Accuracy of Calculated Serum Low-Density Lipoprotein Cholesterol for the Assessment of Coronary Heart Disease Risk in NIDDM Patients

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OBJECTIVE — To evaluate the accuracy of LDL cholesterol calculated with Friedewald's equation in the assessment of cardiovascular risk in NIDDM patients.

RESEARCH DESIGN AND METHODS — The calculation of LDL cholesterol according to Friedewald's formula was compared with the measurement of LDL cholesterol separated by ultracentrifugation in 151 NIDDM patients with fairly good metabolic control (HbA_{1c} ≤10%) and in 405 nondiabetic subjects.

RESULTS — Measured and calculated LDL cholesterol was found to be well correlated in both diabetic ($r = 0.95$) and nondiabetic ($r = 0.97$) subjects. Compared with measured LDL cholesterol, the calculated LDL cholesterol differed by ≥10% in 34% of samples from diabetic patients and in 26% of samples from nondiabetic subjects ($\chi^2 = 3.885$, $P < 0.05$). The percentage of error increased when the serum triglyceride (TG) level was ≥200 mg/dl (2.26 mmol/l) and when the ratio of VLDL cholesterol to TG was <0.20 or >0.29 in both groups of subjects. Although the percentage of error from calculated LDL cholesterol was greater in diabetic than in nondiabetic subjects because of the greater prevalence of hypertriglyceridemia in the former group, the misclassification of coronary heart disease risk, according to the cutoff points of the National Cholesterol Education Program (NCEP), was similar in the two groups (25% in diabetic and 22% in nondiabetic subjects). In both groups of patients, the misclassification of coronary heart disease risk was higher when calculation of LDL cholesterol produced values near the cutoff points.

CONCLUSIONS — Although accuracy in the estimation of LDL cholesterol is less than ideal, Friedewald's equation seems to be of value in the correct assignment of coronary heart disease risk classes in the great majority of diabetic as well as nondiabetic subjects. Caution must be exercised for subjects in whom calculated LDL cholesterol is close to the cutoff points of the NCEP guidelines.

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Coronary heart disease is the leading cause of mortality in NIDDM patients (1,2). Several factors contribute to the increased propensity toward premature atherosclerosis in diabetic patients, among them alterations in serum lipoprotein pattern (3,4). Abnormalities in serum lipids, particularly in LDL cholesterol, must then be carefully evaluated to establish the individual coronary heart disease risk profile and to initiate diet and drug therapy, according to the suggestions of the National Cholesterol Education Program (NCEP) (5). The NCEP Adult Treatment Panel recommends that management of lipid abnormalities be based primarily on LDL cholesterol level (5), and this recommendation has been recently endorsed by the American Diabetes Association (6), underlining the need for an accurate estimation of LDL cholesterol in NIDDM patients.

Because the direct measurement of LDL cholesterol is time-consuming and requires expensive instrumentation that is not available in routine laboratories, LDL cholesterol concentration is usually estimated by numerical calculation. The empirical equation of Friedewald et al. (7) is the most extensively used method. The calculation uses the ratio of VLDL cholesterol to serum triglyceride (TG) level to estimate VLDL cholesterol from serum TG. LDL cholesterol can then be calculated by subtracting from total cholesterol HDL and VLDL cholesterol (TG × 0.20 when values are given in milligrams per deciliter or TG × 0.45 when values are given in millimoles per liter).

The accuracy of LDL cholesterol estimation is related to serum TG level, and it is well known that hypertriglyceridemia and the associated variability of lipoprotein composition represent the main source of error in LDL cholesterol calculation (8–13). Hypertriglyceridemia is common in diabetes (3,4) and may frequently lead to errors in the calculation of LDL cholesterol and, consequently, to inappropriate decisions about therapy.

The purpose of this study was to evaluate the validity of Friedewald's equation in patients with diabetes in comparison with nondiabetic subjects.

RESEARCH DESIGN AND METHODS

— We used sera from 556 patients consecutively submitted to the laboratory for estimation of lipid profile; of these patients, 151 had NIDDM and 405 were nondiabetic. Sera with TG level ≥400 mg/dl (4.52 mmol/l) and sera from diabetic patients with poor glycemic control (HbA_{1c} >10%) were excluded.

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Abbreviations: NCEP, National Cholesterol Education Program; TG, triglyceride.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

Serum lipoproteins were fractionated in duplicate in a Beckman L5-50 preparative ultracentrifuge (Beckman, Fullerton, CA) equipped with an LP 42 Ti rotor. Ultracentrifugation was performed in separate samples with adjusted density 1.006 and 1.063 (the latter obtained by addition of solid KBr). Each run lasted 16 h at 15°C and 202,000*g*. At the end of the run, the supernate was removed by aspiration (Mixer Fractionator; Beckman) and the infranate as well as the total serum was stored at −20°C until assay. VLDL lipids were calculated as the difference between lipid concentration in total serum and in the infranate of density 1.006. LDL lipids were calculated as the difference between lipid concentration in the infranates of densities 1.006 and 1.063. HDL lipids were measured in the infranate of density 1.063. LDL cholesterol was also calculated using Friedewald's formula (7).

Serum total cholesterol and lipoprotein cholesterol were determined with the cholesterol oxidase/*p*-aminophenazone (CHOD-PAP) method (Boehringer Mannheim, Milan, Italy), and total TG and lipoprotein TG were determined with the 3,5-dichloro-2-hydroxybenzenesulfonic acid (DHBS) color method (Ames Miles, Cavenago Brianza, Italy). HbA_{1c} was assayed by high-performance liquid chromatography (Merck-Hitachi, Tokyo).

Data were evaluated by calculation of 95% CIs and by linear regression analysis. Separate multiple linear regression (backward stepwise regression) models examined the bias for calculated LDL cholesterol (Friedewald's formula) as the dependent variable and HbA_{1c} (in diabetic patients), serum TG, lipoprotein lipids, and cholesterol-to-triglyceride ratio of the three lipoprotein classes as independent variables. The χ^2 test was used to compare discrete variables.

RESULTS — As expected, the prevalence of hypertriglyceridemia (serum TG ≥200 mg/dl [2.26 mmol/l]) was greater in diabetic than in nondiabetic patients (48 vs. 24%, respectively; $\chi^2 = 31.021$, $P < 0.001$). As shown in Table 1, diabetic patients had higher serum TG and VLDL lipids, and lower LDL and HDL cholesterol, than nondiabetic subjects. The ratio of VLDL cholesterol to TG varied greatly in the two groups. In 71% of diabetic patients and 76% of nondiabetic patients, the ratio of VLDL cholesterol to TG was <0.20 or >0.29. Mean ratio of VLDL cholesterol to TG was not significantly different between the two groups, whereas the ratios of both LDL and HDL cholesterol to TG were lower in diabetic than in nondiabetic patients. In diabetic patients, HbA_{1c} (mean, 8.18%; SEM, 0.13) was found to correlate with the following variables: serum TG ($r = 0.37$, $P < 0.001$), VLDL cholesterol ($r = 0.28$, $P < 0.001$), VLDL TG ($r = 0.35$, $P < 0.001$), ratio of VLDL cholesterol to TG ($r = -0.19$,

Table 1—*Serum and lipoprotein lipids in nondiabetic and diabetic patients*

	Nondiabetic	Diabetic	95% CI
n	405	151	
Total cholesterol	249.7 ± 3.36	235.9 ± 4.52	1.70 to 25.9
Serum TG	150.3 ± 4.12	202.3 ± 7.19	36.2 to 67.8
VLDL cholesterol	26.7 ± 1.11	38.8 ± 2.13	7.72 to 16.5
VLDL TG	86.6 ± 3.37	133.0 ± 6.22	33.2 to 59.6
VLDL cholesterol/TG	0.39 ± 0.02	0.34 ± 0.03	−0.12 to 0.02
LDL cholesterol	167.8 ± 3.25	149.5 ± 4.02	6.77 to 29.8
LDL TG	40.7 ± 1.09	43.0 ± 1.39	−1.58 to 6.18
LDL cholesterol/TG	4.93 ± 0.13	3.79 ± 0.11	0.70 to 1.58
HDL cholesterol	55.2 ± 0.72	47.6 ± 0.99	4.98 to 10.2
HDL TG	23.1 ± 0.48	26.2 ± 0.77	1.31 to 4.89
HDL cholesterol/TG	2.87 ± 0.08	2.18 ± 0.11	0.39 to 0.99

Data are means ± SEM, expressed in milligrams per deciliter.

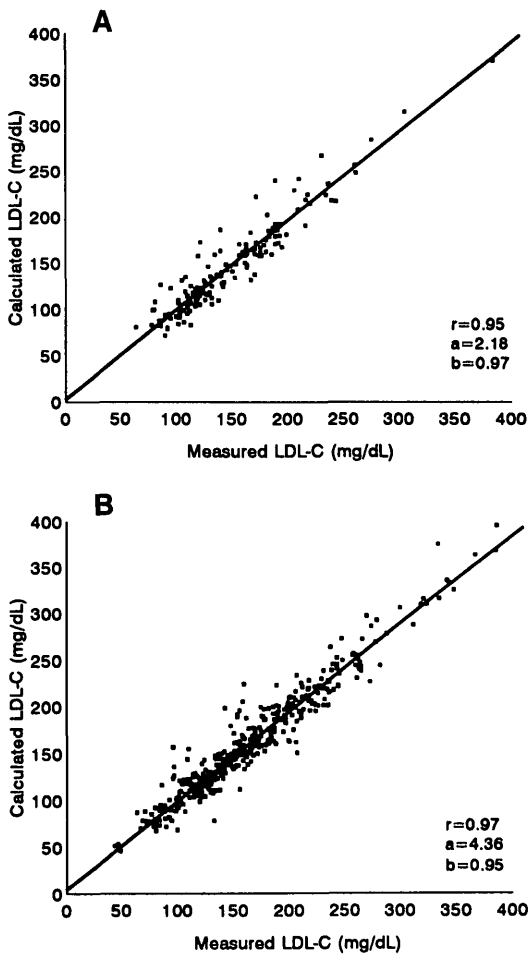

Figure 1—*Correlation between measured and calculated LDL cholesterol (LDL-C) in diabetic (A) and nondiabetic (B) patients. r, correlation coefficient; a, intercept; b, slope.*

$P < 0.05$), LDL TG ($r = 0.26$, $P < 0.01$), ratio of LDL cholesterol to TG ($r = -0.23$, $P < 0.01$), HDL cholesterol ($r = -0.25$, $P < 0.01$), HDL TG ($r = 0.16$, $P < 0.05$), and ratio of HDL cholesterol to TG ($r = -0.22$, $P < 0.01$).

Correlation coefficients between measured and calculated LDL cholesterol in diabetic and nondiabetic patients were 0.95 ($P < 0.001$) and 0.97 ($P < 0.001$), respectively (Fig. 1). The bias plots in Fig. 2 show that the great majority of calculated LDL cholesterol was underestimated over the entire range of measured LDL cholesterol in both groups of patients. However, the mean bias for calculated LDL cholesterol was only −1.1% in diabetic and −2.0% in nondiabetic patients when compared with LDL cholesterol measured after lipoprotein fractionation.

In 34% of samples from diabetic patients and in 26% of samples from nondiabetic subjects, calculated LDL cholesterol differed from measured LDL cholesterol by ≥10% ($\chi^2 = 3.885$, $P < 0.05$) (Fig. 3). The greater error in estimating LDL cholesterol in diabetic than in nondiabetic patients was possibly related to TG level. In diabetic patients, backward stepwise regression analysis ($F = 45.958$, $P < 0.01$) showed that the ratio of VLDL cholesterol to TG (partial $F = 85.465$, $P < 0.01$) and the serum TG (partial $F = 20.576$, $P < 0.01$) were independently associated with the absolute bias for calculated LDL cholesterol. HbA_{1c} was found to be related to the absolute bias for calculated LDL cholesterol after simple regression analysis ($r = 0.26$, $P < 0.01$), but it lost the significant correlation after multiple regression analysis. In nondiabetic patients, backward stepwise regression analysis ($F = 111.026$, $P < 0.01$) showed that the absolute bias for calculated LDL cholesterol was significantly associated with the ratio of VLDL cholesterol to TG (partial $F = 290.844$, $P < 0.01$), LDL TG (partial $F = 36.830$, $P < 0.01$), and serum TG (partial $F = 29.265$, $P < 0.01$).

The influence of serum TG level on the absolute bias for calculated LDL cholesterol is shown in Fig. 3. When serum TG level was <200 mg/dl (2.26 mmol/l), the inaccuracy of Friedewald's formula (absolute bias ≥10%) was found to be similar in the two groups of patients. In both groups, the inaccuracy of the LDL cholesterol calculation significantly increased when serum TG level was ≥200 mg/dl (2.26 mmol/l) ($\chi^2 = 9.191$, $P < 0.005$, between the two diabetic subgroups; $\chi^2 = 5.248$, $P < 0.05$, between the two nondiabetic subgroups).

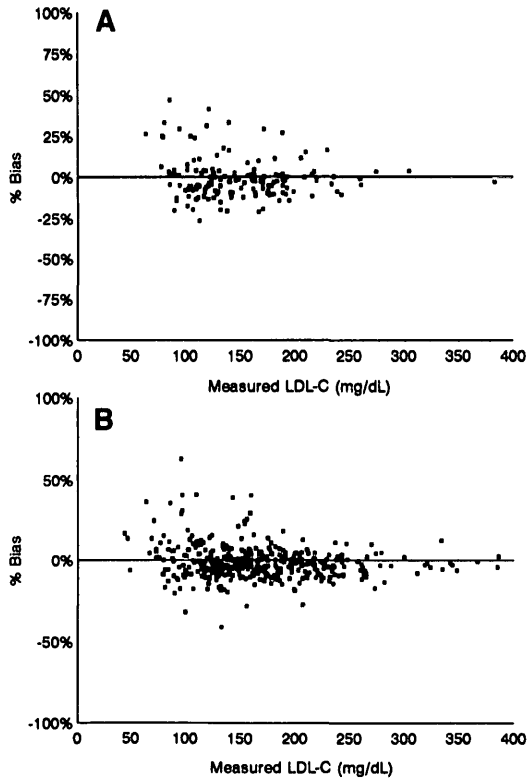

Figure 2—*Bias between the measured and calculated LDL cholesterol (LDL-C) in diabetic (A) and nondiabetic (B) patients.*

The percentage of samples in which calculated LDL cholesterol differed from measured LDL cholesterol by ≥10% was significantly greater in patients of both groups whose ratios of VLDL cholesterol to TG were <0.20 or >0.29 than in those whose ratios were 0.20–0.29 (Fig. 3).

The clinical accuracy of LDL cholesterol calculation is shown in Fig. 4. Samples from both diabetic and nondiabetic patients were subdivided into risk classes according to the cutoff points of NCEP (5). As can be seen, ~90% of patients in the lowest-risk class and >80% of patients in the highest-risk class were correctly classified. On the basis of calculated LDL cholesterol, only 53–66% of patients of the two intermediate-risk classes were correctly classified. Diabetic patients did not show a significant difference in the risk classification with respect to nondiabetic patients. Across the risk classes, 75% of diabetic patients and 78% of nondiabetic patients were correctly classified with calculation of LDL cholesterol; 9% of diabetic patients and 8% of nondiabetic patients were overestimated, and 16% of diabetic patients and 14% of nondiabetic patients were underestimated (Fig. 5).

CONCLUSIONS — The measurement of serum LDL cholesterol level has played an increasingly important role in the assessment of cardiovascular risk. The low-density class of lipoproteins is a heterogeneous population; separation may be performed by sequential and density gradient ultracentrifugation, chromatography, and precipitation procedures (14,15). Methods for LDL separation, however, are too time-consuming and expensive to be used in routine laboratories, and serum LDL cholesterol is currently determined by calculation.

The equation proposed by Friedewald et al. (7) is the first and most extensively used method for calculation of LDL cholesterol. The formula is based on the assumption that there is a fixed relation between VLDL cholesterol and serum TG and that the ratio of VLDL cholesterol to TG is roughly constant. Nevertheless, other lipoproteins contribute to the serum TG pool, and VLDL composition is widely variable, as shown in the present and previous studies (8,11,12,16–18).

Friedewald's formula is not reliable when serum TG is >400 mg/dl (4.52

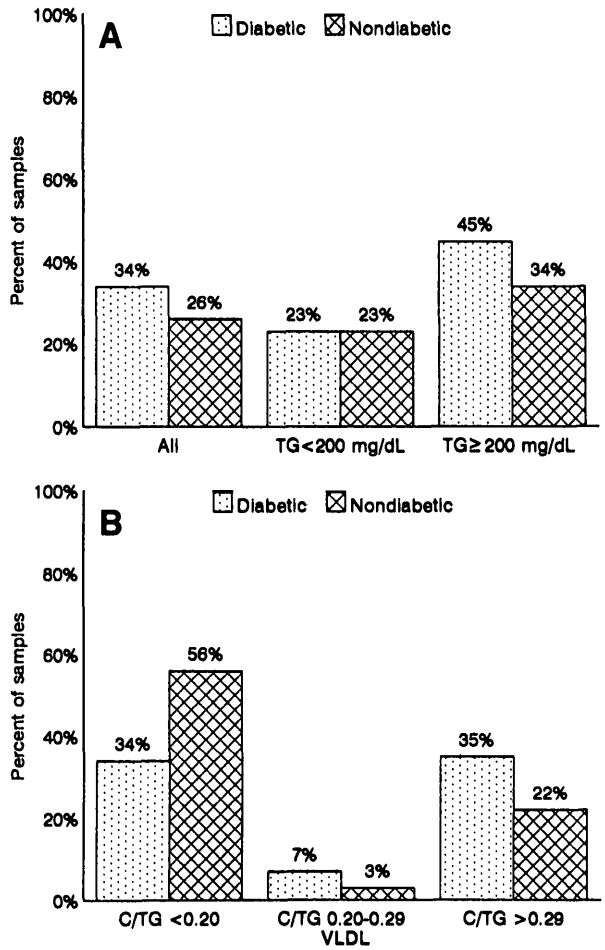

Figure 3—*Percentage of samples for which calculated LDL cholesterol (C) differs by >10% from measured LDL cholesterol in diabetic and nondiabetic patients, according to serum TG concentration (A) and VLDL cholesterol/TG ratio (B).*

mmol/l) and when floating β-lipoproteins and chylomicrons are present (7,11). However, even in the absence of these conditions, the accuracy in estimating LDL cholesterol depends on the concentration of serum TG (8–13), as is also shown in the present study.

Abnormalities in TG metabolism are a common feature in diabetes, especially in NIDDM, and mainly consist of alterations in the ratio of VLDL cholesterol to TG and in TG enrichment of LDL and HDL (3,4). In our series of diabetic patients, the alterations in TG metabolism were related to the metabolic control of diabetes. However, this variable of metabolic control did not appear to contribute independently to the inaccuracy of Friedewald's equation. Unfortunately, other clinical variables that are associated with diabetes, such as obesity and alterations of renal function, were unavailable, and their potential relevance to LDL cholesterol calculation could not be assessed. On the other hand, both obesity (19) and alterations of renal function (20) are associated with abnormalities in lipoprotein composition and concentration, which ultimately account for the inaccuracy of Friedewald's equation in diabetes (8–13).

The reliability of Friedewald's equation in diabetes was studied by Rubies-Prat et al. (18) in 111 diabetic patients. The authors observed a poor concordance between calculated and measured LDL cholesterol, despite a good correlation between the two methods. Hirany et al. (15) showed that in 148 diabetic patients, Friedewald's equation significantly underestimated LDL cholesterol when compared with beta-quantification by ultracentrifugation, especially when serum TG level was >200 mg/dl (2.26 mmol/l). Also, Winocour et al. (21) reported an underestimation (~5.4%) of LDL cholesterol calculated with Friedewald's equation compared with measurement of LDL cholesterol after ultracentrifugation. In contrast, Rubies-Prat et al. (18) reported that LDL cholesterol was overestimated by Friedewald's equation in 39%, and underestimated in 13%, of diabetic patients when compared with measurement of LDL cholesterol after fractionation.

In our series of diabetic patients, the mean difference between calculated and measured LDL cholesterol (–1.1%) was lower than that reported by Winocour et al. (21) and Hirany et al. (15), and calculated LDL cholesterol was found to be underestimated in 19%, and overestimated in 16%, of diabetic patients. A possible explanation of the discrepancies could be the differences in the study populations.

In accord with previous reports (18), the percentage of errors from calculated LDL cholesterol was significantly greater in diabetic (34%) than in nondiabetic (26%) subjects and was possibly related to the greater prevalence of hypertriglyceridemia and of alterations in the ratio of VLDL cholesterol to TG in the former group.

Although in diabetic patients, the percentage of error from calculated LDL cholesterol was significantly greater than in nondiabetic subjects, the percentage of subjects wrongly classified into the NCEP risk classes (5) was similar in both groups. Calculated versus measured LDL cholesterol resulted in misclassification of 25% of diabetic and 22% of nondiabetic patients; most patients were misclassified by one risk class. Only one diabetic patient and one nondiabetic patient were misclassified two categories lower, and four nondiabetic patients were misclassified two categories higher. As expected, the misclassification of the coronary heart disease risk was higher when calculated LDL cholesterol gave values near the cutoff points. The percentage of misclassification from calculated LDL cholesterol was independent of the serum TG level. Hypertriglyceridemia reduced, then, the accuracy of the LDL cholesterol estimation by Friedewald's equation in both diabetic and nondiabetic patients, but this reduction was of minor clinical importance because the erroneous assignment of subjects to the risk classes of coronary heart disease did not appear to increase when the serum TG level was up to 400 mg/dl (4.52 mmol/l).

Although the accuracy in estimations of LDL cholesterol is less than ideal, Friedewald's equation seems to be of value in the correct assignment of coronary heart disease

Ultracentrifugation

Friedewald

LDL-C	<130 n. 65	130-159 n. 25	160-189 n. 36	≥190 n. 25
<130	91%	32%	0%	0%
130-159	8%	56%	33%	0%
160-189	1%	12%	56%	20%
≥190	0%	0%	11%	80%

Diabetic patients

LDL-C	<130 n. 118	130-159 n. 93	160-189 n. 72	≥190 n. 122
<130	93%	19%	0%	0%
130-159	7%	66%	33%	1%
160-189	0%	11%	53%	11%
≥190	0%	4%	14%	88%

Nondiabetic patients

Figure 4—*Coincidence in calculated LDL cholesterol (LDL-C) with respect to measured LDL cholesterol at the NCEP cutoff points of 130, 160, and 190 mg/dl.*

risk classes in the great majority of diabetic as well as nondiabetic patients—independent of serum TG level. Caution must be taken in subjects in whom calculated LDL cholesterol is close to the cutoff points of the NCEP guidelines (5).

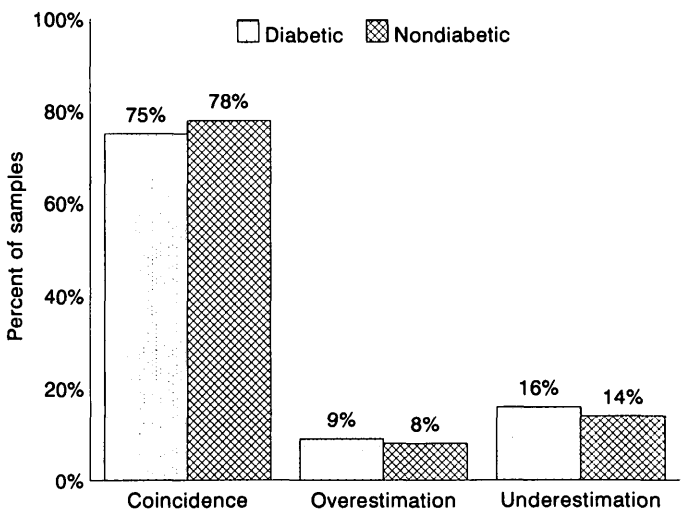

Figure 5—*Total coincidence, overestimation, and underestimation in calculated LDL cholesterol with respect to measured LDL cholesterol in diabetic and nondiabetic patients.*

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References

1. Kannel WB, McGee DL: Diabetes and cardiovascular disease: the Framingham Study. *JAMA* 241:2035–2038, 1979
2. Nathan DM: Long-term complications of diabetes mellitus. *N Engl J Med* 328:1676–1685, 1993
3. Howard BJ: Lipoprotein metabolism in diabetes mellitus. *J Lipid Res* 28:613–628, 1987
4. Taskinen M-R: Hyperlipidemia in diabetes. *Clin Endocrinol Metab* 4:743–775, 1990
5. National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation and Treatment of High Blood Cholesterol in Adults: Summary of the second report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation and Treatment of High Blood Cholesterol in Adults. *JAMA* 269:3015–3023, 1993
6. American Diabetes Association: Management of dyslipidemia in adults with diabetes. *Diabetes Care* 21:179–182, 1998
7. Friedewald WT, Levy RI, Fredrickson DS: Estimation of the concentration of low-density lipoprotein cholesterol in plasma without use of preparative ultracentrifuge. *Clin Chem* 18:499–502, 1972
8. Wilson PW, Abbott RD, Garrison RJ, Castelli WP: Estimation of very-low-density lipoprotein cholesterol from data on triglyceride concentration in plasma. *Clin Chem* 27:2008–2010, 1981
9. DeLong DM, DeLong ER, Wood PD, Lippel K, Rifkind BM: A comparison of methods for the estimation of plasma low- and very low-density lipoprotein cholesterol: the Lipid Research Clinics Prevalence Study. *JAMA* 256:2372–2377, 1986
10. Rao A, Parker AH, El-Sheroni NA, Babelly MM: Calculation of low-density lipoprotein cholesterol with use of triglyceride/cholesterol ratios in lipoproteins compared with other calculation methods. *Clin Chem* 34:2532–2534, 1988
11. Warnick RG, Knopp RH, Fitzpatrick V, Branson L: Estimating low-density lipoprotein cholesterol by the Friedewald equation is adequate for classifying patients on the basis of nationally recommended cutpoints. *Clin Chem* 36:15–19, 1990
12. McNamara JR, Cohn JS, Wilson PWF, Schaefer EJ: Calculated values for low-density lipoprotein cholesterol in the assessment of lipid abnormalities and coronary disease risk. *Clin Chem* 36:36–42, 1990
13. Branchi A, Rovellini A, Fiorenza AM, Torri A, Prandi W, Tomella C, Molgora M, Cardenà A, Velati C, Arcangeli L, Sommariva D: Estimation of cardiovascular risk: total cholesterol versus lipoprotein profile. *Int J*

Clin Lab Res 24:106–112, 1994
14. Rifai N, Warnick GR, McNamara JR, Belcher JD, Grinstead GF, Frantz ID Jr: Measurement of low-density-lipoprotein cholesterol in serum: a status report. *Clin Chem* 38:150–160, 1992
15. Hirany S, Li D, Jialal I: A more valid measurement of low-density lipoprotein cholesterol in diabetic patients. *Am J Med* 102:48–53, 1997
16. Gonzalez Estrada M, Rodriguez Ferrer CR, Astarloa IR, Lahera EM: Use of serum cholesterol/triglyceride ratio to discern for which individuals the Friedewald formula can be used confidently. *Clin Chem* 36: 1673–1675, 1990
17. Senti M, Pedro-Botet J, Nogues X, Rubies-Prat J: Influence of intermediate-density lipoproteins on the accuracy of the Friedewald formula. *Clin Chem* 37:1394–1397, 1991
18. Rubies-Prat J, Reverter JL, Senti M, Pedro-Botet J, Salinas I, Lucas A, Nogues X, Sanmartì A: Calculated low-density lipoprotein cholesterol should not be used for management of lipoprotein abnormalities in patients with diabetes mellitus. *Diabetes Care* 16:1081–1086, 1993
19. Yoshino G, Hirano T, Kazumi T: Dyslipidemia in diabetes mellitus. *Diabetes Res Clin Pract* 33:1–14, 1996
20. Hirano T, Naito H, Kurokawa M, Ebara T, Nagano S, Adachi M, Yoshino G: High prevalence of small LDL particles in non-insulin-dependent diabetic patients with nephropathy. *Atherosclerosis* 123:57–75, 1996
21. Winocour PH, Ishola M, Durrington PN: Validation of the Friedewald formula for the measurement of low density lipoprotein cholesterol in insulin-dependent diabetes mellitus. *Clin Chim Acta* 179:79–83, 1989