methods

Measurement of apolipoprotein B concentration in plasma lipoproteins by combining selective precipitation and mass spectrometry

Laurent Beghin,* Nathalie Duhal,†,§ Philippe Poulain,* Philippe Hauw,* Brigitte Lacroix,†,§ Jean-Michel Lecerf, Jean-Paul Bonte,†,§ Jean-Charles Fruchart,*,§ and Gérald Luc[1],*,****

Department of Atherosclerosis,* Institut Pasteur de Lille, 59019 Lille Cedex, France; Centre Universitaire de Mesures et d'Analyses,† Faculté de Pharmacie, Lille, France; Université de Lille II,§ Lille, France; and Service de Médecine Interne A,** University Hospital of Lille, 59037 Lille, France

Abstract **The measurement of apolipoprotein B (apoB) in purified lipoproteins by immunological assays is subject to criticism because of denatured epitopes or immunoreactivity differences between purified lipoproteins and standard. Chemical methods have therefore been developed, such as the selective precipitation of apoB followed by quantification of the precipitate. In this study, we present the measurement of apoB concentration in lipoproteins purified by ultracentrifugation by combining isopropanol precipitation and gas chromatography/mass spectrometry. Very low density lipoprotein (VLDL; d < 1.006 g/mL); VLDL plus intermediate density lipoprotein (VLDL + IDL; d < 1.019 g/mL); and VLDL, IDL, and low density lipoprotein (VLDL + IDL + LDL; d < 1.063 g/mL) were purified by ultracentrifugation. Apolipoprotein B-100 was selectively precipitated by isopropanol. The leucine content of the pellet was then determined by gas chromatography/mass spectrometry, using norleucine as internal standard. Knowledge of the number of leucine molecules in one apoB-100 molecule makes it possible to calculate the plasma concentration of apoB in the various lipoprotein fractions. ApoB in IDL (d 1.006–1.019 g/mL) and LDL (d 1.019–1.063 g/mL) were then determined by subtracting VLDL-apoB from apoB in lipoproteins d < 1.019 and apoB in lipoproteins d < 1.019 g/mL from apoB in lipoproteins d < 1.063 g/mL, respectively. The isopropanol precipitate was verified as pure apoB (>97%) in lipoprotein fractions isolated from normo- and hyperlipidemic plasma and the method appeared reproducible. The combination of isopropanol precipitation and the GC/MS method appears therefore to be a precise and reliable method for kinetic and epidemiological studies.—** Beghin, L., N. Duhal, P. Poulain, P. Hauw, B. Lacroix, J-M. Lecerf, J-P. Bonte, J-C. Fruchart, and G. Luc. **Measurement of apolipoprotein B concentration in plasma lipoproteins by combining selective precipitation and mass spectrometry. *J. Lipid Res.* 2000.** 41: **1172–1176.**

Supplementary key words apolipoprotein B • lipoproteins • mass spectrometry

Apolipoprotein B (apoB) is the major protein component of a number of lipoproteins such as chylomicrons, very low density lipoproteins (VLDL), intermediate density lipoproteins (IDL), and low density lipoproteins (LDL). The plasma apoB concentration can be determined by a number of immunological methods (1–6), but each of these methods presents drawbacks due to denatured epitopes or immunoreactivity difference between lipoproteins. Therefore, the use of these methods is not suited to quantitating apoB in purified lipoproteins.

The chemical methods developed are based on the selective precipitation of the apoB of human lipoproteins purified by ultracentrifugation (7–9). In these lipoproteins, the apoB content has usually been estimated as the difference between total lipoprotein protein and soluble apolipoproteins in the solvent (10) or directly by measuring the protein content of the pellet after resolubilization (11). In this report, we describe a reproducible procedure to determine the concentration of apoB in plasma purified lipoproteins, using isopropanol precipitation followed by the measurement of the leucine content of the pellet with a mass spectrometer.

MATERIALS AND METHODS

Purification of lipoproteins

Blood drawn directly into EDTA Vacutainer tubes from fasting human subjects was immediately centrifuged at 4°C. Plasma was isolated and supplemented with a concentrated mixture of preservatives containing protease inhibitors and antibiotics that did not modify the results (data not shown). Lipoproteins of d < 1.006 g/mL (VLDL), lipoproteins of d < 1.019 g/mL (VLDL +

Abbreviations: apo, apolipoprotein; VLDL, very low density lipoprotein; IDL, intermediate density lipoprotein; LDL, low density lipoprotein; GC/MS, gas chromatography/mass spectrometry; SDS, sodium dodecyl sulfate.

[1] To whom correspondence should be addressed.

IDL), and lipoproteins of d < 1.063 g/mL (VLDL + IDL + LDL) were separated from aliquots of the same plasma in the 100.4 rotor of a tabletop centrifuge (Beckman, Palo Alto, CA) at 100,000 rpm at 4°C for 2 h and 30 min, 3 h, and 3 h for lipoproteins of d < 1.006, d < 1.019, and d < 1.063 g/mL, respectively. Each lipoprotein fraction was washed by centrifugation under the same conditions as for the density limit. The volumes of the top and bottom fractions were measured with an electronic pipette after ultracentrifugation. The recovery of apoB in the different lipoprotein fractions purified by ultracentrifugation was evaluated by adding known quantities of lipoproteins in plasma and measuring lipoprotein-apoB according to the method described below. Calculated recoveries were 93, 94, and 97% for lipoproteins of d < 1.006, d < 1.019, and d < 1.063 g/mL, respectively, for plasma with triglyceride levels below 400 mg/dl. No correction was made for lipoprotein losses during the ultracentrifugation step.

Measurement of apoB by isopropanol precipitation and GC/MS (isopropanol-GC/MS method)

About 200 μg of lipoproteins was precipitated with isopropanol according to the procedure described by Egusa et al. (12). Lipoproteins were made up to a volume of 750 μL with 0.15 M NaCl, and 750 μL of isopropanol was added. After 12 h at room temperature for lipoproteins of d < 1.006 g/mL or at 4°C for lipoproteins of d < 1.019 g/mL and lipoproteins of d < 1.063 g/mL, the mixture was centrifuged at 1,000 *g* for 30 min at 4°C. The pellet was dried under nitrogen. Norleucine (100 nmol; Sigma, St. Louis, MO) was added as an internal standard. The pellet was hydrolyzed by 1 mL of 6 N ultrapure chlorhydric acid (J. T. Baker, Phillipsburg, NJ) at 110°C for 24 h. The hydrolysate was evaporated and 1 mL of 1 N acetic acid (J. T. Baker) was added to each tube. The acid solution was applied to a 1-mL AG-50W*8 (H^+ form) cation-exchange resin (143-5441; Bio-Rad, Hercules, CA) column. The resin was eluted with 3 M NH_4OH. The eluates containing amino acids were collected in vials. The dried amino acids were esterified and then 100 μL of heptafluorobutyric anhydride (Supelco, Bellefonte, PA) was added to each tube. The sample was heated at 60°C for 20 min, and then dried under nitrogen and dissolved in ethyl acetate (J. T. Baker).

Samples were analyzed by gas chromatography/mass spectrometry (GC/MS) on a Finnigan-MAT SSQ 710 B instrument (Finnigan-MAT, San Jose, CA) under chromatographic conditions previously described (13). Mass spectrometry was used in a negative chemical ionization mode with methane as the reactant gas, and selected charged ions with *m/z* 349 were monitored. This specific *m/z* value corresponds to substituted leucine after the loss of HF and the acquisition of an electron by the substituted molecules. The leucine/norleucine ratio was determined in duplicate. The apoB concentration was calculated by using the known amount of norleucine added to the apoB sample, the number of moles of leucine (n = 524) in 1 mol of apoB-100 (14), and the molecular mass of nonglycosylated apoB-100 estimated at 512,000 Da. This was corrected for lipoprotein aliquot volume and plasma volume.

Analysis of the protein content in the isopropanol precipitate

The proteins of the pellet obtained after lipoprotein precipitation by isopropanol were analyzed by two methods. The former was sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (15), whereby total VLDL, pellet, and supernatant were loaded on gels. As apoE could have been precipitated together with apoB, the relative quantity of apoE compared with apoB-100 was measured in the isopropanol precipitate of VLDL purified from hypertriglyceridemic plasma obtained from four diabetic subjects whose plasma apoE levels were 3- to 4-fold the normal value. After electrophoresis of the pellet in an SDS-polyacrylamide gel, apoB-100 and a large band of gel around the molecular weight of apoE were separately cut. Norleucine was added as an internal standard and the quantities of apoB-100 and apoE were evaluated by GC/MS as described above. The quantity of apoE was evaluated using the number of moles of leucine (n = 37) in 1 mol of apoE and the molecular mass of apoE estimated at 34,145 (16). The second method was amino acid analysis of the pellet. Amino acid analyses were performed on a Beckman 6300 amino acid analyzer after hydrolysis in 6 M HCl under vacuum at 110°C for 24 h in the presence of 0.25% phenol.

Precision of methods

The precision of isopropanol precipitation and GC/MS was estimated by analyzing the variance experiment described in National Committee for Clinical Laboratory Standards (NCCLS) publication EPS-T (17). Eight aliquots of each normolipemic, hypertriglyceridemic, and hypercholesterolemic pool of plasma were measured each day for 4 consecutive days.

Effect of freezing

The effect of freezing was evaluated by comparing apoB measurements with the isopropanol-GC/MS method for 24 different fresh plasma samples and the same plasma stored for 1 month at –80°C.

RESULTS

Analysis of the purity of apoB in isopropanol precipitate

After isopropanol precipitation of VLDL, the pellet contained apoB-100 and possibly albumin (trace); no apoB was detected in the supernatant (**Fig. 1**). Using plasma with a 3- to 4-fold plasma apoE level, no protein corresponding to the molecular mass of apoE was detected in the pellet by SDS-polyacrylamide gel electrophoresis (PAGE). Indeed, apoB-100 represented more than 97% of the sum of apoE + apoB-100 (97.6–102% in VLDL and lipoproteins of d < 1.019 g/mL from four plasma samples).

To assess whether proteins other than apoB-100 were present in the pellet, the amino acid content of the pellet was analyzed by high-performance liquid chromatography (HPLC) after precipitation of VLDL and lipoproteins of d < 1.063 g/mL. The Leu/Gly, Phe/Gly, Val/Ile, Phe/Asp, and Leu/Arg ratios are shown in **Table 1**. Ratios were similar as regards the isopropanol precipitate of VLDL or lipoproteins of d < 1.063 g/mL and those calculated from the published sequence of apoB-100 (18) (Table 1).

Precision of the isopropanol-GC/MS method

Table 2 presents precision data on measurements of apoB in lipoproteins of d < 1.006, d < 1.019, and d < 1.063 g/mL in normolipemic, hypertriglyceridemic, and hypercholesterolemic plasma. Total estimated coefficients of variation are between 3.6 and 9.8%. The coefficients of variation obtained with hypertriglyceridemic plasma were slightly higher (5.5 to 9.8%) than those of normolipemic (4.2 to 6.4%) or hypercholesterolemic plasma (3.6 to 5.4%) for all lipoprotein fractions.

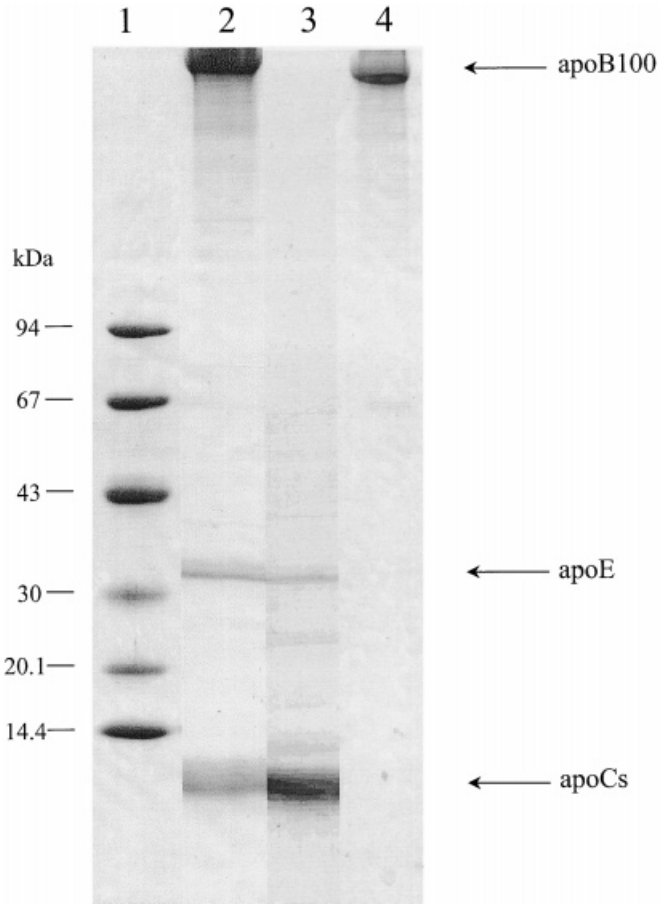

Fig. 1. Polyacrylamide gel electrophoresis, in the presence of SDS, of total VLDL and of supernatant and pellet of VLDL after isopropanol precipitation. Lane 1, molecular weight markers; lane 2, total VLDL; lane 3, supernatant; lane 4, pellet.

TABLE 1. Amino acid ratios in the pellets of VLDL and lipoproteins < 1.063 g/mL obtained after precipitation by isopropanol[a]

Amino Acid Ratio	Isopropanol Pellet of: VLDL	Lipoproteins <1.063 g/mL	Sequence of ApoB-100
Leu/Gly	2.62	2.57	2.60
Phe/Gly	1.09	1.12	1.09
Val/Ile	0.93	0.92	0.89
Phe/Asp	0.49	0.49	0.47
Leu/Arg	3.49	3.49	3.50

[a] These ratios were compared with those calculated from the apoB-100 published sequence (23).

reproducible because of detection precision (GC/MS), the coefficient of variation being <10%, i.e., systematically lower than that achieved with other methods checked (data not shown).

The selectivity of apoB precipitation by isopropanol is important in validating the method described in the present article. The procedures used showed that apoB was totally precipitated and that the amino acid in the precipitate was similar to that of apoB-100. These results are compatible with the great selectivity of lipoprotein-apoB precipitation in the presence of isopropanol as previously described (11, 12). This method is applicable only to purified lipoproteins and not to plasma or serum, because plasma proteins other than apoB were precipitated by isopropanol (19).

The calculation of apoB concentration in lipoproteins by our method depends on the number of moles of leucine in 1 mol of apoB-100, as this is slightly variable depending on the sequence described (20–23) or amino acid composition (14), but the variation in apoB level is

Effect of freezing

The apoB levels in lipoproteins of d < 1.006, d < 1.019, and d < 1.063 g/mL were measured by the isopropanol-GC/MS method in 24 fresh plasma samples and in the same plasma frozen and kept at –80°C for 1 month. The values obtained in fresh and frozen plasma were highly correlated, the coefficients of correlation being 0.96, 0.91, and 0.89 ($P < 0.0001$) for lipoproteins of d < 1.006, d < 1.019, and d < 1.063 g/mL, respectively. However, whereas values of VLDL-apoB and VLDL + IDL-apoB were similar in the usual concentration range, the maximal variation being 3 and 7%, respectively, apoB in lipoproteins of d < 1.063 g/mL could increase or decrease up to 15% after freezing the plasma.

DISCUSSION

In this report, we present a method for measuring the apoB level in VLDL, IDL, and LDL purified from plasma by ultracentrifugation, using a sequence of selective precipitation of apoB by isopropanol and the measurement of leucine content in the pellet by GC/MS. This method is

TABLE 2. Precision of measurement of lipoprotein apoB-100 in normolipemic, hypertriglyceridemic, and hypercholesterolemic plasma by combining isopropanol precipitation and GC-MS[a]

	ApoB	SD_w[b]	SD_b[c]	SD_{total}[d]	CV[e]
	mg/dL				%
Normolipemic plasma lipoproteins					
d < 1.006	4.89	0.09	0.30	0.31	6.4
d < 1.019	9.22	0.34	0.48	0.59	6.4
d < 1.063	76.75	1.70	2.72	3.21	4.2
Hypertriglyceridemic plasma lipoproteins					
d < 1.006	18.78	1.17	1.42	1.84	9.8
d < 1.019	24.22	1.50	1.64	2.22	9.2
d < 1.063	104.49	2.80	5.01	5.74	5.5
Hypercholesterolemic plasma lipoproteins					
d < 1.006	8.99	0.26	0.41	0.49	5.4
d < 1.019	13.82	0.25	0.53	0.59	4.2
d < 1.063	152.83	2.94	4.70	5.54	3.6

[a] Means of eight measurements over 4 days.
[b] SD_w, within-day standard deviation.
[c] SD_b, between-day standard deviation.
[d] $SD_{total} = \sqrt{SD_w^2 + SD_b^2}$.
[e] $CV = (SD_{total} \times 100)/apoB$.

less than 1% when extreme numbers of moles of leucine are used in the calculation. We also used 512,000 as an arbitrary nonglycosylated apoB-100 molecular mass, but these data are debatable as regards the amino acid polymorphisms of apoB (24, 25). These parameters used in the calculation of apoB-100 concentrations in purified lipoproteins left doubt as to accuracy but did not modify measurement precision.

The method described in this article has several advantages. First, high reproducibility is important because better precision can considerably decrease the number of subjects required in kinetic, nutritional, and epidemiological studies. Second, this method does not need standards, antisera, and then standardization.

The great amount of time spent purifying and modifying amino acids for GC/MS analyses is the main drawback of this method. However, with fewer subjects and the use of an autosampler for injection in GC/MS, time can be saved.

Small errors could appear during the measurement of apoB by the isopropanol-GC/MS method; these errors relate to the presence of apoB-48 in the VLDL of hypertriglyceridemic plasma and apoE in the precipitate of VLDL. As this calculation includes the number of leucine molecules per mole of apoB-100, an error related to the different number of leucine molecules and molecular weights of apoB-100 and apoB-48 could appear when measuring apoB in plasma containing apoB-48, because apoB-100 and apoB-48 are simultaneously precipitated by isopropanol. However, this error is relatively small in normolipidemic subjects, as the quantity of apoB-48 when present is always between 1 and 6% of total apoB in VLDL (26–28), but this error could be more significant in hyperlipidemic subjects, for whom apoB-48 in triglyceride-rich lipoproteins can reach 13% of total apoB present in lipoproteins of $d < 1.006$ g/mL (27). However, nonimmunological methods have been described to quantify these apolipoproteins (26, 28, 29). The mass of apoE in pellets of VLDL or lipoprotein of $d < 1.019$ g/mL, evaluated by SDS-PAGE followed by GC/MS, was always less than 3% of the total mass of the pellet. The presence of apoE could be lessened or eliminated by washing the pellet with 50% isopropanol, as described by Egusa et al. (12), but these researchers showed a low recovery of lipoprotein-apoB after washing, which therefore underestimates the concentrations.

Freezing led to a bias in apoB measurement in the different lipoprotein fractions. In the usual concentration ranges, freezing moderately lowered the apoB in VLDL and to a larger extent apoB in lipoprotein of $d < 1.063$ g/mL. The consequence was a decrease in apoB in VLDL and LDL and an increase in IDL-apoB after freezing. It is therefore more reliable to measure apoB in purified lipoproteins in fresh plasma.

To summarize, the method using apoB precipitation by isopropanol followed by the measurement of leucine by GC/MS offers a high degree of precision and is the most reproducible of the methods checked in our laboratory. We conclude that this method could be a useful and convenient research method for measuring VLDL-, IDL-, and LDL-apoB essentially in kinetic and epidemiological studies.

Manuscript received 16 November 1999 and in revised form 14 March 2000.

REFERENCES

1. Labeur, C., J. Shepherd, and M. Rosseneu. 1990. Immunological assays of apolipoproteins in plasma: methods and instrumentation. *Clin. Chem.* **36:** 591–597.
2. Fruchart, J. C., P. Duthilleul, I. Kora, M. Duquesne, V. Clavey, and J. L. Gentilini. 1981. Rapid determination of human plasma apolipoprotein B. *Clin. Chim. Acta.* **114:** 123–126.
3. Curry, M. D., A. Gustafson, P. Alaupovic, and W. J. McConathy. 1978. Electroimmunoassay, radioimmunoassay, and radial immunodiffusion assay evaluated for quantification of human apolipoprotein B. *Clin. Chem.* **24:** 280–286.
4. Ordovas, J. M., J. P. Peterson, P. Santaniello, J. S. Cohn, P. W. Wilson, and E. J. Schaefer. 1987. Enzyme-linked immunosorbent assay for human plasma apolipoprotein B. *J. Lipid Res.* **28:** 1216–1224.
5. Fruchart, J. C., I. Kora, C. Cachera, V. Clavey, P. Duthilleul, and Y. Moschetto. 1982. Simultaneous measurement of plasma apolipoproteins A-I and B by electroimmunoassay. *Clin. Chem.* **28:** 59–62.
6. Fievet, C., A. Dedieu, A. Kandoussi, I. Luyeye, and J. C. Fruchart. 1986. Quantitation of human serum apolipoprotein B by enzyme immunoassay in excess antigen. *Clin. Chim. Acta.* **159:** 269–278.
7. Kane, J. P. 1973. A rapid electrophoretic technique for identification of subunit species of apoproteins in serum lipoproteins. *Anal. Biochem.* **53:** 350–364.
8. Klein, R. L., and D. B. Zilversmit. 1984. Direct determination of human and rabbit apolipoprotein B selectively precipitated with butanol–isopropyl ether. *J. Lipid Res.* **25:** 1380–1386.
9. Holmquist, L., and K. Carlson. 1977. Selective extraction of human serum very low density apolipoproteins with organic solvents. *Biochim. Biophys. Acta.* **493:** 400–409.
10. Kane, J. P., T. Sata, R. L. Hamilton, and R. J. Havel. 1975. Apoprotein composition of very low density lipoproteins of human serum. *J. Clin. Invest.* **56:** 1622–1634.
11. Vega, G. L., and S. M. Grundy. 1996. Quantitation of apolipoprotein B by chemical methods. *In* Plasma Lipoproteins. Part C. W. A. Bradley, S. H. Gianturco, and J. P. Segrest, editors. Academic Press, San Diego, CA. 63–82.
12. Egusa, G., D. W. Brady, S. M. Grundy, and B. V. Howard. 1983. Isopropanol precipitation method for the determination of apolipoprotein B specific activity and plasma concentrations during metabolic studies of very low density lipoprotein and low density lipoprotein apolipoprotein B. *J. Lipid Res.* **24:** 1261–1267.
13. Elkhalil, L., Z. Majd, R. Bakir, O. Perez-Mendez, G. Castro, P. Poulain, B. Lacroix, N. Duhal, J-C. Fruchart, and G. Luc. 1997. Fish-eye disease: structural and in vivo metabolic abnormalities of high-density lipoproteins. *Metabolism.* **46:** 474–483.
14. Hardman, D. A., and J. P. Kane. 1986. Isolation and characterization of apolipoprotein B-48. *Methods Enzymol.* **128:** 262–272.
15. Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature.* **227:** 680–685.
16. Rall, S. C., Jr., K. H. Weisgraber, and R. W. Mahley. 1982. Human apolipoprotein E. The complete amino acid sequence. *J. Biol Chem.* **257:** 4171–4178.
17. NCCLS. 1992. NCCLS Tentative Guideline EP5–T2. User Evaluation of Precision Performance of Clinical Chemistry Devices. NCCLS, Wayne, PA.
18. Kane, J. P., D. A. Hardman, and H. E. Paulus. 1980. Heterogeneity of apolipoprotein B: isolation of a new species from human chylomicrons. *Proc. Natl. Acad. Sci. USA.* **77:** 2465–2469.
19. Yamada, N., and R. J. Havel. 1986. Measurement of apolipoprotein B radioactivity in whole blood plasma by precipitation with isopropanol. *J. Lipid Res.* **27:** 910–912.
20. Chen, S. H., C. Y. Yang, P. F. Chen, D. Setzer, M. Tanimura, W. H. Li, A. M. Gotto, Jr., and L. Chan. 1986. The complete cDNA and amino acid sequence of human apolipoprotein B-100. *J. Biol. Chem.* **261:** 12918–12921.
21. Law, S. W., S. M. Grant, K. Higuchi, A. Hospattankar, K. Lackner,

N. Lee, and H. B. Brewer, Jr. 1986. Human liver apolipoprotein B-100 cDNA: complete nucleic acid and derived amino acid sequence. *Proc. Natl. Acad. Sci. USA.* **83:** 8142–8146.
22. Knott, T. J., R. J. Pease, L. M. Powell, S. C. Wallis, S. C. Rall, Jr., T. L. Innerarity, B. Blackhart, W. H. Taylor, Y. Marcel, R. Milne, D. Johnson, M. Fuller, A. J. Lusis, B. J. McCarthy, R. W. Mahley, B. Levy-Wilson, and J. Scott. 1986. Complete protein sequence and identification of structural domains of human apolipoprotein B. *Nature.* **323:** 734–738.
23. Cladaras, C., M. Hadzopoulou Cladaras, R. T. Nolte, D. Atkinson, and V. I. Zannis. 1986. The complete sequence and structural analysis of human apolipoprotein B-100: relationship between apoB-100 and apoB-48 forms. *EMBO J.* **5:** 3495–3507.
24. Ludwig, E. H., P. N. Hopkins, A. Allen, L. L. Wu, R. R. Williams, J. L. Anderson, R. H. Ward, J. M. Lalouel, and T. L. Innerarity. 1997. Association of genetic variations in apolipoprotein B with hypercholesterolemia, coronary artery disease, and receptor binding of low density lipoproteins. *J. Lipid Res.* **38:** 1361–1373.
25. Ilmonen, M., T. Helio, R. Butler, A. Palotie, P. Pietinen, J. K. Huttunen, and M. J. Tikkanen. 1995. Two new immunogenetic polymorphisms of the ApoB gene and their effect on serum lipid levels and responses to changes in dietary fat intake. *Arterioscler. Thromb. Vasc. Biol.* **15:** 1287–1293.
26. Kotite, L., N. Bergeron, and R. J. Havel. 1995. Quantification of apolipoproteins B-100, B-48, and E in human triglyceride-rich lipoproteins. *J. Lipid Res.* **36:** 890–900.
27. Castro Cabezas, M., D. W. Erkelens, L. A. Kock, and T. W. de Bruin. 1994. Postprandial apolipoprotein B100 and B48 metabolism in familial combined hyperlipidaemia before and after reduction of fasting plasma triglycerides. *Eur. J. Clin. Invest.* **24:** 669–678.
28. Karpe, F., and A. Hamsten. 1994. Determination of apolipoproteins B-48 and B-100 in triglyceride-rich lipoproteins by analytical SDS-PAGE. *J. Lipid Res.* **35:** 1311–1317.
29. Proctor, S. D., and J. C. L. Mamo. 1997. Separation and quantitation of apolipoprotein B-48 and other apolipoproteins by dynamic sieving capillary electrophoresis. *J. Lipid Res.* **38:** 410–414.