A Comparison of Simplified Methods for Lipoprotein Quantification Using the Analytic Ultracentrifuge as a Standard

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ABSTRACT

Two simplified methods for quantitative lipoprotein analysis have been calibrated and compared with each other using analytic ultracentrifugation as a standard reference procedure. The first method was the Friedewald procedure and the second was an automated agarose gel electrophoresis system. Both procedures offer comparable quantitative lipoprotein analysis with potential for large scale screening purposes at low cost ($4.00-$5.00 per analysis). There were advantages and limitations to both procedures. The Friedwald procedure can be used on frozen sera but requires 3 ml sera. In contrast, the electrophoresis system must be used with fresh serum but requires only 50 µl serum and the electrophoretic slides may be quantitatively analyzed several years retrospectively.

INTRODUCTION

During the past decade the Fredrickson, Levy, and Lees "typing" system has allowed classification of most lipoprotein disorders into six types (1). However, a major limitation of this typing system is that it does not provide quantitative lipoprotein data. Such data provide additional information about coronary heart disease (CHD) risk associated with elevated serum cholesterol (TC) and triglyceride (TG) levels. For example, a moderately elevated cholesterol (230-260 mg/100 ml) may be the result of substantial elevations of either the "atherogenic" LDL (2) or the "non-atherogenic" HDL class. Thus, the major classes of lipoproteins, very low density lipoproteins (VLDL), low density lipoproteins (LDL), and high density lipoproteins (HDL) need to be quantitatively measured in clinical laboratories. Measurement of HDL is particularly important since there is recent evidence to suggest that HDL is inversely related to CHD prevalence and that this relationship is largely independent of total cholesterol and LDL levels (2).

Presently the Lipid Research Clinics (LRCs) measure these lipoproteins by a combination of centrifugation and precipitation procedures (3) to obtain the cholesterol content of the three major lipoprotein classes. However, this method requires 5 ml of serum, and because of the centrifugation step, it is severely limited in the number of samples that can be processed.

The purpose of this study was to evaluate simplified quantitative lipoprotein procedures. A simplified $MnCl_2$-heparin precipitation analysis (4) was compared to the analytic ultracentrifuge, which, in this study, was the lipoprotein standard method. The simplified procedures require only 3.0 ml serum and omits the centrifugal step by estimating VLDL cholesterol directly from the serum value TG/5 (4). For comparison as another potentially useful clinical test, a recently automated lipoprotein electrophoresis system (5) was similarly evaluated.

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TABLE I

Comparison of Analytic Ultracentrifuge Lipoprotein Results with Data from a Modesto Normal Reference Population (6)[a]

Population	HDL	VLDL	LDL
This study (n = 37) age 20-39 yrs	357 ± 86	57 ± 50	354 ± 109
Modesto normals (n = 28) age 25-39 yrs	339 ± 58	43 ± 30	319 ± 74

[a]HDL = high density lipoprotein, VLDL = very low density lipoprotein, LDL = low density lipoprotein.

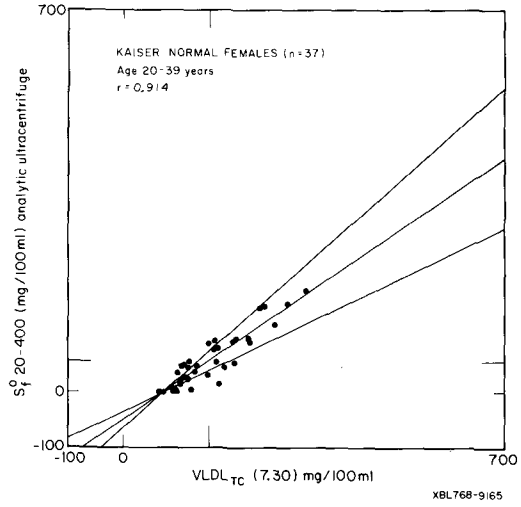

FIG. 1. Comparison of Friedewald very low density lipoprotein (VLDL) data with S_f^0 20-400 analytic ultracentrifuge data.

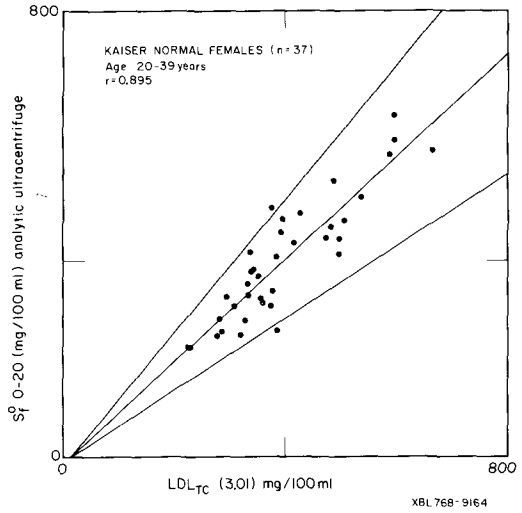

FIG. 2. Comparison of Friedewald low density lipoprotein (LDL) data with S_f^0 0-20 analytic ultracentrifuge results.

METHODS

Lipoprotein analysis was done on serum obtained from 39 fasting volunteer women, aged 20-39 yr, who had multiphasic tests as part of a routine medical examination at the Kaiser Hospital, Walnut Creek, CA (6). Normal, free-living women were selected as subjects since they exhibit a greater range in serum HDL concentration than do men of the same age group (7).

The first method used was the Friedwald procedure (4), as described in detail elsewhere (3). Briefly, 120 µl of heparin solution (5,000 I.U./ml, Riker Laboratories, Northridge, CA) is added to 3 ml serum. After vortex mixing, 150 µl of 1.0M $MnCl_2$ solution is added and again vortexed. After 30 min at 0 C, the VLDL and LDL precipitate was centrifuged at 0 C for 30 min at 1,500 x g. Then a filtrate (Lipo-Frax, Technicraft, San Mateo, CA) was made yielding the HDL containing serum solution for cholesterol analysis. Triglyceride and cholesterol analyses were made by the Technicon AA II procedure (8,9), with satisfactory phase 2 standardization by the Control Disease Center, Atlanta, GA. Lipoprotein concentrations were calculated assuming a mean wt% cholesterol content for VLDL, LDL, and HDL of 14%, 33% and 17%, respectively (10).

Lipoprotein electrophoresis was performed using the Bio-Gram A Lipoprotein Profile Kit (Bio-Rad Laboratories, Richmond, CA) with modifications described earlier (10). Results were internally standardized using both serum triglyceride and cholesterol measurements and

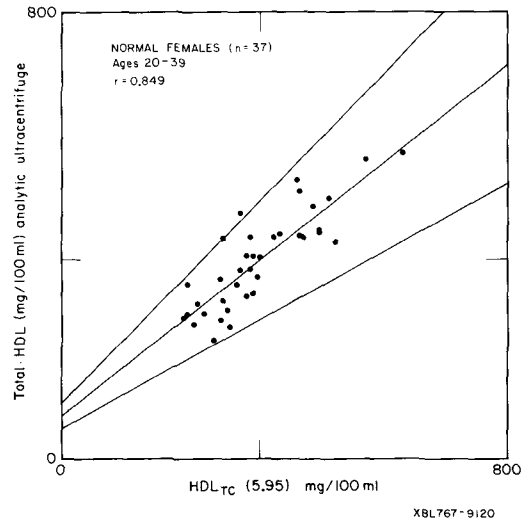

FIG. 3. Comparison of Friedewald high density lipoprotein (HDL) data with $F_{1.20}$ 0-9 analytic ultracentrifuge data.

were independent of the amount of sample (0.75-1.50 µl) applied to the gel. VLDL, LDL, and HDL concentrations were calculated from the relative dye uptake factors, the mean wt% content of TG and TC in each lipoprotein class, and the serum total TG and TC values. The hardware consisted of a densitometer, an analog to digital converter, a cathode ray tube terminal (with connection to a large computer), a teleprinter and a small computer. Full details of this automated microdensitometry facility have

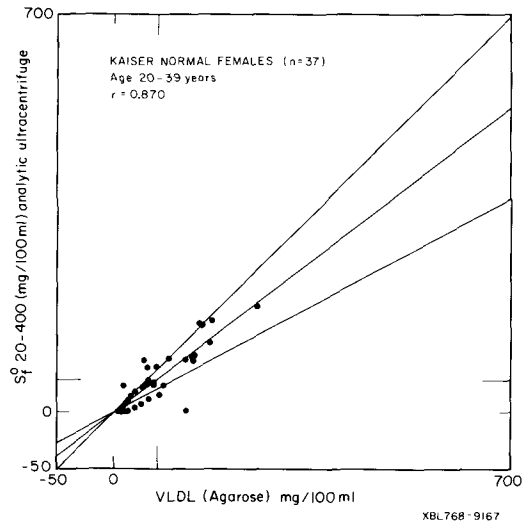

FIG. 4. Comparison of agarose gel electrophoretic pre-beta very low density lipoprotein (VLDL) data with S_f^0 20-400 analytic ultracentrifuge results.

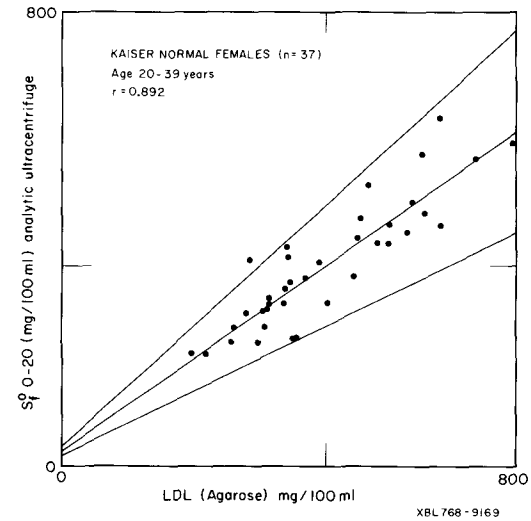

FIG. 5. Comparison of agarose gel electrophroretic beta low density lipoprotein (LDL) data with S_f^0 0-20 analytic ultracentrifuge results.

been given elsewhere (5). Both of the above simplified procedures were compared and calibrated using analytic ultracentrifugation (11) as a standard reference procedure.

RESULTS

Quantitative lipoprotein determinations were obtained using each of the three procedures: complete analytic ultracentrifugation, quantitative agarose gel electrophoresis, and by

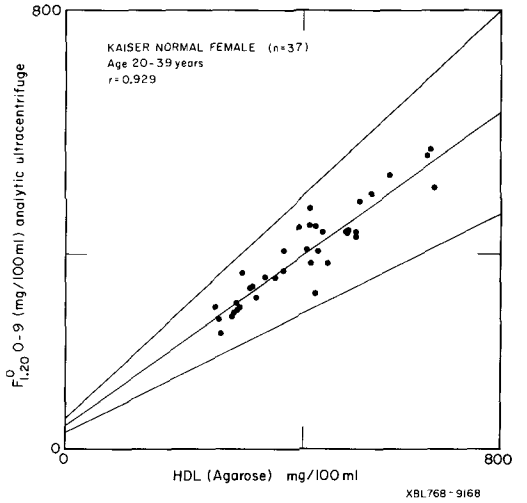

FIG. 6. Comparison of agarose gel electrophoretic alpha high density lipoprotein (HDL) data with $F_{1.20}^0$ 0-9 analytic ultracentrifuge data.

the Friedewald $MnCl_2$-heparin precipitation procedure. Analytic ultracentrifuge results, although somewhat lower for LDL in this study, are given in Table I and were not significantly different from a normal reference population studied earlier (7).

Comparison of the Friedewald-$MnCl_2$-heparin procedure with analytic ultracentrifugation is given in Figures 1, 2, and 3. Similar comparison of automated agarose electrophoresis is given in Figures 4, 5, and 6. Correlation coefficients for all data were 0.849, 0.914, and 0.895 for HDL, VLDL, and LDL, respectively, for the Friedewald proceudre. Analogous correlation for electrophoresis were 0.929, 0.870, and 0.892, respectively. Thus, comparable quantitative lipoprotein data may be expected from these two simplified procedures. Table II gives a more detailed comparison of the two methods, including regression formulae for both procedures allowing calculation of equivalent ultracentrifuge data. Also, comparison of the error of measurement in estimating the assumed true value, i.e., the analytic ultracentrifuge results, is given by Sy.x. Although agarose provides a somewhat better estimation for HDL, no definitive advantage in accuracy is provided by either method.

Evaluation of the frozen standard by the electrophoretic procedure is possible only for a standard very low in VLDL (10). For such a standard over a period of 1 yr with 24 analyses, the mean and SD values for LDL and HDL were 420 ± 33 and 301 ± 24, respectively. In contrast, no such restrictions are involved in a frozen standard for the Friedewald procedure.

TABLE II

Comparison of Electrophoretic and $MnCl_2$-Heparin Procedures with Analytic Ultracentrifugation[a]

Variable	Mean ± SD	r	$S_{y \cdot x}$	b	a
Agarose HDL	438 ± 111	0.929	33	43	0.717
Agarose VLDL	77 ± 57	0.870	26	2	0.770
Agarose LDL	466 ± 138	0.892	51	27	0.702
$MnCl_2$-Hep. HDL[b]	355 ± 95	0.849	47	78	0.785
$MnCl_2$-Hep. VLDL[c]	158 ± 67	0.914	21	−51	0.686
$MnCl_2$-Hep. LDL[d]	398 ± 106	0.895	50	−21	0.920

[a]y = b + ax, where y = Analytic Ultracentrifuge Lipoprotein Values. HDL = high density lipoprotein, VLDL = very low density lipoprotein, LDL = low density lipoprotein.

[b]HDL = 5.95 x HDL_{TC}.

[c]VLDL = Serum TG/5 x 7.30.

[d]LDL = (Serum TC - HDL_{TC} - TG/5) 3.01.

A typical standard analyzed 36 times over a 9 day period gave 304 ± 16, 149 ± 14, and 341 ± 48 for HDL, VLDL, and LDL, respectively.

DISCUSSION

Two methods for quantitative lipoprotein analysis have been calibrated and compared. Each procedure required 50 μl-3 ml of serum, in addition to that needed for serum triglyceride and cholesterol analysis. Both methods gave comparable accuracy and reproducibility for VLDL, LDL, and HDL. Advantages of the Friedewald type procedure were the capability for analyzing frozen serum samples after prolonged storage as well as a single precipitation step for all samples with TG < 400 mg/100 ml. The disadvantage of this procedure was the necessity of performing accurate cholesterol analyses on HDL containing solutions whose cholesterol concentrations were a fraction of the total serum value.

The automated lipoprotein electrophoresis also provided satisfactory quantitative data for VLDL, LDL, and HDL. Two advantages of this method are the capability of analysis on 50 μl or less of serum if enzymatic TG and TC lipid analyses are performed (12). Secondly, the agarose slides are stable for several years, allowing convenient analysis (or re-analysis) at any future time.

Perhaps the main alternatives to the above procedures would be the quantitative lipoprotein measurement now utilized by the Lipid Research Clinics (3). However, only limited numbers of samples can be done by this centrifugation and $MnCl_2$-heparin precipitation procedure. One technician, with the needed preparative ultracentrifuge, can handle only some 36 analyses per week. The estimated cost per complete analysis is $10.00. Furthermore, this procedure requires 5 ml of serum exclusive of that needed for the total serum TG and TC measurements. On the other hand, using the Friedewald procedure and requiring 3 ml serum, one technician can process 85 samples per week with an approximate cost of $4.00 per analysis (exclusive of the serum TC and TG determinations). By comparison, one technician using the automated electrophoresis system, and requiring as little as 50 μl of serum, can process ca. 250 samples per week (exclusive of the serum TC and TG determinations). A rough estimate of this cost (also exclusive of the lipid determinations) is ca. $5.00 per analysis. Considering the above, the two simplified procedures would appear potentially to offer distinct advantages over the LRC procedure in cost, serum volume requirements, and large scale screening capability.

ACKNOWLEDGMENTS

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