superior to HDL-C in CVD risk assessment *(33, 34)*. Our data showed that apoB and apoA-I reclassified 17% and 13%, respectively, into a lower CVD risk category and 22% and 5%, respectively, into a higher CVD risk category compared to rLDL-C and rHDL-C (online Supplemental Tables 3 and 5). Because no clinical outcome data were available in this study, we cannot assess the clinical accuracy of the reclassification by the 2 apo methods. Another limitation of this study was that only 1 apoB and apoA-I method was used, although these methods matched closely the values for the apoB (SP3-08) and apoA-I (SP1-01) IFCC/WHO standards. A recent prospective study, using clinical end points, revealed that apoB and apoA-I (Behring Nephelometer, BNII) did not significantly improve CVD risk score reclassification over that based on cLDL-C or HDL-C (RA-1000 analyzer, Bayer Diagnostics) *(35)*.

It is important to note that this study had several limitations. The β-quantification procedure used to measure rLDL-C can also be sensitive to cholesterol in intermediate-density lipoproteins and lipoprotein (a) *(2)*. dLDL-C methods that are truly specific for LDL may, therefore, show a negative bias compared to rLDL-C done by β-quantification, as observed for most of the dLDL-C methods in this study. These other apoB-containing lipoprotein fractions, which are also proatherogenic *(36, 37)*, however, would contribute to cLDL-C and non–

Table 3. Comparison of results and classification based on direct non–HDL-C vs RMP non–HDL-C.
(Continued from page 498)

Sekisui	Serotec	Sysmex	UMA	Wako
0.997	0.996	0.995	0.973	0.997
0.03 (1.16)	0.05 (1.93)	0.04 (1.55)	0.09 (3.48)	0.04 (1.55)
1.00 (0.96 to 1.05)	1.04 (0.90 to 1.17)	1.00 (0.93 to 1.08)	1.00 (0.93 to 1.08)	0.99 (0.90 to 1.07)
0.05 (−0.06 to 0.16), 1.93 (−2.32 to 6.19)	−0.05 (−0.42 to 0.32), −1.93 (−16.24 to 12.37)	0.09 (−0.11 to 0.28), 3.48 (−4.25 to 10.83)	0.09 (−0.11 to 0.28), 3.48 (−4.25 to 10.83)	−0.05 (−0.28 to 0.18), −1.93 (−10.83 to 6.96)
95% (90% to 98%), 0.94	93% (88% to 97%), 0.91	93% (87% to 96%), 0.90	90% (84% to 95%), 0.87	88% (81% to 93%), 0.83
0%/5%	1%/6%	0%/8%	5%/4%	12%/1%
2%	2%	4%	8%	3%
0.999	0.965	0.996	0.979	0.992
0.02 (0.77)	0.04 (1.55)	0.02 (0.77)	0.06 (2.32)	0.03 (1.16)
1.01 (0.99 to 1.02)	0.99 (091 to 1.06)	1.01 (0.98 to 1.04)	1.01 (0.95 to 1.06)	0.99 (0.96 to 1.03)
−0.01 (−0.06 to 0.05), −0.39 (−2.32 to 1.93)	0.06 (−0.22 to 0.34), 2.32 (−8.51 to 13.15)	0.03 (−0.07 to 0.14), 1.16 (−2.71 to 5.41)	−0.02 (−0.32 to 0.28), −0.77 (−12.37 to 10.83)	−0.10 (−0.26 to 0.06), −3.87 (−10.05 to 2.32)
93% (77% to 97%), 0.91	89% (72% to 98%), 0.87	82% (63% to 94%), 0.78	79% (59% to 92%), 0.74	93% (77% to 99%), 0.91
0%/7%	0%/11%	0%/18%	4%/18%	7%/0%
0%	4%	0%	4%	7%

HDL-C values, which may account, at least in part, for the observed improved performance in cardiovascular risk score classification for these markers. Another limitation of this study was that not all patients were fasting (71 participants fasted <12 h), although a separate analysis of this population did not show a significant difference from fasting individuals in terms of the accuracy of CVD risk score by the various methods (online Supplemental Table 6). In addition, TC and TGs were measured using only 1 routine method each, although the methods used were verified for accuracy in the CDC Lipid Standardization Program. Because approximately 80% of the samples in this study came from patients with dyslipidemias, the results from this study may not apply to other populations; however, these are the types of individuals for whom accurate lipid and lipoprotein testing is the most important. Finally, the sample size of the study was relatively small (n = 175), particularly for the subset of individuals with TG ≥2.26 mmol/L (200 mg/dL) and <4.52 mmol/L (400 mg/dL) (n = 20).

In summary, except for hypertriglyceridemic samples, 7 of 8 dLDL-C methods did not improve the accuracy of CVD risk score classification over cLDL-C. This was attributable, at least in part, to the fact that dHDL-C methods, in general, showed greater concordance with their RMP than did dLDL-C methods. Overall, non–HDL-C, using dHDL-C results, showed the best correspondence to its RMP and better harmonization in CVD risk score classification compared to dLDL-C and cLDL-C methods for both low- and high- TG samples. Future studies with clinical end points should be performed to assess the clinical utility of the various direct measurement methods for LDL-C and HDL-C and to resolve the uncertainty about the clinical significance of the lipoprotein fractions that are being excluded or measured in these direct assays compared to the ultracentrifugation RMPs.

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