Calculated Low-Density Lipoprotein Cholesterol Should Not Be Used for Management of Lipoprotein Abnormalities in Patients With Diabetes Mellitus

JUAN RUBIÉS-PRAT, MD
JORDI L. REVERTER, MD
MARIANO SENTÍ, MD
JUAN PEDRO-BOTET, MD
ISABEL SALINAS, MD
ANNA LUCAS, MD
XAVIER NOGUÉS, MD
ANNA SANMARTÍ, MD

OBJECTIVE — To assess the validity of calculated low-density lipoprotein cholesterol by the Friedewald formula for management of lipoprotein abnormalities in patients with diabetes mellitus.

RESEARCH DESIGN AND METHODS — Calculated LDL cholesterol by the Friedewald formula was compared with measured LDL cholesterol after separation by ultracentrifugation in 61 patients with type I diabetes, 50 patients with type II diabetes, and 116 healthy control subjects.

RESULTS — Calculated LDL cholesterol coincided with measured LDL cholesterol, with <10% error, in 54 (49%) patients with diabetes mellitus, and 85 (73%) control subjects. Calculated LDL cholesterol was overestimated, with an error of ≥10% of measured LDL cholesterol in 39% of patients and 26% of control subjects, and underestimated in 13 and 1%, respectively. Despite a good correlation between calculated and measured LDL cholesterol, the intraclass correlation coefficients demonstrated a poor concordance between calculated and measured LDL cholesterol, both in patients and control subjects. When comparing the mean differences of calculated and measured LDL cholesterol for diabetic subjects versus control subjects, significantly greater differences in type II (but not type I) diabetic subjects were seen.

CONCLUSIONS — Calculation of LDL cholesterol by the Friedewald formula may be inaccurate for assessment of cardiovascular risk in patients with type II diabetes and may not be appropriate for management of lipoprotein abnormalities in those diabetic patients.

FROM THE DEPARTMENT OF MEDICINE, HOSPITAL DEL MAR, UNIVERSIDAD AUTÓNOMA DE BARCELONA, INSTITUTO MUNICIPAL DE INVESTIGACIÓN MÉDICA, BARCELONA; AND THE ENDOCRINOLOGY SECTION, HOSPITAL GERMANS TRÍAS I PUJOL, UNIVERSIDAD AUTÓNOMA DE BARCELONA, BADALONA, SPAIN.

ADDRESS CORRESPONDENCE AND REPRINT REQUESTS TO J. RUBIÉS-PRAT, MD, DEPARTMENT OF MEDICINE, HOSPITAL DEL MAR, PASEO MARÍTIMO 25–29, 08003 BARCELONA, SPAIN.

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LDL, LOW-DENSITY LIPOPROTEIN; HDL, HIGH-DENSITY LIPOPROTEIN; VLDL, VERY-LOW-DENSITY LIPOPROTEIN; IDL, INTERMEDIATE-DENSITY LIPOPROTEIN; TYPE I DIABETES, INSULIN-DEPENDENT DIABETES MELLITUS; TYPE II DIABETES, NON-INSULIN-DEPENDENT DIABETES MELLITUS; NCEP, NATIONAL CHOLESTEROL EDUCATION PROGRAM; ANOVA, ANALYSIS OF VARIANCE; CV, COEFFICIENT OF VARIATION.

Hypercholesterolemia, hypertriglyceridemia, and low levels of HDL cholesterol are common abnormalities in patients with diabetes mellitus and play an important role in increasing the risk of coronary heart disease. NCEP (1) has recommended the determination of LDL cholesterol in individuals with total cholesterol values >6.2 mM, as well as those with borderline-high values (5.2–6.2 mM), who are at high risk because they have definite coronary heart disease or two other cardiovascular risk factors. These conditions are found in most diabetic men, and it has been claimed that diabetic women also warrant lipoprotein analysis considering the high prevalence of coronary heart disease and complex dyslipemias, regardless of the total cholesterol concentration (2).

The targets for management of hypercholesterolemia in the general population, and in patients with diabetes mellitus, are based on LDL-cholesterol levels rather than total cholesterol levels (1,3). Because separation of LDL cholesterol by ultracentrifugation is unavailable in routine laboratories, its concentration is estimated by numerical calculation with the Friedewald formula (4). It is well-known that hypertriglyceridemia and the variability of VLDL composition (5–13), and the abnormalities of IDLs (13), are the main sources of error attributed to this formula.

The aim of this study was to evaluate the validity of the Friedewald formula in patients with diabetes mellitus, given the well-known VLDL changes and IDL abnormalities in these patients (14–19). In addition, we analyze the possible impact of errors of calculated LDL cholesterol on the clinical management of hyperlipidemia in patients with diabetes mellitus.

RESEARCH DESIGN AND METHODS

— Sera from 111 consecutive patients (50 men and 61 women) with diabetes mellitus seen in the outpatient diabetic clinic (61 type I and 50

Table 1—*Measured and calculated LDL cholesterol in healthy control subjects and diabetic patients*

	n	LDL cholesterol (mM)		P value
		Measured	Calculated	
Control subjects	116	3.18 ± 0.89	3.60 ± 0.92	<0.001
All diabetic patients	111	2.95 ± 1.03	3.52 ± 1.32	<0.001
Type I diabetic patients	61	2.69 ± 0.85	3.22 ± 1.03	<0.001
Type II diabetic patients	50	3.29 ± 1.17	3.91 ± 1.53	<0.001

Data are means ± SD.

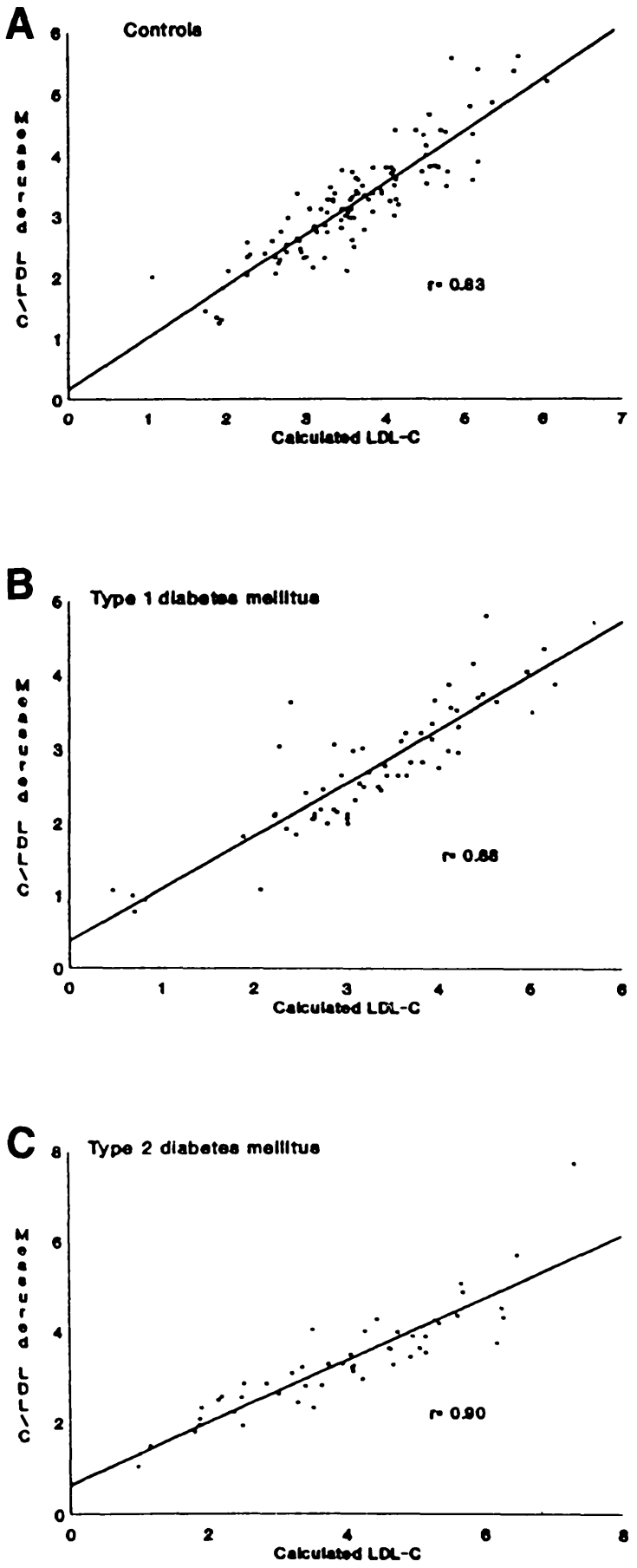
type II) were analyzed. Patients included in the study were acceptably controlled with HbA_{1c} <9.5% measured by a chromatographic method, and triglyceride levels were <3.4 mM in all cases (1.15 ± 0.61 mM) (mean ± SD). Thirty-four patients with type II diabetes were receiving insulin at the time of the study, and the remaining 16 were receiving diet with or without oral antidiabetic agents. One-hundred and sixteen healthy individuals (76 men and 40 women) with serum triglyceride levels <3.4 mM (1.17 ± 0.52 mM) were used as control subjects.

Blood samples were taken in the morning after an overnight fast. Serum was removed by centrifugation, supplemented with a preservative solution (20), and stored at 4°C for no more than 4 days before ultracentrifugation. Lipoprotein isolation was conducted by a double ultracentrifugation procedure (21,22). VLDL fraction (d < 1.006 g/ml) was isolated by preparative ultracentrifugation in an ultracentrifuge Centrikon T–1055 (Kontron Instruments, Milan, Italy) using a TFT 50.38 rotor (Kontron Instruments). The other lipoproteins (IDL, 1.006 < d < 1.019 g/ml; LDL, 1.019 < d < 1.063 g/ml; HDL, d > 1.063 g/ml) were isolated by density-gradient ultracentrifugation in a TST 41.14 rotor. Cholesterol and triglycerides in serum and lipoprotein fractions were assayed by enzymatic methods (Merck, Frankfurt, Germany) using a selective multichannel analyzer Eris (Eppendorf, Hamburg, Germany) and standardized with control sera (Qualitrol, Merck). The mean intra- and interseries CVs for the double ultracentrifugation procedure never exceeded 10%. Lipoprotein recovery was verified by comparing the sum of cholesterol and triglycerides in the fractions with total serum cholesterol and triglycerides. Quantification of HDL cholesterol in the density >1.063 g/ml fraction obtained by ultracentrifugation has been considered a classical method that correlates well with precipitation methods for separating apoprotein B–containing lipoproteins (23,24).

Comparison between measured and calculated LDL cholesterol in each group was made using paired Student's t test. Differences among groups were tested using a one-way ANOVA of mean differences between measured and calculated LDL cholesterol, and Tukey method was used to compare the means of all possible pairs of groups. Multiple linear regression analysis was used to adjust the calculated LDL-cholesterol levels for the variable group. Measured LDL cholesterol was the dependent variable, and calculated LDL cholesterol and the variable group were the independent variables. Concerning the variable group, we assigned the value 1 for the control group and values 2 and 3 for type I and type II diabetic subjects, respectively. The correlation coefficient between continuous variables was calculated by Pearson's correlation test. Contingency tables were compared using χ^2 test. Because correlation coefficient measures the strength of a relation between two variables, not the agreement between them (25), and the slope and the correlation coefficient are not equivalent measures of association (26), approach to concordance between measured and calculated LDL cholesterol in each group was achieved by the intraclass correlation coefficient, R_i, which combines a measure of correlation with a test in the difference of means (27).

RESULTS — Serum cholesterol in patients and control subjects were 5.31 ± 1.37 vs. 5.26 ± 1.01 mM (means ± SD), respectively. Measured and calculated LDL cholesterol in patients and control subjects are shown in Table 1. Means ± SD of differences between calculated and measured LDL cholesterol were 0.39 ± 0.41 mM in the control group, 0.52 ± 0.50 mM in type I diabetic patients, and 0.61 ± 0.70 mM in type II diabetic patients (ANOVA, P < 0.05). Tukey method showed a significant increase mean difference in type II diabetic patients compared with that seen in the control group (P = 0.01), whereas no differences were found either between type I diabetic patients and control subjects, or between type I and type II diabetic patients. The multiple regression model selected to adjust the calculated LDL-cholesterol levels was as follows: measured LDL cholesterol (mM) = 0.53 + 0.76 × calculated LDL-cholesterol −0.09 × group. Multiple R was 0.892. Correlation coefficients between measured and calculated LDL cholesterol in control subjects, patients with type I diabetes, and patients with type II diabetes were 0.83, 0.88, and 0.90, respectively (Fig. 1). The intraclass correlation coefficients R_i in each group between measured and calculated LDL cholesterol were 0.08, 0.09, and 0.14, respectively.

The (cholesterol/triglyceride) VLDL ratio (normal range 0.20–0.30) (7,11, 13) varied greatly in the studied population (Fig. 2). In 80% of patients with type I diabetes and in 62% of patients with type II diabetes, the (cholesterol/



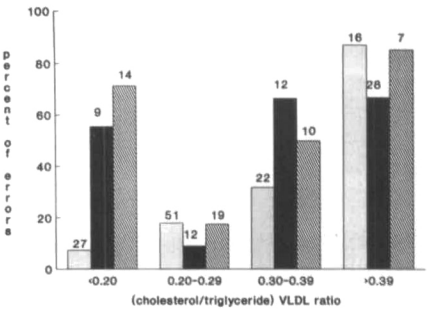


Figure 2—*Percentage of samples in which calculated LDL cholesterol differed from measured LDL cholesterol by ≥10%, according to the different (cholesterol/triglyceride) VLDL ratio (the ratio has been calculated using traditional units) in healthy control subjects (▨), type I diabetic patients (■), and type II diabetic patients (▧). Number of samples appears at the top of each bar.*

triglyceride) VLDL ratio was <0.20 or >0.29. The percentage of samples in which calculated LDL cholesterol differs from measured LDL cholesterol by ≥10% was markedly increased in patients with (cholesterol/triglyceride) VLDL ratio <0.20 or >0.29 than those with normal (cholesterol/triglyceride) VLDL ratio (Fig. 2). However, changes in the (cholesterol/triglyceride) IDL ratio do not substantially influence the accuracy of calculated LDL cholesterol (Fig. 3).

Calculated LDL cholesterol coincided with measured LDL cholesterol with <10% error in 30 of 61 patients with type I and 24 of 50 patients with type II diabetes. In 31 cases of type I and 26 cases of type II diabetes, calculated LDL cholesterol was overestimated or underestimated by >10% compared with measured LDL cholesterol (Table 2). The proportion of over- and under-

Figure 1—*Correlation between measured and calculated LDL cholesterol in healthy control subjects (A), type I diabetic patients (B), and type II diabetic patients (C). LDL cholesterol is given in mM.*

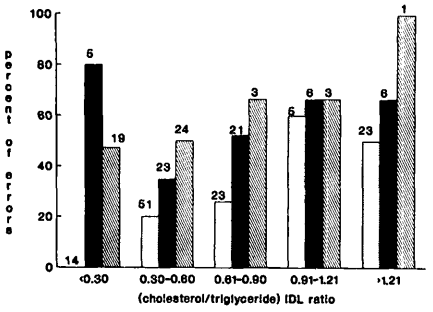


Figure 3—*Percentage of samples in which calculated LDL cholesterol differed from measured LDL cholesterol by ≥10%, according to the different (cholesterol/triglyceride) IDL ratio (the ratio has been calculated using traditional units) in healthy control subjects (▨), type I diabetic patients (■), and type II diabetic patients (▧). Number of samples appears at the top of each bar.*

estimated errors in calculated LDL cholesterol in type I and type II diabetic patients, with 3.4 mM being considered a cut-off point as the highest desirable level according to the recommendations of the NCEP (1), is shown in Table 2. In the 95 patients receiving insulin, the percentage of over- and underestimation of LDL cholesterol was 38 and 10%, respectively.

CONCLUSIONS — Atherosclerotic vascular disease is the leading cause of death in patients with both type I and type II diabetes (28). The high cardiovascular mortality in diabetic individuals is related at least in part to associated lipoprotein abnormalities (14–19,29–34). The lipoprotein abnormalities in type I diabetes usually revert to normal after tight metabolic control. However, treatment of diabetes mellitus with diet alone or associated with oral antidiabetic agents is often unsatisfactory for correcting the atherogenic lipoprotein profile.

Hypertriglyceridemia is an important source of error attributed to the Friedewald formula, although the variability of VLDL composition in normotriglyceridemic sera may also be responsible for inaccuracy (6,7,11–13). The percentage of error of calculated LDL cholesterol is high, both in sera with high (cholesterol/triglyceride) VLDL ratio, as occurs in nondiabetic sera (7,13), and sera with a low (cholesterol/triglyceride) VLDL ratio.

When compared with healthy control subjects in our study and the results of a previous study in a nondiabetic population (13), IDL in patients with diabetes mellitus did not appear to influence the reliability of the Friedewald formula, because the percentage of errors in calculated LDL cholesterol bears no relation to the (cholesterol/triglyceride) IDL ratio.

An overwhelming amount of evidence relates high levels of LDL and low levels of HDL with coronary heart disease. In patients with diabetes, as is stated by the NCEP (1), "the minimal goal of LDL lowering in men is to achieve a reduction of LDL cholesterol to <3.4 mM, for women the minimal LDL-cholesterol goal is <4.1 mM in the absence of coronary heart disease or another risk factor, or <3.4 mM if definite coronary heart disease or another risk factor is present." An important issue of this study is that an error of >10% in calculated LDL cholesterol was found in half of the acceptably controlled patients with diabetes. Despite the good correlation between calculated and measured LDL cholesterol in our patients, as has been described previously (35), the intraclass correlation coefficients both in patients and control subjects demonstrate a poor concordance between measured and calculated LDL cholesterol. Thus, differences between diabetic patients and control subjects are not as great as may have been expected. Nonetheless, higher mean differences between measured and calculated LDL cholesterol were found in type II diabetic patients respective to control subjects. Because diabetes represents an additional risk factor for atherosclerotic vascular disease, we believe that use of the Friedewald formula is less suitable in these patients.

The possible role of hypertriglyceridemia and changes in VLDL composition as risk factors for coronary heart disease in the general population remains a matter of dispute. In recent years, it has been suggested that IDL ab-

Table 2—*Coincidence (with an error <10%), overestimation and underestimation (with an error of ≥10%) in calculated LDL cholesterol respective to actual LDL cholesterol in healthy control subjects and diabetic patients*

	n	Coincidence (%)	Overestimation (%)	Underestimation (%)
Control subjects				
LDL cholesterol < 3.4 mM	73	50 (68)	22 (30)	1 (1)
LDL cholesterol > 3.4 mM	43	35 (81)	8 (18)	0 (0)
All control subjects	116	85 (73)	30 (26)	1 (1)
Diabetic patients				
Type I				
LDL cholesterol < 3.4 mM	48	19 (40)*	24 (50)†	5 (10)
LDL cholesterol > 3.4 mM	13	11 (85)	2 (15)	0 (0)
Type II				
LDL cholesterol < 3.4 mM	28	12 (43)†	8 (28)	8 (28)‡
LDL cholesterol > 3.4 mM	22	12 (54)†	9 (41)	1 (4)
All diabetic patients	111	54 (49)	43 (39)	14 (13)

*$P < 0.005$ vs. control subjects.
†$P < 0.05$ vs. control subjects.
‡$P < 0.001$ vs. control subjects.

normalities in the general population should be considered as atherogenic (36–39). On the other hand, several authors (2,29,32,34,40,41) tend to consider triglyceride-rich lipoproteins a cardiovascular risk factor in diabetic patients. Because VLDL and IDL are implicated in atherogenesis in diabetic patients, Garg and Grundy (2) suggested the use of non-HDL cholesterol rather than LDL cholesterol as a target for treatment of dyslipidemia in these patients.

In conclusion, our results question the routine use of calculated LDL cholesterol by the Friedewald formula in patients with acceptably controlled type II diabetes. Because ultracentrifugation for LDL-cholesterol measurement is expensive and time-consuming, we argue in favor of using non-HDL cholesterol, or alternatively total cholesterol/HDL-cholesterol ratio, or perhaps serum apoprotein B levels, for management of dyslipidemia in diabetic patients.

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