

# Interindividual Variation in the Partition of Lipoprotein(a) Into Lipoprotein Subfractions

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In order to evaluate cardiovascular risk, we analyzed the lipid composition of HDL and the presence of lipoprotein(a) [Lp(a)] by both agarose gel electrophoresis and enzyme-linked immunoassay (ELISA). In 681 plasmas we found a close correspondence between the existence of a visible sinking pre- $\beta$  lipoprotein band and a concentration of Lp(a) higher than 300 mg/L. In the sinking pre- $\beta$ (+) samples, the HDL-cholesterol level obtained by differential ultracentrifugation was significantly higher than that obtained by precipitation with the  $MgCl_2$ -phosphotungstic acid reagent; and the difference between these HDL-cholesterol values was linearly correlated with plasma Lp(a) concentration. Moreover, the other HDL lipid components and the lipid mass ratios of HDL isolated by ultracentrifugation were significantly different from those of HDL isolated by precipitation, and these changes were also correlated with plasma Lp(a). These differences are attributed to Lp(a) because it was detected in the 1.063–1.21 kg/L plasma fractions, whereas it was absent in the plasma supernates after precipitation with  $MgCl_2$ -phosphotungstic acid. Although to a lesser extent, Lp(a) was also present in the LDL and VLDL density ranges and it directly depended on both the Lp(a) and the triglyceride plasma concentrations. The proportion of Lp(a) in HDL as related to that in LDL density fractions decreased as Lp(a) plasma levels increased, reflecting an interindividual variation of Lp(a) density species. Since 90% of our study population had detectable Lp(a) in plasma, the results reinforce the concept that the ultracentrifugation method is not equivalent to precipitation in most samples, and the contaminant effect of Lp(a) cannot be predicted because of Lp(a) partition into the different lipoprotein fractions.

**KEY WORDS:** lipoprotein(a); HDL-cholesterol; ultracentrifugation; polyanion precipitation.

## Abbreviations

Lp(a)	Lipoprotein(a)
VLDL	Very low density lipoprotein
LDL	Low density lipoprotein
HDL	High density lipoprotein
ucHDL	HDL isolated by plasma ultracentrifugation
ppHDL	HDL isolated by plasma precipitation

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## Introduction

In recent years, lipoprotein(a) [Lp(a)] has aroused increasing interest because of its dual proatherogenic and antifibrinolytic character (1). Lp(a) is an independent risk factor for coronary artery disease (2–5). In normolipemic individuals, a plasma Lp(a) concentration exceeding 300 mg/L results in a two-fold increased risk for coronary artery disease when compared to the general population (2,3). In addition, Lp(a) is a powerful determinant for cardiovascular disease in patients with familial hypercholesterolemia (6,7).

Lipoprotein(a) is comprised of one apo(a) molecule linked to a LDL particle by a disulphide bond to apo B-100 (8). Because of the high structural homology between apolipoprotein(a) and plasminogen (9,10), Lp(a) competes with this zymogen for its binding site to fibrin (11,12), fibrinogen (11,13), and different cellular types of receptors (14–16). It has been estimated that at plasma concentration of 300 mg/L, Lp(a) reduces cellular plasminogen binding by 20%, thereby lowering fibrinolytic capacity (15).

Due to the density range of lipoprotein(a), 1.04–1.11 kg/L, (17) and the high sialic acid content of apo(a), this Lp(a) was initially identified as sinking pre- $\beta$  lipoprotein (18). Despite the fact that Lp(a) separates together with both LDL and HDL during centrifugation, it is usual to consider values of HDL-cholesterol obtained by this technique as reference values. This is probably because of the few studies in which the effect of the presence of Lp(a) on those variables has been analyzed. The present work was directed to quantitatively determine the influence of the presence of Lp(a) on the lipid components of HDL, isolated by both ultracentrifugation and polyanion precipitation, and to evaluate the partition of Lp(a) into the different lipoprotein fractions.

## Methods

## SUBJECTS

The study was carried out with plasma samples from 681 individuals, 354 females and 327 males.

Considering the analytical aspects of the work, which required a wide range of lipoprotein levels, we did not apply any exclusion criteria; therefore, the population studied consisted of presumed healthy, hyperlipemic, or endocrinologically affected subjects. Plasma cholesterol and triglyceride levels were  $6.32 \pm 0.08$  (1.96–17.11) mmol/L [mean  $\pm$  SE (range)] and  $1.88 \pm 0.12$  (0.39–50.17) mmol/L, respectively. All the subjects gave consent for this study.

#### LIPOPROTEIN ANALYSIS

Blood was taken from subjects after a 10–12-h fasting period, in tubes containing  $\text{Na}_2\text{-EDTA}$  as anticoagulant (final concentration 1 g/L). A plasma aliquot was precipitated with  $\text{MgCl}_2$  (14.3 mmol/L)-phosphotungstic acid (0.314 mmol/L) reagent (Boehringer-Mannheim GmbH Diagnostic, Mannheim, Germany). Another plasma aliquot was placed in Beckman 50 Ti rotor tubes (Beckman Instruments, Palo Alto, CA, USA); brought to volume with 0.189 mol/L NaCl, 1 mmol/L EDTA ( $d = 1.006$  kg/L); and centrifuged for 18 h at  $134,000 \times g$ , at  $10^\circ\text{C}$ , in a Beckman L7-55 ultracentrifuge in order to isolate VLDL particles in the supernatant. The infranatant fraction was adjusted to a density of 1.063 kg/L with solid KBr (82.1 g/L) and was again centrifuged for 20 h at  $146,000 \times g$ , at  $10^\circ\text{C}$ . LDL particles were recovered in the supernatant and the infranatant was transferred to other tubes, the density was again adjusted to 1.21 kg/L with solid KBr (221 g/L) and the tubes were finally centrifuged for 44 h at  $146,000 \times g$ , at  $10^\circ\text{C}$ . This latter supernatant is called in the present work uHDL.

Supernatant (VLDL-containing fraction) and infranatant (VLDL-free plasma) freshly separated by centrifugation at  $d = 1.006$  kg/L, as well as total plasma were subjected to zonal electrophoresis on 0.5% (w/v) agarose gel plates (Paragon-Lipo, Beckman Instruments, Diagnostic Systems Group, Brea, CA, USA) and stained for lipid with Sudan-black. All the gels were always visually examined by the same person and samples showing a pre- $\beta$  band in the VLDL-free plasma were classified as sinking pre- $\beta$ (+). Despite the limitations of this approach, for the sake of speed and because Lp(a) concentration was to be quantitated by immunoanalysis, densitometric scans of the gels were not performed. Lipoprotein(a) was quantified in frozen samples by a sandwich-type enzyme-linked immunoassay [ELISA; TintElize Lp(a), Biopool AB, Umea, Sweden] with polyclonal antibodies against purified Lp(a), and lyophilized human plasma was used as standard [intraassay coefficient of variation (CV), 4.6%; interassay CV, 6.7%]. The specificity and immunoreactivity of Lp(a) antibody against the different Lp(a) isoforms were evaluated by the manufacturer, and were not further studied herein.

Total cholesterol (Menarini Diagnostics, Firenze, Italy; interassay CV, 2.5%); free cholesterol (Biotrol, Paris, France; interassay CV, 3.4%); triglyceride

(Boehringer Mannheim GmbH Diagnostic, Mannheim, Germany; interassay CV, 1.9%); and choline-containing phospholipids (Menarini Diagnostics, Firenze, Italy; interassay CV, 2.2%) were enzymatically measured in total plasma and fractions using a Hitachi 705 automatic analyzer (Hitachi Ltd, Tokyo, Japan).

#### CALCULATION AND STATISTICAL TREATMENT

Results are expressed as mean  $\pm$  standard error, except for Lp(a) expressed as median and interquartile range. Visual identification of Lp(a) after electrophoresis was compared with Lp(a) values assessed by ELISA and a value of 300 mg/L was taken as the discrimination limit. Specificity was calculated as the percentage of true negative cases as compared to false positive plus true negative cases. Sensitivity was obtained as the percentage of true positive cases as compared to true positive plus false negative cases. Finally, efficiency was calculated as the percentage of true positive plus true negative cases as compared to all cases.

Groups were statistically compared by means of the Student's *t*-test. Mathematical equations and plots were performed by means of both the Statistical graphics statistical package (Statistical Graphics Corporation Inc., Rockville, MD, USA) and the Sigmaplot Scientific Graph System (Jandel Scientific Corporation, Corte Madera, CA, USA) in an IBM PS-2, 55SX computer.

#### Results

##### EFFECT OF Lp(a) ON THE ESTIMATION OF HDL-CHOLESTEROL

Individuals with prominent Lp(a) were first identified by noting a band with a pre- $\beta$  electrophoretic mobility in VLDL-free plasma. This analysis was not intended to quantify Lp(a) but to simply classify samples into two categories. The sinking pre- $\beta$  band was present in 23.1% of the studied subjects who were classified as sinking pre- $\beta$ (+). Lipoprotein(a) was also quantified by ELISA and the distribution of plasma Lp(a) concentration values in this patient population is shown in Figure 1. This is a highly skewed distribution [median (interquartile range): 147 (51–329) mg/L] in which more than 27.5% of the individuals have a plasma Lp(a) concentration higher than 300 mg/L. To analyze the association between the presence of the sinking pre- $\beta$  band and a high plasma Lp(a) concentration, the studied population was subdivided into two groups taking into account the electrophoretic pattern. Figure 2 shows the plasma Lp(a) concentration in both the 524 sinking pre- $\beta$ (-) subjects (upper panel) and 157 sinking pre- $\beta$ (+) subjects (lower panel). In the sinking pre- $\beta$ (-) subjects less than 7% showed Lp(a) values higher than 300 mg/L, while in the sinking pre- $\beta$ (+) subjects more than 96% of the individuals had Lp(a) values higher than 300 mg/L. Classification of the

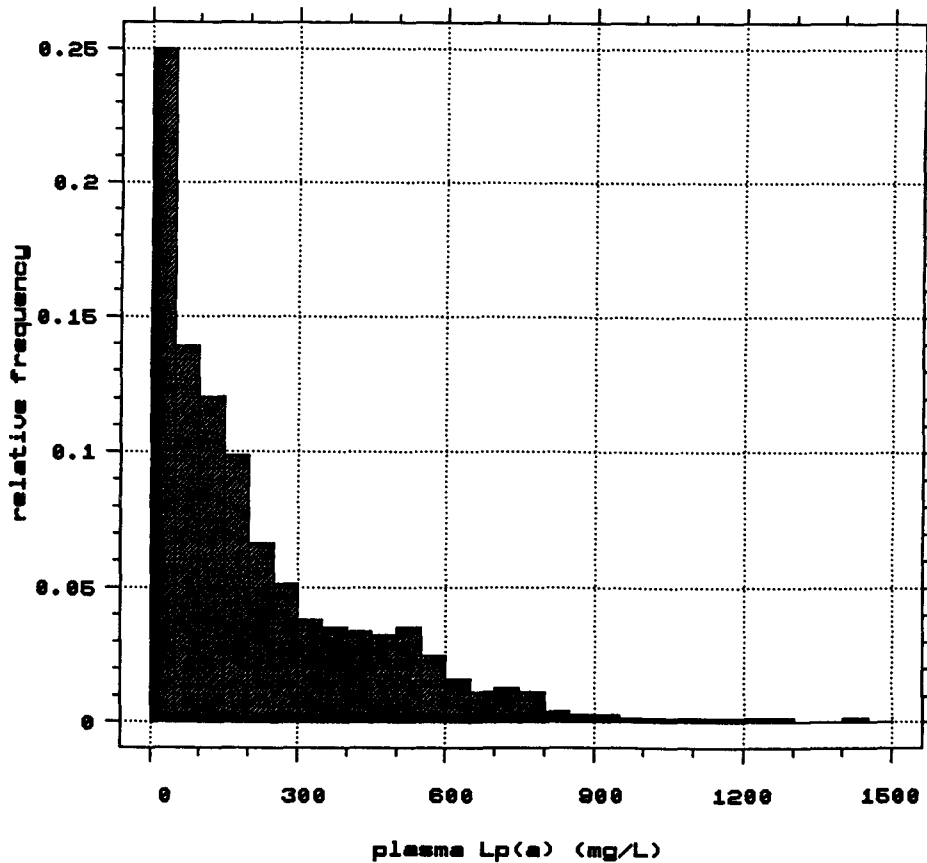


Figure 1 — Frequency distribution of plasma Lp(a) concentration in the study population. The study population consisted of 681 patients suffering from different diseases who were submitted for lipoprotein profile investigations.

subjects according to the appearance of the sinking pre- $\beta$  band as related to Lp(a) quantitation has a specificity of 99%, a sensitivity of 81%, and an efficiency of 96%. Therefore, the classification of individuals into sinking pre- $\beta$ (+) or sinking pre- $\beta$ (-) groups was suitable for our purposes.

A highly significant linear correlation was found between HDL-cholesterol levels from precipitated (ppHDL) and ultracentrifuged (ucHDL) samples. In sinking pre- $\beta$ (-) individuals the regression equation between ucHDL and ppHDL was  $y = 0.939x + 0.100$  mmol/L ( $r = 0.931$ ,  $n = 456$ ,  $p < 0.001$ ); in the sinking pre- $\beta$ (+) group the equation was  $y = 0.954x + 0.253$  mmol/L ( $r = 0.887$ ,  $n = 149$ ,  $p < 0.001$ ). The higher independent value for the latter regression line suggested an additive effect of Lp(a) on HDL-cholesterol values obtained by ultracentrifugation. In order to analyze such a possibility, differences between ucHDL-cholesterol and ppHDL-cholesterol values for the same sample (ucHDL-ppHDL), were calculated for all subjects. These differences were  $0.020 \pm 0.008$  mmol/L and  $0.190 \pm 0.017$  mmol/L in the sinking pre- $\beta$ (-) and sinking pre- $\beta$ (+) groups, respectively ( $p < 0.001$ ). The frequency histogram delineates a normal distribution for sinking pre- $\beta$ (-) cases [Figure 3(A)], with a mode value close to zero, whereas a shift to the right can be seen in the sinking pre- $\beta$ (+) group [Figure

3(B)], where in more than 90% of the cases the difference was greater than zero. Therefore, while the agreement between HDL-cholesterol values obtained by means of ultracentrifugation and precipitation in the sinking pre- $\beta$ (-) group was satisfactory, in the sinking pre- $\beta$ (+) group there existed significant differences because values obtained by ultracentrifugation were higher than those by precipitation.

Regression lines between (ucHDL-ppHDL) differences versus total plasma Lp(a) concentration, were analyzed. For this, both sinking pre- $\beta$ (-) and sinking pre- $\beta$ (+) samples were included. As shown in Figure 4, a positive and significant linear correlation between (ucHDL-ppHDL) cholesterol and Lp(a) ( $r = 0.443$ ,  $n = 592$ ,  $p < 0.001$ ) was found.

#### DISTRIBUTION OF Lp(a) AMONG ULTRACENTRIFUGALLY SEPARATED LIPOPROTEINS

To assess its distribution among the different lipoprotein fractions obtained by ultracentrifugation, Lp(a) concentration was determined in fractions of densities  $< 1.006$  kg/L (VLDL),  $1.006-1.063$  kg/L (LDL), and  $1.063-1.21$  kg/L (ucHDL) from 52 patients with a wide variety of both plasma Lp(a) and VLDL-triglyceride concentrations. Correlations between Lp(a) in ucHDL and LDL fractions and plasma Lp(a) concentration were analyzed sepa-

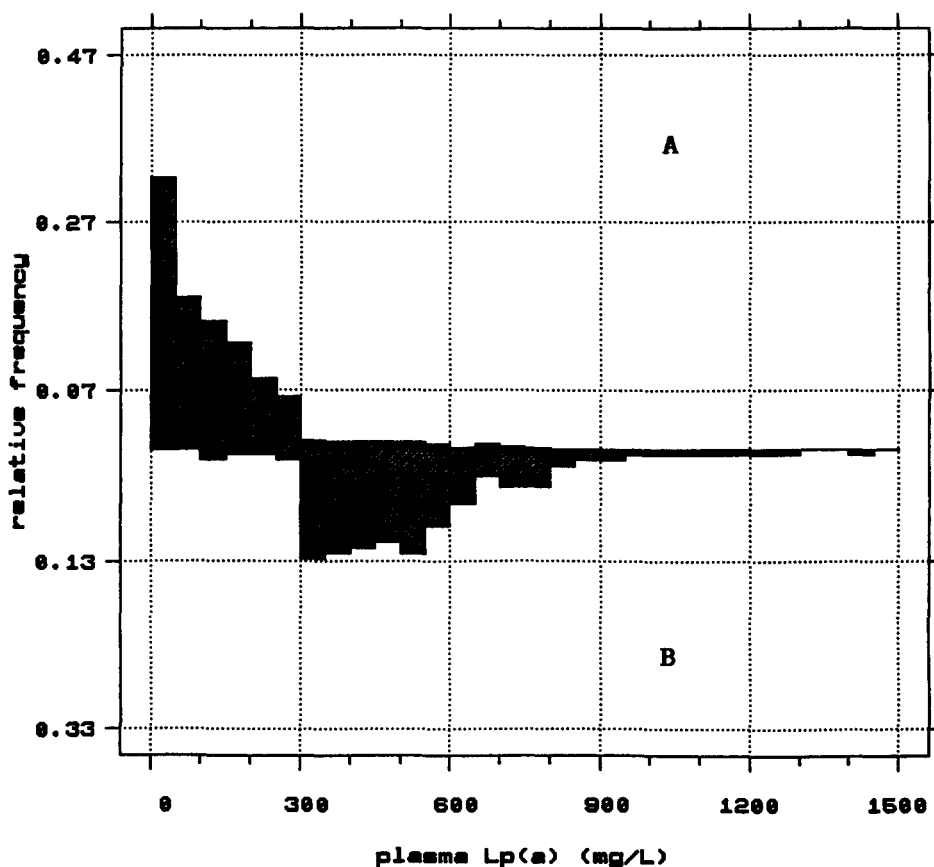


Figure 2 — Frequency distribution of plasma Lp(a) concentration values in sinking pre-β(-) and sinking pre-β(+) samples. Plasma Lp(a) concentration was determined in 681 samples, (A) 524 sinking pre-β(-) and (B) 157 sinking pre-β(+). The classification into sinking pre-β(+) or sinking pre-β(-) was established by the presence of a pre-β band on agarose electrophoresis of VLDL-free plasma (sinking pre-β).

rately. A positive linear correlation between Lp(a) values in ucHDL and plasma Lp(a) concentration ( $r = 0.948, n = 52, p < 0.001$ ) was observed (Figure 5). Lipoprotein(a) values in LDL and plasma Lp(a) concentration were also significantly correlated ( $r = 0.798, n = 52, p < 0.001$ ) but the slope was three times lower than in the previous case (0.217 vs. 0.591; Figure 5). When these values were expressed as the percentage of plasma Lp(a) that was separated into these lipoprotein fractions by ultracentrifugation, it was observed that Lp(a) in ucHDL was negatively correlated to plasma Lp(a) ( $r = -0.466, n = 39, p < 0.01$ ), whereas it was directly correlated in the case of LDL ( $r = 0.404, n = 39, p < 0.01$ ) (Figure 6). In other words, as plasma Lp(a) increased there was a progressive shift in the separation of Lp(a) from HDL to LDL. In every case, however, the proportion of Lp(a) in HDL was higher than in LDL.

Lipoprotein(a) was also analyzed in VLDL fractions from the aforementioned 52 individuals. A value of 5 mg/L (half the sensitivity limit) was assigned to those samples with undetectable Lp(a) in the VLDL fraction. A significant and positive correlation between Lp(a) values in VLDL and plasma triglyceride concentration was observed ( $r = 0.438,$

$n = 52, p < 0.01$ ). When the sample was restricted to subjects with plasma Lp(a) higher than 250 mg/L, a highly significant correlation between the percentage of Lp(a) recovered in the VLDL fraction and the VLDL-triglyceride concentration was found ( $r = 0.743, n = 37, p < 0.001$ ; Figure 7).

Once it was demonstrated that Lp(a) could be separated even within the VLDL density range in some individuals, the possibility that the double pre-β phenotype (19) could correspond to the presence of Lp(a) in the  $d < 1.006$  kg/L fraction was raised. Based on this, Lp(a) concentration from 16 VLDL samples that showed the double band in agarose gel electrophoresis was determined. Contrary to the hypothesis, Lp(a) was undetectable in all of them.

The partition of Lp(a) into the different lipoprotein fractions separated by ultracentrifugation contrasts with its total precipitation along with VLDL and LDL in the presence of the  $MgCl_2$ -phosphotungstic acid reagent (data not shown).

#### HDL-LIPID MASS RATIOS IN DIFFERENT SINKING pre-β PHENOTYPES

In order to assess the implication of Lp(a) on the lipid composition, lipid mass ratios in both ucHDL

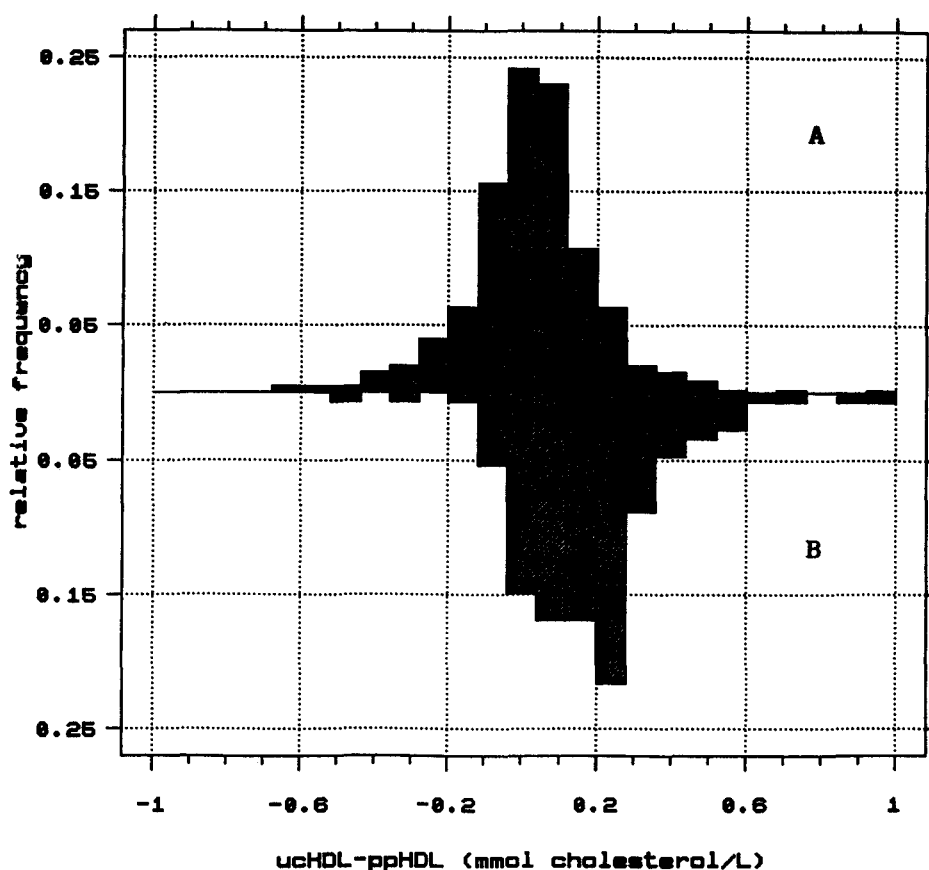


Figure 3 — Frequency distribution of (ucHDL-ppHDL) cholesterol values in sinking pre- $\beta$ (-) and sinking pre- $\beta$ (+) samples. Plasma samples from 681 subjects were processed separately to determine HDL-cholesterol by plasma ultracentrifugation at a density range of 1.063–1.21 kg/L (ucHDL) and plasma precipitation with  $MgCl_2$ -phosphotungstic acid (ppHDL). Samples were subdivided into (A) sinking pre- $\beta$ (-) or (B) sinking pre- $\beta$ (+) with regards to their electrophoretic pattern (see Figure 2).

and ppHDL were analyzed (Table 1). In ucHDL significant differences were observed between the two sinking pre- $\beta$  phenotypes for all the mass ratios considered except esterified-cholesterol/free-cholesterol (EC/FC). As a general observation, ucHDL in the sinking pre- $\beta$ (+) group were proportionally richer in cholesterol and poorer in phospholipids and triglycerides than those from the sinking pre- $\beta$ (-) group. None of these differences were observed in ppHDL (Table 1).

To further evaluate the effect of Lp(a) on lipid composition of ultracentrifugally separated HDL, regression lines between HDL-lipid mass ratios *versus* total plasma Lp(a) concentration were analyzed. For this, both sinking pre- $\beta$ (+) and sinking pre- $\beta$ (-) samples were included. As shown in Table 2 the positive linear correlation between plasma Lp(a) levels and the esterified cholesterol/triglyceride ucHDL-mass ratio (EC/TG) and the inverse linear correlation with the phospholipids/total cholesterol ucHDL-mass ratio (PL/C) *versus* Lp(a) plasma levels were the most significant findings. In contrast, Lp(a) was not correlated with any of the considered mass ratios of ppHDL (Table 2).

## Discussion

In this present work, we evaluated the partition of Lp(a) into the classic lipoprotein fractions isolated by ultracentrifugation. We have confirmed that Lp(a) is separated by ultracentrifugation mainly in the HDL fraction and, as a consequence of this, cholesterol concentration is higher in ucHDL than in ppHDL for samples with elevated Lp(a); the magnitude of this difference depended on the plasma Lp(a) concentration. However, as demonstrated in the present work, a direct extrapolation of this effect cannot be drawn for every sample, because Lp(a) also separates into the other lipoprotein fractions (LDL and VLDL) and this pattern varies as a function of plasma Lp(a).

The presence of Lp(a) in samples was initially identified noting the appearance of a band with pre- $\beta$  mobility in VLDL-free plasma. Identification was carried out by visual examination where samples were classified into sinking pre- $\beta$ (+) and sinking pre- $\beta$ (-), respectively. When comparing this method with ELISA for Lp(a), more than 96% sinking pre- $\beta$ (+) samples have an Lp(a) higher than 300

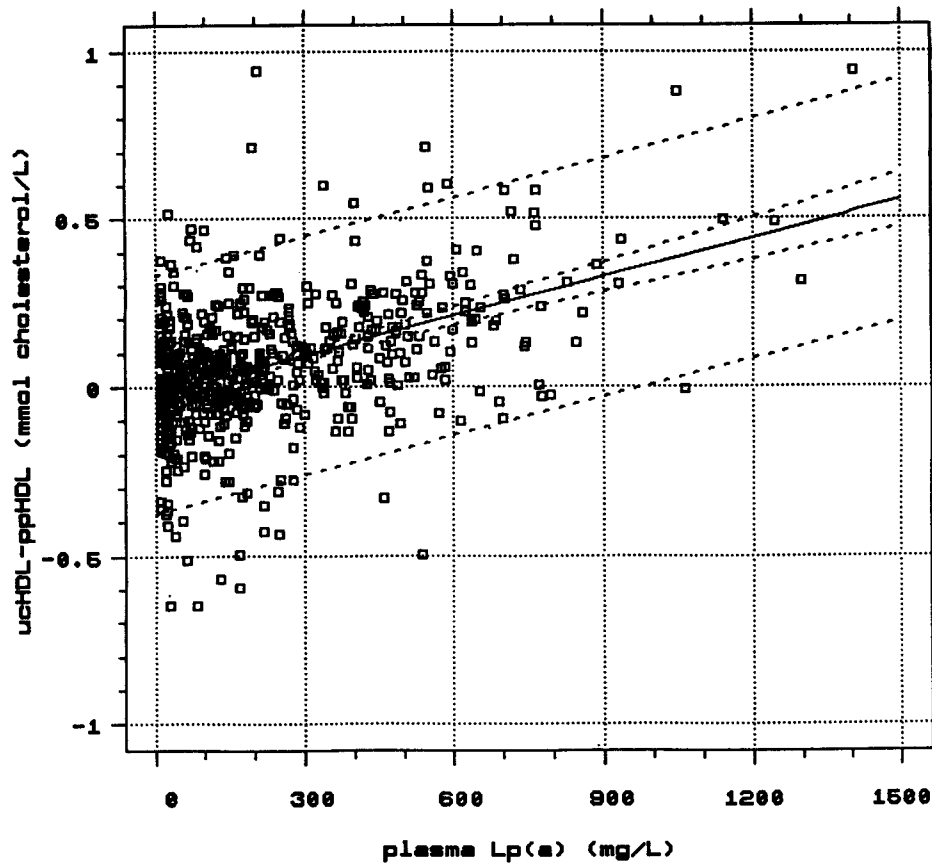


Figure 4 — Linear regression of (ucHDL-ppHDL) cholesterol values on plasma Lp(a) concentration. Regression line:  $y = 0.00039x - 0.023$  ( $r = 0.443$ ,  $p < 0.001$ ,  $n = 592$ ).

mg/L (specificity, 99%). These results agree with previous reports (18,20) that showed the identity of sinking pre- $\beta$  lipoprotein with Lp(a). On the basis of the close relationship between the sinking pre- $\beta$ (+) phenotype and elevated Lp(a) plasma levels, agarose electrophoresis appears to be a valuable technique to confirm the results obtained with immunoassays because reference standards for Lp(a) assays are not available. This is reinforced by the recent observation that Lp(a) with different apo(a) isoforms moves equally in the pre- $\beta$  position (unpublished observations), and therefore, the intensity of this band is solely and directly related to the lipid Lp(a) mass as observed by others (20).

In sinking pre- $\beta$ (+) samples, cholesterol concentration in HDL isolated by ultracentrifugation was significantly higher than in supernates from precipitated plasma, indicating that differential ultracentrifugation separates Lp(a) along with HDL particles, as could be predicted from its physical features. Similar findings were earlier reported by Bachorik *et al.* (21) in some, but not all, of their sample series studied. The HDL-cholesterol values are therefore partially artifactual and, as a consequence, HDL-cholesterol concentration estimated by ultracentrifugation (ucHDL) tends to be higher than that estimated by plasma polyanion precipitation in samples with high Lp(a) plasma levels. As demonstrated in Figure 4, the difference between those values is

highly correlated with plasma Lp(a) concentration and, as inferred from Figure 3, samples with the sinking pre- $\beta$ (+) phenotype, which is equivalent to plasma Lp(a) levels higher than 300 mg/L, showed a value 0.190 mmol/L higher for ucHDL-cholesterol than ppHDL-cholesterol, whereas in sinking pre- $\beta$ (-) samples those values were similar. These results do not support the use of ultracentrifugation as the reference method for separation and quantitation of HDL particles. Moreover, the artifact caused by Lp(a) in ucHDL-cholesterol value is neither irrelevant nor anecdotal because more than 27% of the population studied herein showed elevated Lp(a) levels (higher than 300 mg/L).

The presence of Lp(a) in the ucHDL fraction not only results in an increased HDL-cholesterol value but also in an altered lipid composition of this fraction, as indicated by HDL-lipid mass ratios. As a general observation, ucHDL from subjects with high Lp(a) were proportionally enriched in cholesterol in relation to other lipids, as compared to ucHDL from subjects with low Lp(a) (Table 1), probably because of the fact that Lp(a) is proportionally richer in cholesterol than HDL particles (22). It is worth mentioning that these lipid mass ratios in ucHDL, but not in ppHDL, were correlated with Lp(a) plasma levels (Table 2), which indicates the direct effect of Lp(a) on the variation of ucHDL composition among individuals. Besides these differences in ucHDL be-

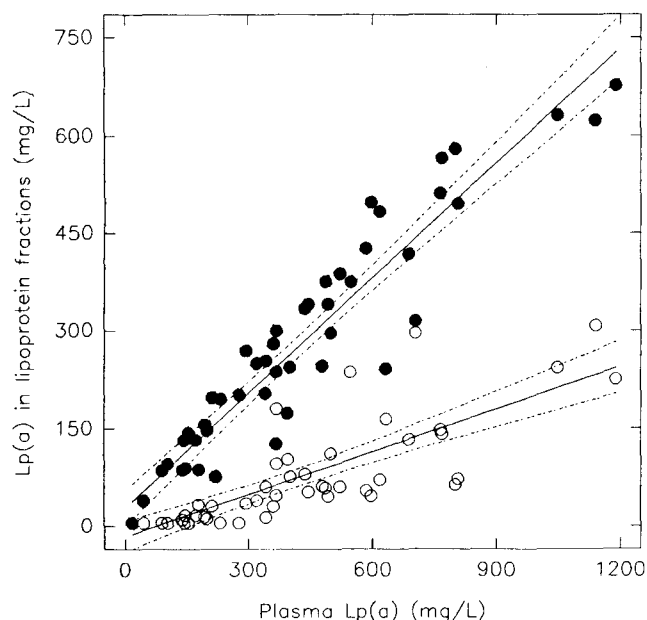


Figure 5 — Linear regression of Lp(a) in lipoprotein fraction values on plasma Lp(a) concentration. (○) Lp(a) in fraction 1.006–1.063 kg/L (LDL), regression line:  $y = 0.217x - 16$  ( $r = 0.798$ ,  $p < 0.001$ ,  $n = 52$ ). (●) Lp(a) in fraction 1.063–1.21 kg/L (ucHDL), regression line:  $y = 0.591x + 26$  ( $r = 0.948$ ,  $p < 0.001$ ,  $n = 52$ ).

tween sinking pre- $\beta$ (+) and sinking pre- $\beta$ (-) groups, an interesting observation of this work is that lipid mass ratios in ucHDL were different than in ppHDL in both sinking pre- $\beta$  groups (Table 1). The partial separation of Lp(a) along with HDL in ultracentrifugation is probably the origin of these differences, not only in the sinking pre- $\beta$ (+), but also in the sinking pre- $\beta$ (-) group. Actually subjects in this latter group also have some Lp(a) in their plasma and ucHDL lipid mass ratios were linearly correlated with plasma Lp(a). This means that ucHDL and ppHDL are not equivalent except in cases where Lp(a) is absent, less than 9% in our study population. For clinical purposes, however, ucHDL-cholesterol is equivalent to ppHDL-cholesterol in subjects with Lp(a) plasma levels lower than 300 mg/L, but even in these cases, lipoprotein particles of ucHDL do not have the same composition as those of ppHDL.

These differences in lipid composition are attributed to Lp(a) because it was mainly isolated in the 1.063–1.21 kg/L density fraction by ultracentrifugation, whereas it was absent in the plasma supernates after precipitation with  $MgCl_2$ -phosphotungstic acid. Although to a lesser extent, Lp(a) was also present in the LDL density range (1.006–1.063 kg/L), the magnitude of this being directly dependent on the Lp(a) plasma levels (Figure 5). This partition of Lp(a) into HDL and LDL was, however, not constant for every Lp(a) plasma concentration. Rather, we have observed that the percentage of Lp(a) in  $d = 1.063$ –1.21 decreases, whereas the percentage of Lp(a) in  $d = 1.006$ –1.063 kg/L increases as plasma

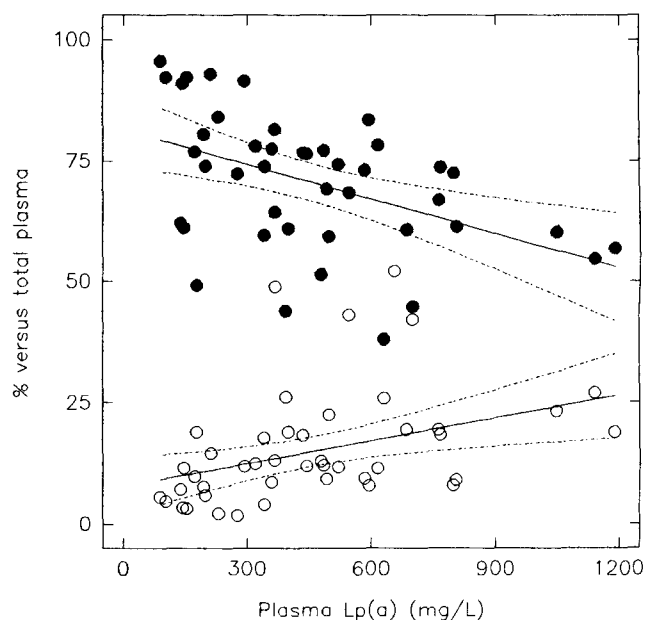


Figure 6 — Percent of Lp(a) in 1.006–1.063 and 1.063–1.21 kg/L density lipoprotein fractions. (○) Lp(a) in fraction 1.006–1.063 kg/L (LDL), regression line:  $y = 0.0156x + 7.8$  ( $r = 0.404$ ,  $n = 39$ ,  $p < 0.01$ ). (●) Lp(a) in fraction 1.063–1.21 kg/L (ucHDL), regression line:  $y = -0.0239x + 81.5$  ( $r = -0.466$ ,  $n = 39$ ,  $p < 0.01$ ). Samples with undetectable Lp(a) in either ucHDL or LDL were discarded.

Lp(a) levels increase (Figure 6), which indicates that Lp(a) particles shift to lower densities as plasma concentration increases. These findings are

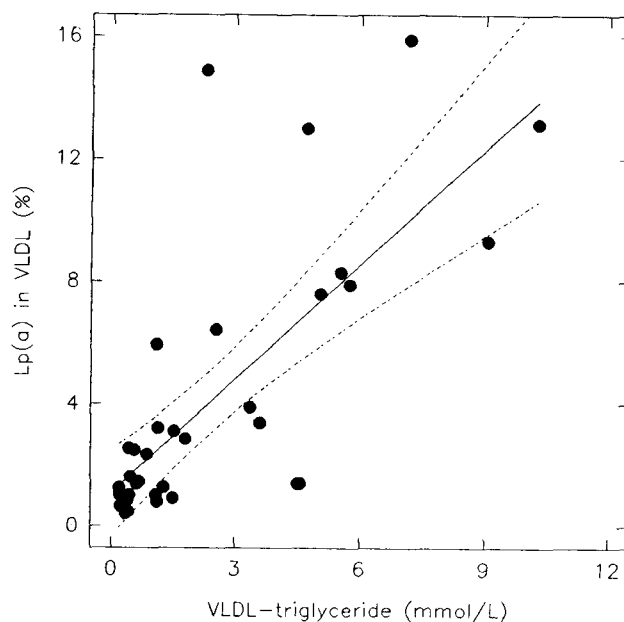


Figure 7 — Linear regression of Lp(a) values in VLDL on VLDL-triglyceride concentration. Lp(a) in  $d < 1.006$  kg/L lipoprotein fraction (VLDL) is expressed as percent of that in total plasma. For this analysis, only samples with Lp(a) higher than 250 mg/L were considered. Regression line:  $y = 1.24x + 1.1$  ( $r = 0.743$ ,  $p < 0.001$ ,  $n = 37$ ).

TABLE 1  
HDL-Lipid Mass Ratios in Sinking pre-β Phenotypes

Mass Ratios	ucHDL <sup>a</sup>			ppHDL <sup>a</sup>		
	pre-β(+)	<i>p</i>	pre-β(-)	pre-β(+)	<i>p</i>	pre-β(-)
C/TG	15.70 ± 0.80	<0.001	13.07 ± 0.305	11.30 ± 0.59	NS	9.97 ± 0.28
EC/TG	12.32 ± 0.64	<0.001	10.19 ± 0.29	8.529 ± 0.44	NS	7.73 ± 0.44
FC/TG	3.38 ± 0.18	<0.05	2.92 ± 0.09	2.58 ± 0.19	NS	2.26 ± 0.07
PL/C	0.804 ± 0.009	<0.001	0.910 ± 0.008	1.07 ± 0.01	NS	1.12 ± 0.01
PL/EC	1.05 ± 0.02	<0.001	1.20 ± 0.02	1.41 ± 0.03	NS	1.49 ± 0.03
PL/FC	3.85 ± 0.06	<0.001	4.18 ± 0.05	5.27 ± 0.22	NS	5.43 ± 0.12
EC/FC	3.82 ± 0.08	NS	3.73 ± 0.06	3.98 ± 0.20	NS	4.06 ± 0.12

C: HDL-cholesterol (mmol/L); EC: HDL-esterified cholesterol (mmol/L); FC: HDL-free cholesterol (mmol/L); TG: HDL-triglyceride (mmol/L); PL: HDL-phospholipids (mmol/L); NS: not significant, *p* > 0.05. Samples were subdivided into sinking pre-β(+) (*n* = 157) or sinking pre-β(-) (*n* = 524) with regards to their electrophoretic pattern (see Figure 2). Results are expressed as mean ± standard error. With the exception of EC/FC all the other lipid mass ratios of ucHDL were significantly different (*p* < 0.01) from those of ppHDL in the two sinking pre-β groups.

<sup>a</sup> ucHDL and ppHDL: HDL lipoprotein fractions separated by ultracentrifugation and precipitation, respectively.

in agreement with recent observations by Guo *et al.* (23) that, though in every case Lp(a) separated mostly in the HDL range, the proportion of Lp(a) particles in the 1.006–1.063 kg/L density range was increased in patients with high Lp(a) plasma levels and low molecular weight forms of apo(a). Therefore, the interindividual differences we observed in the partition of Lp(a) into the different plasma density fractions was probably the result of the distinct physicochemical properties of Lp(a) particles with different apo(a) isoforms. The smaller the apo(a) isoform the lighter the Lp(a) particle. Obviously, this interpretation needs to be confirmed by studying apo(a) phenotypes in our population.

There is a considerable literature emerging on systematic differences in Lp(a) determinations by different assays (24). The Lp(a) standard we used was from a pool of human plasmas. The choice of other calibrators or antibodies would probably lead

to different absolute values of Lp(a) in our samples; and interindividual differences due to specific apo(a) phenotypes could be affected. This effect, however, appears not to be relevant for the partition phenomenon we describe, because it would affect all the fractions from the same individual similarly. This is clear in homozygous subjects for specific apo(a) phenotypes. In the case of heterozygous subjects, the relevance of this effect is not obvious. In subjects with the smaller apo(a) forms, in whom a shift of Lp(a) into the LDL density fraction is expected (23), a change in the calibration method should result in a change of the proportion of Lp(a) particles. However, it would not be very important because the proportion of Lp(a) particles with smaller apo(a) is always greater (25).

Lp(a) was also detected in the VLDL fraction from some individuals. In subjects with plasma Lp(a) concentration higher than 250 mg/L we observed that

TABLE 2  
Linear Regression Coefficients Between Plasma Lp(a) Concentration and HDL-Lipid Mass Ratios

Mass Ratios	ucHDL <sup>a</sup>		ppHDL <sup>a</sup>	
	<i>r</i>	<i>p</i>	<i>r</i>	<i>p</i>
C/TG	0.132	<0.001	0.083	NS
EC/TG	0.145	<0.001	0.064	NS
FC/TG	0.069	NS	0.072	NS
PL/C	-0.325	<0.001	-0.126	NS
PL/EC	-0.220	<0.001	-0.052	NS
PL/FC	-0.176	<0.001	-0.047	NS
EC/FC	0.076	NS	-0.028	NS

C: HDL-cholesterol (mmol/L); EC: HDL-esterified cholesterol (mmol/L); FC: HDL-free cholesterol (mmol/L); TG: HDL-triglycerides (mmol/L); PL: HDL-phospholipids (mmol/L); NS: not significant, *p* > 0.01. Both sinking pre-β(+) and sinking pre-β(-) samples were included (*n* = 681).

<sup>a</sup> ucHDL and ppHDL: HDL lipoprotein fractions separated by ultracentrifugation and precipitation, respectively.



the percentage of Lp(a) present in the VLDL fraction correlated positively with the VLDL-triglyceride concentration (Figure 7). This finding is in agreement with the identification of Lp(a) in  $d < 1.006$  kg/L in hypertriglyceridemic patients (26,27), and suggests that, aside from LDL, apo(a) complexes also with VLDL to a certain extent, being dependent on the number of VLDL particles. This phenomenon is clearly detectable at elevated VLDL concentrations.

The association between high VLDL levels and Lp(a) in  $d < 1.006$  kg/L fraction raised the possibility of a correspondence between double pre- $\beta$  phenotype (19) and the presence of Lp(a) in VLDL fraction in certain patients. However, none of the 16 cases with this phenotype studied here had detectable Lp(a) in the  $d < 1.006$  kg/L fraction. It may then be concluded that the additional pre- $\beta$  band, which is characteristic of this double pre- $\beta$  phenotype, is not Lp(a).

In conclusion, Lp(a) is mostly separated in the 1.063–1.21 kg/L density fraction, but also with the LDL and VLDL fractions. This density range of Lp(a), together with its precipitation by polyanions, is the cause of the differences between HDL lipid values obtained by ultracentrifugation versus precipitation methods. The partition of Lp(a) into the different lipoprotein fractions is, however, not constant for all the individuals but varies as a function of the Lp(a) plasma concentration. Thus, the overestimation of HDL-cholesterol values in samples containing Lp(a) and the altered HDL lipid composition when separated by ultracentrifugation are not readily predictable.

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