

# Estimation of the Concentration of Low-Density Lipoprotein Cholesterol in Plasma, Without Use of the Preparative Ultracentrifuge

William T. Friedewald, Robert I. Levy, and Donald S. Fredrickson

A method for estimating the cholesterol content of the serum low-density lipoprotein fraction ( $S_f$  0-20) is presented. The method involves measurements of fasting plasma total cholesterol, triglyceride, and high-density lipoprotein cholesterol concentrations, none of which requires the use of the preparative ultracentrifuge. Comparison of this suggested procedure with the more direct procedure, in which the ultracentrifuge is used, yielded correlation coefficients of .94 to .99, depending on the patient population compared.

**Additional Keyphrases** *hyperlipoproteinemia classification • determination of plasma total cholesterol, triglyceride, high-density lipoprotein cholesterol • beta lipoproteins*

An important requirement for classification of hyperlipidemia into the different types of hyperlipoproteinemia (1, 2) is the estimation of the concentration of plasma LDL<sup>1</sup> ( $S_f$  0-20; the beta lipoproteins). This quantity is necessary for the assignment of the Type II pattern (1-3), which is defined as an increase in LDL concentration above some arbitrarily selected cut-off limit.

An indirect method is presented here for estimating the plasma LDL concentration in terms of the cholesterol contained in this lipoprotein ( $C_{LDL}$ ). The method requires measurement of the concentrations of plasma total cholesterol, triglycerides, and  $C_{HDL}$ . This information can be obtained without ultracentrifugation and requires only routine lipid analyses in addition to a rapid precipitation of all plasma lipoproteins other than HDL. Two observations are used in the calculation. One is that the ratio of the mass of triglyceride to that of cholesterol in VLDL is apparently relatively

constant and about 5:1 in normal subjects (1, 4) and in patients with all types of hyperlipoproteinemia, except the rare Type III (1, 2). The other is that when chylomicrons are not detectable, most of the triglyceride in plasma is contained in the VLDL. Thus, in the vast majority of plasma samples in which chylomicrons are not present, the cholesterol in plasma attributable to VLDL can be approximated by dividing the plasma triglyceride concentration by five. The justification of this method for estimation of  $C_{LDL}$  is the subject of this paper.

## Methods

Data were obtained from lipid and lipoprotein analyses performed by the Molecular Disease Branch of the National Heart and Lung Institute, on samples from patients with hyperlipidemia and from normal subjects. The results of the laboratory analyses are in the process of transfer to magnetic tapes for rapid retrieval and analysis. At the time of this study, complete lipoprotein analyses from 448 subjects classified as either normal, Type II, or Type IV primary hyperlipoproteinemia had been transferred to tapes and the data from all were used. The data reflect the research interests of the Branch, with most of the data coming from patients with familial hyperlipoproteinemia or their relatives, and as such do not represent an unbiased sample of the general population. The specimen from each patient chosen for analysis in this paper was the first sample on magnetic tape on which a complete lipoprotein analysis had been performed at the National Heart and Lung Institute. The subjects were receiving no dietary or drug treatment for hyperlipoproteinemia at the time of this sampling, except for two patients who were on caloric restriction but who were subsequently classified as Type IV.

The original plasma samples had been obtained 12 to 14 h after the last meal, mixed with EDTA (1 mg/ml), and immediately stored at 4°C until analyzed. Total plasma cholesterol (5) and triglycerides (6) were measured, and data on  $C_{HDL}$ ,  $C_{LDL}$ , and  $C_{VLDL}$  obtained by a combination of ultracentrifugation and precipitation procedures (7). In samples free of chylomicrons the  $C_{VLDL}$  was

From the Biometrics Research Branch (W.T.F.) and the Molecular Disease Branch (R.I.L. and D.S.F.), National Heart and Lung Institute, 9000 Rockville Pike, Bethesda, Md. 20014.

<sup>1</sup> Nonstandard abbreviations used: HDL, high-density lipoprotein; LDL, low-density lipoprotein; VLDL, very low-density lipoprotein; TG, plasma triglycerides;  $C_{subscript}$ , cholesterol concentration (mg/100 ml of plasma) in the fraction identified by the subscript; EDTA, ethylenediaminetetraacetic acid.

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measured in two ways: (a) directly, by measuring the cholesterol content of the supernatant fraction after ultracentrifugation of plasma at D.1006 for 16 h at  $100,000 \times g$  in a Spinco 40.3 rotor, and (b) indirectly, by subtracting the cholesterol content of the infranant fraction (the sum of the  $C_{HDL}$  and  $C_{LDL}$ ) from the total plasma cholesterol. A methodologic error was presumed and the plasma re-analyzed if there was a large disparity between the results of the two methods.

Subjects were classified as normal or as having hyperlipoproteinemia Type I through V according to criteria previously described (1, 2).

## Results

The results of lipid and ultracentrifuge lipoprotein determinations in 232 men and 216 women—96 normal, 204 with Type II, and 148 with Type IV—were analyzed. Various statistics derived from these data are presented in Table 1.

$C_{LDL}$  was also calculated for each person according to the following formula:

$$C_{LDL} = C_{plasma} - C_{HDL} - TG/5$$

Plots of each individual's  $C_{LDL}$  as calculated by this method vs. that obtained after preparative ultracentrifugation (7) are presented in Figures 1 to 3. For normal people and Type II patients the spread of points about the line of equality for the two methods does not appear excessive, as reflected in the high correlation coefficients, .98 and .99, respectively (8). However, in Type IV patients there are many outlying values and the correlation is somewhat lower, namely, .85. Closer scrutiny of these outliers revealed that most such patients had very high plasma triglyceride concentrations, and thus a plot was made of only those Type IV patients with plasma triglycerides less than 400 mg/100 ml (111 of the original 148 people). The spread of values is much smaller after these are

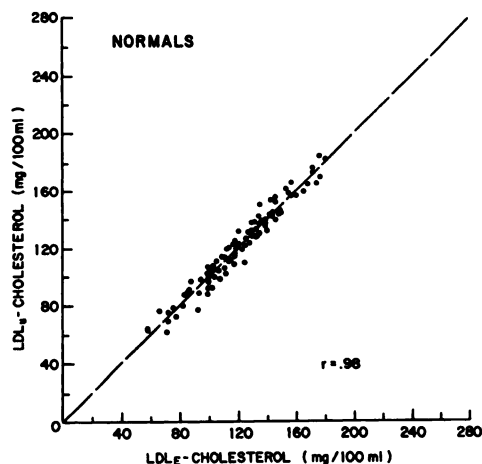


Fig. 1. Comparison of the plasma low-density lipoprotein cholesterol concentration in normal individuals as calculated by the estimation method ( $LDL_E$ ) with that obtained by the ultracentrifuge method ( $LDL_U$ ).

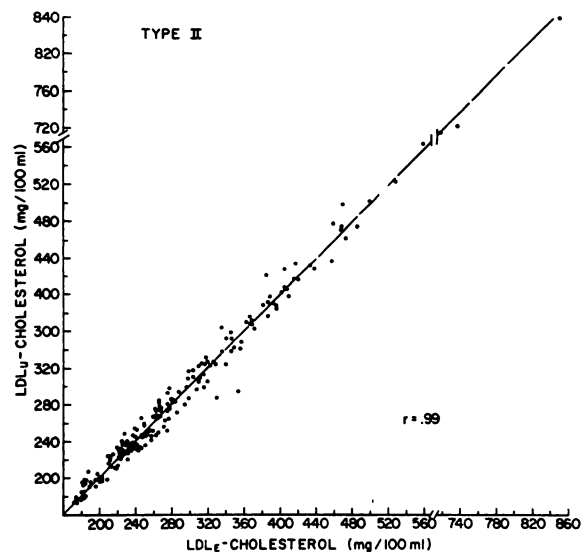


Fig. 2. Comparison of the plasma low-density lipoprotein cholesterol concentration in Type II patients as calculated by the estimation method ( $LDL_E$ ) with that obtained by the ultracentrifuge method ( $LDL_U$ ).

**Table 1. Mean, Standard Deviation, and Range of Plasma Lipids and Lipoproteins**

	Normal (96) <sup>a</sup>	Type II (204) <sup>a</sup>	Type IV (148) <sup>a</sup>
		mg/100 ml	
Total plasma cholesterol	189 ± 33 (166-270)	359 ± 100 (217-888)	241 ± 57 (138-436)
Total plasma triglyceride	73 ± 7 (20-184)	126 ± 16 (25-656)	347 ± 61 (90-2502)
HDL cholesterol	53 ± 13 (29-77)	45 ± 13 (18-82)	38 ± 11 (15-74)
LDL cholesterol	122 ± 28 (62-185)	291 ± 99 (173-840)	135 ± 38 (28-231)
VLDL cholesterol	14 ± 9 (0-40)	24 ± 19 (0-78)	68 ± 55 (6-356)

<sup>a</sup> No. of patients.

excluded (Figure 4), the correlation coefficient then being .94.

Further quantitative expressions for the disagreement between the  $C_{LDL}$  obtained by the two methods are presented in Table 2. Because each of the confidence intervals (9) contains zero, there is no compelling reason based on these data to believe that the estimate of  $C_{LDL}$  will be biased. The tolerance intervals (10) give the range of values that with probability .95 will include 95% of the differences between the two methods of measurement. The per cent error assumes that the  $C_{LDL}$  measured by the ultracentrifuge method is the standard with which the  $C_{LDL}$  estimate obtained by calculation is being compared. The distribution of values by each method was examined for each of the groups, by use of normal probability graph

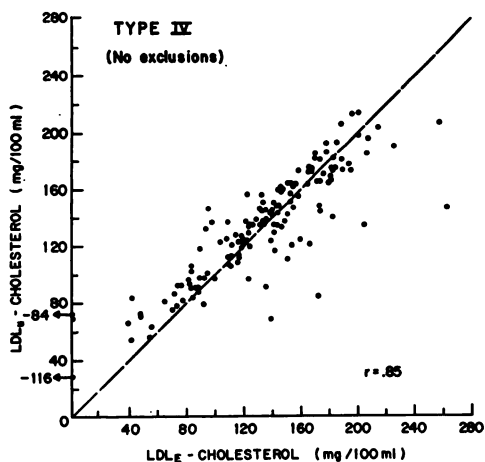


Fig. 3. Comparison of the plasma low-density lipoprotein cholesterol concentration in Type IV patients as calculated by the estimation method ( $LDL_E$ ) with that obtained by the ultracentrifuge method ( $LDL_U$ ); no exclusions

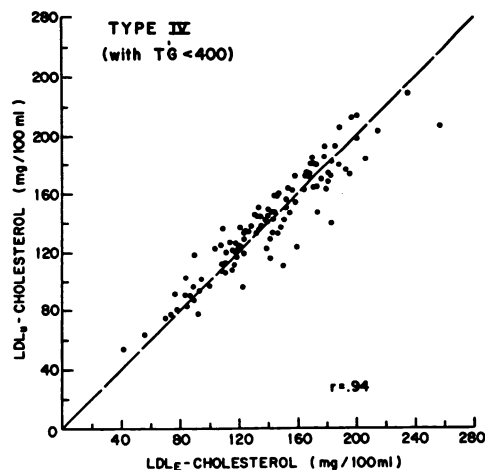


Fig. 4. Comparison of the plasma low-density lipoprotein cholesterol concentration in Type IV patients as calculated by the estimation method ( $LDL_E$ ) with that obtained by the ultracentrifuge method ( $LDL_U$ ), excluding individuals with serum triglycerides  $\geq 400$  mg/100 ml

paper. The values were reasonably normally distributed. Only in the Type IV patients was there evidence of skewness; this was only minimal, and in the negative direction.

We did not attempt to calculate the overall average error or to estimate the probability of misclassification by this estimation procedure because of the unusually large number of Type II and Type IV patients relative to normal people in the sample.

A subsample of 46 Type II patients was randomly chosen, a linear least-squares (11) fit of TG to  $C_{VLDL}$  was performed, and the equation so obtained was used in a separate subset of 55 Type II patients to estimate their  $C_{VLDL}$ . Analysis revealed that simple division of TG by five provided as accurate an estimate of  $C_{VLDL}$  as did this more complicated regression estimate.

## Discussion

The method presented here for estimating plasma LDL concentrations provides a reasonable approximation that is useful for many purposes. There are, however, three important restrictions on its use. First, it is not applicable to plasma samples containing chylomicrons. However, such samples are characterized by a "cream" layer on top of plasma that has been stored at 4°C for 18 h or more. Chylomicrons are characteristic of lipoprotein patterns classified as Types I and V, in which the  $C_{LDL}$  concentration is not abnormally increased. Particles having similar appearance are also sometimes seen in Type III.

Second, the technique for estimating  $C_{LDL}$  gives erroneously high results in the rare patient with Type III hyperlipoproteinemia. In this disorder,

Table 2. Statistics on the Measurement of  $C_{LDL}$  Utilizing the Ultracentrifuge vs. the Estimation Procedure

	No. of values, (n)	$(LDL_U - LDL_E)^b$				$ LDL_U - LDL_E ^c$	
		Mean, ( $\bar{X}$ )	Standard deviation, (SD)	95% confidence interval <sup>d</sup>	95% tolerance interval <sup>e</sup>	Mean	% error/ <sup>f</sup>
Normals	96	.3	5.9	[-.9, 1.4]	[-13.0, 13.5]	4.8	4%
Type II	204	1.3	11.9	[-.4, 2.9]	[-24.3, 26.8]	8.8	3%
Type IV	111 <sup>a</sup>	.4	12.9	[-2.0, 2.9]	[-27.6, 28.6]	9.8	7%

<sup>a</sup> Only people with plasma triglycerides less than 400 mg/100 ml are included.

<sup>b</sup>  $LDL_U = C_{LDL}$  calculated with the preparative ultracentrifuge (see text).

<sup>c</sup>  $LDL_E = C_{LDL}$  calculated by the estimation procedure (see text).

<sup>d</sup>  $|LDL_U - LDL_E|$  = the absolute value of  $(LDL_U - LDL_E)$ .

<sup>e</sup>  $\bar{X} \pm [t\text{-value} (.025, n - 1)] SD/n$ .

<sup>f</sup>  $\bar{X} \pm [tolerance\text{-value} (.95, .95, n)] SD$ .

<sup>f</sup> % error =  $\frac{[\text{mean of } |LDL_U - LDL_E|] \times 100}{(\text{mean of } LDL_U)}$

the VLDL are of two kinds (12). One is the normal variety having the usual triglyceride-to-cholesterol ratio of about five. The other form is unique in having beta mobility on electrophoresis and an abnormally high content of cholesterol relative to triglyceride. Division of the total plasma triglyceride concentration by the factor five yields a falsely low value for the "VLDL" and falsely high value for the "LDL" contribution to the total plasma cholesterol. Thus, when this formula is used, a Type III patient may be falsely classified as a Type II. The anomalous lipoproteins in Type III are detectable with certainty only by ultracentrifugal isolation of VLDL and determination of either its electrophoretic mobility or cholesterol and triglyceride content (1-3).

Third,  $C_{LDL}$  cannot always be accurately estimated when the plasma triglyceride concentration exceeds 400 mg/100 ml. It is noteworthy, however, that only two of the 204 Type II patients in this series had triglyceride concentrations of 400 mg/100 ml or greater. This suggests that few errors in classification would occur if patients with plasma triglycerides exceeding 400 mg/100 ml, in the absence of chylomicrons, were directly classified as Type IV. The frequency of this misclassification will no doubt depend in part on the cut-off limits used in defining an abnormal LDL concentration.

It is noteworthy that despite the good agreement between the estimation and actual measurement of  $C_{LDL}$ , simple division of the plasma triglyceride by five does not give a very accurate estimate of the VLDL cholesterol alone, even in normals or patients with Type II or Type IV. In normals and patients with Type II the average VLDL cholesterol concentration is low (see Table 1), and thus even small absolute errors yield large percentage errors. In Type IV the average VLDL cholesterol concentration is higher, but large percentage errors still result. However, when the estimate of  $C_{VLDL}$  is used to calculate  $C_{LDL}$  the percentage error does decrease to an acceptable level because the absolute error in  $C_{VLDL}$  estimation is small relative to the concentration of  $C_{LDL}$ .

Of some concern is the number of Type IV patients with relatively large values for  $|LDL_{\beta}|$

(see Table 2 and Figure 3), which of course greatly influences the per cent error of the whole group. To examine this problem more closely, we identified all Type IV patients with an  $|LDL_{\beta}|$  value greater than 20 mg/100 ml and their entire laboratory profile on tape was re-evaluated. Of the 16 people so examined, 13 had evidence of an undetected methodological error identified by a large disparity between the actual indirect and direct measurements of  $C_{VLDL}$  (see *Methods*). This suggests the possibility that the larger percentage error seen in the Type IV patients may be due in part to laboratory errors in the ultracentrifuge calculation of LDL cholesterol rather than greater inaccuracy of the estimation procedure.

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