Enzyme-linked immunosorbent assay for human plasma apolipoprotein B

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Abstract A noncompetitive enzyme-linked immunosorbent assay (ELISA) has been developed for measuring total plasma apolipoprotein (apo) B using affinity purified polyclonal and monoclonal antibodies. Microtiter plates from different manufacturers were tested with regard to their IgG binding characteristics; only one plate yielded consistent coefficients of variation of less than 5%. The optimal plasma dilution in this assay was 1:3000. IgG anti-apoB antisera conjugated to alkaline phosphatase was used as a second antibody. *p*-Nitrophenyl phosphate was utilized as substrate for color development, and the absorbance (410 nm) was read utilizing an ELISA reader interfaced with a microcomputer for data processing. Plasma apoB levels in plasma have been determined in 1115 male and female participants in the Framingham Offspring Study. Mean (± SD) plasma concentrations were 89 ± 28 mg/dl. Significant age and sex related differences in apoB levels were noted.—**Ordovas, J. M., J. P. Peterson, P. Santaniello, J. S. Cohn, P. W. F. Wilson, and E. J. Schaefer.** Enzyme-linked inmunosorbent assay for human plasma apolipoprotein B. *J. Lipid Res.* 1987. **28:** 1216–1224.

Supplementary key words coronary artery disease • lipoproteins • radial immunodiffusion

Low density lipoproteins (LDL) as isolated from human plasma in the density region 1.019–1.063 g/ml are particles that contain (weight percent) approximately 25% protein, 40% cholesterol, 25% phospholipid, and 10% triglyceride (1). Apolipoprotein (apo) B-100 is the major protein constituent of LDL comprising over 95% of LDL protein mass. ApoB also accounts for about 30% of the protein present in very low density lipoproteins (VLDL). Increased levels of plasma LDL cholesterol have been associated with an enhanced risk for premature coronary artery disease (CAD) (2, 3). It has been suggested that apoB may be a better predictor for premature CAD than LDL cholesterol (4). The quantitation of apoB allows one to identify those individuals who have elevated apoB levels despite having normal LDL cholesterol levels (hyperapobetalipoproteinemia) (5). These latter subjects are at increased risk for premature CAD (5).

A number of methods have been utilized for quantitation of apoB: radial immunodiffusion (RID), electroimmunoassay (EIA), radioimmunoassay (RIA), and nephelometry (6–12). All of these methods have technical problems that make them somewhat undesirable for use as clinical assays. More recently, several publications have appeared describing apoB measurements based on the use of enzyme-linked immunosorbent assays (ELISA) (13–16). Our purpose in this study was to develop a sensitive, reproducible, and easily performed assay for plasma apoB levels with ELISA methodology utilizing immunopurified polyclonal antibodies. Unlike previously reported assays, our assay requirements included having consistent between-run coefficients of variation of less than 5%. Our assay was optimized to achieve this goal, as well as being standardized with reference material obtained from the Centers for Disease Control, Atlanta, GA. Moreover, this assay has been applied to a large prospective population study of coronary artery disease risk factors (The Framingham Offspring Study), and normal ranges for plasma apoB levels based on 1115 subjects are presented.

METHODS

Subjects

Blood samples were obtained after a 12–14 hr fast from males (n = 536) and females (n = 579), age range 20–71 years, participating in the Framingham Offspring Study. Blood was drawn into tubes containing 0.1% EDTA. The plasma was isolated by centrifugation at 4°C and ana-

Abbreviations: ELISA, enzyme-linked immunosorbent assay; apo, apolipoprotein; LDL, low density lipoproteins; VLDL, very low density lipoproteins; HDL, high density lipoproteins; CAD, coronary artery disease; RID, radial immunodiffusion; EIA, electroimmunoassay; RIA, radioimmunoassay.

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lyzed. Samples that could not be analyzed within 3 days after the phlebotomy were aliquoted in Nunc cryotubes and frozen immediately at −70°C for subsequent analysis.

Isolation of lipoprotein fractions

Human LDL (d 1.030–1.050 g/ml) was isolated by preparative ultracentrifugation from pooled plasma obtained from fasting individuals. Plasma density was adjusted to 1.030 g/ml using KBr. After centrifugation for 18 hr at 39,000 rpm at 4°C in a Beckman L5-80 ultracentrifuge using a Beckman 60 Ti rotor, the infranatant was collected and adjusted to density 1.050 g/ml using KBr. After centrifugation at 45,000 rpm for 18 hr, the top fraction was collected and recentrifuged as before. The LDL recovered in the top fraction was dialyzed overnight at 4°C against 0.85% NaCl, 100 mM EDTA, pH 7.4. LDL preparations contained only apoB in their protein moiety as assessed by 4–22.5% polyacrylamide gradient SDS gel electrophoresis (SDS-PAGE). Other lipoprotein fractions, very low density lipoproteins (VLDL), and high density lipoproteins (HDL) were isolated as previously described (17).

Preparation of antibody

Isolated LDL was used to prepare apoB antibodies by immunization in goats (injections of 5–10 mg of LDL protein with complete and incomplete Freund's adjuvant injected four times over a 4-month period). The specificity of the antisera was assessed by double radial immunodiffusion and two-dimensional immunoelectrophoresis. The IgG fraction was obtained by precipitation with saturated ammonium sulfate. Briefly, two volumes of antibody were precipitated with one volume of the saturated solution of ammonium sulfate. The precipitate was recovered by centrifugation in a Beckman J2-21 centrifuge at 15,000 rpm for 15 min. The pellet was redissolved in half the original volume of antibody utilizing a solution containing borate buffer (0.125 M, pH 8.2) and sodium chloride, 0.15 M. The solution was dialyzed overnight against the same buffer containing 0.1% sodium azide. Monospecific antibodies against apoB were obtained by passing the IgG fraction through an affinity column made by coupling LDL to cyanogen bromide-activated Sepharose 4B (18). After the nonspecific IgG fraction was eluted, the column was washed with 0.1 M borate buffer, pH 8.1, 1 M NaCl, 0.1% Tween 20, until the absorbance (280 nm) of the eluate was less than 0.02. The adsorbed antibodies were eluted with 1 M glycine buffer, pH 3.0, and dialyzed immediately against phosphate-buffered saline (PBS; 8.0 g NaCl, 1.15 g Na_2HPO_4, 0.12 g KH_2PO_4 dissolved in 1 liter). Monoclonal antibodies specific for apoB-100 (B-3) were obtained from RIA Inc. (Scarborough, Canada).

Preparation of antibody-enzyme conjugate

Immunopurified apoB antibodies were used for the preparation of the conjugate. One mg of antibody was mixed with 2,000 units of alkaline phosphatase (Sigma) and dialyzed against PBS. Glutaraldehyde solution (25%) was added to a final concentration of 0.2%. The reaction was carried out at room temperature and in the dark for 2 hr; thereafter the mixture was dialyzed against Tris buffer (0.05 M, pH 8.0) for 2 days and stored at 4°C. The conjugate was stable for up to 6 months.

ELISA procedure

Polystyrene microtiter plates (Nunc Immunoplate I, Nunc, Denmark) were coated with 200 μl of affinity-purified polyclonal (5 μg/ml) or monoclonal (3.9 μg/ml) apoB antibody in 0.2 M sodium bicarbonate buffer, pH 9.6. Other plates tested with our procedure included Immulon II (Dynatech, Vienna, VA), Falcon (Falcon Inc.), Linbro Titertek and EIA (Flow Laboratories), and Corning. The plates were covered with an acetate plate sealer (Flow Laboratories), and incubated overnight at 4°C. The next day the solution containing the unbound antibodies was removed and the remaining binding sites in the plate were blocked by incubating for 5 min with 0.5% bovine serum albumin (RIA grade BSA, Sigma) in PBS. Plates were washed twice with PBS containing 0.05% Tween-20, dried by blotting, sealed, and stored at 4°C in a desiccator until use. Plates are stable for up to 4 weeks.

Control and plasma samples were diluted 1:3000 in PBST-BSA (PBS containing 0.5% BSA and 0.05% Tween-20). Twofold serial dilution was performed for the plasma standard (1:375 to 1:24,000). A secondary plasma standard calibrated against the lyophilized standard provided by the Centers for Disease Control (CDC) (Atlanta, GA) standardization program was utilized. Control samples were obtained from Hyland (Omega and Omega III), or prepared in our laboratory by pooling plasma from different individuals and storing multiple aliquots at −70°C. All the controls were calibrated against the standard supplied by the CDC. Two hundred-μl aliquots of standards, control samples, and plasma samples were added to designated wells in the microtiter plate, after prior dilution and thorough mixing, immediately before the assay. Plasma samples were run in triplicate; five different plasma controls were run in each plate in duplicate or triplicate depending on wells available in the plate. The plate was sealed and incubated at room temperature for 18 to 20 hr. The contents of the plates were then emptied and the wells were washed three times with PBST.

The goat immunopurified apoB antibody conjugated to alkaline phosphatase was diluted in PBST-BSA at a pre-

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determined optimal concentration (1:350), and 200 μl was added to microtiter plate wells. The plate was covered with acetate plate sealer and incubated for 5 hr at room temperature. Thereafter the plate was emptied and wells were washed three times with PBST-BSA. The substrate for the enzymatic color reaction was a 0.1% solution of *p*-nitrophenyl phosphate in 0.1 M glycine buffer. Two hundred μl of this solution was added to each well in the plate. Substrate addition was carried out in a row-by-row fashion at 15-sec intervals. After 20 min at room temperature, the color reaction was stopped by adding 50 μl of 1 M NaOH to all wells, following the same pattern described above (row-by-row, 15-sec intervals). To minimize variability in our assay, we used an automated pipetting system for the various assay steps (Cetus Propette, Emeryville, CA). The plate was placed in a microtiter plate shaker for 10 min. Thereafter the plate was read at 410 nm on a microtiter plate reader (Dynatech MR 600, Dynatech Inc., Vienna, VA). The plate reader was interfaced with an IBM XT microcomputer and the data were analyzed with the Immunosoft program (Dynatech).

RESULTS

Specificity of antisera

Following immunopurification, polyclonal antisera directed against plasma LDL were tested for specificity by radial immunodiffusion, Western blotting analysis, ELISA, and two-dimensional immunoelectrophoresis utilizing total plasma, human albumin, apoA-I, and isolated VLDL (d < 1.006 g/ml), LDL (d 1.030–1.050 g/ml), and HDL fractions (d 1.10–1.21 g/ml). Immunoreactivity was only detected for whole plasma, VLDL, and LDL and gave single immunoprecipitation bands. Western analysis of Lp(a) demonstrated lack of crossreactivity of our antibody with apoLp(a). By ELISA, sample dilution yielded parallel curves for apoB concentrations in plasma, VLDL, and LDL (**Fig. 1**). No color reaction above background was noted with HDL (d 1.10–1.21 g/ml) or human albumin at physiologic concentrations.

Microtiter plates and assay conditions

When LDL was initially bound to the plate, significant variability in binding between LDL from different individuals was noted. This variability resulted in poor coefficients of variation for the assay. For this reason the assay was set up by first coating antibody to the plate. Another source of variability was found to be due to variable plate characteristics. **Table 1** shows the mean, standard deviation, and coefficient of variation obtained in the different plates assayed. Three different plates were tested per each one of the makers, and 39 replicates were run in each plate. Plasma samples used varied for plates of different

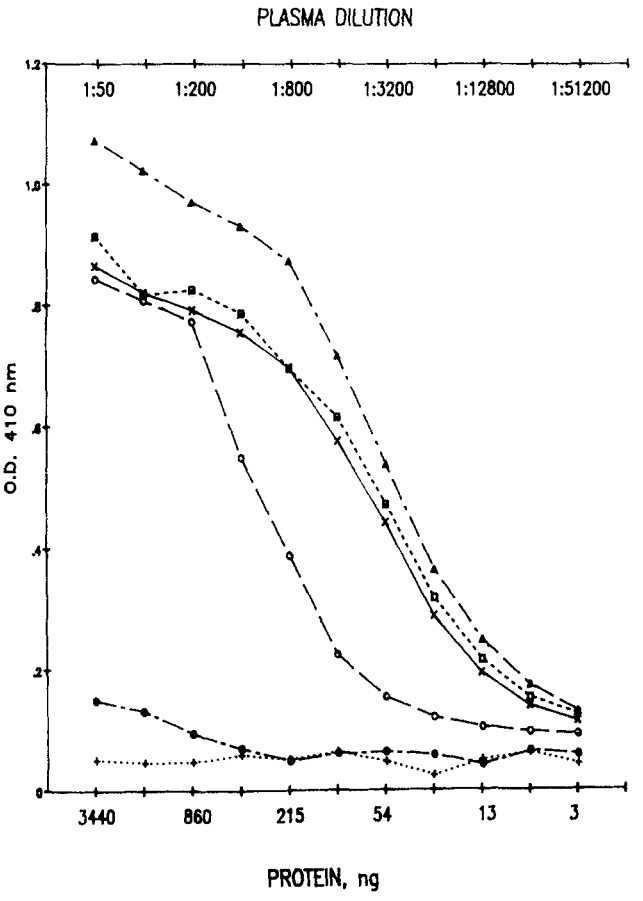

Fig. 1. Calibration curves developed with frozen plasma (—▲—), lyophilized serum (---□---), isolated LDL, d 1.03–1.05 g/ml (—X—), isolated VLDL (— —○—), human serum albumin (• • • + • • •), and isolated HDL, d 1.1–1.2 g/ml (—●—). Various dilutions (200 μl) were incubated overnight with microtiter wells coated with immunopurified polyclonal anti-apoB IgG, and processed as indicated in the Methods section.

makers. Variability at the edge of plates and across plates was noted (**Fig. 2**). In our experience, microtiter plates obtained from Dynatech (Immulon II), Falcon, Limbro (Titertek and EIA), and Corning did not provide adequate well-to-well reproducibility in terms of our assay (coefficient of variation ranging between 3.5 and 10.9%. In contrast, plates obtained from Nunc (Immunoplate I) gave consistently reliable results in all wells (coefficient of variation under 2.5%).

Assay reliability and calibration

No increase in the amount of plasma apoB, detected by ELISA, was noted after heating plasma at either 56°C or 37°C for 2 hr, or by prior incubation with bacterial lipase (Calbiochem) or by adding a larger amount of detergent (0.125% Tween-20) to the sample dilution buffer. Our assay was calibrated with standards obtained from the Centers for Disease Control, Atlanta, GA. The results obtained were in excellent agreement (within 2 mg/dl) with

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TABLE 1. Comparison of different plates for uniform antibody binding[a]

Plates	Concentration	
	Mean ± SD	CV
	mg/dl	
Falcon assay plates (flat bottom)	82.3 ± 5.2	6.3
Linbro, Titertek (round bottom)	50.5 ± 3.0	5.9
Linbro, EIA (flat bottom)	59.4 ± 6.5	10.9
Dynatech, Immulon II (round bottom)	77.7 ± 3.6	4.9
Corning Cell wells (round bottom)	74.1 ± 2.6	3.5
Nunc Immunoplate I (flat bottom)	76.5 ± 1.8	2.3

[a]Mean, standard deviation (SD), and coefficient of variance (CV) of three plates containing 39 replicates per plate. Plasma samples varied from one plate maker to another.

standardization utilizing a protein quantitation of isolated LDL (d 1.030–1.050 g/ml) which contained only apoB-100 on PAGE. To determine the optimal concentration of antibody to use for initial coating of microtiter plates, varying concentrations of apoB immunopurified antisera (0.25–32 μg/ml) were tested using 1:30 dilutions of human plasma. The optimal coating concentration for our immunopurified polyclonal apoB antisera was 5.0 μg/ml (see **Fig. 3**). The optimal sample dilution was determined using the standard curves shown in Fig. 1. The concentration of plasma corresponding to the middle portion of the linear range of the curve was considered as optimal. This dilution was 1:3000 for both plasma and the LDL fraction. Five different plasma control samples were used in duplicate or triplicate in each plate to calculate within-assay as well as between-assay coefficients of variation. The mean values and within-assay coefficient of variation for the different controls from 20 plates run on different days were: 64.3 mg/dl (0.76%), 65.8 mg/dl (0.74%), 50.8 mg/dl (0.95%), 42.8 mg/dl (1.44%), and 72.1 mg/dl (0.79%). Between-assay coefficients of variation were: 3.8%, 4.1%, 4.8%, 4.1%, and 3.6%. Controls were used to correct the concentration of the plasma samples measured in the plates. Samples with a within-assay coefficient of variation higher than 4% were repeated.

Comparison with LDL cholesterol and RID values

A comparison of plasma apoB concentrations obtained by ELISA and radial immunodiffusion (n = 340) yielded a correlation coefficient of 0.743 ($P < 0.001$) (**Fig. 4**). As a whole group, the mean values (apoB ELISA = 91.5 mg/dl, apoB RID = 91.9 mg/dl) were not statistically different as determined by paired *t*-test ($P > 0.1$). In subjects with apoB less than 100 mg/dl (as determined by ELISA), the difference between the means (apoB ELISA = 76.7 mg/dl, apoB RID = 82.0 mg/dl) was significant ($P < 0.001$, paired *t*-test). Conversely, for subjects with plasma apoB values of 100 mg/dl or more, ELISA gave significantly higher values than RID (126 mg/dl versus 115 mg/dl, $P < 0.001$, paired *t*-test). The regression analysis resulted in the following equation: ELISAB = 15.11 + 0.8315 RIDB. The coefficients of variation for the RID for a mean value of 91.87 were 5.2% and 10.5% for within-assay and between-assay, respectively, in samples run in triplicate in ten different assays. ApoB values obtained by ELISA also correlated highly with total cholesterol ($r = 0.701$, $P < 0.001$) and LDL cholesterol ($r = 0.730$, $P < 0.001$).

Comparison of apoB results utilizing fresh and frozen plasma samples

A comparison of values of plasma apoB obtained from fresh samples as compared to aliquots stored over a 6-month period at −70°C (n = 42, analyzed in triplicate) yielded very similar results (98.2 ± 3.5 mg/dl fresh, frozen

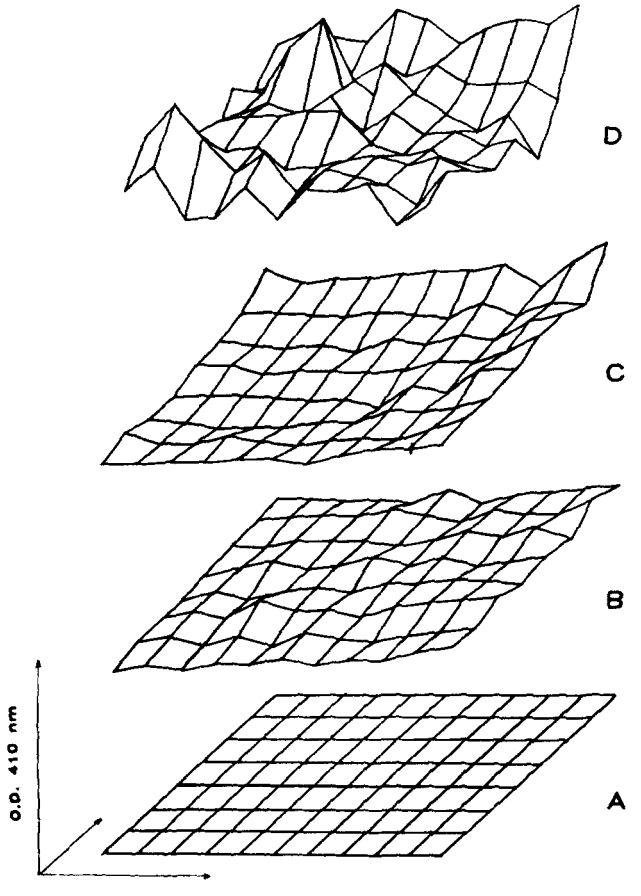

Fig. 2. Three-dimensional representation of the variability of antibody coating to different plates. Plates from different manufacturers were coated in identical conditions, and 200 μl of the same plasma was added to each of the wells of the different plates. The ELISA procedure was performed as described in the Methods section and the absorbance readings were plotted on the Z axis for each one of the wells. Panel A: Nunc Immunoplate I with no sample added; panel B: Nunc Immunoplate I with sample added; panel C: Corning with sample added; panel D: Linbro EIA with sample added.

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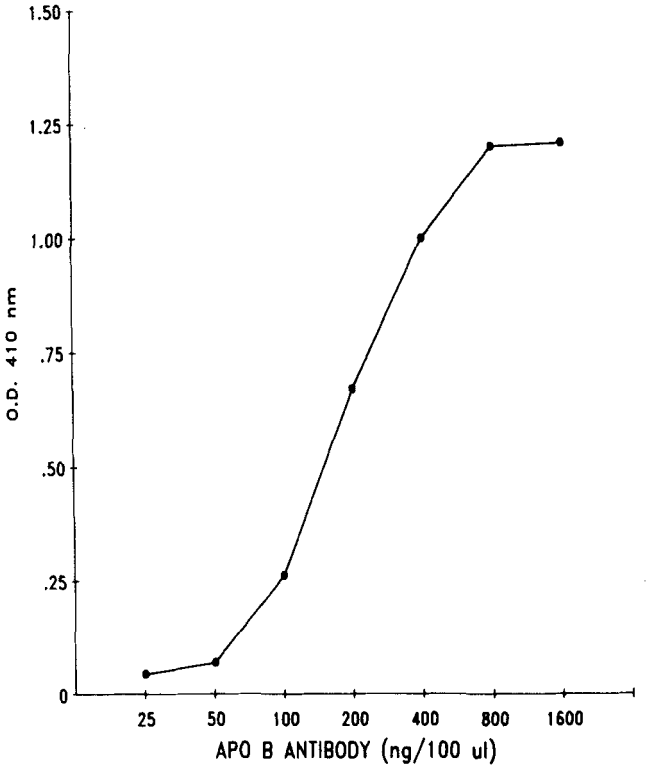

Fig. 3. Titration of the amount of antibodies bound to the microtiter plate (Immunoplate I). Different dilutions of the immunopurified polyclonal anti-apoB antibody (200 μl) were added to the plate and incubated overnight. An excess of human plasma (1:30 dilution) was used to carry out the standard ELISA procedure.

for 6 months 102.1 ± 14.0 mg/dl, and 3 months 99.5 ± 13.0 mg/dl). Different storage conditions were also assayed in one sample analyzed in triplicate utilizing a shorter period of time **(Fig. 5)**. Storage at 4°C or −20°C resulted in lower values of apoB with time. The addition of a protease inhibitor (trypsin inhibitor, Type II-S, Sigma) to the samples stored at −20°C resulted in a higher recovery of the apoB measured after 52 days, but the values were still significantly below the initial readings. Only sample storage at −70°C allowed us to obtain similar results in the fresh state, and after 52 days, 3 months, and 6 months of storage ($P < 0.05$ paired t-test analysis).

Reference values

The mean (± SD) plasma concentration of apoB obtained by ELISA in 1115 male and female participants in the Framingham Offspring Study (ages 20–71 yr) was 89 ± 28 mg/dl. The mean (± SD) of cholesterol in LDL for the same population was 133 ± 37 mg/dl. The distribution of apoB and LDL cholesterol levels for the population are shown in **Fig. 6** and **Fig. 7**. There was increased skewness observed for the apoB distribution (0.93) (Fig. 6) as compared to the skewness for the LDL cholesterol distribution (0.40) (Fig. 7). Mean apoB plasma concentrations by age and sex are presented in **Table 2.** Our data indicate that apoB values were significantly correlated with age ($r = 0.24$, $P < 0.01$), and that males had consistently higher apoB concentrations ($P < 0.01$, t-test

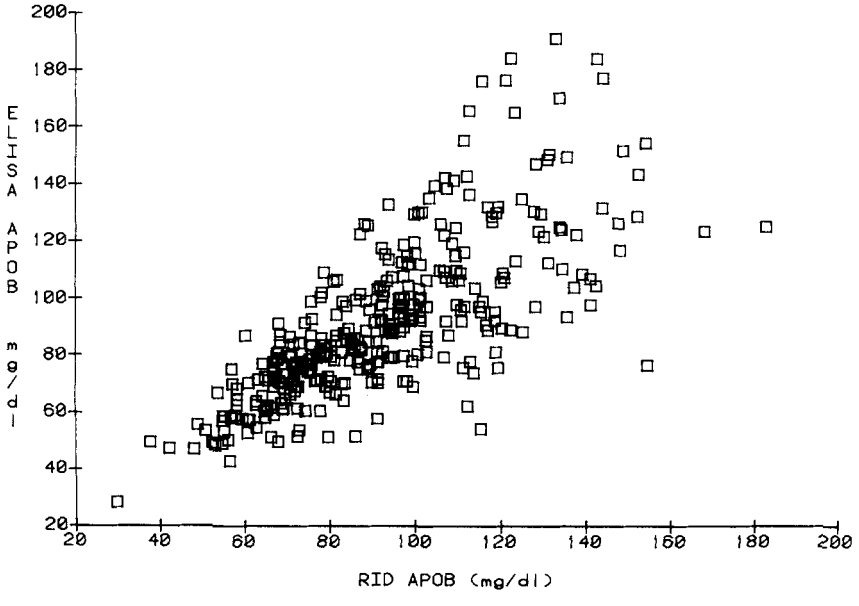

Fig. 4. Comparison of apoB values measured by ELISA with apoB values measured by radial immunodiffusion (RID) in 340 plasma samples. The Pearson's correlation coefficient was $r = 0.743$, and the regression equation was ELISAB = 15.11 + 0.831 RIDB.

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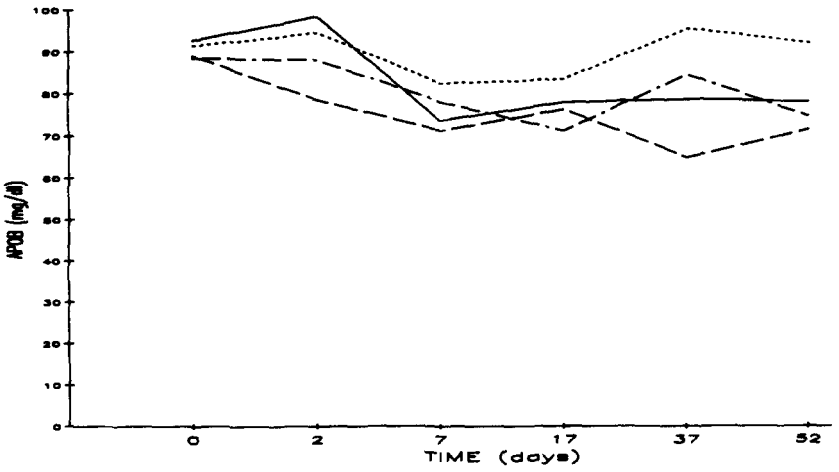

Fig. 5. Effect of different storage conditions on the apolipoprotein B levels measured by ELISA; (— —) 4°C; (-- • --) – 20°C; (———) – 20°C + proteinase inhibitor; (-----) – 70°C.

analysis) than females at ages up to 65 years. Subjects with triglyceride values below 180 mg/dl (mean 91 ± 37) had significantly lower ($P < 0.01$) apoB values (84 ± 24 mg/dl) than subjects with plasma triglycerides over 180 mg/dl (mean 289 ± 166) with a mean apoB concentration of 115 ± 29 mg/dl. The ratios of LDL-cholesterol to apoB for these two groups were, respectively, 1.56 and 1.23, which indicates an increased contribution of apoB VLDL to total plasma apoB in hypertriglyceridemic individuals, and probably a change in the lipid composition of LDL. To determine whether the concentration of apoB in hypertriglyceridemic subjects was under- or overestimated, different amounts of VLDL containing known amounts of apoB were added to plasma samples (n = 12), and apoB was measured by ELISA. The measured apoB in these samples was 98.0 ± 3.4% of the calculated apoB based in the individual concentrations of apoB in the plasmas and in the VLDL fractions.

The values presented in this study are in the same range as data presented in previous studies in various Caucasian populations using different methodologies (**Table 3**). Differences in the ratio of LDL cholesterol to

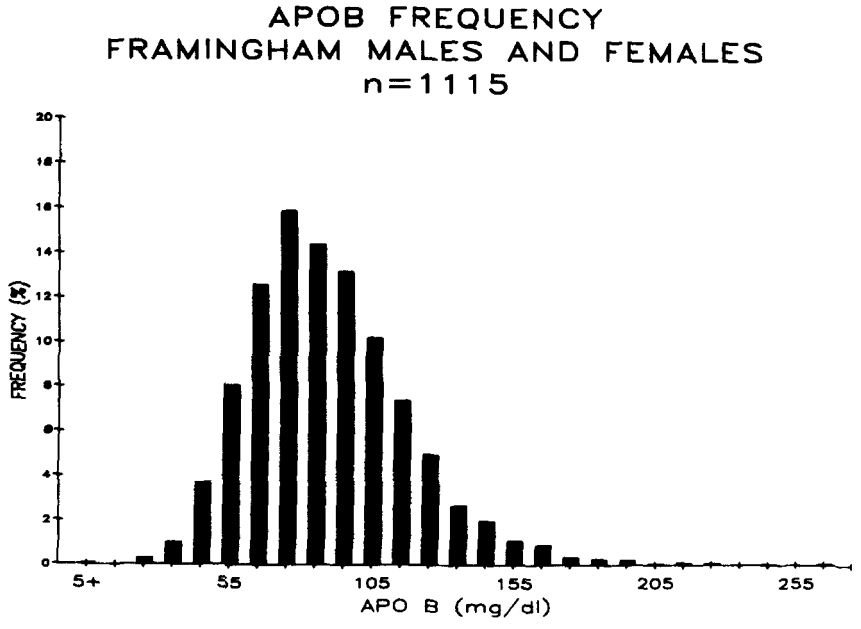

Fig. 6. Frequency of distribution of plasma apoB levels (mg/dl) in 1115 males and females participating in the Framingham Offspring Study.

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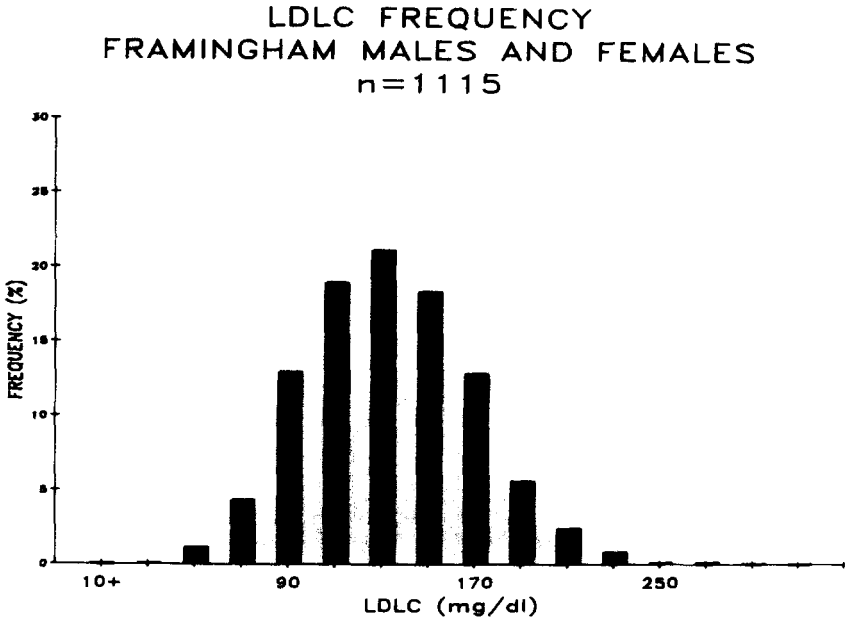

Fig. 7. Frequency of distribution of plasma LDL-cholesterol levels (mg/dl) in 1115 males and females participating in the Framingham Offspring Study.

apoB were noted in these studies (Table 3), which could be due to variability in LDL composition, or plasma triglyceride levels (VLDL-apoB) in various populations, or to differences in methods.

DISCUSSION

Determinations of plasma apoB levels, as well as concentrations of apolipoproteins apoB-100 and apoB-48, will probably become important diagnostic tests in the future in the assessment of premature coronary artery disease risk. ApoB-48 appears to represent a fragment of apoB-100, so that monoclonal antibodies directed specifically at apoB-48 cannot be made. An indirect assessment of apoB-48 levels can be made by calculating the difference between total apoB and apoB-100. In this report we describe the development of a sensitive, specific, and reproducible technique utilizing enzyme-linked immunoassay for determination of total plasma apolipoprotein B. We have also developed an identical procedure utilizing monoclonal antibodies specific for apoB-100 (data not shown) that yields very similar results for plasma apoB levels. With the use of an assay for total apoB and apoB-100 only, one can theoretically quantitate apoB-48 by difference in triglyceride-rich lipoproteins. However, in practice this is not reproducible because of the small amount of apoB-48 present and the error in de-

TABLE 2. Plasma apolipoprotein B levels in the Framingham Offspring Study[a]

Age Group	Males	Sample Size	Females	Sample Size
yr				
20–24	81.1 ± 21.1	7	74.9 ± 27.7	2
25–29	84.9 ± 19.5	20	80.4 ± 20.3	11
30–34	90.1 ± 28.2	39	80.8 ± 19.4	38
35–39	98.2 ± 23.9	69	83.4 ± 22.3	77
40–44	101.5 ± 29.7	85	84.4 ± 20.0	88
45–49	105.0 ± 28.4	82	86.2 ± 25.0	88
50–54	107.4 ± 22.8	75	90.0 ± 24.7	98
55–59	109.3 ± 22.9	81	100.8 ± 27.2	89
60–64	112.7 ± 31.2	62	106.2 ± 31.9	70
65–69	101.5 ± 37.0	13	107.0 ± 29.4	13
> 70	110.5 ± 67.5	3	98.6 ± 28.5	5

[a]Values given in mg/dl as means ± SD; n = 1115.

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TABLE 3.

Reference	Country	Number	Sex	Age[a]	LDL-C	ApoB	LDL-C/ApoB	Method
					mg/dl	*mg/dl*		
3	Italy	40	M,F	40–69	116 ± 28	100 ± 32	1.16	EIA
20	USA	37	M	38 ± 8	114 ± 30	117 ± 35	0.97	EIA
20	USA	35	M	56 ± 5	129 ± 27	121 ± 34	1.07	EIA
21	Italy	89	M	40–60	149 ± 42	125 ± 31	1.19	EIA
22	Sweden	29	M	40–60	158 ± 29	72 ± 16	2.19	EIA
23	UK	116	M,F	20–70	150 ± 12	72 ± 2	2.08	EIA
24	USA	349	M,F	20–65	120 ± 31	81 ± 19	1.48	RIA
5	Canada	30	M,F	49 ± 5	112 ± 30	82 ± 15	1.37	RID
16	USA	20	M,F		111 ± 30	82 ± 22	1.35	ELISA
Our study	USA	1115	M,F	20–71	133 ± 37	89 ± 28	1.48	ELISA

[a]Age is expressed in range or mean ± SD. Abbreviations: EIA, electroimmunoassay; RID, radial immunodiffusion; RIA, radioimmunoassay; ELISA, enzyme-linked immunosorbent assay; LDL-C, cholesterol in low density lipoproteins.

termining total apoB and B-100. Therefore, gel electrophoresis methodology will have to continue to be used.

In the development of these assays, we found that there is only one commercially available plate that gives within-assay coefficients of variation of less than 3% (Immunoplate I, Nunc). The variability in binding characteristics of plates from different manufacturers has been previously discussed (19), however variation of the adsorption of IgG in different regions of the same plate has not received major attention in previous studies. In this report we demonstrate that the choice of the plate is an important factor to achieve the low coefficients of variation necessary for this type of assay. In addition, in our experience the binding of LDL to these plates was not reproducible, so that the assay had to be set up as a non-competitive assay, by first binding antibody to the plate. Moreover, immunopurification of antisera by affinity column chromatography was found to be essential for low assay coefficients of variation. Utilizing the procedures described, we now uniformly obtain between-run coefficients of variation of less than 5% for determinations of plasma apolipoprotein B levels.

Values obtained for plasma apoB levels utilizing our assay are in the same range as those previously reported utilizing other techniques (3, 5, 16, 20–24). The standardization of the different methods of apolipoprotein quantitation has been the subject of a number of studies (25, 26). We have compared the present ELISA methodology with RID. The RID method is known to be strongly influenced by the size of the particle in which the apoB is carried, resulting in underestimation of total apoB due to the impaired migration of VLDL into agarose gel. Our data indicate that the ELISA is not affected in the same measure, as demonstrated by the high recovery (98%) of apoB measured in samples made hypertriglyceridemic by addition of VLDL, as well as by the higher ratio of apoB to cholesterol in hypertriglyceridemic individuals, probably due to increased apoB VLDL, and finally the significant difference between ELISA and RID in samples with high apoB and a higher frequency of hypertriglyceridemic samples. In our hands, the correlation between ELISA and RID was statistically significant. However, the correlation was only at the level of 0.743. The apoB and LDL cholesterol distributions in our population indicate that LDL cholesterol has a more normal distribution with less skewness than that observed for plasma apoB. The correlation between LDL cholesterol and apoB was 0.70. Considering that most of the subjects were normotriglyceridemics, these data support the concept of significant variation in the composition of LDL particles in different individuals as previously reported (5). Reference ranges for apoB based on 1115 individuals from the Framingham Offspring Study are provided. To our knowledge, these data represent the largest population studied with such an assay. The data presented indicate that older individuals have higher levels of apoB than younger subjects, and that males have higher apoB levels than females up to age 65 years. The data reported demonstrate that enzyme-linked immunosorbent assays can provide sensitive and reproducible methodology for determining plasma apolipoprotein B concentrations in population studies.

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