total bilirubin (range 26–58 μmol/L [1.5–3.4 mg/dL], n = 7), or alanine aminotransferase (range 83–110 U/L, n = 4), making it difficult to fully assess interferences from conditions associated with these analytes. One patient with end-stage renal disease who was receiving dialysis treatment, with 37.7 g/L IgG and 25 g/L albumin, had HDL-C values that were 16% to 58% lower than the RMP and LDL-C values that were 22% to 63% lower than the RMP for the different methods, except for the Roche HDL-C and LDL-C methods, which showed no discrepancy with RMP values. Other study participants in this subpopulation had HDL-C and LDL-C results <20% different from the RMPs.

Discussion

The NCEP accuracy goals for HDL-C and LDL-C were based, in part, on the state of the art of laboratory testing at the time the guidelines were developed, and on clinical need for accurate classification of chronic heart disease risk and monitoring of lipid treatment goals. Numerous studies have shown that these accuracy goals were largely met with the precipitation-based HDL-C methods *(19)*, but because errors were compounded in the multiple analytes used for calculating LDL-C by the Friedewald equation, as well as other limitations, these methods were considered unsatisfactory *(3, 4)*.

The composition of lipoproteins in various dyslipidemias influences the ability of direct methods to specifically measure the cholesterol content of one lipoprotein class in the presence of other types of lipoproteins. Consequently, it has been challenging to manufacture direct methods with adequate specificity, because a large number of factors related to genetics, nutrition, disease, and treatment affect the composition of lipoproteins. The direct methods performed well for the nondiseased group, but they all had unacceptable total error for the diseased group (Tables 1 and 2). The primary contributing error components were sample-specific influences. The most likely limitation was nonspecificity for the intended lipoprotein density fraction in the presence of abnormal lipoproteins. Measurements for samples with high or low triglycerides, in particular, were challenged to agree with the RMPs (Figs. 3 and 4). The concentrations of triglycerides in the various lipoprotein fractions are known to be highly variable and to change with lipid disorders and other conditions *(20)*. It is not possible, however, to rule out the potential influence of other interfering substances that may have been present in some of the diseased study participants, most of whom were taking a number of drugs and had various comorbidities. Nonfasting samples, which, according to the manufacturers, are acceptable for use in the direct methods, likely introduced additional confounders that were not investigated in this study.

In many cases the differences between the direct method results and RMPs were sufficiently large (Figs. 1 and 2; Table 2) that they could affect the clinical management of patients. Many of the discordant cases were individuals with low HDL-C, which frequently occurs with hypertriglyceridemia *(21)*. Several patients with a genetic disorder in lipid metabolism, such as LCAT deficiency, had direct-method results that differed so markedly from the RMPs and from the typical values seen in these kinds of patients with HDL-C precipitation–based methods and a calculated LDL-C that they could have been misdiagnosed. Inaccurate HDL-C results may also lead to incorrect cardiovascular risk assessments and to improper choices in drug therapy.

Many of the discordant results were present at lower analyte concentrations. Given the recent interest in more aggressively treating individuals with drugs to lower LDL-C below 1.8 mmol/L (70 mg/dL) to reverse existing atherosclerotic disease *(22)*, accurate measurement of low LDL-C will likely become more important in the future. It is important to note that for some direct methods 30%–45% of test results fell outside the NCEP total error goals for the diseased group (Table 1), which would be expected to reduce the overall effectiveness of screening for cardiovascular risk assessment by direct HDL-C or LDL-C measurements. It is important to note that the frequency and magnitude of the errors observed in this study may be different in other populations that may have different types of dyslipidemias. Several of the patients recruited from the NIH had rare genetic lipid disorders, and the results from these patients may not be representative of the typical performance of the direct methods. However, the majority of the other patients from the NIH and from VCU, who were recruited from a cardiology clinic, had more common forms of lipid disorders (see online Supplementary Tables S1, S7, and S8).

Strengths of this investigation include examination of a range of individuals without disease and patients with various types of lipoprotein disorders and other diseases known to have caused errors in earlier generations of direct measurement reagents. Direct measurement reagents from 7 primary manufacturers were included, and measurements with each method were made at the same time with a single automated analyzer. The RMPs were unmodified and performed by the CDC. Blood collection and processing to obtain serum was performed in the same manner as is typical for clinical testing, and all measurements, including the RMPs, were performed within 2 days of collection on sera stored at 4–8 °C. Sufficient data were available to use an error component model to determine the relative contributions to total error from various sources.

The error component model avoided biased estimates by using differences of ln(concentration) instead of relative percentage differences.

Limitations of this investigation include small changes in results for frozen serum controls from beginning to end of some runs for the direct methods (see online Supplemental Tables S5 and S6 and Supplemental Figs. S1–S16). Examination of the measurement sequence (see online Supplemental Table S2) indicated that any evaporation would not have affected results for patient samples, but may have contributed to the observed CVs for the frozen serum pools. However, the interassay (from controls) and intraassay (from patient sample replicates) CVs (Table 2) were similar for each method, suggesting the magnitude of changes during a run was not an important influence on conclusions. Reagent and calibrator lot changes were not systematically evaluated; however, there were apparent influences of reagent lot on frozen serum pool results for 2 of 3 LDL-C methods that had reagent-lot changes during the study.

A limitation in the error component analysis included exclusion of some outliers either because the values were 0 (logarithm of 0 not possible) or the values were highly discrepant from the RMPs (difference of ln(concentration) exceeded ± 0.8). Outlier exclusion criteria were somewhat arbitrary; those chosen gave reasonable estimates of dominating error sources while excluding results that would have excessively influenced bias and SD terms.

The current NCEP Adult Treatment Panel recommendations for cardiovascular risk assessment were based on epidemiologic results, with use of the Friedewald equation to estimate LDL-C, and HDL-C methods that produced results in agreement with the RMP. Direct HDL-C and LDL-C methods have been used with the same decision points; however, the relationship of direct method results to cardiovascular risk assessment has not been systematically investigated.

In summary, 6 of 8 HDL-C and 5 of 8 LDL-C direct methods met the NCEP total error goals for nondiseased individuals, but all direct methods failed to meet these goals for samples from patients with cardiovascular disease and/or dyslipidemia. Sample-specific effects were the dominant cause of discrepant results.

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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%

Continued on page 494

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%

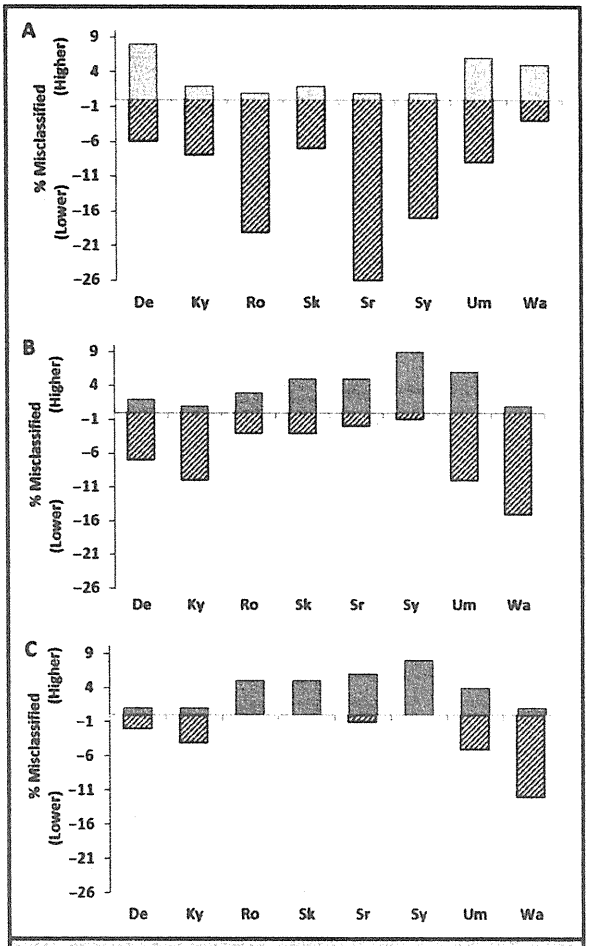

Fig. 1. Misclassification rate for CVD risk for those participants with TG levels <2.26 mmol/L (200 mg/dL), n = 145.

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%

Continued on page 497

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%

Continued on page 499

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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Original Article

Association between Non-High-Density Lipoprotein Cholesterol Levels and the Incidence of Coronary Heart Disease among Japanese: The Circulatory Risk in Communities Study (CIRCS)

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Aim: The aim of this study was to identify the threshold level for non-high-density lipoprotein cholesterol (non-HDL-cholesterol) to raise the risk of coronary heart disease (CHD) incidence in a Japanese general population.
Methods: A total of 8,132 men and women, aged 40 to 69 years with no history of stroke or CHD, completed the baseline risk factor surveys between 1975 and 1987. Systematic surveillance of cardiovascular disease incidence was performed through 2003 (the median follow-up period was 21.9 years), and 155 incidents of CHD were identified.
Results: We found a statistically significant association between non-HDL-cholesterol levels and the risk of CHD with a threshold around 140 mg/dL. After adjustment for potential confounding factors, this association did not change materially. The multivariable hazard ratio of CHD compared with that for levels of <100 mg/dL was 2.49 (95% confidence interval: 1.35 to 4.61) for 140-159 mg/dL and 3.13 (1.58-6.21) for ≥180 mg/dL. Setting the cut-off point at ≥140 mg/dL non-HDL-cholesterol resulted in the greatest improvement of integrated discrimination.
Conclusions: Higher concentrations of non-HDL-cholesterol are associated with an increased risk of CHD with a threshold around 140 mg/dL, suggesting that the optimal cut-off point for healthy persons to prevent increasing the risk of CHD might be around 140 mg/dL non-HDL-cholesterol.

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Key words; Non-HDL-cholesterol, Coronary heart disease, Epidemiology, Primary prevention

Introduction

Non-high-density-lipoprotein cholesterol (non-HDL-cholesterol) as well as low-density-lipoprotein cholesterol (LDL-cholesterol) is a major risk factor for artherosclerotic disease[1-10], and management of these lipids is important for the prevention of coronary heart disease (CHD)[11, 12]; however, current guidelines in the United States and Japan do not stress the importance of non-HDL-cholesterol as much as that of LDL-cholesterol[11-13]. In fact, the National Cholesterol Educational Program (NCEP) Expert Panel recommended the use of LDL-cholesterol as the primary indicator of therapy and primary prevention of CHD, while non-HDL-cholesterol was only a secondary target of therapy for patients with hypertriglyceridemia[11, 12]. The Japan Atherosclerosis Society's guidelines also use a cut-off point for LDL-cholesterol, but not for non-HDL-cholesterol, as an indicator for atherogenic lipid management[13].

However, a recent study has shown that direct measurements of LDL-cholesterol as well as triglycer-

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ides may not be fully standardized in many clinical laboratories[14]. This indicates that LDL-cholesterol estimated with the Friedewald formula[15] as well as directly measured may include measurement errors, which may jeopardize satisfactory lipid monitoring and control in clinical practice.

Non-HDL-cholesterol is easily calculated by using total and HDL-cholesterol concentrations, the determinations of which are well standardized[14, 16]. It has been shown that the predictive value of non-HDL-cholesterol is similar to or better than that of LDL-cholesterol from epidemiological studies[1, 3, 5, 6, 8]; therefore, non-HDL-cholesterol could be a more reliable indicator than LDL-cholesterol for the prevention of CHD in community-based preventive strategies. In Japan, two prospective studies, the Suita study[8] and the JALS-ECC[10], showed a positive association between non-HDL-cholesterol and the incidence of CHD; however, the optimal cut-off point of non-HDL-cholesterol for the primary prevention of CHD remained unclear.

We therefore examined the threshold level of non-HDL-cholesterol to increase the risk of CHD by a prospective cohort study in a Japanese general population in order to estimate the optimal cut-off point for healthy persons to prevent increasing the risk of CHD.

Methods

Study Cohort

The participants consisted of a population-based sample aged 40 to 69 years living in four communities in Japan included in the Circulatory Risk in Communities Study (CIRCS)[17]. They participated in the cardiovascular risk surveys conducted between 1975 and 1980 in Ikawa and Noichi, between 1975 and 1984 in Yao, and between 1981 and 1987 in Kyowa, from which we obtained data for lipid profiles and confounding variables. The proportion of subjects who participated in the surveys was 77% for the total census population.

From the 8,158 participants (3,201 men and 4,957 women), we excluded 26 persons with a confirmed history of CHD and/or stroke at the time of baseline inquiry, because our purpose was to examine the association between non-HDL-cholesterol and the primary incidence of CHD. As a result, 8,132 persons (3,178 men and 4,954 women) were enrolled in the present analysis. The Ethics Committee of Osaka Medical Center for Health Science and Promotion approved this study.

Measurement of Risk Factors

Serum total cholesterol, HDL-cholesterol and triglycerides were measured with enzymatic methods using an automatic analyzer (Hitachi 7250; Hitachi Medical Corp., Hitachi, Japan). These measurements were performed at Osaka Medical Center for Cancer and Cardiovascular Diseases, which has been standardized since April 1975 by the U.S. Centers for Disease Control (CDC)-National Heart, Lung, and Blood Institute (NHLBI) Lipid Standardization Program[14, 16]. Non-HDL-cholesterol was calculated as follows: Non-HDL-cholesterol = Total cholesterol - HDL-cholesterol.

Diabetes was defined as a plasma glucose level of ≥126 mg/dL during fasting or ≥200 mg/dL during non-fasting, or use of medication for diabetes, while borderline diabetes was defined as a plasma glucose level of 110-125 mg/dL at fasting or 140-199 mg/dL at non-fasting, and no use of medication for diabetes. As for blood pressure, mild hypertension was categorized as systolic blood pressure 140-159 mmHg or diastolic blood pressure 90-99 mmHg, while the corresponding values for moderate hypertension were 160-179 mmHg or 100-109 mmHg, and for severe hypertension ≥180 mmHg or ≥110 mmHg, based on World Health Organization-International Society of Hypertension (WHO-ISH) Guidelines[18]. Height in stocking feet and weight in light clothing were measured, and body mass index (BMI) was calculated as weight (kg)/height $(m)^2$. An interview was conducted to ascertain the smoking status, the number of cigarettes smoked per day, and usual alcohol intake per week.

Follow-Up Study

The follow-up was conducted by annual cardiovascular risk surveys to obtain information on incident CHDs from the participants. For non-participants in any of the surveys, these endpoints were ascertained by means of a mailed questionnaire or a death certificate to establish the underlying cause of death (International Classification for Diseases, 9th edition: 410 to 414, 428, 429 and 430 to 438). We also used national insurance claims, ambulance records, reports by local physicians and public health nurses for case ascertainment. To confirm the diagnosis, all living patients were telephoned or visited to obtain their medical history, and their medical records at hospitals were also reviewed. In the case of death, we obtained histories from the deceased's family and reviewed the medical records.

The criteria for CHD used in our study were modified from those of the World Health Organiza-

tion Expert Committee[19]. Definite myocardial infarction (MI) was defined as the presence of typical chest pain lasting for ≥30 minutes accompanied by the appearance of abnormal and persistent Q or QS waves, or changes in cardiac enzyme activity or both. Probable MI was defined as the presence of typical chest pain but for which the findings of electrocardiogram or enzyme activity were not available. Angina pectoris was defined as repeated episodes of chest pain during effort, especially when walking, usually disappearing rapidly after the cessation of effort or the use of sublingual nitroglycerin. The date of the first episode was identified as the date of angina pectoris incidence. We did not include cases whose clinical examination data were negative for MI or angina pectoris, even if clinical symptoms corresponded to our criteria. Sudden cardiac death was defined as death within 1 hour of onset, a witnessed cardiac arrest, or abrupt collapse not preceded by ≥1 hour of symptoms. We excluded sudden cardiac death cases whose cause of death had been diagnosed as lethal arrhythmia, cardiomyopathy, stroke, and other organic heart diseases. CHD was defined as including definite or probable MI, angina pectoris, and sudden cardiac death. The final diagnosis of CHD was made by a panel of three or four physicians, blinded to the baseline data.

For each of the participants, the person-years of follow-up were calculated from the date of the baseline survey to the date of CHD incidence, death, exit from the community, or the end of 2003, whichever occurred first. Participants who moved away from the community (5.9%) were treated as censored. The total person-years studied were 173,025 with a median follow-up period of 21.9 years.

Statistical Analysis

First, sex- and age-adjusted means and proportions of selected cardiovascular risk factors at the baseline survey were identified according to non-HDL-cholesterol categories. Analysis of covariance and Mantel-Haenszel chi-square tests were used to examine differences among non-HDL-cholesterol categories in terms of sex- and age-adjusted mean values and proportions of baseline characteristics.

Second, we examined, non-parametrically and with restricted cubic splines[20], possible non-linear associations between non-HDL-cholesterol levels and risk of CHD. Because sparse tail data may lead to a visual influence (i.e. overestimation of risk difference), predictions from the top and bottom 1% of the analytical distribution are not included in the graph. We used 5 knots, the values of which corresponded to 81 mg/dL, 110 mg/dL, 130 mg/dL 152 mg/dL and 193 mg/dL of non-HDL-cholesterol levels.

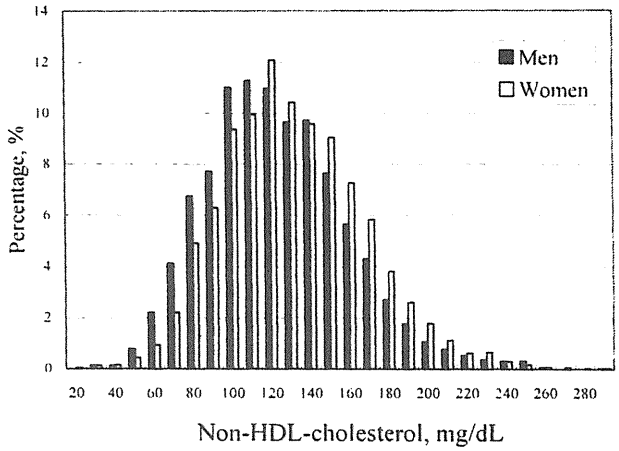

Fig. 1. Sex-specific histogram for distribution of non-HDL-cholesterol.

The distribution percentages for men were 35% for ≥140 mg/dL, 18% for ≥160 mg/dL, 8% for ≥180 mg/dL, and 3% for ≥200 mg/dL. The corresponding percentages for women were 43%, 24%, 11%, and 5%.

Third, categorical analysis was based on the incidence rates of CHD divided by clinical categories of non-HDL-cholesterol (<100, 100-120, 120-139, 140-159, 160-179, ≥180 mg/dL). The Cox proportional hazards model was used to calculate the sex- and age-adjusted and multivariable hazard ratios (HRs) and 95% confidence intervals (95%CI) after adjustment for sex, age and potential confounding factors, which included the blood pressure category (normal, mild, moderate, and severe hypertension), antihypertensive medication use (yes or no), glucose category (normal, borderline diabetes, and diabetes), BMI category (sex-specific quartiles), smoking status (never, ex- and current cigarette smokers at <20 and ≥21 cigarettes per day), alcohol intake category (never, ex-drinker, and current drinker of ethanol at 1 to 22, 23 to 45, 46 to 68, and ≥69 g per day), lipid-lowering medication use (yes or no), HDL-cholesterol category (<40, 40-49, 50-59, 60-69, and ≥70 mg/dL) and triglyceride category (<100, 100-149, 150-199, 200-249, and ≥250 mg/dL), fasting status (<8 hours versus ≥8 hours after last meal), entry year of baseline survey, and study area. We tested the assumption of proportional hazards and found no violation of the proportionality principle. Tests for effect modification by sex or other cardiovascular risk factors were conducted with an interaction term generated by multiplying the continuous variable of non-HDL-cholesterol by sex or other cardiovascular risk factors.

Finally, to confirm whether the threshold of non-

Table 1. Sex- and age-adjusted mean and prevalence as baseline characteristics of participants according to non-HDL-cholesterol categories

	Non-HDL-cholesterol, mg/dL					
	<100	100-119	120-139	140-159	160-179	180+
Median, mg/dL	86	110	129	149	168	197
Range, mmol/L	<2.59	2.59-3.09	3.10-3.61	3.62-4.13	4.14-4.64	4.65+
Number of persons	1,442	1,665	1,771	1,475	964	815
Men, %	48.1	42.5†	37.0†	37.4†	32.8†	31.0†
Age, year	49.9	50.8†	51.9†	52.4†	53.3†	53.9†
Total cholesterol, mg/dL	147.9	168.9†	185.6†	203.1†	221.0†	252.4†
HDL-cholesterol, mg/dL	64.1	59.0†	56.5†	54.1†	52.4†	49.5†
Triglycerides, mg/dL	94.7	110.4†	128.9†	152.4†	173.9†	208.1†
Lipid-lowering medication use, %	0.0	0.0	0.1	0.1	0.1	0.5†
Body mass index, kg/m^2	22.1	22.7†	23.2†	23.7†	24.1†	24.7†
Systolic blood pressure, mmHg	133.1	132.8	133	134.3	135.3†	136.5†
Diastolic blood pressure, mmHg	79.1	79.4	80.1*	81.4†	82.7†	83.4†
Non-hypertension, %	66.2	65.6	65.8	61.4†	58.8†	55.5†
Mild hypertension, %	21.7	22.1	22.3	24.3	24.9	28.9†
Moderate hypertension, %	8.5	8.8	9.2	11.0*	12.3†	12.7†
Severe hypertension, %	3.6	3.5	2.7	3.2	4.0	2.8
Antihypertensive medication use, %	10.1	9.1	9.4	12.4*	10	13.9†
Non-diabetes, %	80	77.8	79.5	78.2	81.4	78
Borderline diabetes, %	4.6	6.4*	6.2	8.2†	7.2*	9.7†
Diabetes, %	2.8	2.3	3.0	3.4	2.8	4.8†
Current smoker, %	29.7	29.1	26.0†	28.6	28.6	32.3
Current drinkers, %	50.6	49.5	45.9†	44.1†	43.6†	37.4†

Test for difference from persons in lowest category; *$p<0.05$, †$p<0.01$

HDL-cholesterol shown in the categorical analysis is the optimal cut-off level, we examined changes in integrated discrimination improvement (IDI)[21] and Akaike's Information Criteria (AIC)[22] at different cut-off points. We selected non-HDL-cholesterol values on the basis of primarily a higher IDI and secondarily a smaller AIC in multivariable Cox proportional hazard models with potential confounding factors as better cut-off points for the prediction of CHD, and used these cut-off points to reduce the misclassification of risk prediction.

All statistical tests were two-sided and $p<0.05$ was regarded as statistically significant. SAS, version 9.13 (SAS Institute, Inc., Cary, NC, USA) was used for all statistical analyses.

Results

Fig. 1 shows a sex-specific histogram of non-HDL-cholesterol distribution at the baseline survey. The percentages were 35% for men with ≥140 mg/dL and 8% for men with ≥180 mg/dL. The corresponding percentages for women were 43% and 11%. The mean value (±standard deviation) was 128.0 mg/dL (±36.1) for men and 136.0 mg/dL (±36.4) for women.

Table 1 shows selected cardiovascular risk factors at the baseline survey according to non-HDL-cholesterol categories. The median value of non-HDL-cholesterol categories was 86mg/dL, 110 mg/dL, 129 mg/dL, 149 mg/dL, 168 mg/dL and 197 mg/dL in each category. Compared with persons in the lowest category of non-HDL-cholesterol (<100 mg/dL), persons in the highest category (≥180 mg/dL) tended to have higher means of total cholesterol levels, triglycerides, body mass index, and systolic and diastolic blood pressures, and lower means of HDL-cholesterol. Also, they were more likely to be female, older, hypertensive, with diabetes, to use of medication for hypertension and hyperlipidemia, and less likely to drink.

During the follow-up period, we identified 155 incidences of CHD, comprising 91 MI, 36 angina pectoris and 28 sudden cardiac death. Higher non-HDL-cholesterol levels were associated with increased

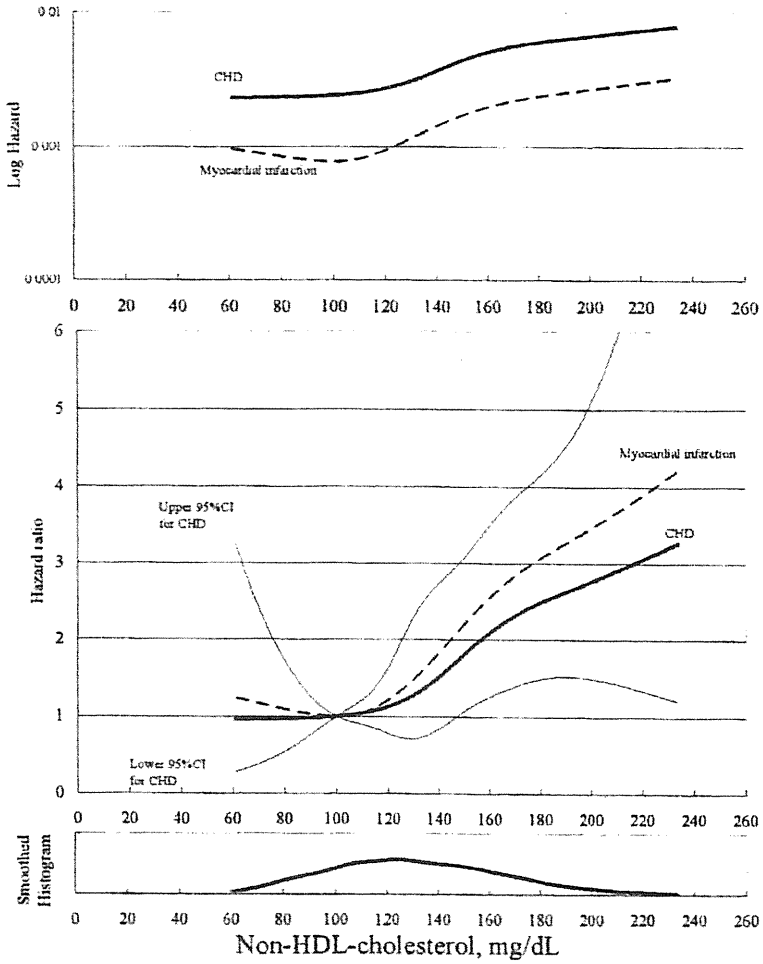

Fig. 2. Multivariable log hazard and multivariable HR for CHD and MI in relation to non-HDL-cholesterol levels.

100 mg/dL of non-HDL-cholesterol was selected as a reference for HR. The values of the five knots corresponded to 81 mg/dL, 110 mg/dL, 130 mg/dL 152 mg/dL and 193 mg/dL of non-HDL-cholesterol levels. The *p*-values for linearity were $p=0.0002$ for CHD and $p=0.001$ for MI. The smoothed histogram shows the distribution of non-HDL-cholesterol levels.

risks of CHD and MI, with a threshold between 120 mg/dL and 140 mg/dL (**Fig. 2**). The HR was fairly flat for non-HDL-cholesterol levels less than 120 mg/dL. The graph suggests that the risk of CHD and MI may start to increase at non-HDL-cholesterol levels between 120 mg/dL and 140 mg/dL.

In the categorical analysis, higher non-HDL-cholesterol levels were found to be associated with increased risks of CHD and MI, with a threshold at around 140 mg/dL (**Table 2**). Adjustment for potential confounding factors did not alter these associations materially. The multivariable HR of CHD compared with that for levels of <100 mg/dL was 2.49 (95% confidence interval (95%CI): 1.35 to 4.61) for 140-159 mg/dL and 3.13 (1.58-6.21) for ≥ 180 mg/dL. The respective multivariable HR of MI was 3.17 (1.40-7.22) and 4.09 (1.64-10.21). These positive associations were similar for men and women with no sex interaction ($p=1.00$ for total CHD, and $p=0.70$ for MI). These results did not alter after exclusion of triglycerides in potential confounding factors (not shown in the tables). There was no interaction of years at entry (1970s versus 1980s) on an association between non-HDL-cholesterol and CHD risk