

A new micromethod for routine measurement of serum LDL-apolipoprotein B

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Summary A new method for low density lipoprotein (LDL) (d 1.019–1.063 g/ml)-apolipoprotein B (apoB) determination has been developed, based on the fact that very low density and intermediate density lipoproteins (VLDL and IDL) contain apolipoprotein C-I (apoC-I), whereas this apolipoprotein is apparently absent in LDL. VLDL and IDL were quantitatively precipitated with a monospecific anti-apoC-I antibody whereafter LDL-apoB in the supernatant was quantitated by Laurell rocket electrophoresis. Over a wide range of cholesterol and triglyceride values there was a linear correlation with LDL-apoB values measured after ultracentrifugation. The method would be useful for routine measurements, especially in children, since only 25 μ l of serum is required, and for making the diagnosis of hyperapobetalipoproteinemia, which at present is complicated. — Andersen, G. E., H. Gry, and G. M. Kostner. A new micromethod for routine measurement of serum LDL-apolipoprotein B. *J. Lipid Res.* 1988. 29: 377–380.

Supplementary key words lipoproteins • apolipoprotein B • apolipoprotein C

Numerous studies have shown that there is an association between elevated serum lipids and lipoproteins and early development of atherosclerosis and myocardial infarction (1, 2). The cholesteryl ester-rich lipoproteins, LDL and Lp[a] have so far been considered to best predict premature vascular disease (3). The apolipoproteins are responsible for the overall metabolism of certain lipoprotein classes and for directing the flow of lipoproteins into the cells via specific receptors. Abnormalities in receptor-mediated cell interaction seem to play a major role in atherogenesis. Therefore, serum apolipoproteins and especially apoB are now considered to be additional or even better discriminators than serum lipoproteins (4, 5). Serum apoB, however, is distributed among five major ultracentrifugally defined types of lipoprotein: chylomicrons, VLDL, IDL, LDL, and Lp[a]. These lipoproteins differ strikingly in chemical composition and physicochemical properties and simple routine procedures for the individual measurement of apoB in distinct density classes are lacking (6). We have focused on developing a method suitable for routine use, to measure serum LDL-apoB, since both this lipoprotein class and apoB seem to be important predictors of premature vascular disease (7). Furthermore, the method can be used to diagnose hyperapobetalipoproteinemia, in which serum LDL-cholesterol concentration is normal but LDL-apoB is elevated (8).

MATERIALS AND METHODS

Serum was obtained from 11 normolipemic, 17 hypercholesterolemic, and 3 hypertriglyceridemic individuals, 3–51 years old, by low speed centrifugation immediately after blood drawing and clotting (range of cholesterol concentrations: 3.89–9.64 mmol/l; range of triglyceride concentrations: 0.56–2.22 mmol/l). Furthermore, serum was obtained from children with low serum triglyceride and from hypertriglyceridemic adults (range of triglyceride concentrations: 0.43–6.46 mmol/l). All individuals had been fasting for 12 hr. Lipoprotein isolation in the ultracentrifuge was begun within 24 hr using the method of Havel, Eder, and Bragdon (9). The protein content of the individual lipoprotein fractions was quantitated by the method of Lowry et al. (10).

Preparation of monospecific anti-apoC-I antibody

ApoC-I was isolated from chylomicrons and light VLDL as described by Kostner and Holasek (11). Five mg of pure protein in 5 ml of 0.15 M NaCl was mixed 1:2 with Freund's adjuvant and injected intraperitoneally into a sheep in five portions at 10-day intervals. The resulting antiserum was monospecific when tested by immunodiffusion and one- and two-dimensional immunoelectrophoresis using purified lipoproteins and apolipoproteins. To remove the apoB-containing lipoproteins from the sheep antiserum, Na-phosphotungstate precipitation was performed, followed by dialysis against 0.15 M NaCl as described by Burstein (12). Sheep apoB-containing lipoproteins may interfere in the immunochemical assay of human apoB if antisera from a different species are used in the next step. Therefore, the γ -globulin fraction of the antiserum against apoC-I was isolated by DEAE cellulose column chromatography (11).

Immunoprecipitation of lipoprotein

In order to separate LDL from VLDL and IDL selectively, immunoprecipitation with the monospecific anti-apoC-I antibody was performed in whole serum and in lipoprotein fractions.

Increasing amounts of antiserum against apoC-I were added to whole serum (range of triglyceride levels: 0.43–6.46 mmol/l) and to the individual lipoprotein fractions. The formed immunoprecipitate was removed by centrifugation for 10 min at 3500 g. After adding anti-apoC-I to whole serum or to the individual lipoprotein fractions, the apoB content of the supernatant was mea-

Abbreviations: (The lipoprotein fractions described in the text were always defined by their density in the ultracentrifuge.) VLDL, very low density lipoproteins (d < 1.006 g/ml); IDL, intermediate density lipoproteins (d 1.006–1.019 g/ml); LDL, low density lipoproteins (d 1.019–1.063 g/ml); HDL, high density lipoproteins (d 1.063–1.215 g/ml); apoB, apolipoprotein B; apoC-I, apolipoprotein C-I; Lp[a], lipoprotein[a].

sured by Laurell rocket electrophoresis as described by Andersen and Gry Nielsen (13). The area of the individual rockets was measured. The coefficient of variation (CV) for the quantitation of apoB after the immunoprecipitation was 2.5% within assay. In one series of experiments, furthermore, anti-apoC-III was added to the supernatant after the LDL fraction had been precipitated with anti-apoC-I to test whether there would be any additional immunoprecipitation of the LDL fraction.

In another series of experiments, the content of LDL-apoB was measured in 31 sera (range of cholesterol concentrations: 3.89–9.64 mmol/l, range of triglyceride concentrations: 0.56–2.22 mmol/l) both after immunoprecipitation with anti-C-I and after VLDL and IDL had been removed by ultracentrifugation at d 1.019 g/ml.

In a final experiment, Lp[a] was quantitated in various sera before and after immunoprecipitation with the anti-apoC-I antibody.

RESULTS

Anti-apoC-I antibody quantitatively precipitated the VLDL and IDL fractions both from whole sera and from ultracentrifugally isolated VLDL ($d < 1.006$ g/ml) and IDL (d 1.006–1.019 g/ml). No reaction with LDL or cross-reaction with our antiserum against apoB was observed in any experiment. Titration experiments showed that 125 μ l of our particular anti-apoC-I antibody solution precipitated all VLDL and IDL from 25 μ l of human serum containing 0.43 to 6.46 mmol/l triglycerides. In addition, the supernatant was completely free of apoC-I when tested immunochemically. **Fig. 1** shows one representative experiment out of more than 20 experiments where the apoB concentrations of different sera were determined in ultracentrifugally isolated VLDL and IDL fractions before (Fig. 1, 1 and 3) and after (Fig. 1, 2 and 4) immunoprecipitation with anti-apoC-I. In both cases, the precipitation was complete. When on the other hand, the anti-apoC-I was added to the LDL fraction, the area of the rockets was not reduced (Fig. 1, 5 and 6). When anti-apoC-III was added to the supernatant after apoC-I immunoprecipitation, no additional immunoprecipitation was observed (Fig. 1, 7). HDL also contains some apoC-I and is co-precipitated upon the addition of anti-apoC-I. This, however, has no influence on the determination of apoB in LDL (Fig. 1, 8 and 9). When whole serum was treated with anti-apoC-I, the formed rockets were reduced (Fig. 1, 11) compared with untreated serum (Fig. 1, 10), corresponding to the amount of apoB in VLDL and IDL. When "Beta-lipoprotein Standard" (Behringwerke, West Germany) was investigated before and after adding anti-apoC-I (Fig. 1, 12 and 13), we observed a mean 10% reduction.

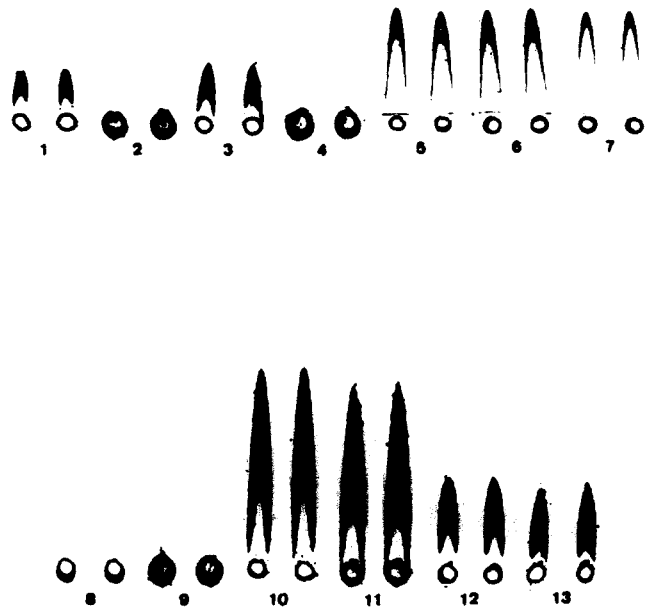


Fig. 1. Apolipoprotein B content of various lipoprotein fractions, human serum, and "Beta-lipoprotein Standard" before and after immunoprecipitation with anti-apoC-I and anti-apoC-III. 1: VLDL, before immunoprecipitation with anti-apoC-I; 2: VLDL, after immunoprecipitation with anti-apoC-I; 3: IDL, before immunoprecipitation with anti-apoC-I; 4: IDL, after immunoprecipitation with anti-apoC-I; 5: LDL, before immunoprecipitation with anti-apoC-I; 6: LDL, after immunoprecipitation with anti-apoC-I; 7: LDL, after immunoprecipitation with anti-apoC-I plus anti-apoC-III; 8: HDL, before immunoprecipitation with anti-apoC-I; 9: HDL, after immunoprecipitation with anti-apoC-I; 10: Whole serum, before immunoprecipitation with anti-apoC-I; 11: Whole serum, after immunoprecipitation with anti-apoC-I; 12: "Beta-lipoprotein Standard," before immunoprecipitation with anti-apoC-I; 13: "Beta-lipoprotein Standard," after immunoprecipitation with anti-apoC-I.

In **Fig. 2** the regression between apoB values in LDL (d 1.019–1.063 g/ml) and after immunoprecipitation of VLDL and IDL in whole serum is presented. Regression analyses showed the values to be distributed around the identity line with a constant CV around the line of 11.45%. The CV for ultracentrifugation contributes 5–10% to this latter value.

In the Lp[a] experiments no reduction of the Lp[a] was observed after immunoprecipitation with the anti-apoC-I antibody.

DISCUSSION

Although most of the apoB in normal serum is found in the LDL fraction, this proportion might be altered considerably in dys- and hyperlipoproteinemia. There are at least five chemically and physicochemically different apoB-containing lipoproteins. Thus, quantitation of serum apoB is not a simple procedure. In order to expose all antigenic determinants to the antibody or to render the

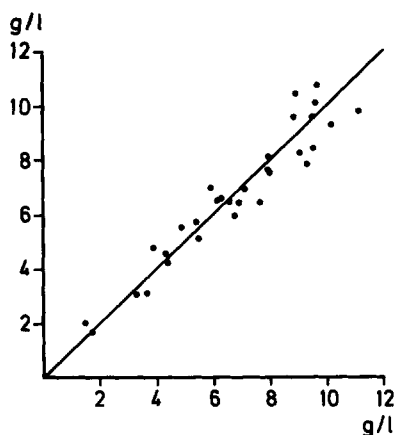


Fig. 2. Regression line for LDL-apolipoprotein B values obtained after ultracentrifugation (x-axis) and after immunoprecipitation with anti-apoC-I (y-axis) in 31 human sera (range of cholesterol concentrations: 3.89–9.64 mmol/l; range of triglyceride concentrations: 0.56–2.22 mmol/l).

apoB-containing fractions into uniform size, the addition of detergents (14) or treatment with lipases (15) have been proposed. The present method is based on the fact that all lipoproteins other than LDL (d 1.019–1.063 g/ml) contain apolipoproteins of the C family (16).

In a preliminary series of experiments, the suitability of antisera against apoE and apoC-III for precipitating VLDL (d < 1.006 g/ml) and IDL (d 1.006–1.019 g/ml) was investigated. None of these antisera resulted in quantitative precipitation of these lipoproteins. However, by using a monospecific anti-apoC-I antibody, we were successful in quantitatively precipitating VLDL and IDL from whole serum as well as from VLDL and IDL fractions isolated in the ultracentrifuge.

The LDL fraction remained unprecipitated both upon the addition of anti-apoC-I and of anti-apoC-I plus anti-apoC-III (Fig. 1, 6 and 7).

The 10% reduction in rocket area after adding anti-apoC-I to "Beta-lipoprotein Standard" suggests that this standard contains some VLDL and/or IDL, and is not pure LDL. Further documentation, however, was not possible for economic reasons, due to the high cost of the standard.

Over a wide range of cholesterol and triglyceride values we were able to demonstrate a linear correlation between the apoB content in LDL (d 1.019–1.063 g/ml) and in whole serum after removal of VLDL and IDL by immunoprecipitation.

In our experiments apoB was quantitated by Laurell rocket electrophoresis. Radial immunodiffusion or nephelometry might be just as useful.

In the Lp[a] experiments, no reduction of Lp[a] was observed after immunoprecipitation. Thus, if the true LDL-apoB content without interference of Lp[a] is of

interest, Lp[a] has to be removed, e.g., by the use of specific antibodies. An alternative would be to calculate the Lp[a]-apoB value from the approximation: $Lp[a]-apoB = Lp[a]-mass \times 0.18$ after quantitation of Lp[a] by Laurell electrophoresis (14, 17).

In summary, we have shown that serum LDL-apoB quantitation can be achieved by immunoprecipitation of VLDL and IDL with a specific antibody against apoC-I. All LDL remained in the supernatant together with Lp[a]. This latter lipoprotein is found in high concentrations only in a few sera. Since Lp[a] is considered highly atherogenic (14), it should be measured together with LDL. ■■

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