Protocol for efficient plasma sampling for low density lipoprotein turnover studies

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Summary Radiolabeled low density lipoprotein (LDL) is commonly used to study the turnover of LDL apolipoprotein B (apoB), the major protein component of LDL. Following an intravenous injection of radioiodinated LDL, typical sampling schedules have including 20–25 samples over a 14-day period with frequent sampling during the first 12 hr and daily samples thereafter. This is a burdensome task for subjects and investigators. To improve acceptance of the procedure, we have examined the effects of reduced sampling schedules upon the estimation of the fractional catabolic rate (FCR) for LDL apoB. Data from 36 different sets of LDL decay curves obtained from investigations of subjects with a variety of lipoprotein phenotypes have been used to test these schedules. Our results indicate that by choosing specific intervals over a 14-day period only 10 samples are sufficient to accurately determine the fractional catabolic rate for LDL in plasma. This reduced sampling schedule should facilitate the study of LDL turnover in large groups of subjects as outpatients.—**Le, N-A., H. N. Ginsberg, and W. V. Brown.** Protocol for efficient plasma sampling for low density lipoprotein turnover studies. *J. Lipid Res.* 1984. **25:** 1387–1391.

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The majority of plasma cholesterol is associated with low density lipoproteins (LDL). Disorders involving both the production and the catabolism of LDL have been associated with premature arteriosclerosis (1, 2). Studies with cultured cells have demonstrated that the interaction of LDL with specific receptors on cell surfaces (through binding to apoB, the major protein of LDL) mediates the uptake and degradation of this lipoprotein (3, 4). Deficiency of these receptors (5, 6) results in a reduced clearance rate of LDL (7) accounting for significant elevations in the levels of plasma LDL in individuals with familial hypercholesterolemia. Increased production of LDL apoB has also been reported for patients with homozygous familial hypercholesterolemia (7, 8). Turnover studies using radiolabeled autologous LDL have also provided information with regard to a number of dietary and pharmacologic factors which affect the clearance and/or secretion of LDL in man (8–13).

The metabolism of apoB in plasma LDL has been extensively investigated using radioiodinated autologous LDL (7–13). The fractional catabolic rate (FCR) for plasma LDL which represents the fraction of the plasma LDL pool that is metabolized per unit time, has been commonly derived from fitting the LDL decay data to a two-pool model (7). This estimate of FCR is equivalent to the curve-peeling method described by Matthews (14). As corroboration, the availability of radioactivity in the urine has been used as an independent estimate of total degradation (7, 15) as well as to indicate some heterogeneity in the clearance of plasma LDL (15). Due to difficulties in the collection of urine data, most studies have limited the analysis to the plasma LDL decay curve. For the majority of the studies reported to date, however, comparison of the values for the FCR estimated from the plasma decay with those determined from the urine/plasma ratio, has resulted in good agreement (10, 11).

A typical protocol schedule for LDL turnover studies requires that subjects be admitted to the metabolic ward for a stabilization period (5–7 days). The subjects then remain in the ward for an additional 2-week period following the injection of the tracer. The protocol in our laboratory has used 22 plasma samples (0.5, 1, 2, 4, 6, 9, 12, 24, 36, 48 hours and daily samples thereafter for the remaining 12 days) for determination of the LDL radioactivity decay (schedule A). The individual experimental values are fitted to a sum of two exponential terms and the FCR is determined from the exponential parameters. The LDL apoB production rate is defined as a simple product of the estimated FCR and a measure of the LDL apoB pool size. In the present work we have shown that similar estimates of FCR can be derived from the analysis of only 10 sample points during the same 2-week period.

METHODS

Subjects

A total of 34 separate LDL turnover studies in 23 different subjects that were completed at our institution during the past several years was used in this analysis. These studies were divided into two groups with the first group (selection group) consisting of 11 subjects who participated in two separate turnover studies each. Thus a total of 22 LDL decay curves was utilized to select the best sampling schedule. Ten of these curves

Abbreviations: LDL, low density lipoprotein; FCR, fractional catabolic rate; TG, triglyceride.

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corresponded to LDL data from five individuals with normal lipid levels studied during two periods of differing dietary cholesterol intake (12). The mean (±SD) plasma triglycerides and cholesterol values for these ten studies were 68 (±16.3) mg/dl and 168 (±20.2) mg/dl, respectively. The remaining twelve LDL studies were carried out in six hyperlipidemic subjects with mean (±SD) TG and cholesterol of 472 (±379.1) mg/dl and 216 (±53.9) mg/dl, respectively, who were studied before and after a period of caloric restriction (13). This initial series of 22 individual LDL decay curves was used to test a number of different sampling schedules.

The best sampling schedule that was arrived at from the analysis of the LDL decay curves from this selection group was then further validated using the decay curves from a second group of subjects (test group). The second group consisted of a single study in each of twelve subjects and included data from i) two individuals with normal lipid levels, ii) eight subjects with hypertriglyceridemia, and iii) two subjects with familial hypercholesterolemia (one with the homozygous and the other with the heterozygous form).

Study protocol

Radiolabeled LDL was prepared using a standard protocol with ultracentrifugal isolation (9) and radioiodination by a modification (16) of the method of MacFarlane (17). The experimental data were derived from 22 points obtained over a 14-day period, as described above. At each time point, apoB specific activity in LDL isolated by ultracentrifugation (d 1.019–1.063 g/ml) was available. Total apoB mass in LDL was obtained from a series of independent samples.

Analysis

Analyses were carried out with the SAAM program (18) using the two-pool compartmental model proposed by Langer, Strober, and Levy (7). A total of four independent parameters was obtained, including: D_1, the initial radioactivity injected into LDL apoB; k(0,1), the fractional rate of irreversible loss from the plasma pool, i.e., k(0,1) is thus the fractional catabolic rate, FCR; k(2,1), the fractional rate of transfer from the intravascular compartment into the extravascular pool; and k(1,2), the fractional transfer rate into the intravascular compartment from the extravascular pool.

Fig. 1 illustrates a typical biphasic decay for LDL apoB radioactivity as generated by the computer. The 22 timed samples corresponding to schedule A are denoted on this graph and include 0.5, 1, 2, 4, 6, 9, 12, 24, 36, 48 hours, and day 3 through day 14. At least three regions of kinetic importance may be noted from this decay curve. The first region, region I, which extends over the first 24 hr following the injection of

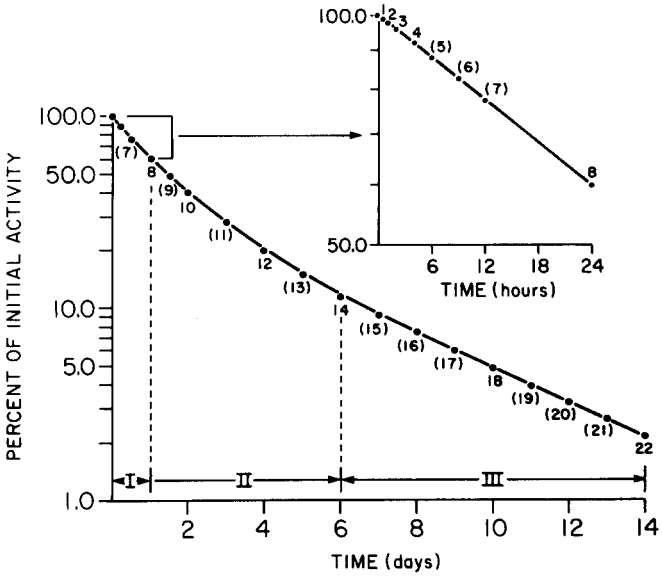

Fig. 1. Kinetic regions of the LDL decay curve. A typical biexponential decay curve for LDL apoB is illustrated over the 14-day sampling period. The insert displays the initial rapid decay associated with equilibration of the label with the extravascular space. After sample #10, all of the remaining samples are taken on a daily basis. The sample numbers that are shown in parentheses are samples that can be omitted from the data set without affecting the estimated value of the fractional catabolic rate of LDL. During the first kinetic region, region I, which extends over the first 24 hr following the tracer injection, at least four samples (0.5, 1, 2, 4 hr) are required (see insert). During the second kinetic region, region II, which starts with the 24-hr sample to the 6-day sample, three additional samples (24, 48, and 96 hr) will be required. During the third and final region, region III, three samples (days 6, 10, and 14) will be needed to accurately define the LDL decay curve.

the tracer, is basically log-linear (see insert, Fig. 1) and corresponds to the phase of rapid equilibration between intravascular and extravascular LDL. The second region, region II, which covers day 1 through day 6 is curvilinear and represents the composite effect of two different exponential rates. The third region, region III, involving day 6 through day 14, is log-linear and represents the slow exponential component reflecting the rate of irreversible removal of LDL from plasma.

For the purpose of investigating the turnover of LDL in human subjects, the most desirable sampling schedule would be one in which the subject has to remain on the metabolic ward for the minimal length of time and in which the intervals between return visits are maximal. The first sampling schedule satisfying these conditions which was tested consisted of a total of nine sample points. Included in these nine samples were four samples within region I (samples 1, 2, 4, and 7), two samples in region II (samples 8 and 10), and three additional samples within region III (samples 14, 18, and 22) of the curve obtained with schedule A (see Fig. 1). With this revised minimum sampling schedule (schedule B), the subject would remain on the clinical ward for 48 hr

after the tracer injection and would have samples taken every fourth day thereafter. Other sampling schedules with additional blood samples were tested, with additional samples taken within region III (schedule C) or within region II (schedule D).

RESULTS

The revised nine-point sampling schedule (schedule B) was used as the simplest scheme to be tested. As depicted in **Table I** for the testing group of 22 individual decay curves, the fractional catabolic rate (FCR) was overestimated by 40% as compared with the values estimated from the analysis of all 22 points in schedule A. In this testing group, the elimination of any of the four samples within region I resulted in changes in the estimation of the parameter D_1. The inclusion of additional samples within this interval did not change the final estimated value for this parameter, nor did it improve the accuracy of the estimate as determined by the computed fractional standard deviation of the estimate.

In schedule C, additional samples were chosen from the interval defined as region III. The FCR values obtained with these points correlated with the estimates from schedule A with a slope of 1.25 (Table I), i.e., an overestimation by only 25%.

In schedule D, an additional sample from region II was included in the analysis; sample 12 corresponding to day 4 (see Fig. 1). With this 10-point sampling schedule, the estimate for the individual FCRs became very similar to values obtained with all 22 timed samples. The slope of the line of regression for FCRs from schedule A versus FCRs from schedule D was 1.028 ($r = 0.974$), indicating an average overestimation of 2.8% of the FCR when the 10-point sampling was compared of the 22-point protocol. The inclusion of additional samples within this region did not result in better agreement of the estimates as determined from the slope of the regression lines.

TABLE 1. Comparison of the fractional catabolic rates estimated from different sampling schedules

Schedule A	Total Number of Samples	Samples Included[a]	Slope of the Regression Line[b]
A	22	all	1.0
B	9	1, 2, 4, 7, 8, 10, 14, 18, and 22	1.40
C	10	Schedule B and 16	1.25
D	10	Schedule B and 12	1.03

[a] The sample points are numbered in sequence as illustrated in Fig. 1.

[b] Regression analysis was carried out in each case between the FCR values obtained with the reduced sampling protocol and the values obtained with the conventional 22-point sampling schedule. The coefficients of correlation of the regression lines presented here all have an r value of 0.950 or greater ($P < 0.001$). The y-intercept in all cases was between 0.05 and 0.01.

Using schedule D, an additional series of LDL decay curves from twelve different individuals was analyzed and the FCR values obtained were compared with those based on the schedule A sampling protocol (**Table 2**). The ratio of the calculated value to the experimentally observed value at each timed point was calculated and the mean values (±SD) were also tabulated for the two sampling schedules. There was no difference in the fit of the calculated curve to the experimental data with the two sampling schedules.

Table 3 presents the regression analysis of the four model parameters derived from sampling schedule A and schedule D. For this analysis, data from all 23 subjects from both study groups were used with each individual contributing only one LDL decay curve (the baseline study). An overestimation of 1.5% was obtained for the FCR for the group as a whole. In this case, of all four parameters estimated from the decay curves, the largest difference (5.9%) was associated with the estimation of the fractional rate k(1,2), the fraction of the extravascular pool that is returned to the plasma space per unit time. Schedules using 12 or more samples did not result in a significant improvement in the fit as determined by a reduction in the sum of squares of the errors.

DISCUSSION

In studying the kinetics of whole body cholesterol, Goodman et al. (19) have proposed a scheme to reduce the number of samples required over the 6-month duration of the study. In this scheme, the frequency of sampling for each subject was based on the data from the first 5 days and a number of assumptions on the kinetics of the tracer. In the case of LDL, however, since the study period only lasts 2 weeks, it would be more practical to have a fixed sampling schedule for all subjects. In the present study, we have divided the 2-week study period into three distinct kinetic regions. By systematically determining the minimum number of data points that are required to characterize each of these regions, we have developed a minimum sampling protocol for LDL turnover study.

The primary parameter to be extracted from the LDL radioactivity decay curve is the fractional rate of clearance (FCR). Whether the curve-peeling method of Matthews (13) or the two-pool model of Langer et al. (7) is used, the same estimate for FCR will be obtained from the actual fitted parameters. The FCR, in this

TABLE 2. LDL kinetic data of the test group comparison of schedule A and schedule D

	Subjects	TG	CHOL	FCR[a] (day^{-1})		QC/QO[b]	
				Schedule A	Schedule D	Schedule A	Schedule D
		mg/dl					
1	N	78	129	0.440 (3.3%)	0.454 (3.9%)	0.997 (5.5%)	1.001 (6.3%)
2	N	55	138	0.382 (3.8%)	0.386 (4.8%)	0.995 (5.8%)	1.000 (5.8%)
3	HTG	313	132	0.715 (2.1%)	0.725 (2.7%)	0.980 (7.9%)	0.977 (8.4%)
4	HTG	215	159	0.374 (3.8%)	0.374 (4.2%)	0.997 (6.3%)	0.996 (4.6%)
5	HTG	289	170	0.564 (7.7%)	0.574 (6.4%)	0.985 (8.2%)	0.990 (7.7%)
6	HTG	264	175	0.646 (2.2%)	0.670 (1.4%)	0.968 (18.8%)	0.994 (8.6%)
7	HTG	526	236	0.655 (6.4%)	0.667 (11.9%)	0.963 (6.4%)	0.951 (15.5%)
8	HTG	459	247	0.564 (4.5%)	0.552 (14.6%)	1.005 (7.3%)	0.987 (7.8%)
9	HTG	624	257	0.504 (3.8%)	0.473 (6.5%)	0.996 (7.4%)	0.952 (8.0%)
10	HTG	421	269	0.730 (3.1%)	0.725 (2.7%)	0.984 (7.8%)	0.997 (2.3%)
11	FH Het.	74	200	0.300 (6.7%)	0.293 (13.0%)	0.995 (7.0%)	0.998 (6.8%)
12	FH Hom.	80	616	0.151 (3.3%)	0.151 (4.1%)	0.998 (3.5%)	0.998 (3.5%)

[a] The fractional catabolic rate (FCR) was determined by nonlinear regression and the confidence limit of the estimate is presented in parentheses.

[b] QC/QO represents the ratio of the calculated value predicted by the model to the observed value obtained experimentally. From all of the individual time points, the mean ratio was calculated as well as the standard deviation (in parentheses).

case, thus represents a unique combination of the kinetic parameters that describe the decay curve.

We have shown that for over 30 different LDL radioactivity decay curves, identical estimates for FCR can be derived from a 10-point sampling scheme as compared to the standard 22-point sampling period. Using this 10-point sampling schedule we have demonstrated that, on the average, the FCR values obtained with the reduced sampling protocol would result in an overestimation of less than 3% when compared to values determined with the conventional 22-sample protocol. The reduced sampling schedule described in this report should reduce the time and expense required for the study of LDL apoB turnover, making possible studies in a large number of individuals. The fewer samples required with the proposed protocol should also allow a larger volume of blood samples to be taken at each time point. This increased sample volume is expected to improve the confidence in the data by allowing replicates to be analyzed at each time point. Alternatively, as in the case of LDL turnover study in which only whole plasma is counted for total radioactivity, the larger sample volume should provide adequate radioactivity with a smaller initial injected dose. More importantly, the proposed sampling schedule may lead to a greater patient cooperation since, except for the initial 48-hr sampling period, the remaining 12 days of the study may be continued on an outpatient basis.

In some individuals, in particular hyperlipidemic subjects, it has been suggested that the urine/plasma radioactivity ratio may be a better parameter for irreversible clearance since it reflects heterogeneity in the degradative pathways (15). This heterogeneity is characterized by a declining slope in the urine/plasma ratio after the 3–4-day sample. Thus a limitation in our sampling protocol would be the dependence of this declining slope on only urine data from 3 days (day 6, 10 and 14). However, to-date studies that have systematically compared the two estimates of clearance rates have resulted only in modest differences (10, 11) and further studies involving LDL subfractions would be required to fully characterize the heterogeneity in LDL.

TABLE 3. Regression analysis on the model parameters: comparison of schedule A and schedule D

	Dose	FCR	k(2,1)	k(1,2)
Slope	1.010	1.015	0.994	1.059
r	1	0.993	0.984	0.886
P	0.001	0.001	0.001	0.001
n[a]	23	23	23	23

[a] All 23 subjects studied were included in this analysis. When a subject participated in more than one LDL study, only the data from the baseline period was used. Thus, each individual contributed only one set of data points in this regression analysis.

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