Determination of apolipoproteins B-48 and B-100 in triglyceride-rich lipoproteins by analytical SDS-PAGE

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Abstract The present work describes a procedure for determining apolipoproteins (apo) B-100 and B-48 in subfractions of triglyceride-rich lipoproteins by analytical sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) with Coomassie staining. The chromogenicity of the two apoB species was found to be almost equal, and independent of lipoprotein particle size. Both proteins were sensitive to overloading of the gel, which resulted in low dye uptake. This was particularly evident for apoB-48. The precision of this analytical SDS-PAGE-based procedure to determine the plasma concentrations of apoB-100 and B-48 in triglyceride-rich lipoproteins was found to be appreciably low (coefficients of variation ranging between 3.1 and 14.1%).—**Karpe, F., and A. Hamsten.** Determination of apolipoproteins B-48 and B-100 in triglyceride-rich lipoproteins by analytical SDS-PAGE. *J. Lipid Res.* 1994. **35:** 1311–1317.

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Apolipoprotein (apo) B normally exists in two molecular variants (1), apoB-100, which is synthesized in the liver and secreted into plasma as the major protein component of very low density lipoproteins (VLDL), and apoB-48, which is secreted from the intestine in chylomicrons after absorption of dietary lipids. ApoB is a marker of lipoprotein particle number, as there is only one apoB molecule per lipoprotein particle and, in contrast to other lipoprotein-bound apolipoproteins and lipids, apoB does not exchange between different lipoprotein particles (2). For several reasons determination of apoB-100 and apoB-48 concentrations in plasma is difficult. First, the amino acid sequence of apoB-48 is identical to the N-terminal 48% of apoB-100. Second, apoB-48 is present in very low concentrations in plasma compared with apoB-100. Third, the expression of apoB epitopes varies depending on the lipid content of the particle (2). These circumstances may restrict the use of immunochemical techniques for determining the two apoB species. Attempts to measure the apoB-100 and apoB-48 content of triglyceride-rich lipoproteins have therefore focused on analytical sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) (3, 4). However, disagreement prevails as to whether differences in chromogenicity between the two molecular variants and between one and the same apoB species in lipoproteins of different particle size are factors to take into account when using SDS-PAGE. The present study describes a sensitive method to determine the plasma concentrations of the two forms of apoB in subfractions of triglyceride-rich lipoproteins with the use of analytical SDS-PAGE and an evaluation of the procedure.

MATERIAL AND METHODS

Plasma samples and isolation of triglyceride-rich lipoproteins

Venous blood was drawn into precooled tubes (Vacutainer, Becton Dickinson, Meylan Cedex, France) containing Na_2EDTA (final conc. ≈4 mM) and immediately put into ice-water. Lipoprotein fractions were prepared from plasma samples by density gradient ultracentrifugation, essentially as described by Redgrave and Carlson (5) and with minor modifications by Karpe et al. (6). Plasma was immediately recovered by low speed centrifugation (1,750 *g*, 20 min, 1°C). To minimize proteolytic degradation of apoB, 1.0 μl per ml plasma of phenylmethylsulfonylfluoride (PMSF, Sigma) 10 mM dissolved in *iso*-propanol and 5 μl per ml plasma of aprotinin (Trasylol, Bayer, Leverkusen, Germany) 1,400 μg/l were added. Subsequently, 702 mg solid NaCl was added to 5.0 ml of plasma to increase the density to 1.10 kg/l. A total volume of 4.0 ml of the d 1.10 kg/l plasma was put in the bottom of a 13.4-ml polyallomer ultracentrifuge tube (Ultra-

Abbreviations: LDL, low density lipoprotein; VLDL, very low density lipoprotein.

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Clear, Beckman Instr., Palo Alto, CA). A density gradient consisting of 3.0 ml each of 1.065, 1.020, and 1.006 kg/l NaCl solutions was then layered on top of the plasma. Ultracentrifugation was performed in a SW40 Ti swinging bucket rotor (Beckman) at 40,000 rpm and 15°C (Beckman L8-55 ultracentrifuge). Consecutive runs calculated to float $S_f > 400$ (32 min), S_f 60–400 (3 h 28 min), and S_f 20–60 (16 h) particles were then made. After each centrifugation, the top 0.5 ml of the gradient containing the respective lipoprotein subclasses was aspirated, and 0.5 ml of d 1.006 kg/l salt solution was used to refill the tube before the next run. The S_f 12–20 fraction was recovered after the last ultracentrifugal run by slicing the tube 29 mm from the top after the S_f 20–60 lipoproteins had been aspirated. All salt solutions were adjusted to pH 7.4 and contained 0.02% NaN_3 and 0.01% Na_2EDTA. Densities were verified to the fourth decimal place (densitometer; Paar, Graz, Austria).

Analytical SDS-PAGE

Preparation of samples. Isolated samples of apoB-containing lipoproteins were delipidated in a methanol–diethyl ether solvent system. A volume of 50–750 μl of the sample was injected into 4.0 ml of methanol in a 10-ml round-bottom glass tube with polyethylene stoppers. Ideally, the sample should contain 1–25 μg apoB after delipidation. A glass syringe with Teflon gaskets was used, the needle-tip of which was slightly deformed in order to spread the sample in the methanol to ensure efficient delipidation. A volume of 4.0 ml ice-cold diethylether was then added to the methanol, and the delipidation mixture was centrifuged for 30 min at 4,000 *g* at 1°C. After gentle removal of solvent with a water suction device, another 4.0 ml diethyl ether was immediately added and the sample was vortexed, pressure was equalized, and the sample was centrifuged for another 20 min under the same conditions, whereafter the diethyl ether was removed. Typically, a whitish haze was now seen at the bottom of the tube. The protein material was dissolved in 100–500 μl of 0.15 M sodium phosphate, 12.5% glycerol, 2% SDS, 5% β-mercaptoethanol, and 0.001% bromphenol blue, pH 6.8, at room temperature over a period of 30 min. The dissolved protein mixture was subsequently denatured at 80°C for 10 min. After denaturation, the tubes were quickly centrifuged to retain the condensed water on the inner wall of the tube in the sample. Most samples were then frozen at −20°C until analytical SDS-PAGE was performed.

Preparation of reference apoB-100. ApoB-100 derived from low density lipoprotein (LDL) was used as a reference protein and for standard curve dilutions. The apoB-100 preparation was derived from LDL ($1.030 < d < 1.040$ kg/l) that was isolated from fasting plasma samples by the density gradient ultracentrifugation procedure described above. The LDL subfraction was recovered from a 1.0-ml portion located between 39 and 46 mm from the top of the tube after 16 h of ultracentrifugation. Desalting was achieved by passing the LDL through a PD-10 column (Kabi-Pharmacia, Stockholm, Sweden). The total protein content was then determined with bovine serum albumin (BSA) as standard (7) with addition of SDS (final concentration 1%) to the reagent mixture to reduce turbidity. The mean of 12 determinations was taken as the final protein value. The desalted LDL preparation was delipidated and dissolved in the SDS-buffer described for the samples. The properties of the LDL protein preparation were then tested on analytical SDS-PAGE before it was accepted as an apoB-100 standard. The following criteria were used. First, to ensure that a successful delipidation had been accomplished, the amount of protein that was larger than the sharply focused apoB-100 band was not allowed to exceed 1% of the apoB-100 band using the laser scan densitometer. Second, signs of degradation of apoB-100 resulting in several sharp bands smaller than apoB-100 with an added protein density exceeding 1% of the apoB-100 band were not accepted. A band localized in the size range of apoB-48 was never seen, whereas a thin band with an R_f corresponding to apoE could occasionally be encountered on extremely overloaded gels. However, the absorbance of this band never exceeded 0.5% of the apoB-100 band. Due to these criteria, more than 97.5% of the total LDL protein was confined to one single band on analytical SDS-PAGE, which according to comparison with a commercially available high molecular weight standard protein mixture had an apparent molecular mass of about 500 kDa, corresponding to apoB-100. Furthermore, the linear regression of the absorbance of the apoB-100 band on the known amount of protein applied on the analytical SDS-PAGE gel, the latter ranging from 0.125 to 2.00 μg, was required to have an r value exceeding 0.95 and pass through the origin to be accepted.

A batch of the apoB-100 protein standard was frozen at −80°C in 100-μl portions in small vials and thawed immediately before use.

Gel casting. Polyacrylamide gels with a short stacking gel consisting of 3% acrylamide followed by a linear gradient from 3 to 20% acrylamide were cast using a two-chamber gradient mixer (GM-1, Pharmacia) connected to a gel-casting cassette (Hoefer Scientific, San Francisco, CA) prepared for 10 gels. The 3% acrylamide solution contained acrylamide (29.25 g/l), bisacrylamide (0.75 g/l, Bio-Rad, Hercules, CA), Tris (0.375 M), SDS (0.1%, Bio-Rad), Temed (1.0 μl/ml), and ammonium persulfate (≈0.4 g/l, prepared on ice), whereas the 20% acrylamide solution contained acrylamide (195 g/l), bisacrylamide (5 g/l), Tris (0.375 M), SDS (0.1%), Temed (0.6 μl/ml), and ammonium persulfate (≈0.1 g/l). The polymerization time was adjusted to 40 min by varying the volume of added ammonium persulfate.

Electrophoresis. Electrophoresis was performed using a

vertical Hoefer Mighty Small II electrophoresis apparatus connected to an EPS 400/500 (Pharmacia) power supply. The upper and lower electrophoresis buffers contained 25 mM Tris, 192 mM glycine, and 0.2% SDS adjusted to pH 8.5. Pre-electrophoresis was performed for 20 min at 60 V. Normally, both 2 and 20 μl of each sample were applied to obtain at least one protein band within the range of the apoB-100 standard curve. The outer wells were not used. Six amounts (0.10–2.0 μg) of the apoB-100 standard were applied on most gels. When two gels were run simultaneously and thereafter fixed, stained, and destained in the same bowl, a standard curve was applied to only one of these gels. Electrophoresis was first run at 60 V for 20 min, and then at 100 V for 2 h. Gels were fixed in 12% trichloroacetic acid for at least 30 min and stained in 0.2% Coomassie G-250 (Serva, Heidelberg, Germany), 40% methanol, 10% acetic acid, for at least 4 h in a glass petri dish. Destaining was in 12% methanol, 7% acetic acid with four changes of destainer during 24 h.

Gel scanning. Gels were scanned using a laser scanner (Ultroscan XL, Pharmacia-LKB) connected to a personal computer which was equipped with software providing automatic integration of areas under scanning curves (Gelscan XL, Pharmacia). The laser beam was adjusted for each lane to cover the whole band, which in most cases resulted in a laser track with a width of 5.6 mm and a length of about 15 mm. Background intensity was withdrawn after scanning an empty lane. Boundaries for start and stop of integration were set for each lane. Bands with an obvious absorbance greater than the highest standard point were not evaluated. In most instances, the 2-μl application was used for evaluation of the content of apoB-100 and the 20-μl application for evaluation of apoB-48 in the sample. A standard curve was fitted by linear regression using the CricketGraph® software (Apple, Cupertino, CA).

Determination of the chromogenicities of apoB-100 and apoB-48. ApoB-100 was prepared from S_f 60–400, S_f 20–60, and S_f 12–20 lipoproteins and the LDL (d 1.019 < d < 1.061 kg/l) fraction obtained from fasting plasma samples as described. ApoB-48 was prepared from chyle obtained from a 48-year-old man with obstruction of the thoracic duct. The density of the chyle was increased to approximately 1.10 kg/l by addition of solid NaCl (140.4 mg/ml chyle), essentially as described for plasma, and thereafter subjected to density gradient ultracentrifugation for 2 h. Chylomicron particles larger than approximately S_f 200 were then harvested from the top of the tube and delipidated by the procedure used for triglyceride-rich lipoproteins derived from plasma samples. After delipidation as described above, the fractions were dissolved in 2% SDS, 10 mM sodium phosphate, pH 7.4, and applied to a 1.6 × 50 cm Superose 6 column connected to a fast protein liquid chromatography system (FPLC, Pharmacia). Elution was made with 0.1% SDS, 10 mM sodium phosphate, pH 7.4, at a rate of 0.05 ml/min. The eluent was collected in 0.5-ml fractions and monitored at 280 nm to indicate the boundaries of recorded peaks. Fractions with pure apoB-100 and apoB-48 as determined by inspection of overloaded analytical SDS-PAGE were subjected to amino acid analysis as were the reference apoB-100 (prepared directly from LDL, 1.030 < d < 1.040 kg/l) and the BSA used for the Lowry protein determination. Volumes estimated to contain 2–6 μg apoB-100 or apoB-48 (20–100 μl) from the molecular sieve column were evaporated under a gentle stream of N_2. The reference apoB-100 was dialyzed against 0.1% SDS, 10 mM sodium phosphate, pH 7.4, prior to evaporation. A volume of 100 μl of the eluent buffer was treated in the same way to provide a background sample. The dried protein samples were hydrolyzed by constant boiling HCl under vacuum, in the presence of one crystal of phenol at 110°C for 24 h. A Pico Tag Work station from Waters (Milford, MA) was used. After hydrolysis the samples were dried and dissolved in citric acid buffer (0.1 M, pH 2.2) containing α-amino-β-guanido propionic acid-HCl as internal standard. The amino acid analysis was performed on a Biotronic LC5000 amino acid analyzer. The values were calculated by an on-line Shimadzu C-R2AX integrator. The background sample, which, in fact, contained less than 0.5% material recorded as amino acids compared with the apoB samples, was subtracted from the amino acid content of the samples. Aliquots of the apoB fractions (containing 0.2–1.0 μg of protein) subjected to amino acid analysis were also applied in triplicate to an analytical SDS-PAGE gel together with the standard apoB-100 for determination of the chromogenicity of each protein. The gels were stained with Coomassie G-250, and the dye uptake was recorded by laser densitometry as described.

Calculation of apoB-100 and B-48 concentrations. As the chromogenicities of apoB-100 and apoB-48 were found to be equal, the plasma concentrations of the two apoB species contained in triglyceride-rich lipoproteins were calculated from the equation derived from the linear regression line between the absorbance and the known apoB-100 content of the standard preparation. Quantification of the reference apoB-100 derived from isolated LDL was made according to Lowry et al. (7) with BSA used as standard, as a good correspondence was obtained with determination based on amino acid analysis (**Table 1**).

Reproducibility of the assay. The inter-assay variability of the apoB-48 and apoB-100 determinations was estimated by treating six identical 5-ml portions of plasma as six separate samples through all steps. Samples were ultracentrifuged separately and subsequently run on separate gels. By this approach, the combined error of the preparation of plasma, the ultracentrifugation procedure, the delipidation, and the analytical SDS-PAGE was tested. Furthermore, after the ultracentrifugation the total

TABLE 1. Comparison between protein mass determinations of isolated apoB-48 and apoB-100, the reference apoB-100, and BSA made by amino acid analysis and analytical SDS-PAGE with subsequent Coomassie staining

	1st Determination		2nd Determination
ApoB-48[a]			
Amino acid analysis (μg)	3.03		4.65
Determination with SDS-PAGE (μg)	2.19	3.20	4.21
Recovery (%)	72	106	90
ApoB-100 from S_f 60–400			
Amino acid analysis (μg)	4.07		1.97
Determination with SDS-PAGE (μg)	4.23	3.96	2.48
Recovery (%)	104	97	126
ApoB-100 from S_f 20–60			
Amino acid analysis (μg)	3.78		
Determination with SDS-PAGE (μg)	4.17	4.14	
Recovery (%)	110	109	
ApoB-100 from S_f 12–20			
Amino acid analysis (μg)	3.24		
Determination with SDS-PAGE (μg)	3.27	3.24	
Recovery (%)	101	100	
ApoB-100 from LDL[b]			
Amino acid analysis (μg)	2.89		82.0
Determination with SDS-PAGE (μg)	3.17		88.3
Recovery (%)	110		108
ApoB-100 from the reference LDL[c]			
Amino acid analysis (μg)	1.57		
Determination with SDS-PAGE (μg)	1.68		
Recovery (%)	107		
BSA			
Amino acid analysis (μg)	2.20		
Gravimetrical analysis (μg)	2.40		
Recovery (%)	109		

Values for SDS-PAGE masses are mean of triplicate determinations; apo, apolipoprotein; S_f, Svedberg flotation rate; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; LDL, low density lipoprotein; BSA, bovine serum albumin.

[a] ApoB-48 was isolated from chyle recovered from a chylothorax patient with a lipoprotein flotation rate of about $S_f > 200$.

[b] LDL was isolated from the same gradient ultracentrifugation as the triglyceride-rich lipoproteins, yielding a density cut of $1.019 < d < 1.061$ kg/l.

[c] ApoB-100 derived directly from delipidated LDL ($1.030 < d < 1.040$ kg/l) without previous isolation by molecular sieve chromatography.

protein content (7) of the isolated fractions was determined in order to discriminate the error of the ultracentrifugation step from that inherent in the later steps of the procedure. Finally, serial application of six identical samples was made on the same gel in order to test the intra-assay variability of the analytical SDS-PAGE per se.

Application on postprandial triglyceride-rich lipoproteins. A standardized oral fat load of a mixed-meal type (6) was given to 14 healthy men, 39–45 years of age, who were recruited from a population survey. Participants were admitted early in the morning to the Clinical Research Unit. They had been fasting for 12 h and had been asked to refrain from smoking during the fasting period and from alcohol intake during the preceding 3 days. Blood samples were obtained through an indwelling catheter inserted into the antecubital vein. A fasting blood sample was taken before the test meal. Subsequent blood samples were drawn hourly and the last sample was taken 9 h after ingestion of the emulsion. Samples for fractionation of triglyceride-rich lipoproteins and determination of apoB-100 and apoB-48 with the present method were drawn before and 3, 6, and 9 h after intake of the test meal.

The study protocol was approved by the Ethics Committee of the Karolinska Hospital, and all subjects gave oral informed consent.

RESULTS AND DISCUSSION

The present method provides a fairly simple, sensitive, and reliable means of determining the content of apoB-100 and apoB-48 in $S_f > 12$ lipoproteins by analyt-

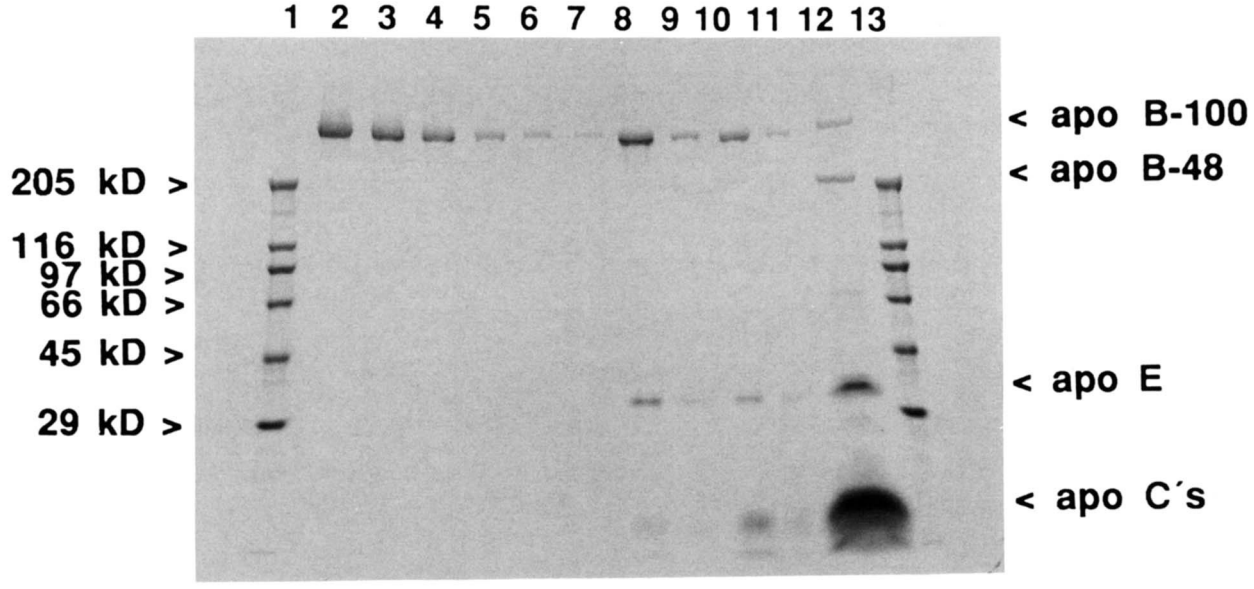

Fig. 1. A 3–20% acrylamide SDS-PAGE slab gel stained with Coomassie showing: (1) high molecular weight standard including myosin, 205 kD; β-galactosidase, 116 kD; phosphorylase B, 97 kD; bovine serum albumin, 66 kD; egg albumin, 45 kD, and carbonic anhydrase, 29 kD; (2–7) a reference apoB-100 standard curve: (2) 2.00 μg, (3) 1.50 μg, (4) 1.00 μg, (5) 0.40 μg, (6) 0.20 μg, and (7) 0.10 μg; (8) S_f 20–60 fraction 3 h after fat intake, 20-μl application; (9) same as (8) but 2-μl application; (10) S_f 60–400 fraction 3 h after fat intake, 20-μl application; (11) same as (10) but 2-μl application; (12) $S_f > 400$ fraction 3 h after fat intake, 20-μl application; (13) same as (1).

ical SDS-PAGE. A gel showing the results of representative samples and a standard curve of apoB-100 is shown in **Fig. 1.** ApoB-100 is distinctly separated from apoB-48. Scanning with evaluation of the area under curve for the absorbance of the standard curve is shown in **Fig. 2.** In our experience the standard curve is always linear if a maximum of 2 μg apoB-100 is applied to the gel. Application of larger amounts results in a biphasic relationship between protein content and dye uptake (**Fig. 3**). Interestingly, apoB-48 seems to be even more sensitive to overloading than apoB-100. No more than 0.8 μg could be applied to preserve the linear relationship between protein content and dye uptake. Using the present dimensions of slab gels and wells it was therefore possible to calculate the maximal apoB-100 content per area of resolution medium (polyacrylamide) that gives a linear relationship between apoB mass and dye uptake. The polyacrylamide surface area of the well used for standard curves is 3.75 mm^2, which gives a maximal resolution of approximately 0.5 μg/mm^2 for apoB-100 and a corresponding value of 0.2 μg/mm^2 for apoB-48. These measures might be critical when determining apoB mass by Coomassie staining of an analytical SDS-PAGE slab gel.

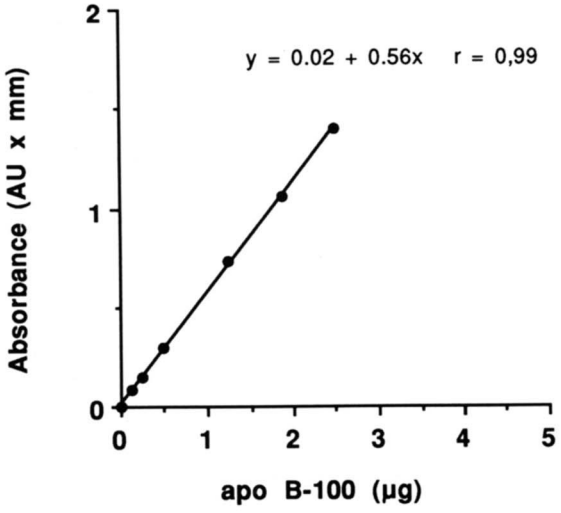

Fig. 2. Absorbance expressed as area under curve (AUC) (absorbance units (AU) × mm) for the laser densitometry of the apoB-100 band as a function of the reference apoB-100 mass.

When applying such small amounts of protein, there was no obvious difference in chromogenicity between apoB-100 and apoB-48 or between apoB-100 derived from triglyceride-rich lipoproteins of varying particle size (Table 1). This is in agreement with a recent study by Zilversmit and Shea (4), but seemingly contradictory to the findings of Poapst, Uffelman, and Steiner (3). The main difference between our method for determining apoB-100 and apoB-48 and that of Poapst et al. (3) is our use of slab gels on which a small amount of apoB is applied in contrast to rod gels on which at least a tenfold greater protein mass is applied. As clearly pointed out by Poapst et al. (3), the different chromogenicity of the various dilutions of apoB-100 resulted in an intersection of the ordinate far above the origin. Theoretically, this may cause problems in the determination of apoB-100 mass. However, Poapst et al. (3) circumvented this problem by taking account of

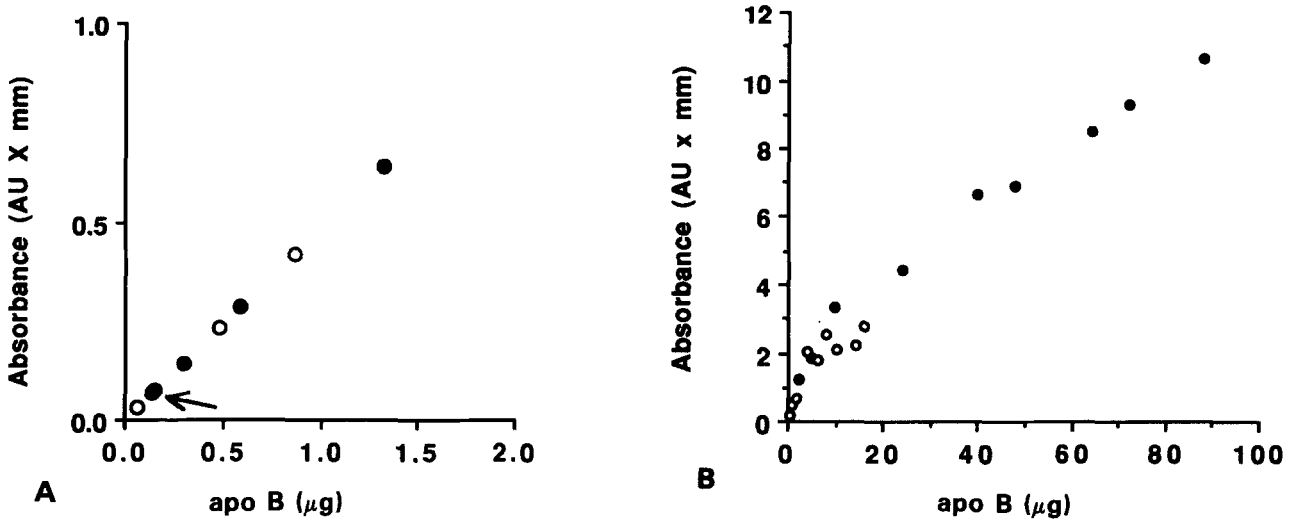

Fig. 3. A: Relationship between absorbance on laser densitometry of minute masses of isolated apoB-100 (filled circles) and apoB-48 (open circles) obtained from a molecular sieve column. The arrow indicates an open circle that is superimposed by a filled one. B: The effect of massive overloading of isolated apoB-100 (filled circles) and apoB-48 (open circles) on densitometric absorbance upon laser scanning.

the relative difference in chromogenicity between the two molecular variants of apoB and between the different preparations of apoB-100 by an analysis involving mass determination on several dilutions of the same sample.

It can thus be concluded that overloading of apoB on the SDS-PAGE gel may easily cause a non-linear relationship between protein content and staining intensity due to a restricted dye-protein interaction. Consequently, certain steps have to be taken to ensure accurate determination of the protein mass when staining with Coomassie. With the use of a slab gel, two applications of the same sample (typically 2 and 20 μl) can be used, one for determining the component that is present at a high concentration, and one for determining the component present at a low concentration. In the lane with the smaller protein amount, the band of the compound present at a low concentration will have an absorbance below the standard curve. In contrast, in the lane where the larger amount of protein has been applied, the high-concentration component will show signs of overloading. Use of rod gels may introduce an inherent bias, as only one sample can be applied to the gel.

The chromogenicity of apoB-100 derived from large apoB-containing lipoproteins (S_f > 60) has been reported to be increased compared with apoB-100 derived from smaller lipoprotein particles (S_f 0–12) (3). The present study cannot confirm this finding. The discrepancy may be explained by the obvious fact that apoB-100 is present at low concentrations in S_f > 60 lipoproteins, whereas the major proportion is contained in LDL (S_f 0–12). Therefore, overloading of a sample derived from LDL may easily occur, resulting in an inaccurately low estimation of the apoB-100 content and a seemingly lower chromogenicity of the apoB-100 preparation.

The precision of the apoB-48 and apoB-100 measurements including all steps (handling of plasma, ultracentrifugation, delipidation, and SDS-PAGE) was calculated as the coefficients of variation (CVs) for six separate determinations. ApoB-100 in the S_f > 400 and apoB-48 in the S_f 12–20 fractions were below the limit of detection (between 0.01 and 0.02 mg/l in plasma concentration). This yielded CVs of 9.8%, 7.4%, and 7.7% for apoB-48 in the fractions larger than S_f 12–20, and 5.3%, 3.1%, and 14.1% for apoB-100 in the fractions smaller than S_f > 400. To establish the error inherent in the ultracentrifugation step, a protein determination was made on isolated fractions of triglyceride-rich lipoproteins. In this analysis CVs of the S_f > 400, S_f 60–400, S_f 20–60, and S_f 12–20 fractions were 8.3%, 4.3%, 4.1%, and 7.8%, respectively. The error of the SDS-PAGE step was finally evaluated by analysis of six identical samples providing a CV of 4.4% for apoB-48 and 4.2% for apoB-100. It can thus be concluded that a major proportion of the analytical error is accounted for by the procedure for isolating the triglyceride-rich lipoprotein fractions.

ApoB-48 increased in response to the test meal in the S_f > 20 fractions, whereas the low initial level was unchanged in the S_f 12–20 fraction (**Table 2**). Fasting values of apoB-100 were in good agreement with values previously reported by Cohn et al. (8). A prominent increase was seen in the S_f 60–400 fraction, which is in agreement with findings reported from our own group (6) and other groups (8, 9). ApoB-100 remained very low in the S_f > 400 fraction after fat intake, whereas it approximately

TABLE 2. Fasting and postprandial plasma concentrations of triglycerides and of apoB-48 and apoB-100 in $S_f > 400$, S_f 60–400, S_f 20–60, and S_f 12–20 lipoprotein fractions in 14 healthy males

	0 Hour	3 Hours	6 Hours	9 Hours
Plasma triglycerides (mmol/l)	1.04 ± 0.41	2.55 ± 1.06[a]	2.25 ± 1.11[a]	1.32 ± 1.10
$S_f > 400$				
ApoB-48 (mg/l)	tr	0.3 ± 0.2	0.1 ± 0.1	0.1 ± 0.1
ApoB-100 (mg/l)	tr	0.2 ± 0.2	0.2 ± 0.2	0.1 ± 0.2
S_f 60–400				
ApoB-48 (mg/l)	0.2 ± 0.3	1.1 ± 0.6[a]	0.8 ± 0.7[a]	0.4 ± 0.6
ApoB-100 (mg/l)	15.6 ± 11.3	28.0 ± 15.1[b]	26.0 ± 19.3[c]	14.3 ± 15.3
S_f 20–60				
ApoB-48 (mg/l)	0.3 ± 0.3	0.6 ± 0.5[c]	0.4 ± 0.3	0.2 ± 0.3
ApoB-100 (mg/l)	28.9 ± 24.6	27.4 ± 13.3	29.9 ± 16.7	22.5 ± 14.3
S_f 12–20				
ApoB-48 (mg/l)	0.2 ± 0.3	0.1 ± 0.3	0.2 ± 0.3	0.2 ± 0.3
ApoB-100 (mg/l)	40.1 ± 28.5	30.8 ± 16.8[c]	37.8 ± 22.6	44.5 ± 25.7

Values are mean ± SD, n = 14; S_f, Svedberg flotation rate; apo, apolipoprotein; tr, trace.
[a] $P < 0.001$; [b] $P < 0.01$; [c] $P < 0.05$, compared with baseline (0 h); Student's paired *t*-test.

doubled between 0 and 3 h in the S_f 60–400 fraction (Table 2). At 6 h, the S_f 60–400 apoB-100 level was still elevated, but had returned to base-line values at 9 h. In the S_f 20–60 fraction no significant change was seen in response to the oral fat load. In contrast, a 25% decrease of apoB-100 was seen at 3 h in the S_f 12–20 fraction. Recently, a specific polyclonal antibody for detection of apoB-48 has been described (10, 11). The production of this antibody was achieved by immunizing New Zealand white rabbits with a synthetic oligopeptide of identical sequence as the C-terminal portion of apoB-48. This holds prospects for the future in terms of specific quantification of apoB-48-containing lipoproteins. However, so far no wider application of this antibody has been published.

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