Quantification of apolipoproteins B-100, B-48, and E in human triglyceride-rich lipoproteins

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Abstract We have developed a procedure to quantify apolipoprotein (apo) B-100, apoB-48, and apoE in human triglyceride-rich lipoproteins. This procedure permits delipidation of small amounts of triglyceride-rich lipoproteins without appreciable losses, and quantification of these apolipoproteins in samples containing as little as 10 μg of protein. Delipidated triglyceride-rich lipoproteins are subjected to sodium dodecyl sulfate polyacrylamide slab gel electrophoresis, and the mass of apolipoproteins is estimated after densitometric scanning and volume integration of Coomassie blue-stained bands. The chromogenicities of apoB-100 and apoB-48 are virtually identical, and twofold lower than that of apoE. The standard curve for each apolipoprotein follows a power function over a wide protein range, permitting quantification of as little as 0.2 μg of apoB-48 and as much as 30 μg of apoB-100 from a single application of triglyceride-rich lipoproteins to the gels. This method is suitable for routine use in studies of the intestinal and hepatic contributions to triglyceride-rich lipoproteins and their responses to postprandial lipemia.—**Kotite, L., N. Bergeron, and R. J. Havel.** Quantification of apolipoproteins B-100, B-48, and E in human triglyceride-rich lipoproteins. *J. Lipid Res.* 1995. **36:** 890–900.

Supplementary key words SDS slab gel electrophoresis • apotransferrin • chylomicrons • very low density lipoproteins • postprandial lipemia

Despite accumulating evidence that remnants of chylomicrons and VLDL are atherogenic (1), few methods to quantify the structural proteins of these particles (apoB-48 and apoB-100) have been proposed. Recently, we described a method for quantifying these proteins in triglyceride-rich lipoproteins (TRL) by densitometric scanning after SDS electrophoresis in slab gels and reported the absolute concentration of apoB-100 and apoB-48 in healthy young men before and after ingestion of an ordinary meal (2). A key feature of this method, based upon the observations of Zilversmit and Shea in the rat (3), is that the chromogenicities of the two proteins stained with Coomassie blue R-250 are equal and that a linear response of staining intensity to apolipoprotein mass is obtained over a useful range of concentration.

Earlier, Poapst, Uffelman, and Steiner (4) reported a method for quantifying apoB-48 and apoB-100 in TRL, based upon SDS electrophoresis in tube gels. They found different chromogenicities for the two proteins that varied with the mass applied, and devised a method for circumventing this problem involving the use of several dilutions of each sample for analysis. Several reports have appeared in which this method has been applied by Karpe and associates (5–8) to studies of postprandial changes in the concentration of apoB-48 and apoB-100 in TRL in health and disease. In one of these studies (8), the reported concentrations of apoB-48 in healthy normolipidemic subjects were several-fold higher than we found with our method (2). Very recently, Karpe and Hamsten (9) have described another method quite similar to ours and presented results in healthy young men that are much closer to those that we reported earlier (2).

Here we describe modifications of our method that permit delipidation of very small amounts of TRL without appreciable losses, and analysis of the concentration of apoB-48, apoB-100, and apoE in TRL over a wide range of applied mass so that all three proteins can be quantified from a single application of the solubilized apolipoproteins to a slab gel. This method, which utilizes a laser densitometer that permits volumetric integration of protein-bound dye, is suitable for routine use.

METHODS

Materials

Electrophoresis purity grade acrylamide, bisacrylamide, SDS, TEMED (N,N,N',N'-tetramethylethylenediamine), and ammonium persulfate were obtained from Bio-Rad

Abbreviations: apo, apolipoprotein; TRL, triglyceride-rich lipoproteins; VLDL, very low density lipoproteins; LDL, low density lipoproteins; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; TMU, tetramethylurea; BSA, bovine serum albumin; PBS, phosphate-buffered saline.

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(Richmond, CA). Reducing agents (mercaptoacetic acid, dithiothreitol), benzamidine, Tris, apotransferrin, crystalline bovine serum albumin (BSA), and Coomassie Brilliant Blue R-250 were from Sigma (St. Louis, MO). JIH and 4G3 monoclonal antibodies against apoB-100 were kindly provided by Dr. K. Nakajima (Japan Immunoresearch Laboratories; Takasaki, Japan) and Dr. Y. Marcel (Montreal, Canada), respectively.

Apolipoprotein standards

ApoB-100. Because less than 3% of "narrow-cut" low density lipoprotein (LDL)-protein is soluble in tetramethylurea (10), the apolipoprotein of ultracentrifugally isolated LDL can be used directly as a B-100 standard. ApoB-100 can also be separated from other apolipoproteins by gel permeation chromatography. Both preparations were used here.

To prepare LDL, blood from normolipidemic donors was drawn into tubes containing disodium EDTA (0.05%), and in the presence of benzamidine (0.03%) to prevent scission of apoB-100 (11). Plasma was then separated by centrifugation (720 g) at 4°C, followed by sequential ultracentrifugation (12) to isolate LDL ($1.025 < \varrho < 1.055$ g/ml). The LDL were recentrifuged at the upper density limit, and then dialyzed overnight at 4°C against 0.15 M NaCl, 0.05% EDTA, 0.02% NaN_3, pH 7.4.

To isolate apoB-100 by gel filtration, the dialyzed LDL were first delipidated with 20 volumes of ice-cold ethanol–ether 3:1 overnight at −20°C, followed by centrifugation for 20 min at 720 *g*, −10°C to precipitate apolipoproteins. After decanting the organic phase, the protein pellet was resuspended in ethanol–ether, kept at −20°C for at least 4 h, and then washed twice with cold anhydrous diethyl ether (13). The moist protein pellet was then solubilized in phosphate-buffered saline (PBS; 0.01 M sodium phosphate, pH 7.2, 0.15 M NaCl) containing glycerol (10%), SDS (2%), and EDTA (0.01%), and a gentle stream of nitrogen was applied to the sample to remove remaining traces of ether. The solubilized LDL-apolipoprotein sample was applied to a 1.2 × 90 cm column of Ultrogel AcA-22 (Pharmacia) and eluted at room temperature with 0.15 M NaCl, 0.01 M Na-phosphate, pH 7.2, containing 0.1% SDS and 0.01% EDTA, at a flow rate of 4 ml/h. The absorbance of the eluent fractions was monitored at 280 nm (**Fig. 1**). Purity of the apoB-100 was examined by loading SDS slab gels with 20 μg apoB-100 and verifying that the gels stained

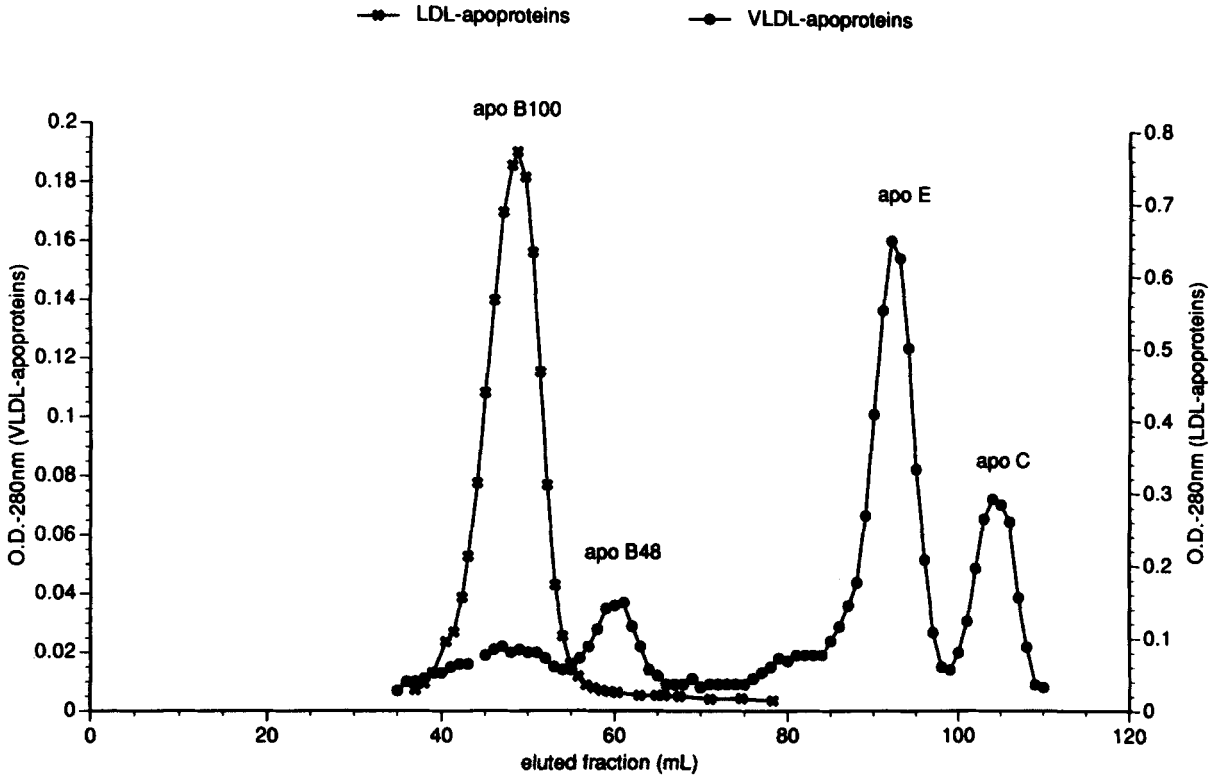

Fig. 1. Elution profile of VLDL and LDL-apolipoproteins separated on a 1.2 × 90 cm column of Ultrogel AC-22, equilibrated in 0.1% SDS, 0.15 M NaCl, 0.01 M PO_4, 0.01% EDTA, pH 7.2. The VLDL were subjected to sequential immunoaffinity chromatography as described in Methods to remove the bulk of apoB-100.

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with Coomassie R-250 had no visible bands other than apoB-100.

ApoB-48. Human apoB-48 was separated from apoB-100 by sequential immunoaffinity chromatography using two monoclonal antibodies to apoB-100 (JIH and 4G3), followed by gel filtration to separate apoB-48 from the remaining VLDL apolipoproteins. For sequential immunoaffinity chromatography, fresh plasma from a patient with familial dysbetalipoproteinema was first applied to the JIH column to separate the bulk of apoB-100-containing lipoproteins from the other lipoproteins (2, 14). The unbound apoB-48-enriched TRL were separated by ultracentrifugation (ϱ = 1.006 g/ml) at 93000 *g*, 12°C, 18 h and then applied to the 4G3 column (2, 15) to remove most of the remaining apoB-100. The unbound lipoproteins were then delipidated and the protein pellet was solubilized as described for apoB-100. Finally, this solubilized material was applied to a 1.2 × 90 cm column of Ultrogel AcA-22 eluted under the same conditions as described above, to separate apoB-48 from apoE and any remaining apoB-100 (Fig. 1). The purity of apoB-48 was then examined by loading 0.5–3.0 μg onto SDS-gels and verifying that no bands other than apoB-48 were visible on Coomassie-stained gels.

To test the linearity of apolipoprotein chromogenicity when larger amounts of apoB-48 are applied to SDS gels, we used rat lymph chylomicrons in which apoB-48 constitutes 25–30% of the total protein mass. The lymph was collected from the mesenteric lymph duct of anesthetized rats receiving a constant intraduodenal infusion of 0.15 M NaCl containing glucose (555 mM) (16). After its nonprotein solvent density was adjusted to 1.019 g/ml with D_20, the lymph was layered under 0.15 M NaCl (ϱ = 1.006 g/ml) in 6.5 ml centrifuge tubes and subjected to ultracentrifugation in a 40.3 Beckman rotor for 1 h at 93000 *g*, 22°C to separate chylomicrons. The floating chylomicrons were recentrifuged at 93000 *g*, 12°C for 18 h to remove albumin and other proteins.

ApoE. ApoE was prepared from ultracentrifugally separated VLDL of a patient with familial dysbetalipoproteinemia, as described (17). The purity of apoE eluted from the column was then verified by SDS-PAGE and isoelectric focusing.

Measurements of apolipoprotein mass

The mass of apoB-100 from narrow-cut LDL ($1.025 < \varrho < 1.055$ g/ml) and apoB-100 isolated by gel filtration was estimated. For narrow-cut LDL, apoB-100 was calculated as the difference between total LDL-protein and TMU-soluble protein (18) measured by a modification (19) of the method of Lowry et al. (20) with crystalline bovine serum albumin (BSA) (Sigma, St. Louis, MO) as standard. For apoB-100, apoB-48, and apoE isolated by gel filtration in the presence of 0.1% SDS, the apolipoprotein mass was also determined by the Lowry procedure (20). SDS up to a concentration of 0.4% had no effect on the absorbance of the BSA standard at 750 nm. The mass of apoB-100, apoB-48, and apoE so calculated was identical to that obtained by measuring the absorbance of these apolipoproteins at 220 nm against the BSA standard (3).

To quantify the mass of apoB-48 in rat chylomicrons, the total protein content was first measured by the modified Lowry procedure (19, 20). ApoB-48 was then precipitated with isopropanol (21), and the protein pellet was washed once with 50% isopropanol in 0.15 M NaCl. Finally, the pellet was solubilized in 2% SDS-PBS and the mass of apoB-48 was estimated (19, 20). The percentage of apoB-48 in the total protein was then used to estimate the mass of apoB-48 in chylomicron protein applied to the SDS slab gels (see below).

Preparation of apolipoproteins and lipoproteins for electrophoresis

ApoB-100, apoB-48, and apoE were diluted in Sample Buffer (3% SDS, 0.12 M Tris pH 6.8, 10% glycerol, 1.5% dithiothreitol, 1% mercaptoacetic acid, and 0.002% bromophenol blue). Samples were then heated for 3 min in a boiling water bath and cooled to room temperature.

In addition, LDL (800 μg total protein) and chylomicrons (500 μg total protein) were delipidated as described above and the total protein content was measured (19). The solubilized apolipoproteins were then diluted with Sample Buffer and heated as above.

Electrophoresis procedure

Three to 10% linear polyacrylamide slab gels, 1.5 mm thick, were prepared with a gradient mixer (Buchler, Fort Lee, NJ). Gel electrophoresis was carried out in the Laemmli system (22) at 50 volts/gel for 30 min and then at 75 volts/gel for 60–90 min in a Mini-Protean II vertical gel apparatus in which 2 mini gels are run simultaneously (Bio-Rad Laboratories, Hercules CA). Each gel can accommodate 10 sample wells (1.3 cm × 0.5 cm). For certain purposes a vertical gel apparatus for large gels, 1.5 mm thick (Hoefer; model SE600), which can accommodate 15 sample wells (2.7 cm × 0.6 cm) per gel was used. This system was run at a constant current of 15 mA/gel for 90 min and then at 25 mA/gel for 4 h. At least six amounts of each apolipoprotein were applied in duplicate: apoB-100, 0.5 to 12 μg; LDL-apolipoprotein containing 0.2 to 30 μg protein; human apoB-48, 0.44 to 2.0 μg; rat chylomicron-apolipoproteins containing 0.07 to 4.4 μg apoB-48 and apoE, 1 to 8 μg. Gels were stained for 18–22 h in trays containing 100 ml 0.25% Coomassie Brilliant Blue R-250 in methanol–water–acetic acid 5:5:1 and destained for 7–8 h with at least four changes of 150 ml methanol–water–acetic acid 5:5:1, at room temperature and under constant agitation on an orbital shaker. Finally, gels were placed between two sheets of cellophane gel

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wrap (Biodesign Inc., New York, NY) and allowed to dry completely in a fume hood at room temperature overnight.

Measurement of dye uptake

Dried gels were scanned with a laser densitometer (Molecular Dynamics, Sunnyvale, CA, model PD-020) equipped with an Image Quant software package and computer. Gels were placed on the glass plate of the densitometer and secured with cellophane tape. Apolipoprotein bands were integrated by volume, which allows quantification of widely varying masses of apolipoproteins in bands of irregular shape. Each apolipoprotein band was first enclosed by a rectangle drawn with a "mouse." To correct for uneven destaining of the acrylamide gels, a second rectangle, representing the background, was drawn immediately above each band, and the density of each rectangle was integrated. The average density per pixel for the background is automatically subtracted from each pixel within the stained band and the summed value is calculated in volume units. For samples containing very low amounts of apoB-48 relative to apoB-100, the average density of rectangles just above and below the apoB-48 band was used as background. To construct standard curves, the chromogenicities (volume units per μg apoprotein) of apoB-100, apoB-48, and apoE were then evaluated by the method of least squares for the power function $y = ax^b$.

Quantification of apoB-100, apoB-48, and apoE in TRL from human blood plasma

The total protein concentration of isolated triglyceride-rich lipoproteins (TRL) from normal and hypertriglyceridemic humans varies widely and is also a function of the extent to which the TRL are concentrated. Because apolipoproteins are insoluble in most organic solvents, delipidation of TRL is a commonly used procedure not only to extract their lipid moiety but also to precipitate and concentrate their apolipoproteins. When the concentration of total protein in TRL is low, we have found that it is often impossible to obtain a solid pellet after delipidation. In this event, more protein is liable to adhere to the side of the delipidation tube or to be lost when the organic phase is removed by aspiration. This problem can be greatly mitigated by including a suitable carrier protein (that does not co-migrate with the apolipoproteins of interest) to TRL before delipidation.

Evaluation of the recovery of delipidated apolipoproteins

To determine the amount of carrier protein (apotransferrin, 80 kDa) required to precipitate apolipoproteins completely when the concentration of TRL is very low, portions of TRL from a patient with familial dysbetalipoproteinemia and containing 22 μg total protein

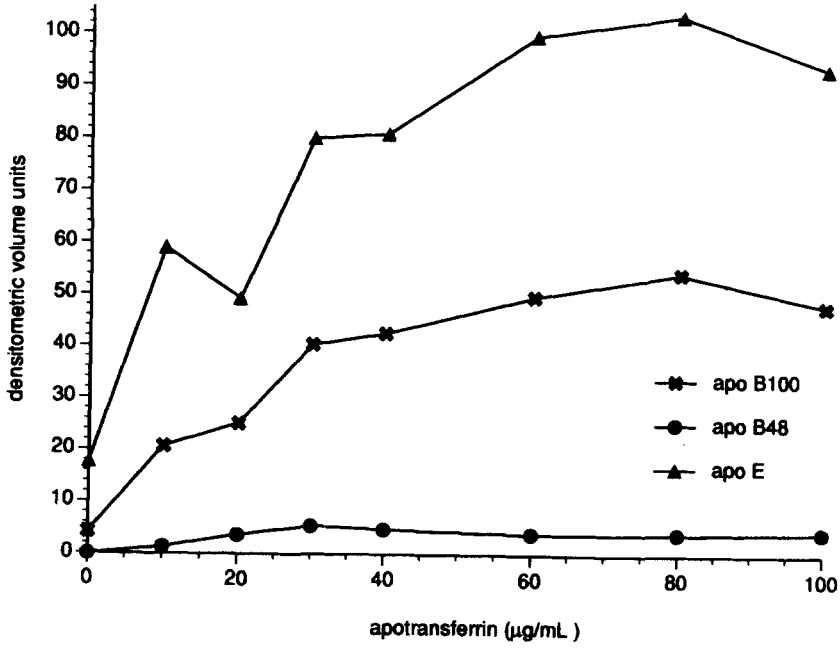

Fig. 2. Effect of adding increasing amounts of apotransferrin (from stock solution: 2 mg/ml 0.15 M Nacl) to 11 μg/ml of TRL protein before delipidation on the recovery of TRL-apolipoproteins (volume units) on SDS slab gels.

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were mixed with 0, 20, 40, 60, 80, 120, 160, and 200 μg of apotransferrin and diluted to a final volume of 2 ml with 0.15 M NaCl. One ml of each mixture (containing 11 μg total protein and 0 to 100 μg apotransferrin) was delipidated with 10 ml ice-cold ethanol–ether 3:1 (v/v) in 15 ml siliconized glass centrifuge tubes and stored at −20°C overnight. We have found that delipidating TRL with 10 volumes of ethanol–ether (13) is as effective in extracting lipids and precipitating apolipoproteins as 20 volumes (unpublished data) and allows TRL to be delipidated in smaller centrifuge tubes, thus facilitating protein pelleting and reducing the likelihood of apolipoprotein losses. After centrifugation for 20 min at −10°C, 720 *g*, the organic phase was removed by aspiration and the protein pellet was resuspended in cold anhydrous diethyl ether and recentrifuged under the same conditions. The moist pellet was solubilized in 200 μl of Sample Buffer and heated for 3 min in a boiling water bath. Portions of 50 μl (containing 2.75 μg TRL-protein and 0 to 25 μg apotransferrin) were applied to duplicate lanes of the gels and run as described above. Gels were stained overnight, destained for 7–8 h and the dried gels were scanned to quantify apolipoproteins by volume integration. When 60, 80, or 100 μg apotransferrin was added to 11 μg TRL before delipidation and when 25% of the precipitated TRL-protein was applied to SDS gels, there was no further increase in the mass (volume units) of the apolipoprotein bands on the gels (**Fig. 2**). In contrast, when 11 μg TRL was delipidated without apotransferrin and 25% of the precipitated TRL-protein was applied to the gels, apoB-48 was undetectable and the mass of apoB-100 and apoE was only 15% of that found when TRL was delipidated with 60–100 μg apotransferrin. Thus, when dilute TRL are delipidated as described, at least 60 μg of apotransferrin must be added before delipidation to facilitate protein precipitation and thereby prevent apolipoprotein losses.

To determine the minimum concentration of TRL-protein that can be delipidated without loss, portions of TRL from the same patient and containing 22, 44, 66, 88, 100, 132, 150, 200, 300, 400, and 600 μg total protein were mixed with 0 or 120 μg apotransferrin as carrier and diluted to a final volume of 2 ml with 0.15 M NaCl. One ml of each of the above mixtures (containing 11 to 300 μg total protein and 0 or 60 μg apotransferrin) was delipidated with 10 ml ethanol–ether 3:1 (v/v) as described above. The moist protein pellet from each TRL sample was dissolved in 200 μl of sample buffer, and treated as above. Fifty μl of each sample (containing 2.75 to 75 μg total protein and 0 or 15 μg apotransferrin) was applied to duplicate lanes of SDS-gels. Gels were then stained and apolipoproteins were quantified as described above. When 66 to 300 μg TRL-protein was delipidated without apotransferrin, the observed mass (volume units) of apoB-100, apoB-48, and apoE was the same as that found when delipidation was carried out in the presence of 60 μg apotransferrin (**Fig. 3**). In contrast, when 11 to 50 μg TRL-protein was delipidated without apotransferrin, the observed mass of apoB-100, apoB-48, and apoE ranged from 15 to 75% of that found when the same amounts of TRL were delipidated with 60 μg apotransferrin. These results indicate that when no carrier protein is used, TRL

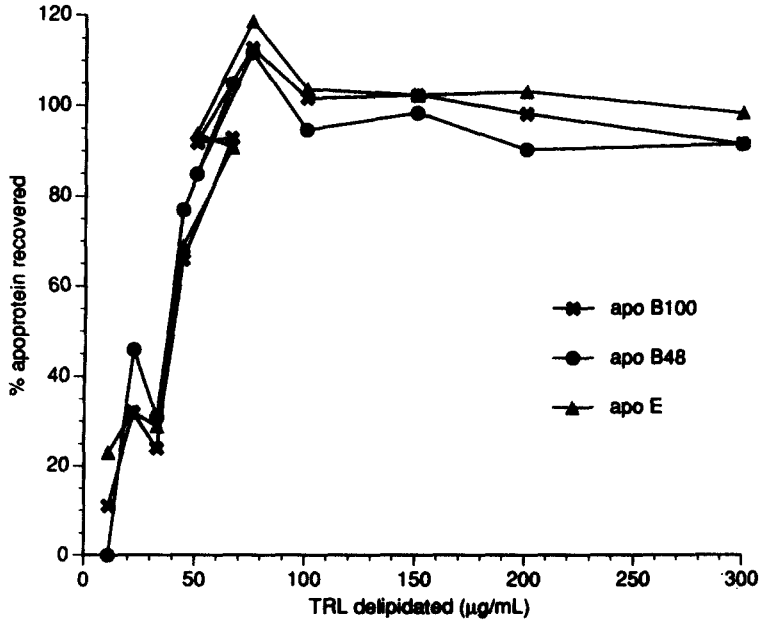

Fig. 3. Recovery of apolipoproteins on SDS-PAGE slab gels. Effect of adding apotransferrin (60 μg/ml) to increasing concentrations of TRL (11–300 μg protein/ml); % apolipoprotein recovered = (volume units TRL-apolipoprotein without apotransferrin/volume units TRL-apolipoprotein with 60 μg/ml apotransferrin) × 100.

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must contain at least 66 μg total protein/ml in order to precipitate TRL-protein completely during delipidation. Based upon these observations, when 75 μg TRL-protein is available, 60 μg apotransferrin is routinely added before delipidation. The described procedure for electrophoresis, staining, and scanning of purified apolipoproteins is then used to quantify apoB-100, apoB-48, and apoE in TRL. As described under Results, quantification is based on the standard curves constructed for each protein that relate densitometric volume units to apolipoprotein mass.

Evaluation of the variation in dye uptake by apolipoproteins

To minimize between-run variation in dye uptake of apolipoproteins, a secondary standard, placed in lanes 2 and 9 of each slab gel, was included with each electrophoretic run. The secondary standard was TRL collected from a patient with familial dysbetalipoproteinemia and was prepared as follows. TRL containing 1–2 mg of total protein/ml were delipidated with 20 volumes of ethanol–ether, as described above for LDL. The moist protein pellet was then solubilized in 2% SDS-PBS, and 50 μl portions of the standard, containing 75 μg of total protein, were stored in 1.5 ml microcentrifuge tubes at −70°C. For use, a vial of the secondary standard was thawed at room temperature and diluted to a final volume of 250 μl with Sample Buffer, boiled for 3 min, and 50 μl (15 μg) was applied to slab gels and run simultaneously with unknown TRL samples. After 80 runs (320 applications of the secondary standard), the average mass of apoB-100, apoB-48, and apoE in the secondary standard was calculated to determine the between-run variation in the dye uptake of each apolipoprotein. Routinely, this average mass for each apolipoprotein is also used to correct for between-run variation in apolipoprotein dye uptake in unknown TRL samples.

Quantification of apolipoproteins in normal TRL

Blood samples were obtained from 16 healthy nonsmoking Caucasian men, 25–36 years of age, who had fasted for 12 h, overnight. They had been on measured diets containing 38% of calories from fat, with a P/S ratio of 0.2. Plasma was obtained by centrifugation at 720 *g*, 4°C, and TRL were separated by ultracentrifugation of 6 ml of plasma ($\varrho < 1.006$ g/ml) at 93000 *g*, 12°C for 18 h. Because all samples contained > 160 μg/ml of total protein, no apotransferrin was added to TRL before delipidation. Delipidated TRL were solubilized with 200–300 μl of Sample Buffer and 50 μl portions were applied in duplicate to slab gels. Electrophoresis and quantification of TRL-apolipoproteins were performed as described above.

RESULTS

Because the concentration of apoB-48 in postabsorptive TRL is very low and because sensitivity has been an important limitation to the existing procedures used to quantify apolipoproteins in dilute solutions, we developed a procedure that permits quantification of apoB-100, apoB-48, and apoE in TRL containing as little as 0.2 μg of a given protein in 50 μl of Sample Buffer. As described

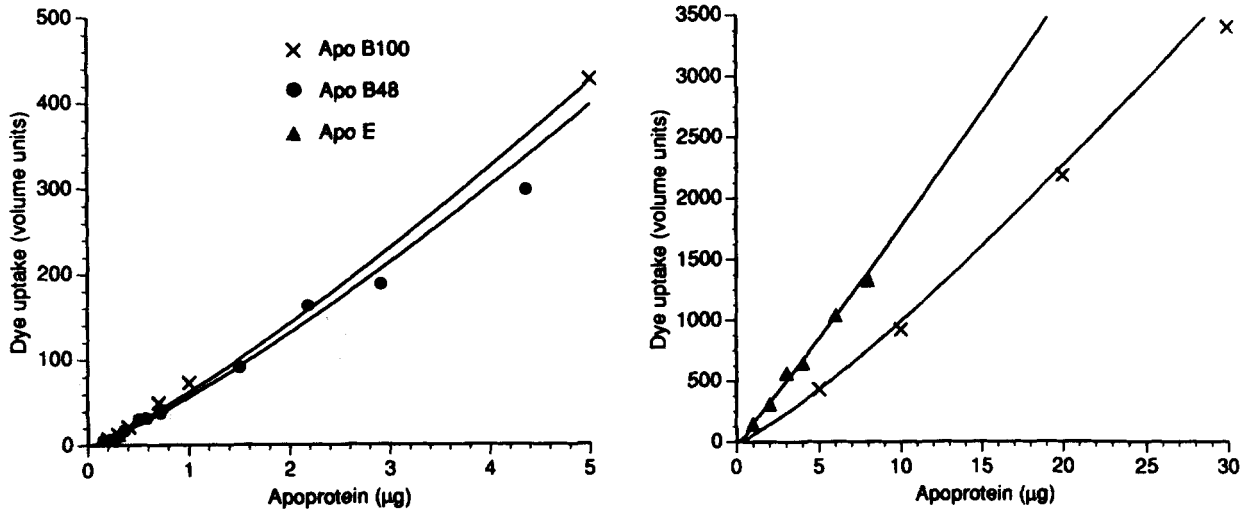

Fig. 4. Chromogenicities of apoB-100 (0–5 μg) and apoB-48 (0–4.4 μg) (left panel) and apoB-100 (0–30 μg) and apoE (0–8 μg) (right panel). The apolipoproteins were solubilized as described in Methods and applied to mini slab gels (8.5 × 5.5 × 0.15 cm). LDL-apoB-100: $y = 61.28 \times^{1.20}$ ($r^2 = 0.998$); rat chylomicron-apoB-48: $y = 55.96 \times^{1.22}$ ($r^2 = 0.989$); human-apoE: $y = 150.64 \times^{1.06}$ ($r^2 = 0.992$).

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above, 60 μg of apotransferrin is added to TRL samples containing < 75 μg of protein to minimize apolipoprotein losses during the delipidation step that precedes electrophoresis.

In slab gels stained with Coomassie blue R-250, we found that the chromogenicity of apoB-100 from human LDL is almost the same as that of apoB-48 from rat lymph chylomicrons (**Fig. 4**), as previously reported by Zilversmit and Shea (3) under similar electrophoretic conditions. By contrast, the chromogenicity of apoE was approximately 2-fold higher than that of apoB-100 and apoB-48. For each apoprotein, we found that the relation between dye uptake and the mass of apoB-100, apoB-48, or apoE deviated slightly from linearity and followed a power function ($y = ax^b$) over a wide range of protein mass. Each standard curve intersected the abscissa at zero volume units (Fig. 4). In their study, Zilversmit and Shea (3) found that the relation between dye uptake and the mass of rat apoB-48 and human and rat apoB-100 was linear ($y = bx$). Differences between the best fit equations in our study and that of Zilversmit and Shea (3) may be related to differences in the two-dimensional scanning method used to quantify dye uptake, which in our study was estimated by volume integration as opposed to area integration. Our standard curves for apoB-100, apoB-48, and apoE each followed a power function over a wide range of applied protein which enables the quantification of very small amounts of apoB-48 together with large amounts of apoB-100 and apoE from the same application of an unknown TRL sample. Apolipoprotein standard curves that cover a wide protein range are advantageous because it is often necessary to apply large amounts of TRL-protein to SDS gels in order to quantify apoB-48, which constitutes only 1–2% of the total protein mass in normal TRL.

We found that the chromogenicity of human LDL-apoB-100 separated by ultracentrifugation was slightly higher than that of apoB-100 isolated by gel filtration (**Fig. 5**). The chromogenicity of rat apoB-48 from lymph chylomicrons was comparable to that of human apoB-48 isolated by gel filtration (**Fig. 6**). Consequently, human LDL and rat lymph chylomicrons respectively, are suitable alternatives to human apoB-100 and apoB-48 isolated by gel filtration to prepare apolipoprotein standard curves, provided that the methods used to quantify apolipoprotein mass in lipoproteins are reliable.

To validate our method against standard assays, the total apoB mass (apoB-100 + apoB-48) measured by SDS-gel electrophoresis in 21 TRL samples (containing 300–500 μg total protein) was compared with the total apoB mass measured directly in the isopropanol-precipitated pellets of the same TRL samples. We found a close correlation ($r^2 = 0.91$) between the total apoB mass estimated by SDS gel electrophoresis and the mass of protein measured directly in the isopropanol-precipitated pellet (**Fig. 7**). We were unable to compare the total apoB mass in TRL at very low protein concentrations because isopropanol was not effective in precipitating apoB in TRL containing < 300 μg total protein per ml. The mass of apoE estimated by SDS-gel electrophoresis in 26 TRL samples (containing 40–500 μg total protein) was highly correlated ($r^2 = 0.93$) with the mass of apoE estimated by RIA (17) (**Fig. 8**). Mass measurements estimated by the two methods were usually in good agreement for TRL samples containing as little as 0.3 μg and as much as 25 μg apoE.

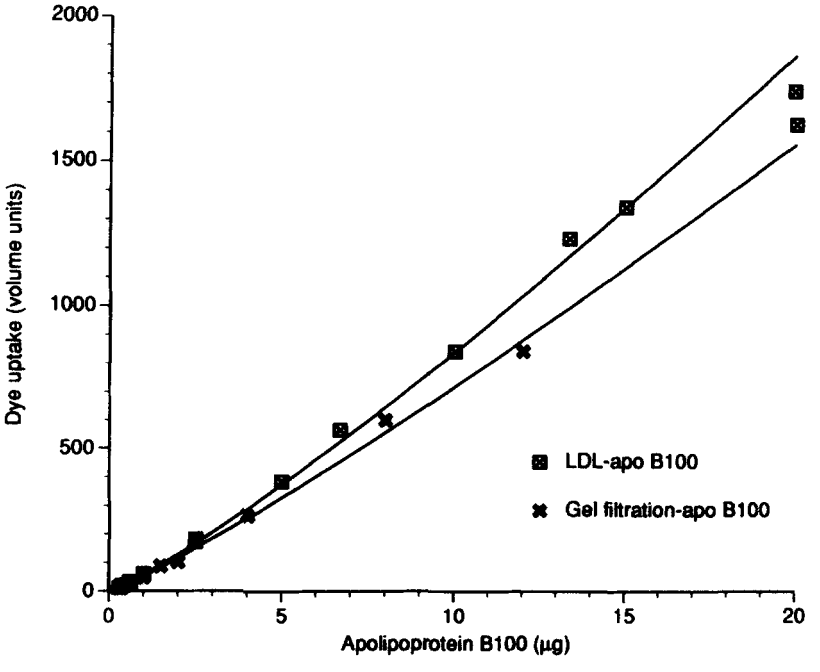

Fig. 5. Chromogenicities of human apoB-100 isolated by gel filtration and LDL-apoB-100 isolated by ultracentrifugation. The apolipoproteins were applied to large slab gels (14 × 12.5 × 0.15 cm). Gel filtration apoB-100: $y = 52.95 \times ^{1.13}$ ($r^2 = 0.997$); LDL-apoB-100: $y = 57.16 \times ^{1.17}$ ($r^2 = 0.998$).

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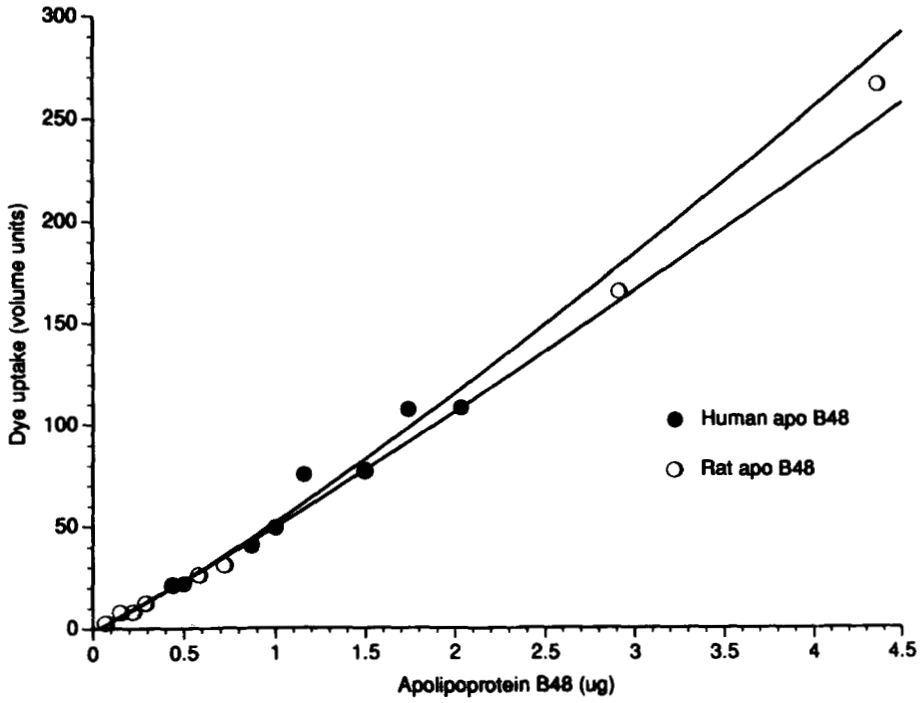

Fig. 6. Chromogenicities of human apoB-48 isolated by gel filtration and rat apoB-48 from lymph chylomicrons isolated by ultracentrifugation. The apolipoproteins were applied to large slab gels (14 × 12.5 × 0.15 cm). Human apoB-48: $y = 51.80 \times^{1.16}$ ($r^2 = 0.997$); rat apoB-48: $y = 49.90 \times^{1.10}$ ($r^2 = 0.992$).

The between-run coefficients of variation in dye uptake of apoB-100, apoB-48, and apoE in our secondary standard were 10.6%, 10.2%, and 10.1%, respectively, for 80 separate electrophoretic runs. Between-run variations in dye uptake for each protein were well correlated and presumably reflected differences in dye uptake and pipetting errors. Because the coefficient of variation in dye uptake was the same for each apolipoprotein, LDL could also be used as a secondary standard and the average densitometric volume units of the LDL-apoB-100 would then be taken to correct for variation in dye uptake of all three apolipoproteins in TRL samples.

In 16 healthy men, the average fasting concentrations of TRL-apoB-48, apoB-100, and apoE were 0.25 ± 0.12, 4.85 ± 2.19 and 0.88 ± 0.47 mg/dl, respectively (mean ± SD). The mass of TRL-apoB-48 was well correlated with that of TRL-apoB-100 ($r^2 = 0.76$) (**Fig. 9**).

DISCUSSION

We have developed procedures for delipidation of very small amounts of TRL without appreciable losses, and quantification of apoB-48, apoB-100, and apoE in TRL by SDS-PAGE over a range as large as 150-fold (for apoB-100) so that all three apolipoproteins can be quantified from a single application of TRL-protein to slab gels. An important modification to our previously described method (2) is the addition of apotransferrin (60 μg) to TRL containing < 75 μg total protein to prevent apolipoprotein losses during delipidation. Under these

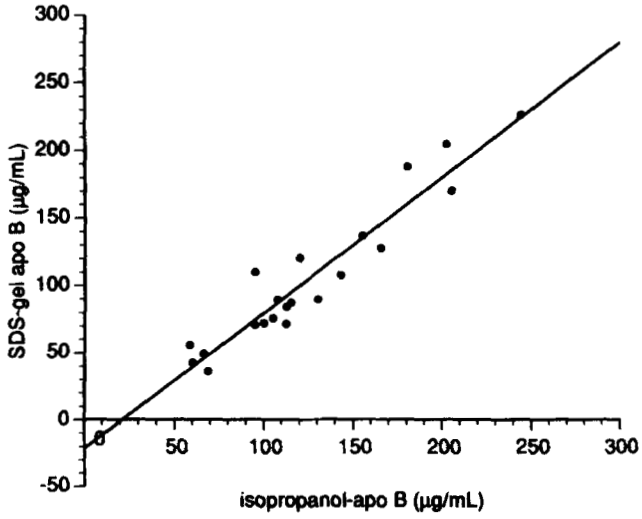

Fig. 7. Correlation between total apoB concentrations in TRL determined by isopropanol precipitation and SDS gel electrophoresis. The TRL contained 300–500 μg/ml of total protein. The mass measurements were compared by simple linear regression analysis for the equation $y = ax + b$; $y = 1.01 \times -21.28$, $r^2 = 0.91$ ($n = 21$).

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modified conditions it is possible to quantify apoB-100, apoB-48, and apoE in samples of apo-TRL containing as little as 10 μg of total protein, providing a sensitive method to measure these apolipoproteins, particularly apoB-48 whose concentration in normal TRL is typically 20-fold and 4-fold lower than that of apoB-100 and apoE, respectively. Another modification to our previous method is the measurement of apolipoprotein dye uptake by volume integration which, in contrast to area integration, enables quantification of widely varying masses of apoB-48, apoB-100, and apoE in bands of irregular shape. Apolipoprotein standard curves relating dye uptake to apolipoprotein mass can thereby be constructed over a wide range, permitting quantification of apoB-48, apoB-100, and apoE from a single application of TRL-protein to slab gels.

We validated our method for each protein. First, for total apoB (and thus apoB-100 which constitutes 95–98% of the total apoB mass in normal VLDL), we used the measured chromogenicities of apoB-48 and apoB-100 to calculate the mass of these proteins in TRL samples and found that our method compares favorably with the isopropanol precipitation method (Fig. 7). Furthermore, expressed as the percentage of the total protein mass in 14 TRL samples, we found that apoB-48 + apoB-100 measured by our method constituted 32.4 ± 7.6% (mean and standard deviation) of the protein mass, as compared to 37.1 ± 6.3% when total apoB was measured in the isopropanol-precipitated pellet of the same TRL. These findings are in good agreement with those of Kane and associates (10) who reported that total apoB, precipitated with TMU, constituted 36.9 ± 7.9% of the protein mass in 43 TRL samples. The slightly lower values obtained with our procedure may be the result of incomplete solubilization of apoB in the sample buffer. Despite these small differences, obvious advantages to our electrophoretic method over the isopropanol method include the ability to estimate both apoB-48 and apoB-100, and the much greater sensitivity of our method that permits quantification of these apolipoproteins in samples containing about 10 μg of total protein. The above comparisons validate our electrophoretic method for apoB-100 but do not provide direct validation for quantification of apoB-48 which constitutes the remaining 2–5% of the total apoB mass, and only 1–2% of the total protein mass in normal TRL.

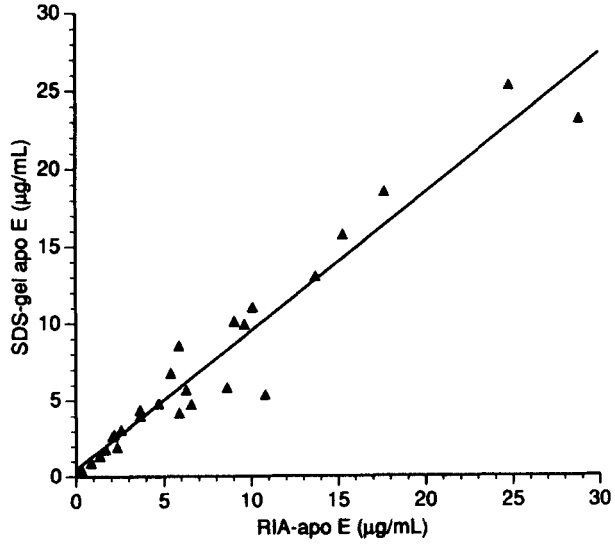

Fig. 8. Correlation between apoE concentrations in TRL determined by RIA and SDS gel electrophoresis. The TRL contained 40–500 μg/ml of total protein. The mass measurements were compared by simple linear regression analysis for the equation $y = ax + b$; $y = 0.91 \times +0.49$, $r^2 = 0.93$ ($n = 26$).

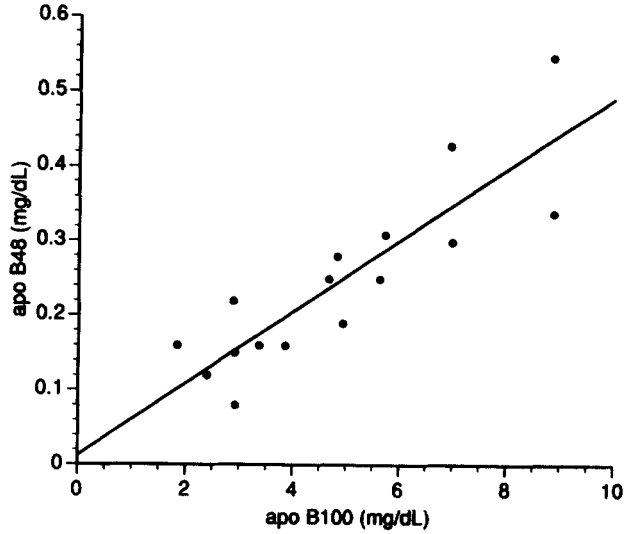

Fig. 9. Correlation between the masses of apoB-100 and apoB-48 in TRL samples from fasting, healthy young men; $r^2 = 0.76$ ($n = 16$).

We validated our method for quantification of apoB-48 in TRL by comparing the chromogenicities of rat and human apoB-48. This validation is based upon our mass measurement of apoB-48 in rat lymph chylomicrons precipitated with isopropanol (21) and of human apoB-48 isolated by gel filtration. Using these values to estimate the amount of apoB-48 applied to slab gels, we found that the chromogenicities of rat and human apoB-48 are virtually identical. This finding is expected given the close similarity in the derived amino acid sequences of rat and human apoB-48 (23, 24). Because we used distinct procedures to estimate the amount of rat and human apoB-48 applied to slab gels, our finding of equal chromogenicities for apoB-48 from these species indicates that the mass measurements are correct, and provides indirect validation for our electrophoretic method to quantify apoB-48 in TRL.

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Finally, we validated our method to quantify TRL-apoE in 26 samples, and found that it compares favorably with a radioimmunoassay (Fig. 8). The advantage of SDS gel electrophoresis for the current application derives from the fact that our method allows us to quantify apoE simultaneously with apoB-100 and apoB-48.

Several other quantitative (2–4, 9) and semi-quantitative (25, 26) methods have been proposed to measure apoB-48 and apoB-100 in plasma lipoproteins. The quantitative methods are based upon SDS-electrophoresis in polyacrylamide tube (4) or slab (2, 3, 9) gels. With 3.3% polyacrylamide tube gels, Poapst, Uffelman and Steiner (4) reported a lower chromogenicity for apoB-48 than for apoB-100, and a non-linear relationship between the intensity of dye uptake and the mass of apolipoprotein applied to the gels. The authors attempted to circumvent these problems by devising a tedious analysis involving several dilutions of a given sample to permit quantification of apoB-48 and apoB-100 within the linear portion of the standard curves. Applying this method to quantify apoB-48 and apoB-100, Karpe and associates (8) reported absolute levels of apoB-48 in TRL from fasting subjects that are several-fold higher than those we previously reported in healthy subjects with comparable plasma triglyceride levels (2). Their values for TRL apoB-100 are also considerably higher. These discrepancies, which are almost certainly methodological, suggest that data obtained with tube gels should be interpreted cautiously.

With slab gel electrophoresis to quantify apoB-48 and apoB-100 in rat TRL, Zilversmit and Shea (3) found equal chromogenicities between rat apoB-48 and rat and human apoB-100, and a linear response between dye uptake and apolipoprotein mass over a range of 0.35–35 μg of apoB applied to slab gels. These observations are consistent with our previous (2) and current findings and have also been confirmed by Karpe and Hamsten (9), who very recently reported a slab gel electrophoretic method quite similar to ours to quantify apoB-48 and apoB-100 in human TRL. Based upon their confirmation that the chromogenicities of apoB-48 and apoB-100 are equal, the authors calculated the mass of the two apoB species from a standard curve for apoB-100 in LDL. Using this method, they reported fasting concentrations and postprandial changes in TRL-apoB-48 and apoB-100 that are closer to those we reported previously (2). Our current values for apoB-100, apoB-48, and apoE in TRL from fasting, healthy young men are also similar to those previously reported by Schneeman et al. (2). An important limitation to the method of Karpe and Hamsten (9) derives from their finding that the standard curves were linear only within a narrow protein range of 0.1–0.8 μg for apoB-48 and 0.1–2.0 μg for apoB-100. Consequently, their method requires two applications of the same sample, a large load to measure apoB-48 and a small load to measure apoB-100. Our standard curves cover a wide protein range and permit quantification of apoB-48, apoB-100 as well as apoE from a single application of TRL-protein to slab gels.

The semi-quantitative methods developed to measure apoB-48 and apoB-100 include an high pressure liquid chomatographic method and an immunoblotting procedure. In the high pressure liquid chomatographic method (25), TRL are delipidated, the B apolipoproteins are precipitated with TMU, then solubilized in a reducing buffer containing 1% SDS and applied to a silica gel column to separate apoB-48 and apoB-100. Although this procedure appears to provide good separation of the two apoB species and a linear response up to 20 μg of applied protein mass, the relative amount of apoB-48 measured in fasting TRL (13% of total apoB) is difficult to reconcile with our current electrophoretic data and those reported previously (2, 9). This inconsistency may be the result of incomplete solubilization of apoB, particularly apoB-100, in the SDS buffer before its injection onto the high pressure liquid chomatography column.

The described immunoblot analysis uses an antiserum to the C-terminal sequence of apoB-48 to quantify apoB-48 in TRL during alimentary lipemia (26). The immunoblots show the specificity of the antiserum for apoB-48, and suggest a pattern of increased apoB-48 mass after a test meal. Although currently only semi-quantitative, this antiserum holds promise for absolute quantification of apoB-48.

In summary, we have developed and validated a sensitive method that permits delipidation of small amounts of TRL, and quantification of apoB-48, apoB-100, and apoE in as little as 10 μg of TRL-protein. Furthermore, by measuring dye uptake by volume integration of stained apolipoprotein bands separated by SDS-PAGE on slab gels, we have constructed standard curves that cover a wide protein range, permitting quantification of as little as 0.2 μg of apoB-48 and as much as 30 μg of apoB-100 from a single application of TRL. Given these key features, our method provides a sensitive and reliable means to quantify these apolipoproteins in TRL from postabsorptive and postprandial subjects.

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