papers on methodology

Determination of triglycerides in lipoproteins separated by agarose gel electrophoresis

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Abstract We developed a simple method for the quantitation of triglycerides in electrophoretically separated lipoproteins by specific enzymatic staining. After electrophoresis, glycerol is liberated from triglycerides by the action of cholesterol esterase. Glycerol is oxidized by a sequence of enzymatic reactions. Due to the presence of triosephosphate isomerase and glyceraldehyde-3-phosphate dehydrogenase in the reaction mixture, two moles of the precipitating dye formazane are generated per mole glycerol. The relative amounts of α, pre-β, and β lipoproteins are determined by densitometric scanning at 570 nm. Absolute triglyceride concentrations of the respective lipoprotein fractions are calculated from total triglycerides. When tested with purified very low density lipoproteins, the electrophoresis assay was linear between 0.08 and 6.5 g/l pre-β lipoprotein triglycerides. The intra-assay and inter-assay coefficients of variation were between 5.2% and 9.8%, and between 3.2% and 12.9%, respectively. Comparison of the electrophoresis method with a combined ultracentrifugation/precipitation method in 172 sera resulted in the following correlation coefficients: α lipoprotein versus high density lipoprotein triglycerides, $r = 0.847$; pre-β lipoprotein versus very low density lipoprotein triglycerides, $r = 0.989$; β lipoprotein versus low density lipoprotein triglycerides, $r = 0.815$. This method is easy to perform, and is a precise and accurate technique for the determination of lipoprotein triglycerides. It is the first reliable method that allows the direct quantification of LDL triglycerides without ultracentrifugation.—**Winkler, K., M. Nauck, R. Siekmeier, W. März, and H. Wieland.** Determination of triglycerides in lipoproteins separated by agarose gel electrophoresis. *J. Lipid Res.* 1995. **36:** 1839–1847.

Supplementary key words lipoprotein electrophoresis · enzymatic staining · atherosclerosis

Many epidemiological and clinical studies have shown the significance of serum lipoproteins for the development of coronary artery disease (CAD) (1–4). Lipoproteins are usually quantified by their content of cholesterol (CH). Aufenanger et al. (5, 6) reported a method for the separation of lipoproteins by means of electrophoresis and subsequent enzymatic staining of CH. In their method, the relative amounts of β, pre-β, and α lipoproteins were derived from densitometric scans and absolute values were calculated from total plasma CH.

In recent years, in addition to CH, plasma triglycerides (TG) have attracted increasing interest as a risk factor of coronary heart disease (7). There is a close correlation between plasma TG and the prevalence of small dense low density lipoproteins (LDL), referred to as LDL subfraction pattern B. Pattern B increases the risk of myocardial infarction (8). In the development of coronary artery disease, LDL TG appear to be of particular interest. In a report of 36 young survivors of myocardial infarction, only the level of TG in the LDL fraction was associated with the angiographic estimates of severity and the rate of progression of coronary heart disease (9). Additional evidence for the significance of LDL TG to atherogenesis came from a study showing that normolipidemic patients with CAD have a marked enrichment of LDL in TG compared to controls (10). Regnström et al. (11) found a correlation between the TG content of LDL and their susceptibility to oxidation, an observation which might provide a mechanistic link between LDL TG and atherosclerosis. TG of very low density lipoproteins (VLDL) are estimated with sufficient accuracy by measuring the total plasma TG content; high density lipoprotein (HDL) TG may be deter-

Abbreviations: apo, apolipoprotein; CH, cholesterol; CV, coefficient of variation; EDTA, ethylenediaminetetraacetate; HDL, high density lipoproteins; LDL, low density lipoproteins; Lp, lipoproteins; LRC, Lipid Research Clinics; 4-NBT, 4-nitroblue tetrazolium; TG, triglycerides; VLDL, very low density lipoproteins; REP, rapid electrophoresis system; TIM, triosephosphate isomerase; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

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mined after precipitating apolipoprotein (apo) B-containing lipoproteins with polyanionic agents (12–15). However, simple means to quantify LDL TG are lacking. LDL TG concentrations cannot be determined by precipitation techniques alone, as even those reagents tailored to selectively precipitate LDL have been shown to coprecipitate VLDL at significant amounts (16–18). The only means so far to determine LDL TG has been a combined ultracentrifugation/precipitation method in which VLDL are floated by ultracentrifugation and LDL TG are calculated from the difference of TG before and after precipitating LDL from the d > 1.006 kg/l bottom fraction (19, 20). As this procedure involves ultracentrifugation, it is not suitable for routine use. We developed a simple method to determine the TG content of lipoproteins by agarose gel electrophoresis and specific enzymatic staining.

MATERIALS AND METHODS

Patients

Blood was drawn from healthy individuals and hyperlipidemic patients after an overnight fast. The blood was allowed to clot at room temperature and serum was obtained by centrifugation at 1500 *g* for 10 min.

Lipoprotein separation by ultracentrifugation/precipitation

CH and TG were determined enzymatically with the CHOD-PAP and the GPO-PAP method (Boehringer Mannheim, Germany), respectively. To isolate individual lipoprotein fractions required in the studies of the analytical recovery, sequential preparative ultracentrifugation was performed at the following densities: d < 1.006 kg/l for VLDL, 1.019 < d < 1.063 kg/l for LDL, and 1.063 < d < 1.21 kg/l for HDL (21).

As the reference method in the intermethod comparison (n = 172), a combined technique of preparative ultracentrifugation and precipitation was used. In this method, VLDL were isolated by ultracentrifugation and LDL were precipitated from the d > 1.006 kg/l infranate with phosphotungstic acid/$MgCl_2$. Recoveries of the 1.006 kg/l spin were determined routinely and always exceeded or equalled 94%. VLDL CH and VLDL TG were then calculated as the differences between the concentrations in whole serum and the d > 1.006 kg/l infranatant; LDL lipids (CH and TG) were calculated as the differences between the concentrations in the d > 1.006 kg/l infranatant before and after LDL precipitation (19, 20).

Electrophoresis

Electrophoresis, drying, and densitometric scanning were performed on a Rapid Electrophoresis System (REP) from Helena (Helena, Hartheim, Germany). All enzymes, chemicals, and reagents were purchased from Boehringer Mannheim, Germany, Sigma-Aldrich, Deisenhofen, Germany, and Helena Laboratories, Hartheim, Germany. Without further treatment, 1 µl of sample was applied directly to a commercially available agarose gel (REP-HDL Plus from Helena, Hartheim, Germany) and electrophoresis was performed for 15 min at 400 V and 20°C. One gel accomodates up to 30 samples. TG were visualized enzymatically according to the sequence of reactions shown in **Fig. 1.** Details of the staining procedure are as follows. After completion of the electrophoresis, the gels are incubated for 30 min at 30°C with a reaction mixture containing 6 kU/l cholesterol esterase (EC 3.1.1.13), 4.8 kU/l glycerokinase (EC 2.7.1.30), 48 kU/l glycerol-3-phosphate dehydrogenase (EC 1.1.1.8), 300 kU/l triosephosphate isomerase (EC 5.3.1.1), 24 kU/l glyceraldehyde-3-phosphate dehydrogenase (EC 1.2.1.12), 4.8 kU/l diaphorase (EC 1.8.1.4), 5 mM ATP, 5 mM NAD^+, 0.5 mM Na_2-EDTA, 2 mM Na-cholate, 10 mM $MgCl_2$, 3 mM 4-nitroblue tetrazolium (dissolved in 70% (v/v) aqueous dimethylformamide) and 0.5% (v/v) Genapol® in 0.2 M glycylglycine, pH 8.5. The enzymes were kept separately on ice. Immediately before incubation, the enzymes and the substrate-solution were mixed, brought to incubation temperature, and applied to the gel. The Helena REP system possesses a gantry, holding the vessel with the staining reagent. After completion of the electrophoresis, the gantry moves over the gel and pours the reagent over the gel surface. Two glass rods spread the reagent evenly over the gel. Excess reagent is pushed over the edge of the gel by the glass rods and does not interfere with the staining procedure, thus providing reproducible staining results. The volume of staining reagent applied was always 1.5 ml. This is sufficient for all of the differently sized gels used in this study.

According to Aufenanger et al. (5), background staining increases when the gel is not incubated in the dark. Therefore, the gels were developed in the covered incubation chamber of the REP system. After staining, excess reagent was removed by washing and the stained bands were fixed in 10% (v/v) acetic acid for 30 min. Finally, the gels were soaked in distilled water for another 30 min and dried. Staining for CH was performed with a commercially available reagent (Helena, Hartheim, Germany). The relative amounts of β, pre-β, and α lipoprotein (Lp) lipids (TG or CH) were determined by densitometric scanning at 570 nm. Due to their tremendous polydispersity, the VLDL produced a broader band on lipoprotein electrophoresis than the

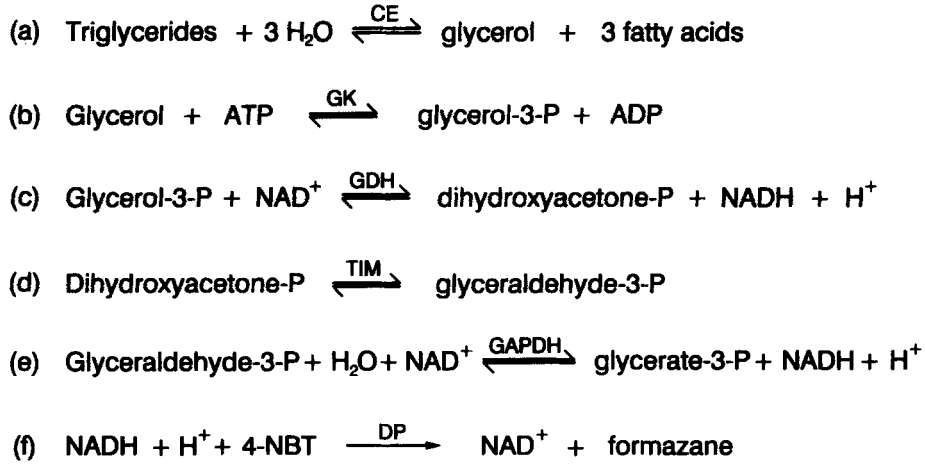

Fig. 1. Enzymatic determination of TG after electrophoretic separation: sequence of enzymatic ractions. Glycerol is liberated by the action of cholesterol esterase (CE) (eq. a), and converted to glycerol-3-phosphate by glycerokinase (GK) (eq. b). Glycerol-3-phosphate is further oxidized by glycerol-3-phosphate dehydrogenase (GDH), resulting in NADH and dihydroxyacetone-phosphate (eq. c). Dihydroxyacetone-phosphate is then converted to NADH and glycerate-3-phosphate via glyceraldehyde-3-phosphate by the action of the enzymes triosephosphate isomerase (TIM) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (eq. d and eq. e). The four reduction equivalents generated (i.e., 2 NADH, eq. c and eq. e) are then transferred to 4-nitroblue-tetrazolium by the enzyme diaphorase (DP). This results in the formation of one mole of the precipitating dye formazane per mole NADH (eq. f).

LDL and, incidentally, the pre-β and β Lp overlapped. Hence, the position separating β and pre-β Lp could not always be determined from the TG scans alone. In every individual sample lipoprotein bands were, therefore, delimited referring to corresponding gels stained for CH. The resulting areas of each fraction (β, pre-β, α) were expressed in percent of the total area. To obtain absolute values of triglycerides, the area percentages of the triglyceride staining were multiplied with the total plasma triglyceride concentration. To obtain absolute values of cholesterol, the area percentages of the cholesterol staining were multiplied with the total plasma cholesterol concentration.

Statistical methods

TG concentrations of lipoprotein fractions determined either by the electrophoresis technique and enzymatic staining or by the ultracentrifugation/precipitation method were compared by least squares linear regression analysis.

RESULTS

Enzymatic staining of triglycerides in electrophoretically separated lipoproteins

In our enzymatic staining method, TG are hydrolyzed by cholesterol esterase and the liberated glycerol is processed to dihydroxyacetone-phosphate resulting in the formation of one mole NADH per mole glycerol. If triosephosphate isomerase (TIM) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) are present, dihydroxyacetone-phosphate is further oxidized to glycerate-3-phosphate yielding a second mole NADH per mole glycerol. This approximately doubles the sensitivity of the staining method. **Figure 2** shows an experiment in which varying amounts of ultracentrifugally prepared VLDL were subjected to agarose gel electrophoresis and then stained for TG in the presence or in the absence of TIM and GAPDH. The slopes of the response versus concentration lines were 55.3 and 33.2 in the presence or absence of TIM and GAPDH, respectively.

The time kinetics of color formation was examined with VLDL (pre-β Lp). As shown in **Fig. 3,** the reaction is complete within 30 min in a sample containing as much as 5.4 g/l pre-β Lp TG. No background staining was observed, even when the optimum incubation time was exceeded. After fixation and drying, the gels could be stored for months without losing information. Therefore, densitometric scanning may be performed at any convenient time.

Sera from patients with different types of hyperlipidemias according to Frederickson and Lees (22) were analyzed by electrophoresis and subsequent staining for CH and TG, respectively (**Fig. 4**). In both staining methods, three lipoprotein fractions were detected, which will henceforth be referred to as α, pre-β, and β Lp. In gels stained for TG, unesterified, i.e., `free', glycerol is

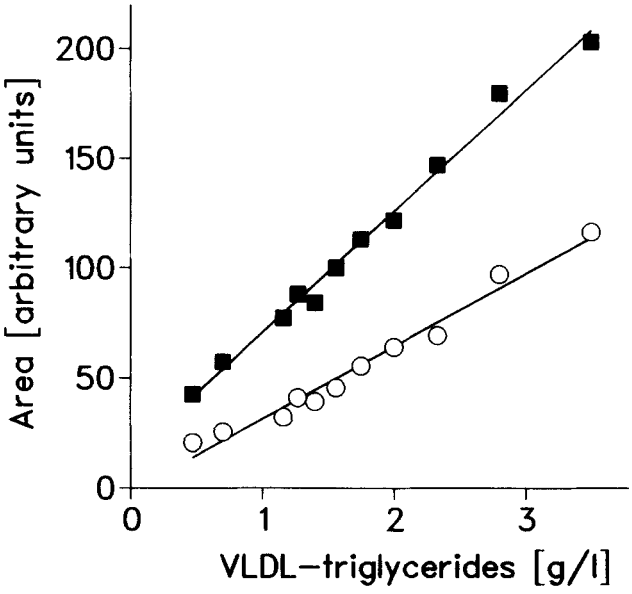

Fig. 2. Enzymatic staining of TG in agarose gels with or without TIM/GAPDH: Increasing concentrations of VLDL were applied to two gels. One gel was stained for TG with a staining solution containing TIM/GAPDH (■, $y = 55.31 \times +15.34$, $r = 0.995$); the other gel was stained without TIM/GAPDH (○, $y = 33.17 \times -1.74$, $r = 0.988$) for 30 min at 30°C. The amounts of formazane generated were measured by densitometry and are reported in arbitrary units.

detectable as an additional fourth fraction. Due to electroendosmosis, free glycerol migrates to the cathode. In normal serum, the enzymatic TG reagent stained the pre-β Lp most intensely, followed by the β Lp. Due to the low TG content in HDL, only a weak signal was obtained for the α Lp. As expected, the highest concentrations of pre-β Lp TG were present in type IV and type V hyperlipidemias. In type III hyperlipidemia, pre-β Lp TG were increased, but the β Lp fraction was also markedly enriched in TG, a finding that is compatible with the well-documented accumulation of TG-rich particles with β electrophoretic mobility in this disorder (23).

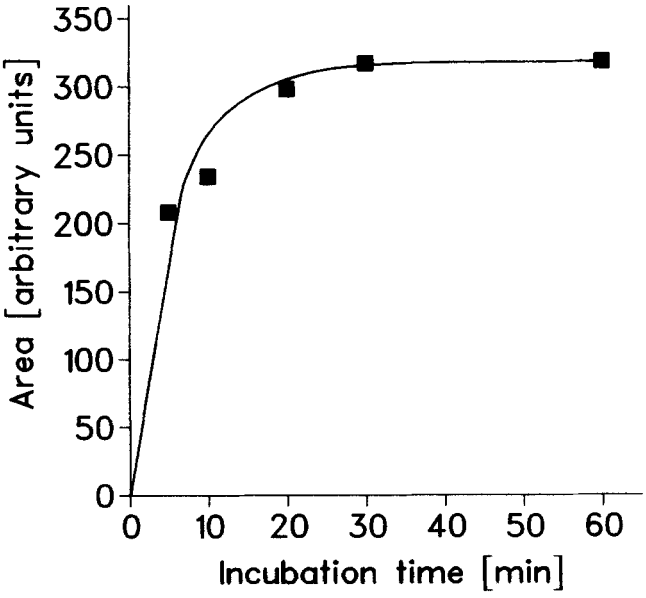

Fig. 3. Enzymatic staining of TG after electrophoretic separation: time course of the color development. After electrophoresis, VLDL (5.4 g/l TG) were stained at 30°C for the time intervals indicated on the abscissa. The gels were scanned densitometrically; the amounts of formazane generated are reported in arbitrary units.

Linearity range and recovery

To determine the analytical range and the recovery of the method, we added VLDL and LDL at increasing concentrations to sera with low TG. Thus, we generated samples containing between 0.15 and 5.4 g/l and between 0.08 and 0.56 g/l VLDL and LDL TG, respectively. When we plotted the VLDL TG and the LDL TG added against the expected concentrations, we obtained straight lines ($y = 0.95 \times +0.01$, $r = 0.998$, and $y = 0.90 \times +0.08$, $r = 0.999$, for VLDL and LDL, respectively), indicating that the assay was linear within the analytical range. The slopes of the regression lines were 0.95 and 0.90, corresponding to average percentage recoveries of 95% and 90% of the added VLDL TG and LDL TG, respectively. The recovery of HDL TG was determined in an analogous fashion; it was 89%.

We showed previously that Lp[a] migrated to the pre-β position in the agarose gels used in this study (24, 25). This raises the possibility that Lp[a] TG might interfere with the determination of the pre-β TG. To assess the effect of Lp[a] on pre-β TG, VLDL or Lp[a] were mixed with VLDL-deficient serum to yield different concentrations of VLDL and Lp[a]. VLDL and VLDL-deficient serum were separated by ultracentrifugation at d = 1.006 kg/l. Lp[a] was purified from the eluate of a dextran sulphate-based LDL apheresis system by preparative ultracentrifugation and size exclusion chromatography as described (26). We generated mixtures containing VLDL TG at final concentrations of 0.5 to 2.0 g/l; Lp[a] was added at particle mass concentrations of 0.18 to 0.36 g/l, the latter value corresponding to 0.12 g/l Lp[a] CH and 0.04 g/l Lp[a] TG. In none of these mixtures did Lp[a] discernibly affect the results obtained for pre-β TG, indicating that Lp[a] TG do not affect our assay significantly in the clinically relevant concentration range.

Imprecision

To determine intra-assay imprecision, we analyzed two sera containing 1.14 g/l and 2.83 g/l total TG, respectively. The samples were run 15 times on the same gel. As shown in **Table 1,** the coefficients of variation (CV) ranged between 5.2% (pre-β Lp TG in the sample

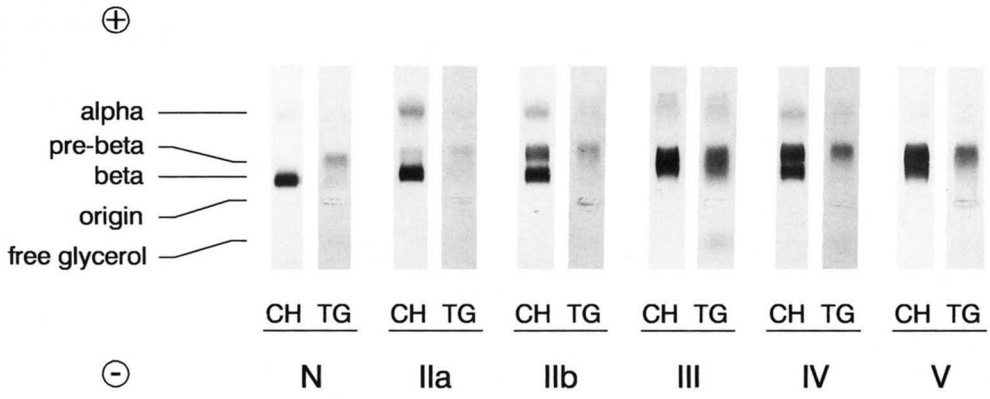

Fig. 4. Detection of TG after electrophoresis of normal serum and of sera with different types of hyperlipidemias. Three lipoprotein bands corresponding to β, pre-β, and α Lp are obtained. The TG staining solution also reacts with nonesterified (i.e., free) glycerol which migrates to the cathode due to electroendosmosis. N, Normal serum (CH: 2.07 g/l, TG: 0.74 g/l); type IIa (CH: 2.61 g/l, TG: 1.10 g/l); type IIb (CH: 2.38 g/l, TG: 4.45 g/l); type III (CH: 3.63 g/l, TG: 8.76 g/l); type IV (CH: 2.65 g/l, TG: 6.16 g/l) and type V (CH: 2.41 g/l, TG: 14.14 g/l).

with 1.14 g/l TG) and 9.77% (β Lp TG in the sample with 2.83 g/l TG). Serum pools with 0.7 g/l, 1.27 g/l, and 2.03 g/l total TG, respectively, were used to assess the inter-assay imprecision. At least 19 analyses were carried out on consecutive working days. Inter-assay imprecision was calculated from single measurements of each sample on each day. As the electrophoretic mobility of Lp may change during prolonged periods of storage, we stabilized the serum pool used for the determination of the between-run imprecision by adding sucrose at a final concentration of 15% (w/v) (27). The inter-assay CVs are shown in **Table 2;** they ranged between 3.2% (pre-β Lp TG in the sample with 2.03 g/l total TG) and 12.9% (α Lp TG in the sample with 2.03 g/l total TG). No systematic trend over time was observed, suggesting that sucrose prevented major alterations of the samples during the observation period.

TABLE 1. Electrophoretic determination of lipoprotein triglycerides: intra-assay precision

	Serum A[a]		Serum B[b]	
Triglyceride	Mean	CV	Mean	CV
	g/l	%	*g/l*	%
α Lp TG	0.28	9.42	0.45	5.59
Pre-β Lp TG	0.50	5.20	1.42	5.65
β Lp TG	0.37	5.66	0.97	9.77

[a]Serum A was run 15 times on the same electrophoretic plate. Total TG was 1.14 g/l; total CH was 1.76 g/l.

[b]Serum B was run 15 times on the same electrophoretic plate. Total TG was 2.83 g/l; total CH was 1.87 g/l.

Comparison with an ultracentrifugation/precipitation method

One hundred seventy-two sera were used to compare the electrophoresis and enzymatic staining method (α Lp TG, pre-β Lp TG, and β Lp TG, respectively) with an LRC-type combined ultracentrifugation and precipitation assay for lipoprotein triglycerides (the results of which will be denoted HDL TG, LDL TG, and VLDL TG, respectively). The scatterplots are shown in **Fig. 5.** α Lp TG and HDL TG correlated well (r = 0.847); the slope of the regression line was 1.33, suggesting that HDL TG were overestimated by α Lp TG. An excellent correlation was found between pre-β Lp TG and VLDL TG (r = 0.989); pre-β Lp TG were measured slightly (8%) lower than VLDL TG. The correlation of β Lp TG and LDL TG was also good (r = 0.815). The slope of the regression line was 1.25, indicating that LDL TG were overestimated by β-Lp TG.

Triglyceride and cholesterol distribution in normolipidemic sera

To obtain estimates for the distribution of TG among lipoproteins in normolipidemic individuals (Chol ≤ 2 g/l; TG ≤ 2 g/l), sera from 25 women and 19 men were analyzed (**Table 3**). Total CH (1.52 g/l female, 1.63 g/l male) was slightly higher in the men than in the women. Total TG (1.07 g/l female, 1.03 g/l male), α Lp TG (0.18 g/l female, 0.19 g/l male), pre-β Lp TG (0.49 g/l female, 0.52 g/l male) and β Lp TG (0.40 g/l female, 0.33 g/l male) were almost identical in females and males (Table 3). The cholesterol distribution among individual lipoprotein fractions determined by electrophoresis and

TABLE 2. Electrophoretic determination of lipoprotein triglycerides: inter-assay imprecision

Triglyceride	Serum A[a]		Serum B[b]		Serum C[c]	
	Mean	CV	Mean	CV	Mean	CV
	g/l	%	*g/l*	%	*g/l*	%
α Lp TG	0.218	10.76	0.347	8.95	0.320	12.94
Pre-β Lp TG	0.220	6.26	0.464	6.16	1.151	3.20
β Lp TG	0.262	6.91	0.459	6.53	0.560	5.03

[a]Serum A was analyzed once each day on 19 consecutive working days. Total TG was 0.7 g/l; total CH was 1.12 g/l.
[b]Serum B was analyzed once each day on 23 consecutive working days. Total TG was 1.27 g/l; total CH was 1.69 g/l.
[c]Serum C was analyzed once each day on 20 consecutive working days. Total TG was 2.03 g/l; total CH was 1.53 g/l.

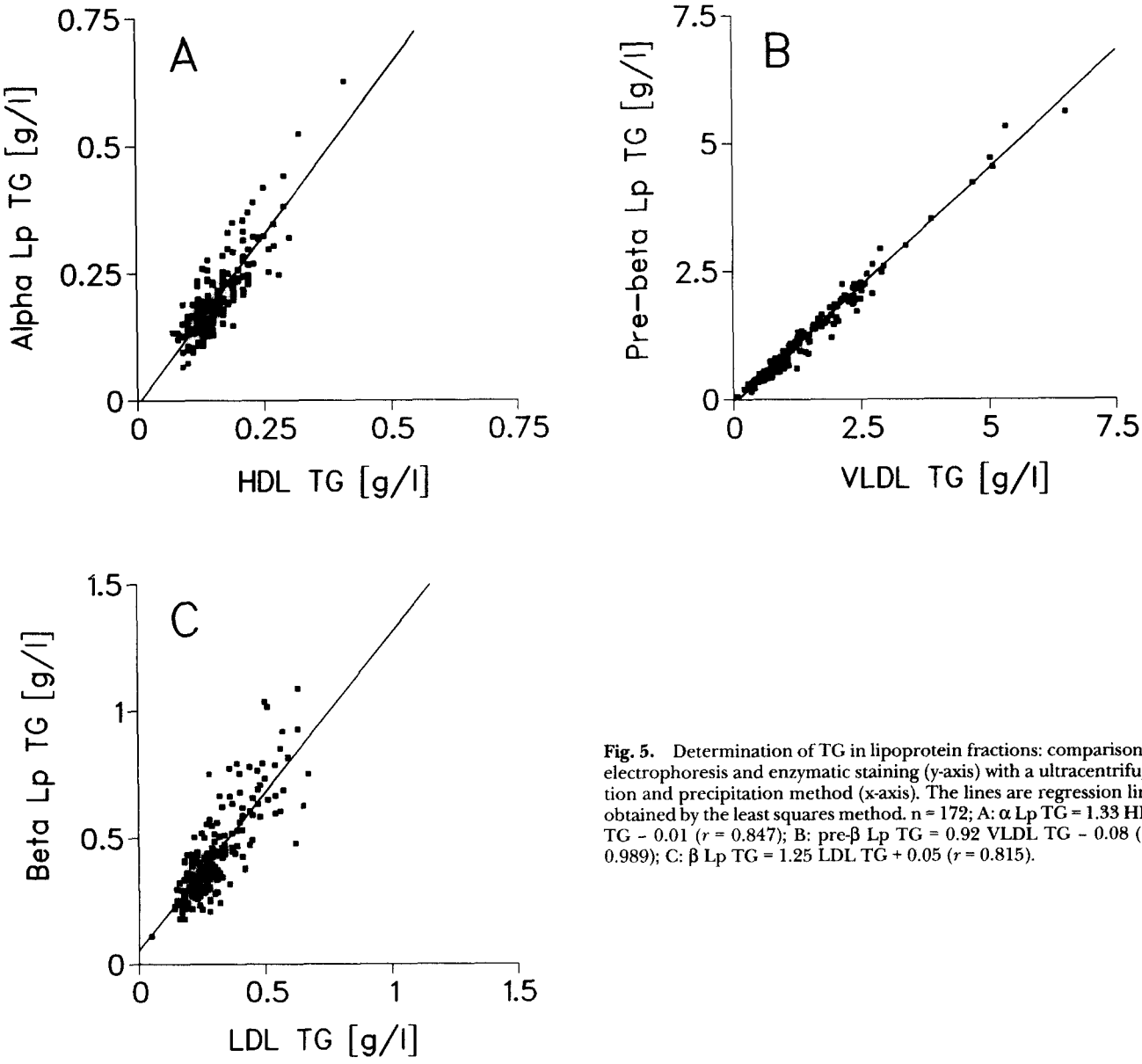

Fig. 5. Determination of TG in lipoprotein fractions: comparison of electrophoresis and enzymatic staining (y-axis) with a ultracentrifugation and precipitation method (x-axis). The lines are regression lines obtained by the least squares method. n = 172; A: α Lp TG = 1.33 HDL TG - 0.01 (r = 0.847); B: pre-β Lp TG = 0.92 VLDL TG - 0.08 (r = 0.989); C: β Lp TG = 1.25 LDL TG + 0.05 (r = 0.815).

TABLE 3. Distribution of triglycerides and cholesterol among lipoprotein fractions in normolipidemic (CH ≤ 2 g/l; TG ≤ 2 g/l) females and males

	Women (n = 25)		Men (n = 19)	
	Mean ± SD	Median (Range)	Mean ± SD	Median (Range)
	g/l	*g/l*	*g/l*	*g/l*
Total TG	1.07 ± 0.42	1.00 (0.47-1.88)	1.03 ± 0.36	1.01 (0.47-1.67)
α LP TG	0.18 ± 0.09	0.16 (0.06-0.46)	0.19 ± 0.05	0.18 (0.10-0.29)
Pre-β Lp TG	0.49 ± 0.22	0.45 (0.19-1.07)	0.52 ± 0.28	0.47 (0.19-1.07)
β LP TG	0.40 ± 0.17	0.37 (0.13-0.68)	0.33 ± 0.10	0.32 (0.19-0.55)
Total CH	1.52 ± 0.28	1.53 (0.89-2.00)	1.63 ± 0.25	1.67 (0.94-1.95)
α LP CH	0.47 ± 0.08	0.45 (0.18-0.58)	0.43 ± 0.10	0.42 (0.33-0.77)
Pre-β Lp CH	0.12 ± 0.06	0.11 (0.03-0.38)	0.12 ± 0.07	0.13 (0.04-0.33)
β Lp CH	0.93 ± 0.26	0.93 (0.40-1.23)	1.08 ± 0.25	1.11 (0.42-1.41)

subsequent enzymatic staining was similar to values reported previously (15).

DISCUSSION

We describe the first method for the quantification of TG in electrophoretically separated lipoproteins. In this method, TG are detected by enzymatic staining. TG are cleaved enzymatically to produce free glycerol. The latter is detected in a sequence of reactions ultimately leading to the formation of the insoluble dye formazane. To hydrolyze TG we preferred cholesterol esterase (EC 3.1.1.13) over lipase (triacylglycerol acylhydrolase) (EC 3.1.1.3) which is present in most enzymatic TG reagents. Lipase alone or in combination with cholesterol esterase gave less satisfactory results. With cholesterol esterase, TG and cholesteryl esters are cleaved at the same time. As a consequence, lipoprotein particles are effectively denaturated and the access of the enzyme to its TG substrate is obviously facilitated. An additional feature of the enzymatic staining method is that glycerol is oxidized twice, resulting in the formation of two molecules of formazane per molecule glycerol. Thus, even lipoprotein fractions containing low amounts of TG (i.e., α Lp) are detectable after electrophoresis. The enzyme diaphorase (EC 1.8.1.4) functions as an electron-carrier between NADH and 4-nitroblue tetrazolium, transforming the latter into the precipitating dye formazane.

Enzyme activities and substrate concentrations needed for TG detection after electrophoresis are notably higher compared to those typically used for the determination of total TG in solution (28). This is due to the fact that the reaction mixture is spread over the gel and is thus diluted, whereas the TG substrate is concentrated in the lipoprotein bands.

The new method is precise and accurate. The intra-assay and inter-assay CVs are similar to those reported for electrophoresis assays of plasma lipoproteins involving precipitation of lipoproteins with polyanions (15) or enzymatic detection of CH (6, 29). When we compared the new electrophoresis method with a combined ultracentrifugation and precipitation method to separate lipoproteins, we obtained excellent correlation coefficients. For VLDL TG and pre-β Lp TG, the slope of the regression line was close to unity. However, the electrophoresis method resulted in concentrations of LDL TG and HDL TG 25% and 33% higher, respectively, than the combined ultracentrifugation and precipitation method. One explanation for the differences might be that the enzymatic conversion of pre-β Lp TG was not quantitative at high concentrations. Because in the electrophoresis method final concentrations are derived from densitometrically determined relative peak areas, underestimation of pre-β Lp TG would have caused β Lp TG and α Lp TG to be overestimated. For two reasons, however, this is not very likely. First, when we supplemented serum with purified VLDL, the concentration of TG recovered increased linearly up to 5.4 g/l VLDL TG, the slope of the line indicating an analytical

recovery of 95%. Second, the scatterplot showing VLDL TG and pre-β Lp TG was perfectly linear within the entire range of concentrations (0.08 to 6.51 g/l VLDL TG) likewise ruling out that incomplete analytical recovery occurred at high concentrations. From these data we infer that the trend to overestimate LDL TG and HDL TG was not attributable to analytical characteristics of the TG staining procedure itself, but to the fact that the two methods used to separate lipoproteins, electrophoresis on the one hand and ultracentrifugation and precipitation on the other, rely on completely different physicochemical characteristics of lipoprotein particles.

On electrophoresis of lipoproteins, the pre-β band not only contains VLDL, but also Lp[a] (24, 25). Theoretically, the electrophoresis assay might, therefore, overestimate VLDL TG in samples with high Lp[a] concentrations. We were, however, not able to detect a significant effect of Lp[a] on the results obtained for pre-β TG when we added purified Lp[a] at particle mass concentrations up to 0.36 g/l, which is the upper limit of the reference range for Lp[a]. This is obviously due to the low TG content of Lp[a]. In a normolipidemic individual, fasting TG and VLDL TG are virtually never less than 0.7 g/l and 0.6 g/l, respectively. Assuming a total Lp[a] particle mass concentration of 0.3 g/l, the Lp[a] TG would be 0.03 g/l. This corresponds to 5% of the assumed VLDL TG of 0.6 g/l and is within the random analytical error of our method. Even Lp[a] particle mass concentration of 0.6 g/l would lead to an overestimation of VLDL TG by not more than 10% in this sample. As fasting triglycerides are often higher than 0.7 g/l, percentage increase of VLDL TG brought about Lp[a] will be even less. Along with our experimental data, these considerations illustrate that interference resulting from high Lp[a] concentrations would have only minor, if any, practically relevant influence on the electrophoretic assessment of VLDL TG.

In summary, we present a simple, precise, and accurate technique to determine the TG content of plasma lipoprotein fractions. It is the first reliable method allowing the direct determination of LDL TG without ultracentrifugation. The method takes little time and does not require special equipment; it is hence suitable for research and clinical use as well. As we have demonstrated, it is easily automated and may, therefore, become a valuable tool in future investigations on the role of TG in the development of atherosclerosis.

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