Influence of Remnant Accumulation Markers on Plasma Concentrations of Two Lipoprotein(a) Subspecies (containing or free of apoE)

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Lipoprotein(a) [Lp(a)], an atherosclerosis marker, has 2 subspecies differing in structure and composition that can easily be distinguished by the presence or absence of apolipoprotein E (apoE). The subspecies containing apo E [Lp(a):B:E] is found mainly in the very–low-density lipoprotein (VLDL) size range, while that free of apoE [Lp(a):B] is found mainly in the LDL size range. As little is known about the physiologic function of these subspecies, this study investigated Lp(a):B and Lp(a):B:E concentrations in a population of normotriglyceridemic and moderately hypertriglyceridemic subjects in fasting state and attempted to determine the parameters influencing their plasma concentrations. The subjects studied (n = 98) had a mean total Lp(a) concentration of 108 mg/dL (28 to 252, minimum to maximum), a mean Lp(a):B concentration of 92.6 mg/dL (5 to 254), and a mean Lp(a):B:E concentration of 15.6 mg/dL (0 to 137). These results indicate that Lp(a):B:E, even in normolipidemic subjects, constitutes a detectable part of total Lp(a), ie, a mean percentage of 16.2% (0% to 96%). Multiple stepwise regression analyses showed that triacylglycerol has no independent effect on the concentration of Lp(a) subspecies, and that remnant accumulation markers, such as the E/LpB:E molar ratio (number of apoE per particle containing both apoB and apoE) and apoE-LpB (mass of apoE bound to particles containing both apoB and apoE), have a strong independent effect on this concentration. A strong positive influence of E/LpB:E on Lp(a):B:E subspecies was noted, as well as a negative influence of apo E-LpB on Lp(a):B subspecies. Taken together, these results suggest that the apoE bound to LpB:E particles plays a dominant role in the concentration of Lp(a) subspecies and that a redistribution of Lp(a) subspecies occurs under the influence of the apoE content of triacylglycerol-rich lipoprotein particles.

HUMAN LIPOPROTEIN(a) [LP(a)], discovered by Berg in 1963,[1] is of particular interest as a marker of atherosclerosis (a plasma Lp[a] level above 30 mg/dL is an independent cardiovascular risk factor).[2-7] Its physiologic function and metabolism have not been thoroughly elucidated, and most of the information concerning its structure and composition relates to particles rich in cholesterol ester. Lp(a) is currently regarded as a low-density lipoprotein (LDL)-like lipoprotein containing a single copy of apolipoprotein B (apo B) cross-linked to apo(a) by 1 or more disulfide bonds and exhibiting a density between that of LDL and high-density lipoprotein (HDL) (1.055 g/mL $< d <$ 1.120 g/mL).[8-10] However, several studies have shown that apo(a) is also found in association with less dense triacylglycerol-rich lipoproteins in the density fraction ($d <$ 1.006 g/mL) of plasma. This has been shown in normolipidemic subjects after ingestion of a fat-rich meal[11-13] or intravenous infusion of a lipid emulsion.[14] Apo(a) has also been found in association with triacylglycerol-rich lipoproteins in patients with type III[14] or IV[15] hyperlipoproteinemia or lipoprotein lipase deficiency,[14] as well as in those in the fasting state.[12,16] It has been determined[12] that 0% to 17% of total plasma apo(a) in the fasting state is associated with triacylglycerol-rich lipoproteins, while this percentage increases (0% to 83%) in the fed state in correlation with high plasma triacylglycerol levels.[12]

These results indicate that Lp(a) can be found in the very–low-density lipoprotein (VLDL), as well as the LDL density range. Two subspecies of plasma Lp(a) differing in density and lipid-protein composition[13,16] can be distinguished, depending on the absence or presence of apoE: (1) Lp(a) free of apoE [Lp(a):B], found mainly in the LDL size range, is enriched in cholesterol ester, but has low triacylglycerol content; and (2) Lp(a) containing apoE [Lp(a):B:E], found mainly in the VLDL size range, is enriched in triacylglycerol, but has low cholesterol ester.[13,16] The pathophysiologic role of these subspecies and the factors influencing them have not been determined.

The purpose of the present study was to determine the concentration of Lp(a):B and Lp(a):B:E subspecies in a population of normolipidemic and moderately hypertriglyceridemic subjects in the fasting state and define the lipid or clinical parameters that influence their plasma concentrations.

MATERIALS AND METHODS

Subjects and Sampling

The population included in our study was screened among subjects undergoing a medical checkup in a preventive medical center in Western France sponsored by an agricultural insurance company (Institut pour la Santé de l'Ouest, Carquefou, France). Controls and hypertriglyceridemic subjects were selected among men over 18 years of age without hepatic or renal disease or any history of coronary heart disease, as assessed by a medical questionnaire, and whose aspartate transaminase (ASAT), alanine transaminase (ALAT), creatinine, and fasting blood glucose values were in the normal range. Plasma cholesterol and triacylglycerol concentrations below 250 mg/dL and 400 mg/dL, respectively, were required. The population studied was divided into 2 groups according to triacylglycerol levels: normotriglyceridemic (NTG) (<150 mg/dL) and hypertriglyceridemic (HTG) (>150 mg/dL). Subjects taking medications known to affect plasma lipid levels were excluded. The 98 subjects selected among the 756

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meeting these criteria had Lp(a) plasma levels greater than 25 mg/dL. After an overnight fast, venous blood was collected in tubes containing EDTA, and plasma was immediately separated by centrifugation. After addition of preservatives [final concentrations: EDTA 0.01%, NaN_2 0.05%, Pefabloc (4-(2 aminoethyl) benzenesulfonyl fluoride hydrochloride) 1 mmol/L (Interchim, Montluçon, France)], plasma samples were stored at +4°C until analysis.

Analytical Methods

Biochemical parameters and lipoproteins. Cholesterol and triacylglycerol were determined by automated procedures (Bayer Diagnostic, Puteaux, France) adapted to an Axon analyzer (Bayer Diagnostic). Cholesterol was measured in HDL containing supernatant after phosphotungstate/magnesium chloride precipitation of apoB-containing lipoproteins (Boehringer, Mannheim, Germany), using a Hitachi 747 analyzer (Roche Diagnostic, Meylan, France). LDL-cholesterol was calculated with the Friedewald formula. Lp(a) screening in human plasma was performed by electrophoresis on agarose gel using the Hydragel Lipo+Lp(a) kit (Sebia, Issy-les-Moulineaux, France). This gel detects an Lp(a) band once the Lp(a) concentration is above 10 to 25 mg/dL.[17] This preselection procedure was also intended to avoid inclusion of any samples containing chylomicrons.

Lipoprotein particles. Total plasma Lp(a), apoB, and apoE were measured by electroimmunoassay (Sebia). Separation of the 2 subpopulations of apo(a) containing lipoprotein particles [Lp(a):B and Lp(a):B:E], as well as the 2 subpopulations of apoB containing lipoprotein particles [LpB:E and LpB], was achieved by immunoprecipitation (as adapted from Alaupovic).[18] Briefly, the plasma sample was subjected to immunoprecipitation using polyclonal antibodies purified from rabbit antiserum against human apoE (Dako, Trappes, France). The absence of residual apoE in supernatant after immunoprecipitation was checked by immunoblot. Antibody specificity, as checked by Dako, showed that only the apoE precipitation arch appeared after staining with Coomassie brilliant blue in crossed immunoelectrophoresis when 12.5 μL of antibody per square centimeter of gel area were used against 2 μL of human plasma. The specificity of the antibodies was further checked in our laboratory by immunoblot on VLDL and HDL. A monoclonal antibody known to recognize all 3 apoE isoforms (E01, Serlia, Institut Pasteur, Lille, France) was also used to check that no residual apoE remained in the supernatant after immunoprecipitation of E4-E3 and E3-E2 phenotype sera with Dako antibodies. PEG 6000 (Fisher, Elancourt, France) was added to the mixture to facilitate precipitation. The precipitation step was performed by mixing 1 vol of plasma, 1 vol of anti-apoE antibody, and 1 vol of an isotonic saline solution containing 5% (wt/vol) PEG 6000. After 2 hours at 4°C, the mix was centrifuged for 15 minutes at 4,000 rpm. The supernatant fraction after immunoprecipitation contained LpB and Lp(a):B. Lp(a) and apoB contents were determined by the same assays used to measure these variables in whole plasma. Lp(a):B:E was then calculated as the difference between Lp(a) and Lp(a):B, while LpB:E was calculated as the difference between plasma apoB and LpB. The precipitation procedure was validated by enrichment of a plasma sample with apoE-rich VLDL isolated by ultracentrifugation at $d < 1.006$ g/mL of a hypertriglyceridemic plasma sample. The apoE concentration of the enriched plasma was determined by electroimmunoassay (Sebia). The maximum capacity of the precipitation procedure was considered to have been reached when an apoE rocket appeared for the supernatant. Inter- and intraassay precision was estimated according to NC-CLS (National Committee for Clinical Laboratory Standards) recommendations.[19]

ApoE bound to apoB (apoE-LpB) and that not bound to apoB (apoE-Lp non-B) were obtained by precipitation with an anti-apoB antibody using a similar procedure.[20]

The molar ratio between apoE LpB and LpB:E (E/LpB:E) was used to estimate the number of apoE molecules in LpB:E particles. This calculation was performed according to a molecular weight of 34,000 for apoE and 500,000 for apoB.

ApoE isoforms were determined in plasma delipidated with ethanol: ether (3 vol:1 vol) by preparative isoelectric focusing (IEF) on 7.5% polyacrylamide-urea (8 mol/L) gels (pH gradient, 4 to 7).[21]

Statistical Analysis

Results were analyzed using SAS statistical software (SAS Institute, Cary, NC). For non–normally-distributed variables [glucose, triacylglycerol, HDL-cholesterol, Lp(a), Lp(a):B, Lp(a):B:E, apoB, LpB:E, apoE, apoE Lp non-B, E/LpB:E, and body mass index (BMI)], values were log-transformed before linear correlation and multiple regression analyses. Univariate analysis was used to study correlations between Lp(a), Lp(a):B, Lp(a):B:E, triacylglycerol levels, and other lipids. A multiple stepwise regression procedure was performed to determine the biological or clinical parameters that might influence the concentrations of Lp(a) subspecies. Two models were tested for each subspecies. The first included parameters related to triacylglycerol accumulation (triacylglycerol levels, LpB:E, apoE LpB, E/LpB:E), cholesterol, glucose, and BMI). The second took into account Lp(a) and all of the parameters previously included in the first model. In both cases, parameters were kept in the model when they satisfied a .5 significance level. The NTG and HTG groups were compared using the Wilcoxon rank sum test. For all analyses, a P value of less than .05 was considered significant.

RESULTS

Validation of the ApoE Precipitation Procedure

Assay precision was estimated according to the analysis of variance experiment described in NC-CLS recommendations.[19] Pooled plasma containing normal concentrations of the variables to be tested was divided into aliquots, stabilized with preservatives (as described in Materials and Methods), and stored at 4°C. Ten aliquots were then measured as total and precipitated plasma each day for 4 consecutive days. For each parameter, the coefficient of variation was less than 10%. The coefficients of variation obtained were 6.72%, 9.8%, 3.79%, and 4.19%, respectively, for total Lp(a), Lp(a):B, total apoB, and LpB.

The maximum precipitation capacity of our antibodies against apoE was determined by adding to the normolipidemic serum various quantities of VLDL ($d < 1.006$ g/mL) obtained from a hypertriglyceridemic pool of plasma samples. The apoE of each fraction was immunoprecipitated with anti-apoE antibodies. The precipitation limit of our antibody was reached for an apoE plasma level of 13.3 mg/dL.

Lipid, Lipoprotein and Lipoprotein Particle Levels of the Study Populations

The results obtained are shown in Table 1. Mean plasma levels for the population studied were 108 mg/dL (28 to 254, minimum to maximum) for total plasma Lp(a), 15.6 mg/dL (0 to 137) for Lp(a):B:E, and 92.6 mg/dL (5 to 254) for lp(a):B. Thus, even in the fasting state, the 2 subspecies of Lp(a) could be quantified in this healthy population with Lp(a) above 25 mg/dL. The triacylglycerol-rich subspecies of Lp(a) was quantitatively important because Lp(a):B:E represented a mean 16.2% of total Lp(a) (0% to 96%).

Table 1. Biological Characteristics of the Study Population

	Cholesterol (mg/dL)	Triacylglycerol (mg/dL)	LDL-C (mg/dL)	Lp(a) (mg/dL)	Lp(a):B:E (mg/dL)	Lp(a):B (mg/dL)	Lp(a):B:E/Lp(a) (%)
Mean	219.9	126.5	137.2	108.0	15.6	92.6	16
Minimum	124	21	65	28	0	5	0
Maximum	312	368	205	254	137	254	96
	LpB:E (mg/dL)	ApoE-LpB (mg/dL)	ApoE-Lp non-B (mg/dL)		E/LpB:E (molar ratio)	BMI (kg/m^2)	Age (yr)
Mean	11.0	1.9	4.3		3.3	25	41
Minimum	0	0	0.2		1	18	23
Maximum	135	14	13		22	33	64

Abbreviations: LDL-C, LDL-cholesterol; Lp(a):B:E, Lp(a) subspecies containing apo(a), apo B, and apo E; Lp(a):B, Lp(a) subspecies containing only apo(a) and apoB.

Relationship Between Main Lipids and Lipoprotein Particles and Lp(a) Subspecies

Univariate analysis showed a strong relation between Lp(a):B:E subspecies and plasma triacylglycerol. In fact, plasma Lp(a):B:E and plasma triacylglycerol levels strongly correlated together ($r = .36$, $P = .0002$), while a negative correlation was found between triacylglycerol and Lp(a):B ($r = -.20$, $P = .05$) and no correlation between this parameter and total Lp(a). A strong correlation was also found between (1) Lp(a):B:E lipoprotein particles and apoE bound to apoB (apoE-LpB) ($r = .30$, $P = .003$) and (2) Lp(a):B:E and the molar ratio between apoE and LpB:E (E/LpB:E) ($r = .36$, $P = .001$). Lp(a):B:E was also positively correlated with total cholesterol ($r = .31$, $P = .002$) and LDL-cholesterol ($r = .21$, $P = .03$).

The Wilcoxon rank sum test showed no significant difference between NTG and HTG groups, either for total Lp(a) or Lp(a):B subspecies. Conversely, our correlation study showed that Lp(a):B:E levels were significantly higher in the HTG than the NTG group (Table 2). Although LpB:E lipoprotein level (corresponding to the number of lipoprotein particles containing both apoB and apoE) did not differ between the 2 groups, the apoE contained in LpB (apoE-LpB), which reflected the mass of apoE in these particles, was significantly higher in the HTG than the NTG group (Table 2). Similarly, the molar ratio between apoE and LpB:E particles (E/LpB:E), which represented the number of apoE molecules per LpB:E particle, was significantly higher in the HTG than the NTG group (Table 2).

Determinants of Lp(a):B and Lp(a):B:E Concentrations

Multiple stepwise regression analyses assessed the independent contribution of lipids and clinical variables to plasma Lp(a):B:E and Lp(a):B levels (Table 3). Two models were tested for each subspecies. Model 1 took the following parameters into account: cholesterol levels, triacylglycerol levels, apoE-LpB levels, LpB:E levels, the E/LpB:E ratio, glucose, and BMI. Model 2 took the same parameters into account, as well as total plasma Lp(a) levels. Our data show that, among all of the parameters included in our models, the E/LpB:E molar ratio (representing the number of apoE per LpB:E particle) was the single variable contributing independently to Lp(a):B:E levels ($\beta = .70$, $P = .003$). Neither triacylglycerol nor the mass of apoE bound to LpB:E particles (apoE-LpB) had an independent effect on Lp(a):B:E subspecies. The relationship between E/LpB:E and Lp(a):B:E was still significant when total Lp(a) was included in the model, and multiple stepwise regression analysis still showed an independent contribution of E/LpB:E to Lp(a):B:E ($\beta = .70$, $P = .003$).

Contrasting results were obtained with Lp(a):B subspecies. A significant contribution of apoE-LpB to Lp(a):B subspecies was only found when Lp(a) was not included in the regression model (Table 3). Moreover, a negative correlation was found between apoE-LpB and Lp(a):B level. Contrary to its effect on Lp(a):B:E subspecies, this suggests that an increase in plasma apoE-LpB level could induce a reduction of Lp(a):B concentration. When Lp(a) was included in the model, the parameters related to the apoE content of triacylglycerol-rich lipoproteins did not appear to be independent contributors to the Lp(a):B concentration. However, it should be kept in mind that the negative effect of E/LpB:E on Lp(a):B was close to statistical significance ($P = .06$).

These data suggest that apoE associated with LpB:E particles could play a major role in concentrations of Lp(a) subspecies.

Table 2. Comparison of apoE- and Non–apoE-Containing Particles in NTG and HTG Subjects

Subjects	Lp(a)	Lp(a):B	Lp(a):B:E	LpB:E	ApoE LpB (mg/dL)	E/LpB:E	Cholesterol	Triacylglycerol
NTG (n = 69)	106 (54)	98 (54)	8 (8)	10 (19)	0.7 (0.6)	1.8 (2)	210 (35)	74.3 (29.4)
HTG (n = 29)	113 (58)	81 (62)	33 (32)	13 (11)	4.8 (3.7)	6.6 (6.5)	243 (31)	250.1 (60)
*P**	NS	NS	.0001	NS	.0001	.0017	NS	.0001

NOTE. Note that the results are means (SD).

Abbreviations: NS, not significant; Lp(a):B, Lp(a) subspecies containing only apo (a) and apo B; Lp(a):B:E, Lp(a) subspecies containing apo(a), apoB, and apoE; LpB:E, lipoprotein containing both apoB and apoE; apoE LpB, apoE of lipoproteins containing apoB.

*Wilcoxon rank sum test.

Table 3. Influence of Different Parameters on Lp(a):B:E and Lp(a):B as Determined by Multiple Stepwise Regression Analysis

Variable	$\beta \pm$ SE	*P*
Lp(a):B:E		
Model 1 ($r^2 = .18$)		
Cholesterol	0.02 ± 0.01	NS
Glucose	2.51 ± 3.20	NS
E/LpB:E	0.70 ± 0.23	.003
Model 2 ($r^2 = .20$)		
Cholesterol	0.01 ± 0.01	NS
Glucose	2.46 ± 3.18	NS
E/LpB:E	0.70 ± 0.23	.003
Lp(a)	0.91 ± 0.68	NS
Lp(a):B		
Model 1 ($r^2 = .10$)		
Triacylglycerol	0.28 ± 0.17	NS
Glucose	0.71 ± 0.80	NS
ApoE-LpB	−0.12 ± 0.05	.02
BMI	−1.65 ± 0.74	.03
Model 2 ($r^2 = .63$)		
Cholesterol	−0.002 ± 0.002	NS
Triacylglycerol	−0.10 ± 0.13	NS
BMI	−0.47 ± 0.49	NS
E/LpB:E	−0.08 ± 0.04	NS
Lp(a)	1.19 ± 0.11	.0001

NOTE. β, standardized partial regression coefficient. Model 1, cholesterol, triacylglycerol, apoE-LpB, LpB:E, E/LpB:E, glucose, BMI. Model 2, cholesterol, triacylglycerol, apoE-LpB, LpB:E, E/LpB:E, glucose, BMI.

Abbreviations: Lp(a):B:E, Lp(a) subspecies containing apo(a), apoB, and apoE; Lp(a):B, Lp(a) subspecies containing only apo(a) and apoB; LpB:E, lipoproteins containing both apoB and apoE; ApoE/LpB, molar ratio between apoE and apoB in lipoproteins containing apoB and apoE[2]; BMI, body mass index.

Determination of apoE isoforms showed that 21%, 70%, 7.5%, and 1.5% of our subjects, respectively, had E4-E3, E3-E3, E3-E2, and E2-E2 phenotypes. No difference was found between the 3 main phenotypes for Lp(a), Lp(a):B:E, and Lp(a):B (data not shown).

DISCUSSION

Ultracentrifugation[11-15] or immunoaffinity chromatography[13,16] have generally been used to study Lp(a) in the VLDL density range or Lp(a) subspecies. However, these 2 methods are less convenient for Lp(a):B:E detection than the one described and validated here. Both require extensive plasma treatment, which may reduce the detection of Lp(a):B:E. Ultracentrifugation for long periods (24 to 48 hours) at high speed (40,000 rpm) may weaken the association between apo(a) and lipoproteins found at VLDL size and cause a loss of apoE. Affinity chromatography can cause dilution of the sample, which may also limit the capacity of Lp(a):B:E detection. The sensitivity of our procedure is much greater because plasma sample processing is simpler. For the first time, Lp(a):B and Lp(a):B:E subspecies can be quantified directly in a total plasma sample after immunoprecipitation of apoE alone. This allows easy detection of very small quantities of Lp(a):B and Lp(a):B:E subspecies. In the fasting state, this simple procedure clearly showed the existence of both circulating Lp(a) subspecies [Lp(a):B and Lp(a):B:E] in NTG and moderately HTG subjects. These results are in agreement with previous data obtained by Bard et al[16] and Scanu et al,[13] whose studies showed a contrasting distribution of these 2 subspecies. Lp(a):B, found in the LDL density range, was rich in cholesterol ester and triacylglycerol-poor, while Lp(a):B:E corresponded to a less dense Lp(a) fraction in the VLDL density range and was poor in cholesterol ester and triacylglycerol-rich. However, our immunoprecipitation method does not allow additional analysis of the so-called Lp(a):B:E, which may contain other apolipoproteins than apoB and apoE, namely apoCII and apoCIII, as shown by Bard et al.[16] However, the purpose of our study was not to quantify apolipoprotein contents in each Lp(a) subspecies differing by the presence or absence of apoE, but to study the influence of various parameters on their concentration. Our results are also in agreement with studies showing the presence of apo(a) in triacylglycerol-rich lipoproteins when plasma was enriched with triacylglycerol during the fed state[11-13] or when a lipid emulsion was infused intravenously in type III[14] or IV[15] hyperlipidemia or lipoprotein lipase deficiency.[14] However, to our knowledge, this is the first study clearly showing that a detectable amount of apoE-containing Lp(a) subspecies is present in fasting healthy subjects.

The influence of various biological factors on total Lp(a), Lp(a):B, and Lp(a):B:E concentrations was studied. Most published reports have shown an inverse relationship between triacylglycerol levels and total Lp(a).[22-26] Our study suggests more precisely that the influence of plasma triacylglycerol levels is not really on the level of total Lp(a), but on the concentration of a subspecies associated or not with triacylglycerol-rich lipoproteins. This result is concordant with the findings of Cohn et al,[12] who showed that the enrichment of patient plasma with triacylglycerol-rich lipoproteins is associated with a switch of apo(a) towards the VLDL density range. Our data show that moderately elevated triacylglycerol levels in a healthy population can modify the concentrations of Lp(a) subspecies, producing a significant increase of Lp(a):B:E levels with no significant change in total Lp(a) and Lp(a):B. This relationship between triacylglycerol and Lp(a):B:E levels was shown by univariate analysis and comparison between NTG patients and moderately HTG patients.

The measurement of lipoprotein particles that differ in their apolipoprotein composition represents another means of distinguishing between lipoproteins. LpB:E reflects the mass of apoB associated with apoE. Assuming that there is 1 apoB molecule per lipoprotein particle, the LpB:E level would correspond to the number of particles containing both apoB and apoE. This number is increased in various forms of dyslipidemia, including those induced by diabetes.[18,27] The mass of apoE bound to apoB (apoE-LpB) was also measured by another means in our study. Therefore, the molar ratio between apoE-LpB and LpB:E represents the number of apoE molecules per lipoprotein particle containing both apoB and apoE. Interestingly, multiple stepwise regression analysis indicated that the apoE bound to particles containing both apoE and apoB was a major determinant of the concentration of Lp(a) subspecies. First, the mass of apoE bound to LpB:E particles (apoE-LpB) had an influence on Lp(a):B level. Secondly, the number of apoE per LpB:E particle (E/LpB:E) had an influence on Lp(a):B:E con-

centration. Moreover, apoE contributed in an opposite manner to the concentration of each of the Lp(a) subspecies measured: Lp(a):B:E increased with the increase in E/LpB:E, whereas Lp(a):B decreased with the increase in apoE-LpB:E. However, the influence of apoE-LpB on Lp(a):B concentration was not strong enough to be still apparent once total Lp(a) concentration (obviously a main contributor to Lp(a):B concentration) was included in the model. This observation, together with the fact that total Lp(a) concentration did not contribute significantly to Lp(a):B:E concentration, is indicative of a simple redistribution of Lp(a) subspecies under the influence of the apoE content of triacylglycerol-rich lipoprotein particles, as suggested by others.[12,28-30] The metabolism of each Lp(a) subspecies could differ because the presence of apoE in 1 of them could enhance its hepatic clearance.[31,32] Kinetic studies are required to clarify this point. In fact, the presence of apoE in an Lp(a) subspecies could inhibit its lipolysis,[33] thus, partly accounting for the relationship between triacylglycerol levels and Lp(a):B:E concentration.

In summary, the method described here allows rapid, direct determination of the 2 Lp(a) subspecies (1 containing and the other free of apoE). A detectable amount of apo(a) was found in apoE-containing lipoproteins, even in normolipidemic fasting subjects. The major determinant of the plasma concentration of this subspecies appears to be apoE accumulation in triacylglycerol-rich lipoproteins.

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