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Triglyceride Metabolism Modifies Lipoprotein(a) Plasma Concentration

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Abstract

Background: Lipoprotein(a) (Lp(a)) is a significant cardiovascular risk factor. Knowing the mechanisms that regulate its concentration can facilitate the development of Lp(a)-lowering drugs. This study analyzes the relationship between triglycerides (TGs) and Lp(a) concentrations, cross-sectionally and longitudinally, and the influence of the number and composition of TG-rich lipoproteins, and the *APOE* genotype.

Methods: Data from Aragon Workers Health Study (AWHS) (n = 5467), National Health and Nutrition Examination Survey III phase 2 (n = 3860), and Hospital Universitario Miguel Servet (HUMS) (n = 2079) were used for cross-sectional TG and Lp(a) relationship. Lp(a) intrasubject variation was studied in AWHS participants and HUMS patients with repeated measurements. TG-rich lipoproteins were quantified by nuclear magnetic resonance in a subsample from AWHS. Apolipoproteins B and E were quantified by Luminex in very low-density lipoprotein (VLDL) isolated by ultracentrifugation, from HUMS samples. *APOE* genotyping was carried in AWHS and HUMS participants. Regression models adjusted for age and sex were used to study the association.

Results: The 3 studies showed an inverse relationship between TG and Lp(a). Increased VLDL number, size, and TG content were associated with significantly lower Lp(a). There was an inverse association between the apoE concentration in VLDL and Lp(a). No significant association was observed for apolipoprotein (apo)B. Subjects carrying the apoE2/E2 genotype had significantly lower levels of Lp(a).

Conclusion: Our results show an inverse relationship Lp(a)-TG. Subjects with larger VLDL size have lower Lp(a), and lower values of Lp(a) were present in patients with apoE-rich VLDL and apoE2/E2 subjects. Our results suggest that bigger VLDLs and VLDLs enriched in apoE are inversely involved in Lp(a) plasma concentration.

Key Words: lipoprotein(a), TG-rich lipoproteins, VLDL, lipoprotein subclasses, apolipoprotein E

Abbreviations: apo, apolipoprotein; AWHS, Aragon Workers Health Study; CETP, cholesterylester transfer protein; HTG, hypertriglyceridemia; HUMS, Hospital Universitario Miguel Servet; IQR, interquartile range; K, kringles; LDL, low-density lipoprotein; Lp(a), lipoprotein(a); NHANES, National Health and Nutrition Examination Survey; NMR, nuclear magnetic resonance; TG, triglyceride; VLDL, very low-density lipoprotein

Lipoprotein(a) (Lp(a)) is an apolipoprotein B (apoB)-containing lipoprotein bound to a hydrophilic and highly glycosylated protein called apo(a) (1, 2). Apo(a) is composed of a variable number of repeating amino acid sequences, called kringles (K) (3, 4). The *LPA* locus, coding for apo(a), is responsible for most of Lp(a) variability in plasma concentration (5, 6). The mechanisms for Lp(a) synthesis and catabolism have not been fully elucidated, but they seem to be mostly independent of low-density lipoprotein (LDL) metabolism (1, 6). Newly synthesized apo(a) reaches the Golgi apparatus and is secreted in its mature form as a glycoprotein. The genetically determined size of apo(a), depending on the number of K-IV type 2 repeats, affects Lp(a) processing and secretion by HepG2 cells (5). Small isoforms are secreted much faster compared with large isoforms, which would explain the inverse relationship between the number of apo(a) K-IV type 2 repeats and Lp(a) plasma concentration. The assembly of Lp(a) is a 2-step process. First, apo(a) initially binds to apoB lysine residues on LDL via its lysine-binding sites in KIV7 and KIV8. Second, a covalent disulfide bridge is established between K-IV type 9 of apo(a) and apoB probably in the blood although the final mechanism has no conclusive

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evidence (7, 8). Apo(a) preferentially associates LDL particles; however, there is evidence that can also link to VLDL particles (9). Lp(a) particle seems to be eliminated from the plasma by a receptor-dependent pathway in the liver and kidney. Different receptors, such as macrophage scavenger receptor-B1, LDL receptor, or VLDL receptors, have shown some affinity for Lp(a), although the actual uptake in vivo is not well established (7, 10).

Many studies have confirmed that Lp(a) is an independent risk factor for atherosclerotic vascular disease (11, 12). However, the mechanisms that promote arteriosclerosis and many pathophysiology aspects of Lp(a) are not well understood yet (10). Lp(a) plasma concentration has a great interindividual variation, with a range from < 1 mg/dL to > 500 mg/dL, and more than 90% of it is genetically determined (6). Nevertheless, triglyceride (TG) synthesis may also be critical for the synthesis of apo(a) in hepatoma cells (13), and 1 study identified some association between Lp(a) and TG blood concentrations (14). Furthermore, Lp(a) concentration varies depending on the *APOE* genotype (15), and apoE concentration in very LDL (VLDL) is directly related to Lp(a) concentration (16). However, it has not yet been studied whether TG variation can be associated with Lp(a) concentration or whether the composition and size of VLDL particles affect Lp(a) levels.

In this study, we analyzed: (1) the cross-sectional association between TG and Lp(a) concentrations in 3 independent samples; (2) the effects of TG intra-subject variation on Lp(a); (3) the lipoprotein particle number and composition in a large range of Lp(a) levels; and (4) the relationship of *APOE* genotype with Lp(a) concentration, which we believe can help to shed light onto some aspects of Lp(a) metabolism.

Material and Methods

Design

This research uses data from 3 sources, approaching each aim with the necessary cross-sectional or longitudinal methods.

Cross-sectional association between TG and Lp(a)

We analyzed cross-sectional TG and Lp(a) data from 3 independent samples: Aragon Workers' Health Study (AWHS), a sample of workers from a car factory in Spain, from which the first available Lp(a) measurement was used; National Health and Nutrition Examination Survey III phase 2 (NHANES III), a representative sample of the US population; and a sample of patients examined in the Lipid Clinic of Hospital Universitario Miguel Servet (HUMS) in Zaragoza, Spain.

Effects of TG intra-subject variation on Lp(a)

Blood draws were performed in the AWHS study on approximately a yearly basis, providing the opportunity for repeated measurements. Also, Lp(a) in some patients from the HUMS sample was measured twice. For participants with repeated measurements, we selected 2 of them with the highest and lowest TG concentrations.

Lipoprotein particle number and composition association with Lp(a)

An AWHS subsample was selected for nuclear magnetic resonance (NMR) serum analysis, providing metabolomics lipid data. A subsample of HUMS was selected for VLDL isolation and apolipoprotein quantification.

ApoE genotype association with Lp(a)

A cross-sectional association was performed with AWHS and HUMS participants after determining *APOE* genotype in large subsamples.

Participants

AWHS

The AWHS is a longitudinal cohort study, started in 2009 based on the OPEL Spain automobile assembly plant (Zaragoza, Spain) and involves workers' annual medical examinations and biological samples. All workers were offered to participate in the study, and the response rate was 94.5%. The sample is predominantly male (>95%). Exclusion criteria include a history of cardiovascular disease or the presence of clinical conditions that limit survival to less than 3 years. The study was approved by the central institutional review board of Aragón (Comité Ético de Investigación Clínica de Aragón). All study participants provided written informed consent (17).

NHANES III

The National Center for Health Statistics conducted the second phase of the NHANES III between 1991 and 1994. This cohort was composed of a civilian US noninstitutionalized population. A representative sample was obtained through a multistage complex sampling process (18). Subjects that participated in NHANES were asked to sign a consent form, so they knew the nature and purpose of the survey. In that phase, 3992 participants 20 years and older completed fasting blood samples. Subjects with missing Lp(a) (n = 128) and missing TG (n = 4) were excluded.

HUMS

All consecutive unrelated patients 18 to 80 years old studied in the Lipid Clinic of HUMS from January 2006 to December 2020 were recruited for lipid research. The study was approved by the central institutional review board of Aragón (Comité Ético de Investigación Clínica de Aragón). All study participants provided written informed consent. Among these, analytical determinations performed in the HUMS laboratory between 2014 and 2019 that included simultaneously Lp(a) and TG were screened for this analysis.

Biochemical Data

AWHS and HUMS serum lipids

AWHS and HUMS used the same biochemical protocols. Patients had blood drawn after overnight fasting. Laboratory tests were performed on the same day of blood sampling. TG levels were measured in fresh serum by standard enzymatic automated methods in an AU5800 (Beckman Coulter Inc). Lp(a) concentration was measured by rate nephelometry using LPAX reagent in conjunction with IMMAGE Immunochemistry Systems and Lp(a) Calibrator (OMS/IFCC SRM 2 B; Beckman Coulter), following manufacturer instructions. This assay is not completely isoform insensitive, but uses the Denka Seiken method, which has been shown to have very good concordance with reference material from the World Health Organization/International Federation of Clinical Chemistry (19, 20). To rule out an interference between the

determination of Lp(a) in subjects with hypertriglyceridemia (HTG), the concentration of Lp(a) in the serum of 10 subjects with hypertriglyceridemia was analyzed and the samples were diluted to concentrations < 200 mg/dL of TG, without observe significant differences in the concentration of Lp(a) with a coefficient of variation < 10%. Four quality control samples were used daily with a coefficient of variation < 3.5% in all cases. When Lp(a) was below the lower detection limit, its value was imputed to 1 mg/dL, which is one-half the lower detection limit (AWHS n = 571, HUMS n = 271).

NHANES III serum lipids

Laboratory protocols and analytical methods were established at the Centers for Disease Control and Prevention. Fasting blood samples were tested for plasma lipids. TGs were analyzed with a Hitachi 704 Analyzer using the Boehringer Mannheim Diagnostics instrumentation and Lp(a) was analyzed using the ELISA assay with Strategic Diagnostics Venture, Inc. instrumentation in the Lipoprotein Analytical Laboratory at Johns Hopkins University of Baltimore, MD. When Lp(a) was below the lower detection limit, its value was imputed to 0.5 mg/dL, half the lower detection limit (n = 521).

TG-rich lipoprotein particle measurement

AWHS

TG-rich lipoproteins were quantified from serum samples in a subset of 664 volunteers from AWHS using high-throughput NMR metabolomics (Nightingale Health Ltd., Helsinki, Finland). This method provides simultaneous quantification of lipids and lipoprotein subclass profiling with lipid concentrations within 14 subclasses. Details of the experimentation and applications of the NMR metabolomics platform have been described previously (21, 22). In this study, for each lipoprotein subclass in the LDL to VLDL range (small, medium, large LDL, intermediate density lipoprotein, very small, small, medium, large, very large, and extremely very large LDL), we analyzed particle concentration (fmol/L) and lipid TG concentration (mmol/L).

VLDL apolipoprotein composition

HUMS

Apolipoprotein composition was measured in serum samples of 185 untreated subjects with TG > 200 mg/dL from HUMS, which underwent procedures to quantify apoB and apoE in VLDL previously isolated by ultracentrifugation. Briefly, the VLDL separation was carried out by centrifugation at 100,000 rpm for 150 minutes at 16°C. After centrifugation, the upper lipid with a density of 1.006 g/mL containing VLDL was removed. ApoB and apoE were determined with MILLIPLEX MAP Kits (Cat. #APOMAG-62K, Merck) according to the manufacturer's instructions. Assay sensitivities were 1.91 ng/mL for apoB and 0.49 ng/mL for apoE. Intra-assay precision (mean of % coefficient of variation) was < 10% for both metabolites, and inter-assay precision (mean of % coefficient of variation) was < 20% for all of them.

Genetic analysis

AWHS and HUMS

Genomic DNA from whole blood samples was isolated using standard methods. Exon 4 of *APOE* gene was amplified by PCR and purified by ExoSap-IT (USB), as previously described (23). Amplified fragments were sequenced by the Sanger method using the BigDye 3.1 sequencing kit (Applied Biosystems) in an automated ABI 3500xL sequencer (Applied Biosystems). DNA sequences were analyzed using Variant Reporter software (Applied Biosystems).

Statistical analysis

Continuous variables are expressed as mean (SD) or median [25th percentile-75th percentile] as applicable and categorical (nominal) variables are reported as percentages of the total sample. The relationship between TG variation and Lp(a) variation was studied by age- and sex-adjusted regression models. All statistical analyses were performed with R version 3.5.0 and significance was set at $P < 0.05$.

Results

Cross-sectional association between TG and Lp(a)

Lp(a) and TG were measured in 5467 AWHS participants. Median Lp(a) was 16 mg/dL. Figure 1A shows that in the relationship between Lp(a) and TG, median Lp(a) decreases to above 300 mg/dL of TG. An abnormally high median is seen for the group between 400 and 500 mg/dL of TG. However, because of the small sample in this group, a small number of participants with high values implies a wider distribution of the values in this group. However, an age- and sex-adjusted model estimates an Lp(a) reduction of ×0.94 (95% CI, 0.90-0.97) per each 100 mg/dL of TG increase (Supplementary Figure 1A) (24).

In the NHANES III study, phase 2, 3860 participants had both Lp(a) and TG data. Median Lp(a) was 14 mg/dL. Figure 1B similarly shows that in the relationship between Lp(a) and TG, median Lp(a) decreases as TG increases when both are above 300 mg/dL. Higher medians are seen for the groups above 400 mg/dL of TG, but these groups are small, too. The age- and sex-adjusted model estimates an Lp(a) reduction of ×0.88 (90% CI, 0.80-0.97) per each 100 mg/dL of TG increase (Supplementary Figure 1B) (24).

Among the patients tested in HUMS in the considered period, 2079 subjects underwent blood tests in which Lp(a) and TG were determined. Median Lp(a) was 28.8 mg/dL. Figure 1C shows a decrease in Lp(a) for TG values above 300 mg/dL. Age- and sex-adjusted Lp(a) reduction per each 100 mg/dL of TG increase was ×0.89 (95% CI, 0.87-0.92) (Supplementary Figure 1C) (24).

The correlation coefficients (r value and P values) between plasma TG and the logarithm (as this transformation was applied in all analyses) of Lp(a) concentrations were: AWHS: $r = -0.0484$, $P = 0.0003$; NHANES III $r = -0.0890$, $P < 0.0001$; and HUMS $r = -0.1943$, $P < 0.0001$. The concentration of TG stratify by Lp(a) levels is presented in Supplementary Figure 2 (24). There was a nonsignificant trend of higher TG in subjects with the lowest Lp(a) concentrations.

Effect of TG intra-subject variation on LP(A)

In AWHS, 2381 participants had TG and Lp(a) measured at least twice. Median (interquartile range [IQR]) variation of Lp(a) was ×1.05 (0.92, 1.30) and median (IQR) variation of TG was 1 (-32, 36) mg/dL. For every 100 mg/dL TG increase, Lp(a) was reduced by 8%, it changed ×0.92 (95% CI, 0.91-0.94), once adjusted for age and sex. The association was similar in all TG strata at the first measurement (Supplementary Figure 3A) (24).

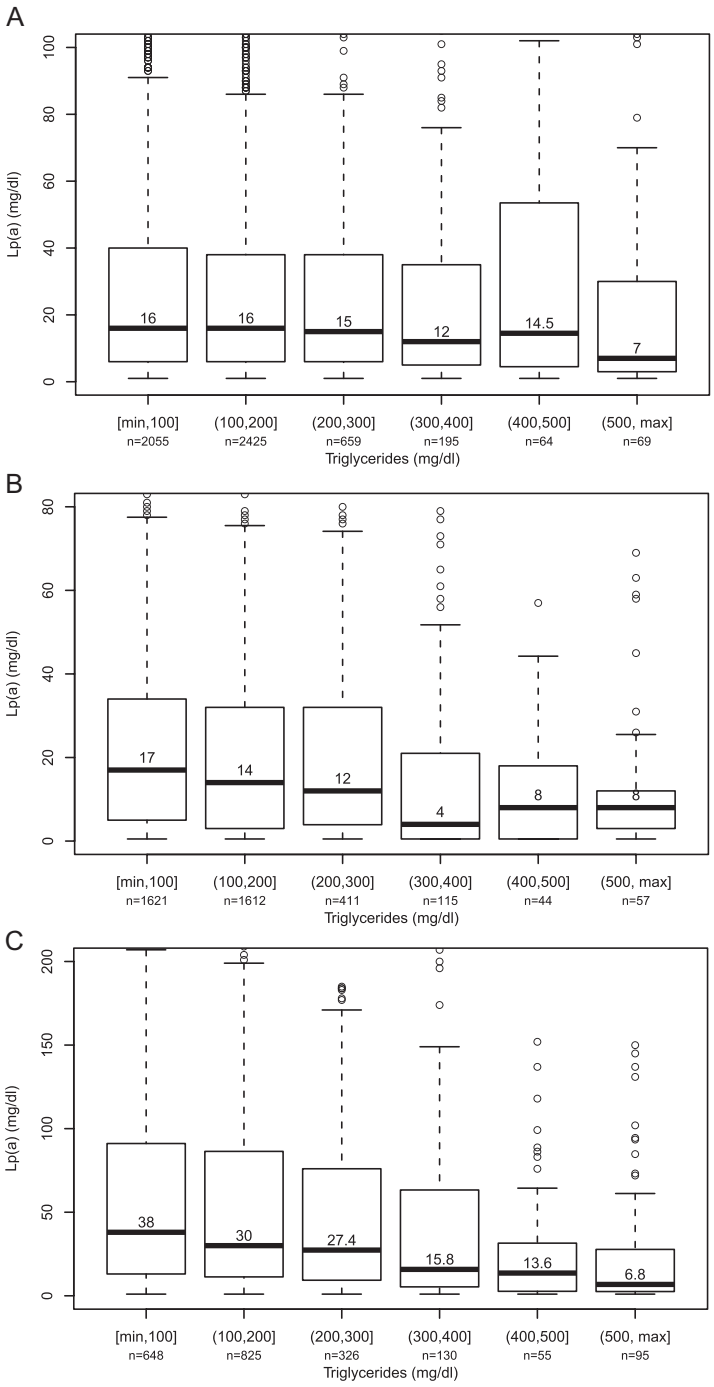

Figure 1. Boxplots showing the relationship between TG and Lp(a) in AWHS (A), NHANES (B), and HUMS (C).

TG and Lp(a) concentrations were measured at least twice in 441 HUMS patients. Median (IQR) variation of Lp(a) was ×1.07 (0.89, 1.35) and median (IQR) variation of TG was -22 (-61, 15) mg/dL. Age- and sex-adjusted Lp(a) reduction per each 100 mg/dL of TG increase was ×0.92 (95% CI, 0.90-0.95). Those with higher TG on the first measurement showed stronger association of TG variation with Lp(a) variation (Supplementary Figure 3B) (24).

TG-rich Particle Concentration, Distribution, and Composition and Lp(a) Concentration

TG-rich lipoprotein subclasses were analyzed in 664 subjects from AWHS by NMR with an Lp(a) range between 1 and 223 mg/dL. Global VLDL characteristics were considered first, the independence of the characteristics was explored next, and, finally, their influence by VLDL size stratum. Global VLDL characteristics included: total VLDL particle count, VLDL size, and triglyceride mass transported in the VLDL. The amount of total VLDL mass depends on the particle count and the size of each particle and these parameters grow together with the triglyceride mass transported in the VLDL fraction. Correlation coefficients between VLDL count and VLDL size was $r = 0.800$, $r = 0.927$ between VLDL count and TG transported in VLDL, and $r = 0.922$ between VLDL size and TG transported in VLDL ($P < 0.001$, in all cases) indicating their high collinearity.

The association with Lp(a) concentration was studied per standard deviation change of each characteristic. Each VLDL

characteristic was negatively associated with Lp(a) concentration, and the intensity of the coefficients of association were quite similar when estimated with separate regressions (Table 1). Once mutually adjusted, none of the 3 variables was a statistically significant independent predictor of Lp(a) change because of their high collinearity (Table 2). However, considering the 3 together, their joint influence on Lp(a) was statistically significant. Lp(a) decrease could depend more on the particle count and size than on the amount of TG transported in VLDL, and the coefficient for size seems to be the one less affected by mutual adjustment (Table 2). In the size-stratified analysis, and according with the observed major role of VLDL particle size in the association, Lp(a) concentration was reduced significantly when the count of VLDL particles in most of the size strata increased (Fig. 2A) and to a lesser extent when the amount of TG transported in each lipoprotein increased (Fig. 2B). Interestingly, these inverse associations appear mainly in VLDL and with a positive magnitude gradient dependent on size after adjustment for total TG, similarly for the 2 parameters, particle count (Fig. 2C), and TG transported in each lipoprotein size stratum (Fig. 2D).

To further characterize the association of TG-rich lipoproteins and Lp(a), 3 approaches are shown in the Lp(a) variation: per absolute change in the predictor variable units, which allows direct interpretation, but the comparability of the influence is impaired when ranges of variation of strata differ; per predictor variable doubling, which helps compare strata if it is assumed that all strata rise in parallel; and per SD, which is best for comparing strata relative influence. The coefficients were similar among them (Supplementary Table A) (24), and VLDL size showed to be the VLDL variable most associated and less affected after mutual adjustment (Supplementary Table B) (24). The inclusion of the *APOE* genotype in the regression models did not modify the total VLDL particle concentration, triglyceride content of VLDL, and VLDL size effects on the Lp(a) concentration.

ApoB and ApoE VLDL Composition and Lp(a) Concentration

The apolipoproteins quantified in VLDL particles of HUMS patients were associated with Lp(a) as follows: Lp(a) decreased ×0.822 (95% CI, 0.685-0.986; $P = 0.037$) per mg/dL apoE in the particles, but it was unrelated to apoB ×1.001 (95% CI, 0.978-1.024) per mg/dL.

APOE Genotype and Lp(a)

Among those participants in the cross-sectional analyses, 5008 AWHS participants and 1544 HUMS patients had *APOE* genotype available. The APO E2/E2 genotype carriers had significantly lower Lp(a) values than E3/E3, the most common genotype, in both samples. Lp(a) values were approximately half than those of carriers of the rest of *APOE* genotypes. Lp(a) was ×0.300 (95% CI, 0.170-0.530) that of E3/E3 in AWHS and ×0.426 (95% CI, 0.208-0.873) in HUMS (Table 3).

Discussion

Our study shows that plasma concentrations of Lp(a) and TG are inversely correlated when TGs are increased. The slope is particularly apparent above 300 mg/dL. Moreover, this inverse association seems primarily driven by the number and the size of VLDL particles. The higher the number of larger particles and their TG content, the lower the concentration of Lp(a). In addition, VLDL apoE content was also inversely associated with Lp(a).

Table 1. Effect of total VLDL particle concentration, triglyceride content of VLDL, and VLDL size on Lp(a) concentration

VLDL variable	Lp(a) change per standard deviation
VLDL particle concentration.	×0.793 (95% CI, 0.682-0.922)
Triglycerides in VLDL	×0.783 (95% CI, 0.677-0.906)
VLDL size	×0.770 (95% CI, 0.664-0.892)

Abbreviations: Lp(a), lipoprotein a; VLDL, very low-density lipoprotein.

Table 2. Mutually adjusted effect of total VLDL particle concentration, triglyceride content of VLDL and VLDL size on Lp(a) concentration

VLDL variable	Lp(a) change per SD
VLDL particle concentration.	×0.913 (95% CI, 0.575-1.449)
Triglycerides in VLDL	×1.069 (95% CI, 0.537-2.127)
VLDL size	×0.778 (95% CI, 0.512-1.181)
P (likelihood ratio test)	0.007

Abbreviations: Lp(a), lipoprotein a; VLDL, very low-density lipoprotein.

The relationship between TG and Lp(a) was first described by Bartens et al (25)., studying a small number of subjects with HTG. They showed that Lp(a) levels were significantly lower in 60 HTG subjects vs 128 control subjects. Besides, they found that plasma Lp(a) concentrations in the HTG patients correlated negatively with TG levels, suggesting that increased levels of TG-rich lipoproteins may influence the metabolism of Lp(a). More recently, this inverse relationship was also found in diabetic patients (26, 27).

The concentration of Lp(a) has been described to be genetically determined although a recent report indicates that Lp(a) levels increase with age (28). Furthermore, different circumstances and drugs modify the concentration of Lp(a), although there is some controversy among studies, and the mechanisms of this modification are largely unknown. For example, different studies have demonstrated that Lp(a) levels change throughout life, especially in females during puberty, pregnancy, or after menopause, indicating their relationship with hormonal factors (29). Besides, severe weight loss induced by bariatric surgery or severe weight reduction in obese children have been demonstrated to produce a reduction of Lp(a) concentrations (30-32). Nevertheless, other studies showed that the diet-induced weight loss was accompanied by an increase in Lp(a) levels in obese individuals with and without type 2 diabetes (33). Statins modestly increase Lp(a) levels, possibly because of higher expression of the *LPA* gene and, therefore, higher apo(a) concentration (34). Interestingly, some drugs that substantially modify TG concentration, such as fibrates, niacin, omega 3 fatty acids, and cholesterylester transfer protein (CETP), or apoCIII inhibitors, modify the Lp(a) concentration (35, 36). For example, in 1 randomized study, Wi et al (34) showed that although the treatment with fenofibrate 160 mg and niacin 1500 mg achieved a significant reduction on TG, the Lp(a) levels only decreased after the treatment with niacin. In the case of CETP inhibitors, these have been shown to decrease the Lp(a) levels because of the reduction in the apo(a) rate production (37). These data show

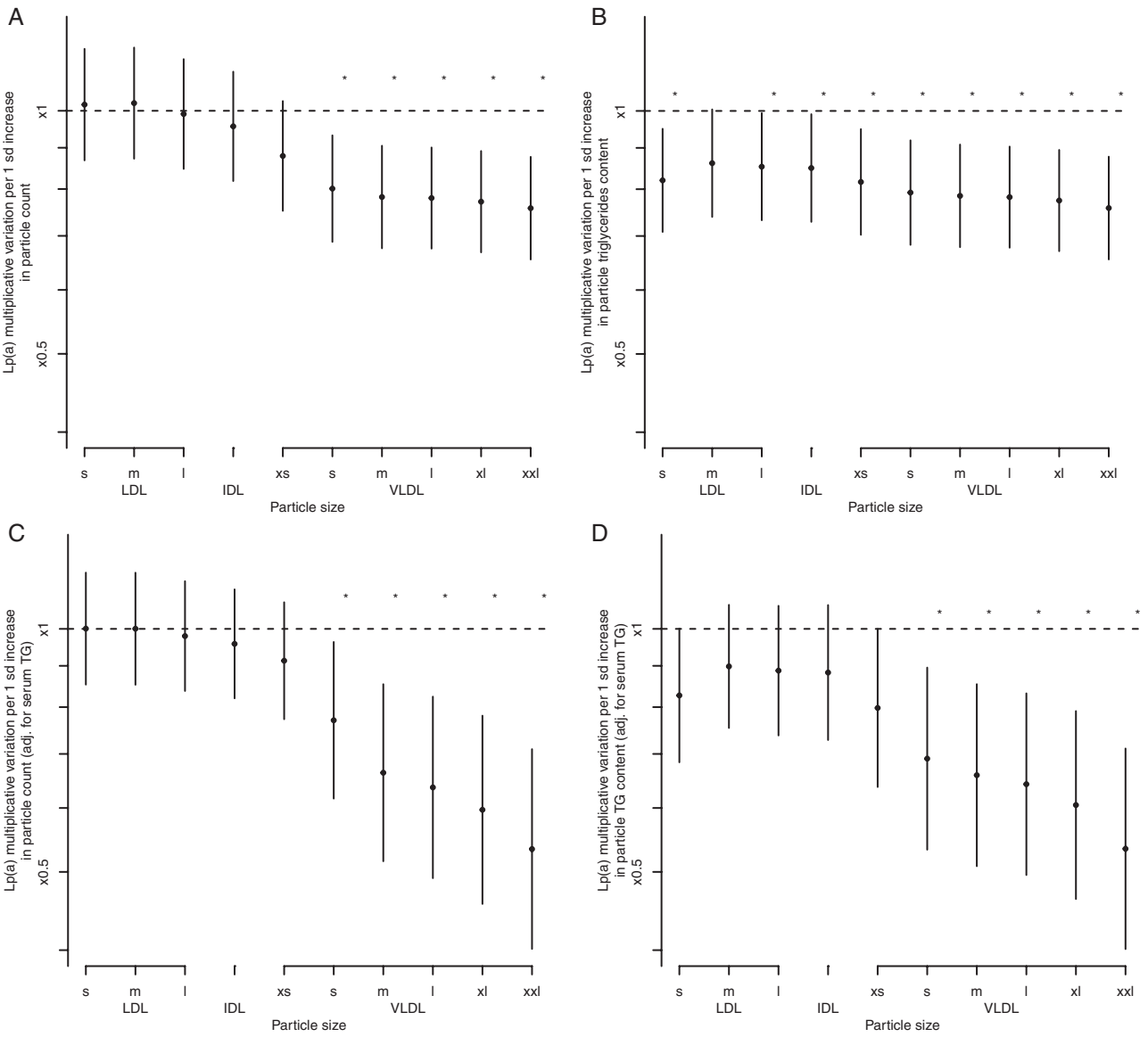

Figure 2. Effect on Lp(a) concentration per 1 SD in: the count of the different LDL, IDL, and VLDL particles sizes (A), TG transported in each lipoprotein particle size (B), lipoprotein count adjusted for total TG (C), and lipoprotein count adjusted for TG content in each particle size (D). IDL, intermediate-density lipoprotein; l, large; LDL denotes low-density lipoprotein; m, medium; s, small; VLDL, very-low density lipoprotein; xl, extra-large; xxl, extra-extra-large.

the large complexity of the regulation of Lp(a) concentration. The mechanism proposed in this article as result of the inverse association Lp(a)/TG may be only one of the mechanisms and it may be relevant only above a certain threshold of TG. NMR data indicate that those with severe HTG and larger VLDL particles have less Lp(a) and the opposite occurs in the presence of very small VLDL. This inverse relationship between Lp(a) and extra-large, large, and medium-sized VLDL particles has been previously described as associated with 1 single nucleotide polymorphism in the *LPA* gene by Kettunen et al (38).

Overall, it can be suggested that some apo(a)-VLDL assembly occurs in the presence of a sufficient number of small VLDLs and that the presence of larger VLDLs hinders the formation of Lp(a), or can extract free apo(a) on the surface of the liver before it is complexed with LDL-apoB to form Lp(a). A small amount of Lp(a) is present in the VLDL density range in plasma of hypertriglyceridemic subjects and individuals with normal TGs after a high-fat meal (9), but this incorporation of apo(a) into VLDL is not accompanied by an increase in the concentration of total Lp(a), which indicates a lower incorporation into particles in the density of LDL (39). Although LDLs are the main acceptors for the formation of Lp(a), when VLDL was infused into apo(a) transgenic mice, circulating Lp(a) appeared delayed in relation to LDL injection, which suggests that apo(a) preferentially associated with a metabolic product of VLDL (40). Our data indicate that TG enrichment of VLDL probably reduces this formation of Lp(a). This phenomenon has been described with CETP inhibitors. By preventing the exchange between HDL and VLDL of cholesterol esters and TG, VLDL is enriched with TG, which causes the synthesis of Lp(a) to decrease without modifying its fractional catabolic rate (36). Therefore, it seems that this inverse relationship would be related to a decrease in the synthesis of Lp(a) rather than an increase in its catabolism.

Table 3. Lp(a) concentration according to *APOE* genotype in AWHS and HUMS

	APOE genotype					
	E3E3	**E2E2**	**E2E3**	**E2E4**	**E3E4**	**E4E4**
AWHS						
n	3535	21	526	59	824	43
G-mean Lp(a), mg/dL	13.6	4.1	13.1	11.5	14.4	13.8
Model (x-times)	1.000 (ref.)	0.300 (95% CI, 0.170-0.530)	0.963 (95% CI, 0.852-1.087)	0.837 (95% CI, 0.595-1.178)	1.053 (95% CI, 0.953-1.165)	1.007 (95% CI, 0.676-1.501)
P	-	<0.001	0.538	0.308	0.311	0.972
HUMS						
n	1014	16	124	24	332	34
G-mean Lp(a), mg/dL	27.9	11.1	21.9	21.4	26.3	14.9
Model (x-times)	1.000 (ref.)	0.426 (95% CI, 0.208-0.873)	0.807 (95% CI, 0.615-1.058)	0.777 (95% CI, 0.432-1.398)	0.933 (95% CI, 0.779-1.117)	0.538 (95% CI, 0.328-0.884)
P	-	0.020	0.121	0.399	0.450	0.014

Model adjusted for age and sex.
Abbreviations: AWHS, Aragon Workers Health Study; G-mean, geometric mean (unadjusted); HUMS, Hospital Universitario Miguel Servet; Lp(a), lipoprotein a.

Interestingly, apoE2/2 subjects have a lower concentration of Lp(a) than the subjects carrying the rest of *APOE* genotypes. This finding was described in a large group of subjects studied by Moriarty et al (15), in whom they determined the *APOE* genotype, the Lp(a) mass, and several apoB-related lipoproteins. These authors speculated that apoE4 proteins prefer large triglyceride-rich lipoproteins, whereas apoE3 and apoE2 proteins preferentially bind to smaller lipoproteins that could affect the metabolism of Lp(a). The apoE2/2 genotype is associated with higher apoE levels in plasma and dysbetalipoproteinemia, a disorder characterized by the accumulation of remnants of TG-rich lipoproteins, enriched in cholesterol and apoE (41). Using NMR, we have not been able to identify substantial changes in the amount of cholesterol in very small VLDL in relation to the concentration of Lp(a), but we have identified that the apoE in VLDL is inversely associated with Lp(a) concentration. One hypothesis would be that apoE enrichment in VLDL remnants, as occurs in dysbetalipoproteinemia, would be responsible for the decrease in Lp(a), preventing apoB and apo(a) from binding.

Our study has limitations. First, our data are observational and do not imply causality. Therefore, other factors different from TG could contribute to the described association. Second, the cross-sectional analysis of lipid clinic data might be affected by some selection bias, although it does not affect the longitudinal analysis. The amount of Lp(a) explained by TG concentrations, although significant, is quantitatively small and the clinical significance is probably minor given the changes around 70 to 100 mg/dL necessary to have a clinical impact (42, 43). Recently, the validity of some NMR determinations has been questioned, more precisely concentrations of LDL and HDL particles, and VLDL cholesterol. However, these potential limitations of the method do not affect the main results of the study because NMR was basically used to quantify the number of VLDL particles (44).

In summary, elevated TG concentrations above 300 mg/dL are associated with lower Lp(a) concentrations. Subjects with a higher number of larger VLDL particles and enriched in TG have lower Lp(a). APOE2/2 genotype is associated with lower Lp(a), and there is an inverse relationship between apoE in VLDL and Lp(a). This would suggest that VLDLS enriched in apoE are not a good substrate for the formation of Lp(a). In conclusion, the synthesis of Lp(a) is closely related to the structure and composition of VLDL particles. The reduction and/ or modification of those VLDL particles that are good receptors of apo(a) could be a tool to reduce the concentration of Lp(a) in the future.

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Author Contributions

Conceptualization, M.L. and F.C.; data curation, M.R.-C., M.L., V.M.B., I.L.-M., J.M.O., and F.C.; formal analysis I.L.-M., F.C., and M.L.; funding acquisition, F.C. and A.C.; investigation, M.R.-C., A.M.B., R.M.-G., P.C., and A.C.; methodology, J.P., A.C., J.M.O., and A.M.B.; project administration, F.C.; resources, J.M.O. and F.C.; software I.L.-M., M.L., and M.R.-C.; supervision F.C., M.L., and A.C.; validation I.L.-M., M.L., and F.C.; visualization, A.C., F.C., M.L.; writing—original draft preparation, M.R.-C., I.L.-M., F.C., and M.L.; writing—review and editing, M.L., A.C., J.M.O., and F.C. All authors have read and approved the final manuscript.

Conflict of Interest

The authors declare no conflict of interest.

Data Availability

Some or all datasets generated during and/or analyzed during the current study are not publicly available but are available from the corresponding author on reasonable request.

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