



HAL
open science

VLDL (Very-Low-Density Lipoprotein)-Apo E (Apolipoprotein E) May Influence Lp(a) (Lipoprotein [a]) Synthesis or Assembly

Mikaël Croyal, Valentin Blanchard, Khadija Ouguerram, Maud Chétiveaux,
Léa Cabioch, Thomas Moyon, Stéphanie Billon-Crossouard, Audrey Aguesse,
Karine Bernardeau, Cedric Le May, et al.

► To cite this version:

Mikaël Croyal, Valentin Blanchard, Khadija Ouguerram, Maud Chétiveaux, Léa Cabioch, et al.. VLDL (Very-Low-Density Lipoprotein)-Apo E (Apolipoprotein E) May Influence Lp(a) (Lipoprotein [a]) Synthesis or Assembly. *Arteriosclerosis, Thrombosis, and Vascular Biology*, 2020, 40 (3), pp.819-829. 10.1161/ATVBAHA.119.313877 . hal-03098122

HAL Id: hal-03098122

<https://hal.science/hal-03098122>

Submitted on 5 Jan 2021

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

ORIGINAL RESEARCH

VLDL (Very-Low-Density Lipoprotein)-Apo E (Apolipoprotein E) May Influence Lp(a) (Lipoprotein [a]) Synthesis or Assembly

Mikaël Croyal,* Valentin Blanchard,* Khadija Ougueram, Maud Chétiveaux, Léa Cabioch, Thomas Moyon, Stéphanie Billon-Crossouard, Audrey Aguesse, Karine Bernardeau, Cédric Le May, Laurent Flet, Gilles Lambert, Samy Hadjadj, Bertrand Cariou, Michel Krempf, Estelle Nobécourt-Dupuy

OBJECTIVE: To clarify the association between PCSK9 (proprotein convertase subtilisin/kexin type 9) and Lp(a) (Lipoprotein [a]), we studied Lp(a) kinetics in patients with loss-of-function and gain-of-function *PCSK9* mutations and in patients in whom extended-release niacin reduced Lp(a) and PCSK9 concentrations.

APPROACH AND RESULTS: Six healthy controls, 9 heterozygous patients with familial hypercholesterolemia (5 with low-density lipoprotein receptor [*LDLR*] mutations and 4 with *PCSK9* gain-of-function mutations) and 3 patients with heterozygous dominant-negative *PCSK9* loss-of-function mutations were included in the preliminary study. Eight patients were enrolled in a second study assessing the effects of 2 g/day extended-release niacin. Apolipoprotein kinetics in VLDL (very-low-density lipoprotein), LDL (low-density lipoprotein), and Lp(a) were studied using stable isotope techniques. Plasma Lp(a) concentrations were increased in *PCSK9*-gain-of-function and familial hypercholesterolemia-*LDLR* groups compared with controls and *PCSK9*-loss-of-function groups (14 ± 12 versus 5 ± 4 mg/dL; $P=0.04$), but no change was observed in Lp(a) fractional catabolic rate. Subjects with *PCSK9*-loss-of-function mutations displayed reduced apoE (apolipoprotein E) concentrations associated with a VLDL-apoE absolute production rate reduction. Lp(a) and VLDL-apoE absolute production rates were correlated ($r=0.50$; $P<0.05$). ApoE-to-apolipoprotein (a) molar ratios in Lp(a) increased with plasma Lp(a) ($r=0.96$; $P<0.001$) but not with PCSK9 levels. Extended-release niacin-induced reductions in Lp(a) and VLDL-apoE absolute production rate were correlated ($r=0.83$; $P=0.015$). In contrast, PCSK9 reduction (-35% ; $P=0.008$) was only correlated with that of VLDL-apoE absolute production rate ($r=0.79$; $P=0.028$).

CONCLUSIONS: VLDL-apoE production could determine Lp(a) production and/or assembly. As PCSK9 inhibitors reduce plasma apoE and Lp(a) concentrations, apoE could be the link between PCSK9 and Lp(a).

Key Words: apolipoprotein E ■ isotopes ■ kinetics ■ loss of function mutation ■ niacin

Lp(a) (Lipoprotein [a]) consists of one molecule of apo(a) (apolipoprotein [a]) bound covalently to the apoB100 (apolipoprotein B100) of a LDL (low-density lipoprotein)-like particle. An elevated plasma Lp(a) concentration is regarded as a strong cardiovascular risk factor.^{1,2} However, metabolic pathways governing Lp(a) concentrations remain poorly understood.

The LDL receptor (LDLR) was first proposed as a potential route for Lp(a) catabolism, as individuals with familial hypercholesterolemia (FH) and *LDLR* mutations display higher plasma Lp(a) concentrations.³ The inhibition of PCSK9 (proprotein convertase subtilisin/kexin type 9), a circulating endogenous inhibitor of the LDLR, provided data supporting this hypothesis. PCSK9 inhibitors restored LDLR expression at hepatocyte surfaces,

Correspondence to: Mikaël Croyal, PhD, 8 quai Moncoussu, F-44000 Nantes, France. Email mikael.croyal@univ-nantes.fr

*M. Croyal and V. Blanchard contributed equally to the article.

The online-only Data Supplement is available with this article at <https://www.ahajournals.org/doi/suppl/10.1161/ATVBAHA.119.313877>.

For Sources of Funding and Disclosures, see page XXX.

© 2020 American Heart Association, Inc.

Arterioscler Thromb Vasc Biol is available at www.ahajournals.org/journal/atvb

Nonstandard Abbreviations and Acronyms

ApoE	apolipoprotein E
APR	absolute production rate
CTL	control
ERN	extended-release nicotinic acid
FCR	fractional catabolic rate
FH	familial hypercholesterolemia
GOF	gain-of-function
HDL	high-density lipoprotein
IDL	intermediate-density lipoprotein
LDL	low-density lipoprotein
LDL-C	LDL-cholesterol
LOF	loss-of-function
LRP-1	LDLR-related protein
PCSK9	proprotein convertase subtilisin/kexin type 9
VLDL	very-low-density lipoprotein

thereby markedly reducing LDL-cholesterol (LDL-C) concentrations.^{4,5} In parallel, PCSK9 inhibitors generated an unexpected 20% to 30% reduction in plasma Lp(a) concentrations, proportional to the treatment dose and the baseline level of plasma Lp(a).⁶

PCSK9 inhibition with monoclonal antibodies (ie, alirocumab and evolocumab) or small interfering RNA (ie, inclisiran) similarly reduces Lp(a) plasma levels, albeit through different mechanisms of action.^{7,8} However, unlike LDL-C reduction, widely varying responses were observed among individuals in whom Lp(a) was reduced, with no clear relationship with the apo(a) polymorphic size, or the baseline PCSK9 plasma concentration.^{9–11} Statins also increase LDLR expression but, in contrast to PCSK9 inhibitors, they have no effect on or only slightly increase the plasma Lp(a) concentration.¹²

Controversial in-vitro results have been also reported regarding the mechanism of action of Lp(a) reduction with PCSK9 inhibitors, ranging from a key role of the LDLR in Lp(a) catabolism¹³ to a key role of PCSK9 in Lp(a) production.¹⁴ In humans, Lp(a) fractional catabolic rates (FCR) were found to be similar in patients with FH and controls.¹⁵ In nonhuman primates, we reported that alirocumab strongly reduced the circulating Lp(a) concentration by acting on apo(a) production rate.¹⁶ In humans, in vivo lipoprotein kinetics studies with PCSK9 monoclonal antibodies have also led to conflicting data. While alirocumab induced a nonsignificant increase in the Lp(a) FCR in one study,¹⁷ evolocumab led to a significant decrease in the Lp(a) production rate in another one.¹⁸ However, since the combination of evolocumab with atorvastatin increased Lp(a) FCR, the role of an overexpressed LDLR at the hepatocyte surface in Lp(a) catabolism could be probable.

Highlights

- PCSK9 (proprotein convertase subtilisin/kexin type 9) inhibitors reduce Lp(a) (lipoprotein [a]) concentrations by 20% to 30% via incompletely understood mechanisms.
- Despite strong variability in plasma PCSK9 and low-density lipoprotein cholesterol concentrations, Lp(a) fractional catabolic rates were similar between patients displaying loss- or a gain-of-function *PCSK9* mutations.
- VLDL (very-low-density lipoprotein)-apoE (apolipoprotein E) absolute production rates were positively correlated with Lp(a)-apo(a) absolute production rate.
- Proteomic analysis of isolated Lp(a) particles showed that the apoE content of Lp(a) increased significantly with plasma Lp(a) but not with PCSK9 concentration.
- Under extended-release niacin-induced reductions of Lp(a), PCSK9, and VLDL-apoE concentrations, the relative changes in the VLDL-apoE and Lp(a)-apo(a) absolute production rates were strongly correlated, whereas changes in plasma PCSK9 concentration correlated only with changes in VLDL-apoE absolute production rate.

The apo(a) size, defined as the number of Kringle IV-2 copies on the *LPA* gene, governs 30% to 70% of circulating Lp(a) levels.¹⁹ Recent studies showed that the *APOE* and *PCSK9* genes may also influence Lp(a) levels.^{20,21} We showed that single-nucleotide polymorphisms of apoE (apolipoprotein E), a ligand for the LDLR and the LRP-1 (LDLR-related protein), influence plasma Lp(a) concentrations in patients with FH with *LDLR* mutations.²² However, the underlying mechanisms remain unknown.

To gain further insight into mechanisms governing Lp(a) concentrations, we studied patients carrying rare loss-of-function (LOF) and gain-of-function (GOF) mutations of *PCSK9*.^{23,24} We previously showed that *PCSK9* mutations strongly modulate LDL catabolic rates, independently of any pharmacological inhibitor.^{24,25} In the present study, we aimed to extend this research by analyzing Lp(a) kinetics in these patients and their relationships with plasma PCSK9 and other lipoprotein components. We also compared our results with those obtained in an interventional study examining the ability of extended-release niacin (ERN) to reduce LDL-C, PCSK9, and Lp(a) concentrations.²⁶

MATERIALS AND METHODS

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Patients and Controls

Lp(a) kinetics were explored in patients with various phenotypes: 3 subjects with the dominant-negative heterozygous

R104C/V114A *PCSK9* LOF mutation (*PCSK9*-LOF group),²⁴ and 9 patients with FH, consisting of 4 heterozygous *PCSK9* GOF mutation carriers (3 with S127R, 1 with R218S; *PCSK9*-GOF group),²⁵ and 5 controls with FH and heterozygous *LDLR* mutations (FH-*LDLR* group).²⁷ The control (CTL) group was composed of 6 healthy volunteers.²⁸ The clinical characteristics of the patients are detailed in Table 1. The Ethics Committee of Nantes approved the clinical protocol, and written informed consent was obtained from all subjects (protocol referenced as no. 15/06-BRD 06/3-E).

ERN Intervention

We supported our study findings by performing additional analyses of data from a previously reported intervention trial.²⁶ Eight nondiabetic overweight male subjects (Table 1) were enrolled in a randomized crossover controlled study (8 weeks/phase and a 4-week washout interval) comparing the effects of placebo and 2 g/day ERN. In that study, ERN resulted in the reduction of plasma Lp(a) and *PCSK9* concentrations.²⁶ The Ethics Committee of Nantes approved the clinical protocol (trial no. NCT01216956), and written informed consent was obtained from all subjects.

Kinetic Protocol

After an overnight fast, each subject received a bolus of 10 μ mole/kg ²H₃-leucine (Cambridge Isotope Laboratories, Tewksbury, MA), followed immediately by constant infusion (10 μ mole/kg per h) for 14 hours. Blood samples were collected at 0, 0.5, 1, 2, 4, 6, 8, 10, 12, and 14 hours, and plasma was separated by centrifugation at 4°C for 30 minutes. Sodium azide, an inhibitor of bacterial growth, and Pefabloc SC (Interchim, Montluçon, France), a protease inhibitor, were added to the blood samples at final concentrations of 1.5 and 0.5 mmol/L, respectively. Plasma lipoprotein fractions, including VLDL (very-low-density lipoprotein), IDL (intermediate-density lipoprotein), LDL, and HDL (high-density lipoprotein) were separated by sequential ultracentrifugation.²⁵ Samples were stored at -80°C until use.

Biochemical Measurements

Cholesterol and triglyceride (TG) concentrations were measured using enzymatic kits from Boehringer Mannheim GmbH (Mannheim, Germany). Plasma *PCSK9* and Lp(a) concentrations were measured by ELISA (R&D Systems, Lille, France and Aviva Systems Biology Corporation, CA).

Table 1. Characteristics of Patients

Patient	Sex	Age, y	BMI, kg/m ²	<i>PCSK9</i> Mutation	<i>LDLR</i> Mutation	ApoE Genotype
<i>PCSK9</i> -LOF 1	F	22	22	R104C/V114A	None	E3/E3
<i>PCSK9</i> -LOF 2	M	49	29	R104C/V114A	None	E3/E3
<i>PCSK9</i> -LOF 3	F	29	23	R104C/V114A	None	E3/E3
<i>PCSK9</i> -GOF 1	F	43	22	S127R	None	E3/E3
<i>PCSK9</i> -GOF 2	M	23	19	S127R	None	E3/E3
<i>PCSK9</i> -GOF 3	F	39	33	S127R	None	E3/E3
<i>PCSK9</i> -GOF 4	M	32	27	R218S	None	E3/E3
FH- <i>LDLR</i> 1	F	64	19	None	F105S, exon 4	E3/E3
FH- <i>LDLR</i> 2	M	41	23	None	Frameshift, exon 4	E3/E3
FH- <i>LDLR</i> 3	M	28	28	None	Frameshift, exon 9	E3/E4
FH- <i>LDLR</i> 4	F	50	31	None	W66G, exon 3	E3/E3
FH- <i>LDLR</i> 5	M	57	25	None	A370T, exon 8	E3/E3
CTL 1	M	31	24	None	None	E3/E3
CTL 2	M	21	18	None	None	E3/E3
CTL 3	M	31	24	None	None	E3/E3
CTL 4	M	31	20	None	None	E3/E3
CTL 5	M	26	20	None	None	E3/E3
CTL 6	M	18	20	None	None	E3/E3
OW 1	M	34	32	None	None	E3/E4
OW 2	M	52	34	None	None	E2/E3
OW 3	M	26	32	None	None	E3/E3
OW 4	M	63	29	None	None	E3/E3
OW 5	M	48	30	None	None	E3/E3
OW 6	M	37	32	None	None	E3/E3
OW 7	M	59	29	None	None	E3/E3
OW 8	M	62	31	None	None	E2/E3

ApoE indicates apolipoprotein E; BMI, body mass index; C, cysteine; CTL, control; F, female; FH-*LDLR*, familial hypercholesterolemia with mutation of *LDLR*; GOF, gain of function; *LDLR*, low-density lipoprotein receptor; LOF, loss of function; M, male; OW, overweight (ERN study); *PCSK9*, proprotein convertase subtilisin/kexin type 9; R, arginine; and V, valine.

Plasma Leucine Measurements

Plasma leucine (endogenous leucine and exogenous 2H_3 -leucine) concentrations were measured by electron-impact gas chromatography–mass spectrometry as described previously.²⁵ Briefly, amino acids were purified by cation exchange chromatography and then derivatized by heptafluorobutyric acid. The isotopic ratio was determined by selected ion monitoring at mass-to-charge ratios of 282 (leucine) and 285 (2H_3 -leucine).

Lp(a) Immunoprecipitation

Lp(a) was immunoprecipitated from plasma samples (50 μL) using the precoated wells of an Lp(a) ELISA kit (Aviva Systems Biology Corporation, monoclonal antibody). After the performance of blocking procedures according to the supplier's instructions, the wells were washed and trypsin digested for mass-spectrometry analyses.

Apolipoprotein Quantification

Apolipoproteins were quantified in plasma and lipoprotein fractions using trypsin proteolysis and subsequent analysis of proteotypic peptides by liquid chromatography–tandem mass spectrometry as described previously.^{26,29,30} Quantification was performed with 3 replicates at all kinetic time points. Apo(a) polymorphic sizes were determined using 2 proteolytic peptides,²⁶ and apoE phenotypes were determined using a combination of 5 proteolytic peptides.³⁰ The intra- and inter-assay variabilities did not exceed 7.1%.

Apolipoprotein Enrichment Measurement

The isolation and measurement of leucine enrichment in apoB100 were performed as described previously.²⁵ Briefly, apoB100-containing lipoprotein fractions (VLDL, IDL, LDL) were isolated by sodium dodecyl sulfate polyacrylamide gel electrophoresis and then hydrolyzed with concentrated hydrochloric acid solution. Amino acids were purified by cation exchange chromatography, derivatized (with heptafluorobutyric acid), and analyzed by gas chromatography–mass spectrometry as described above. VLDL-apoE and Lp(a)-apo(a) leucine enrichments were measured in concentrated lipoprotein fractions by liquid chromatography–tandem mass spectrometry, as detailed previously.^{26,29} As the majority ($\approx 95\%$) of Lp(a) particles are located in the density range of 1.019 to 1.210 g/mL following ultracentrifugation of plasma samples, equal volumes of ultracentrifuged fractions of LDL (1.019–1.063 g/mL), and HDL (1.063–1.21 g/mL) were combined. Leucine enrichment measurements were performed on 3 replicates at all kinetic time points, and the intra- and inter-assay variabilities did not exceed 6.8%.

Modeling

Lp(a)-apo(a) and VLDL-apoE FCRs were estimated using the SAAM II modeling program (Epsilon Group, Charlottesville, VA) and by fitting of a mono-compartmental model.^{26,29} Kinetic data for VLDL- and LDL-apoB100 were calculated using a multiple compartmental model including IDL-apoB100 enrichment measurements, as described previously.²⁵ Due to the heterogeneity of patients' lipid profiles, the asymptotic plasma leucine plateau (tracer-to-tracee ratio) was used as a pool precursor,

instead of the VLDL-apoB100 asymptotic plateau. Pool sizes were considered to be constant, as no significant variation was observed in apolipoprotein concentrations at different sampling times (concentration variations within 8.2%). At this steady state, the FCR was equal to the fractional synthetic rate. Therefore, absolute production rates (APRs) were calculated as the products of the FCR and the pool sizes of apolipoproteins in lipoprotein fractions, and assuming a plasma volume of 4.5% of body weight.

Statistical Analysis

Results are expressed as mean \pm SDs, unless otherwise specified. Statistical analyses were performed with GraphPad Prism software (version 6.0; GraphPad Software, Inc, La Jolla, CA). Due to the small number of patients, only *P* for trend values (obtained by the Kruskal-Wallis test) are presented for group comparison. Pairwise statistical analyses were conducted with Spearman correlation tests, and the nonparametric Wilcoxon matched-pairs signed rank test was used to evaluate ERN effects. Results were considered to be significant at $P < 0.05$. Data were finally explored with a principal component analysis and a principal component analysis regression circle (SIMCA-P+, version 13.0; Umetrics, Umeå, Sweden). A correlation network was also established between all biochemical data with only positive correlations and *r* coefficients > 0.5 . The correlation network was calculated and visualized using the Cytoscape software (<http://www.cytoscape.org/>).

RESULTS

Biochemical Findings

Baseline lipid parameters of the patients are provided in Table 2. As expected, patients in the *PCSK9*-LOF group displayed dramatically reduced plasma total cholesterol, LDL-C, and *PCSK9* concentrations compared with the other groups. Plasma concentrations of apoB100 and apoE were also reduced in these patients. In contrast, LDL-C and plasma apoB100 concentrations were increased in the *PCSK9*-GOF and FH-*LDLR* groups, associated with a trend of higher plasma apoE concentrations. Plasma Lp(a) and apo(a) concentrations were significantly correlated ($r = 0.60$; $P = 0.009$). Plasma Lp(a) concentrations were increased in patients with *PCSK9*-GOF and *LDLR* mutations (14.0 ± 12.1 mg/dL, $n = 9$) compared with the others (5.3 ± 3.6 mg/dL, $n = 9$; $P = 0.039$). Analysis of lipoprotein apolipoprotein content showed reduced concentrations of apoB100 and apoE in the VLDL and LDL fractions in the *PCSK9*-LOF group (Tables I and II in the [online-only Data Supplement](#)). After specific immunoprecipitation of Lp(a) particles, apo(a) concentrations were close to the sum of recovery in the VLDL, LDL, and HDL fractions. In isolated Lp(a) particles, the apoB100-to-apo(a) molar ratio was close to 1, excluding possible contaminations with other apoB-containing lipoproteins, whereas the apoE-to-apo(a) molar ratio ranged from 0.5 to 3 and correlated with plasma Lp(a) concentrations ($r = 0.96$, $P < 0.0001$;

Table 2. Biochemical Parameters of Patients

Parameter	PCSK9-LOF (n=3)	PCSK9-GOF (n=4)	FH-LDLR (n=5)	CTL (n=6)	P Value for Trend
TC, mg/dL	114 (100–143)	361 (292–408)	440 (388–461)	165 (125–198)	0.0001
TG, mg/dL	49 (34–71)	213 (75–268)	164 (131–189)	71 (47–114)	0.0012
LDL-C, mg/dL	44 (16–58)	281 (241–326)	327 (237–415)	86 (64–136)	0.0001
HDL-C, mg/dL	75 (63–83)	38 (36–42)	35 (31–50)	52 (41–66)	0.0009
ApoA-I, mg/dL	174 (158–198)	103 (96–110)	102 (94–105)	109 (95–134)	0.0377
ApoB100, mg/dL	21 (20–37)	175 (155–196)	169 (104–208)	80 (45–114)	0.0001
ApoC-II, mg/dL	1.6 (0.8–2.3)	2.0 (0.8–2.2)	2.0 (1.3–2.8)	1.5 (1.3–2.1)	0.5610
ApoC-III, mg/dL	5.8 (2.9–9.4)	8.6 (3.5–12.7)	4.0 (2.6–7.1)	4.2 (3.1–5.4)	0.3288
ApoE, mg/dL	4.2 (3.1–4.5)	9.1 (6.4–11.4)	6.7 (3.6–11.2)	4.4 (3.0–6.6)	0.0147
PCSK9, ng/mL	38 (0–89)	181 (100–398)	288 (142–303)	232 (194–322)	0.0292
Lp(a), mg/dL	3.6 (3.3–6.6)	6.5 (4.8–29.5)	12.2 (3.2–33.4)	4.0 (3.1–14.6)	0.2234
Apo(a), nmol/L	16 (14–23)	32 (15–114)	16 (10–141)	14 (4–54)	0.5939
Kringle IV repeat, n	22 (21–33)	20 (13–30)	22 (15–32)	24 (16–38)	0.4207

Values are presented as median (range). *P* for trend values were obtained by Kruskal-Wallis tests. Apo indicates apolipoprotein; CTL, control; FH-LDLR, familial hypercholesterolemia with mutation of the low-density lipoprotein receptor; GOF, gain of function; HDL-C, high-density lipoprotein cholesterol; Kr-IV, Kringle IV; LDL-C, low-density lipoprotein cholesterol; LOF, loss of function; Lp(a), lipoprotein (a); PCSK9, proprotein convertase subtilisin/kexin type 9; TC, total cholesterol; and TG, triglyceride.

Figure 1) but not with plasma PCSK9 concentrations ($r=0.30$, $P=0.222$). The apoE-to-apoB100 ratio calculated in VLDL and LDL particles was not correlated with plasma Lp(a) levels, suggesting a specific role of apoE in Lp(a) production/assembly irrespective of both VLDL and LDL pools. Of note, Lp(a) plasma concentrations were not correlated with plasma TG ($r=0.35$, $P=0.157$).

Kinetics of Lp(a) and Other Apolipoproteins

The asymptotic plasma leucine plateaus used as precursor pools were of $10.1 \pm 0.2\%$ (PCSK9-LOF), $11.0 \pm 1.2\%$ (PCSK9-GOF), $10.4 \pm 0.5\%$ (FH-LDLR), and $8.6 \pm 0.5\%$ (CTL). The reduced precursor pool of CTL patients could explain the marked difference in VLDL-apoB100 asymptotic plateaus between CTL and PCSK9-LOF patients (Figure 2A). But the opposite was observed for VLDL-apoE. This could stem from the sharply distinct lipid

profiles of those patients. Since CTL individuals displayed elevated plasma TG and reduced HDL-C (Table 2) compared with PCSK9-LOF subjects, apoE could for instance be differently exchanged between VLDL and HDL particles in such patients. Lp(a)-apo(a) FCR were similar in all groups (Figure 2). The APR of Lp(a)-apo(a) was within the same lower range in the CTL and PCSK9-LOF groups and was slightly increased in the FH-LDLR and PCSK9-GOF groups. The VLDL-apoE FCR was lower in the FH-LDLR and PCSK9-GOF groups and was slightly decreased in the PCSK9-LOF group compared with the CTL group. The VLDL-apoE APR was within the same range in the CTL, PCSK9-GOF, and FH-LDLR groups and decreased in the PCSK9-LOF group. The VLDL- and LDL-apoB100 FCRs were dramatically increased in the PCSK9-LOF group, and the LDL-apoB100 FCR was decreased in the FH-LDLR and PCSK9-GOF groups. Of note, plasma PCSK9 concentrations correlated negatively with the VLDL-apoB100 ($r=-0.57$; $P=0.015$) and LDL-apoB100 ($r=-0.47$; $P=0.049$) FCRs, but not with their APRs. Unfortunately, we were not able to detect Lp(a)-apoB100 and Lp(a)-apoE enrichments in isolated Lp(a) particles because of liquid chromatography–tandem mass spectrometry limits of detection. Due to this methodological limitation, we were not able to develop a multicompartamental model to evaluate the kinetics of Lp(a) components (apoB100, apo(a) and apoE).

Parameters Governing Lp(a) Kinetics

Lp(a)-apo(a) concentrations and APR were strongly and negatively correlated with the mean repeats of Kringle IV ($r=-0.64$, $P=0.004$ and $r=-0.71$, $P<0.001$, respectively; Table 3). No significant correlation was found between Lp(a)-apo(a) kinetic parameters and plasma PCSK9 concentrations or VLDL- or LDL-apoB100

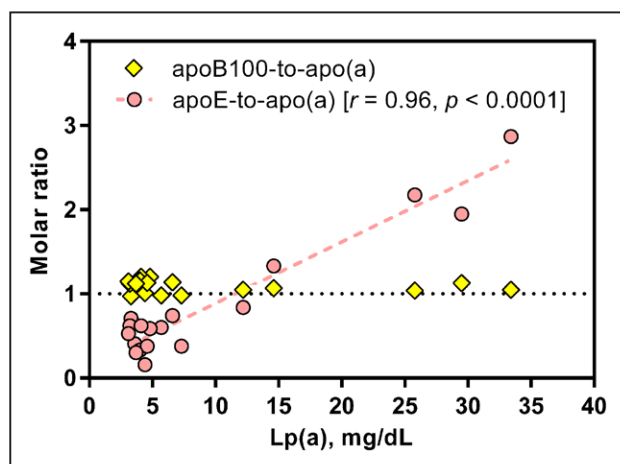


Figure 1. Lp(a) (Lipoprotein [a]) composition.

ApoB100-to-apo(a) and apoE (apolipoprotein E)-to-apo(a) molar ratios in Lp(a), isolated by immunoprecipitation (Spearman test).

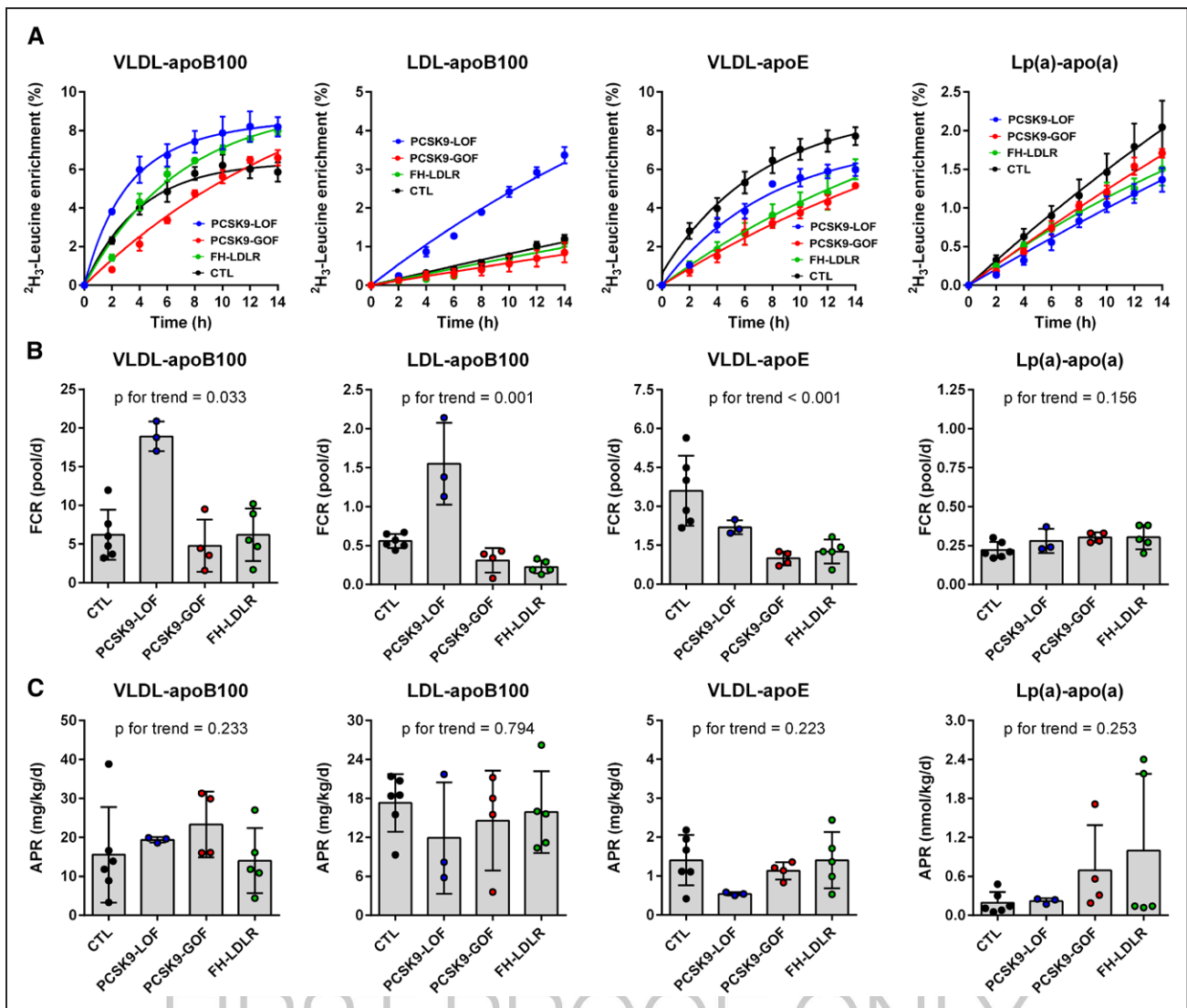


Figure 2. Kinetics of targeted apolipoproteins.

A, Mean±SEM kinetic curves obtained over the course of tracer infusion, **(B)** fractional catabolic rates (FCRs), and **(C)** absolute production rates (APRs) deduced from kinetic curves. *P* for trend values were obtained by Kruskal-Wallis tests. FH indicates familial hypercholesterolemia; GOF, gain-of-function; LOF, loss-of-function; and PCSK9, proprotein convertase subtilisin/kexin type 9.

kinetics. The main finding was the significant correlations observed between Lp(a)-apo(a) and VLDL-apoE kinetics parameters. Total apoE and VLDL apoE concentrations and VLDL-apoE APR correlated positively with Lp(a)-apo(a) concentrations and APR (Table 3) but not with the Lp(a)-apo(a) FCR. The VLDL-apoE FCR correlated with the Lp(a)-apo(a) FCR. No significant correlation was found between the plasma PCSK9 concentration and any concentration or kinetics parameters of apoE. Additional correlations between apoB100, apoE, and apo(a) kinetic parameters are shown in Table III in the [online-only Data Supplement](#). As expected, VLDL-apoE was significantly correlated with plasma TG ($r=0.59$; $P=0.009$) and VLDL-apoB100 ($r=0.73$; $P=0.001$). Only a nonsignificant trend was found for the correlation between VLDL-apoB100 and Lp(a)-apo(a) ($r=0.45$; $P=0.063$).

Modulation of Lp(a), apoE, and PCSK9 With ERN

To further evaluate the association between Lp(a)-apo(a) and VLDL-apoE APRs, we studied the effects of ERN on the lipoprotein kinetics of patients with mild hypertriglyceridemia. As reported previously and summarized in Table IV in the [online-only Data Supplement](#), ERN improved the lipid profile by reducing Lp(a)-apo(a) by 20% ($P=0.008$), apoB100 by 21% ($P=0.008$), TG by 44% ($P=0.023$), and LDL-C by 16% ($P=0.039$). ERN also increased HDL-C by 22% ($P=0.008$). Plasma PCSK9 and VLDL-apoE concentrations were also reduced with ERN by 35% ($P=0.008$) and 42% ($P=0.008$), respectively, whereas no significant change was observed in total apoE concentrations. We previously showed that the ERN-induced reduction of Lp(a)-apo(a) concentrations was related to

Table 3. Associations Between Kinetics of Lp(a)-apo(a) and Other Lipid Parameters

Parameter	Lp(a)-apo(a) Kinetic Parameters		
	Concentration	FCR	APR
Kr-IV repeat	-0.64 (0.004)	ns	-0.71 (<0.001)
PCSK9	ns	ns	ns
ApoE	0.54 (0.020)	ns	0.60 (0.009)
VLDL-apoE	0.57 (0.011)	ns	0.63 (0.005)
VLDL-apoE FCR	Ns	0.57 (0.013)	ns
VLDL-apoE APR	0.48 (0.045)	ns	0.50 (0.035)
ApoB100	Ns	ns	ns
VLDL-apoB100	Ns	ns	ns
VLDL-apoB100 FCR	Ns	ns	ns
VLDL-apoB100 APR	Ns	ns	ns
LDL-apoB100	Ns	ns	ns
LDL-apoB100 FCR	Ns	ns	ns
LDL-apoB100 APR	Ns	ns	ns

Values are presented as r (P) values (Spearman test). apo(a) indicates apolipoprotein (a); Apo, apolipoprotein; APR, absolute production rate; FCR, fractional catabolic rate; Kr-IV, Kringle IV; LDL, low-density lipoprotein; Lp(a), lipoprotein (a); ns, not significant ($P > 0.05$); PCSK9, proprotein convertase subtilisin/kexin type 9; and VLDL, very-low-density lipoprotein.

a balance between a 50% reduction in the Lp(a)-apo(a) APR and a 37% reduction in the Lp(a)-apo(a) FCR. In contrast, we observed that the decrease in VLDL-apoE concentrations was related to the reduction of its APR by 47% ($P=0.008$; Figure 3A). Additional correlations between changes related to ERN are shown in Table V in the [online-only Data Supplement](#) and Figure 3B. Interestingly, changes in the VLDL-apoE APR correlated with changes in the Lp(a)-apo(a) APR ($r=0.83$; $P=0.015$) and plasma PCSK9 concentration ($r=0.79$; $P=0.028$). A nonsignificant trend for a positive correlation between changes in the Lp(a)-apo(a) APR and plasma PCSK9 concentration ($r=0.69$; $P=0.070$) was also noted (Figure 3B). Of note, in isolated Lp(a) particles, the apoE-to-apo(a) molar ratio was still correlated with plasma Lp(a) concentrations ($r=0.82$; $P=0.0132$, Figure I in the [online-only Data Supplement](#)). Again, no significant correlation was found between Lp(a) concentrations and the apoE-to-apoB100 ratios calculated in VLDL/LDL particles.

Multivariate Analysis Findings

To further assess and confirm the determinants of apo(a)/Lp(a) concentrations, we performed multivariate analyses of the lipid and lipoprotein parameters of all studied patients. Principal component analysis clearly identified all groups (Figure 4A) and explained 55% of the variance in apo(a) concentrations. The correlation cluster identified variables associated positively with apo(a), including age, TG, VLDL-apoB100, plasma apoE, and PCSK9, whereas apo(a) Kringle IV repeats were strongly and negatively correlated. Importantly, the

correlation network clearly associated VLDL-apoE concentrations and APR as main determinants of plasma apo(a) concentrations (Figure 4B).

DISCUSSION

We showed that plasma Lp(a) concentrations were slightly increased in patients with FH with *LDLR* or *PCSK9*-GOF mutations compared with healthy subjects and those with *PCSK9* LOF mutations, but no significant effect was observed on the Lp(a)-apo(a) production and catabolic rates. We also showed a reduction in plasma apoE concentration in patients with *PCSK9* LOF mutation, related mainly to a decrease in its APR into VLDL. Interestingly, VLDL-apoE APR was positively correlated with Lp(a)-apo(a) APR. In addition, the specific analysis of Lp(a) particles revealed that the apoE-to-apo(a) molar ratio increased significantly with plasma Lp(a), but not PCSK9, concentration. Under ERN-induced reductions of Lp(a), PCSK9 and VLDL-apoE concentrations, the relative changes in the VLDL-apoE and Lp(a)-apo(a) APRs were strongly correlated, whereas changes in plasma PCSK9 concentration correlated only with changes in VLDL-apoE APR.

This finding is somewhat unexpected as patients with type 2 diabetes mellitus often exhibit elevated TG concentrations, attributed to an overproduction and a reduced clearance of triglyceride-rich lipoproteins,³¹ and reduced Lp(a) concentrations.³² In addition, other studies showed a negative association between Lp(a) and TG plasma levels³³ and between plasma Lp(a) and extra-large, large, and medium-sized VLDL particles.³⁴ Another study reported a delayed catabolism of a portion of Lp(a) in a homozygous apoE-deficient subject, resulting in elevated Lp(a) plasma concentration.³⁵ However, this work aimed to clarify the relationship between PCSK9 and Lp(a) metabolisms, as PCSK9 inhibitors reduce plasma Lp(a) levels.³⁶ To achieve this goal, we included patients with rare *PCSK9* LOF and GOF mutations. The patients with *PCSK9* LOF mutations originated from the same family and carried the same mutation (R104C/V114A). Patients with heterozygous *PCSK9* GOF mutations came from 2 families with different mutations (S127R and R218S) but displayed homogenous kinetic parameters. As reported previously, VLDL- and LDL-apoB100 FCRs were strongly increased in the *PCSK9*-LOF group, and LDL-apoB100 FCR was decreased in the *PCSK9*-GOF group, similarly to those in patients with heterozygous FH-*LDLR* mutations.^{24,25} Despite strong differences in plasma PCSK9 concentrations or LDL-apoB100 FCR, Lp(a)-apo(a) FCR was similar in patients carrying *PCSK9* LOF and GOF mutations. These results challenge the role for PCSK9 in Lp(a) catabolism, as observed previously with PCSK9 inhibitors in human hepatocytes in vitro¹⁴ and in nonhuman primates or healthy volunteers in vivo.^{16,18} However, Lp(a)-apo(a) APR did not correlate

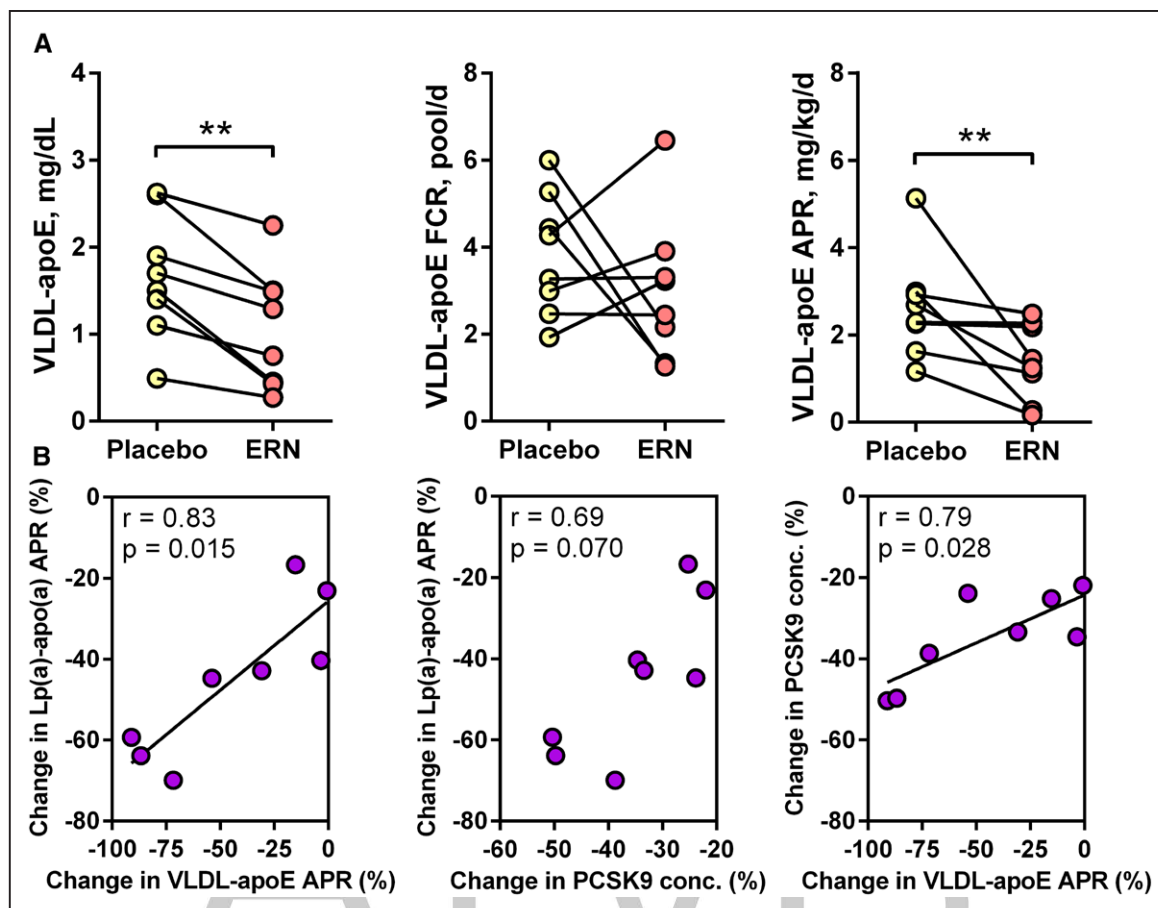


Figure 3. Modulation of VLDL (very-low-density lipoprotein)-apoE (apolipoprotein E) kinetics by extended-release niacin (ERN) treatment and associations with PCSK9 (proprotein convertase subtilisin/kexin type 9), and apo(a) changes.

A, Effect of ERN on VLDL-apoE kinetics (Wilcoxon test). **B**, Spearman correlations between changes in the VLDL-apoE absolute production rate (APR), Lp(a)-apo(a) APR, and PCSK9 concentration.

with plasma PCSK9 concentrations, likely due to the small number of subjects.

Beyond Lp(a) levels, PCSK9 inhibitors also reduce plasma apoE concentrations.^{17,37} In this study, total plasma and VLDL apoE concentrations were lowered in the *PCSK9*-LOF group compared with the *FH-LDLR* and *PCSK9*-GOF groups. Interestingly, the Lp(a)-apo(a) APR was strongly correlated with total plasma and VLDL apoE concentrations and VLDL-apoE APR. As previous studies using PCSK9 inhibitors did not explore apoE kinetics, we gained further insights from additional analyses from an ERN study that previously showed reduction of plasma Lp(a) and PCSK9 concentrations.²⁶ Here, we showed that ERN strongly reduced the VLDL-apoE concentration as a consequence of a sharp reduction in its APR. The reduction in VLDL-apoE production was correlated with that of Lp(a)-apo(a), whereas the interaction with that of the plasma PCSK9 concentration was more puzzling. Therefore, apoE and especially VLDL-apoE may influence Lp(a) production, synthesis, or assembly, but the role of PCSK9 in this phenomenon remains to be determined. It could be related to similar modulations of production rates of VLDL carrying apoE and apo(a),

as suggested by reduced VLDL-apoE and Lp(a)-apo(a) APRs in patients with *PCSK9* LOF mutations. However, the VLDL-apoB100 APR did not differ in these patients compared with the others.

Lp(a) and apoE interactions have not been explored in depth, probably because Lp(a) synthesis and regulation are not clearly understood.³⁶ Whether Lp(a) assembly occurs in hepatocytes, at hepatocyte surfaces, or in the Disse space remains a matter of debate, but several kinetic studies suggested that a large part of Lp(a) assembly takes place outside of hepatocytes.^{38,39} Lp(a) synthesis requires the covalent binding of Kringle IV-9 of apo(a) [C4057] to apoB100 [C4326] by a disulfide bond.³⁰ However, the role of the native lipoprotein composition for final Lp(a) synthesis remains unclear. Proteomic analyses identified numerous proteins associated with Lp(a) beyond apo(a) and apoB100, such as apoA-I, apoC-I, apoC-II, apoC-III, and apoE.⁴⁰ In addition, correlation networks indicated strong interactions between apo(a), apoB100, and apoE.^{40,41} Hence, we can speculate that changes in apoE concentrations in Lp(a) precursors act on apo(a)-apoB100 binding. In support of this hypothesis, epidemiological studies demonstrated

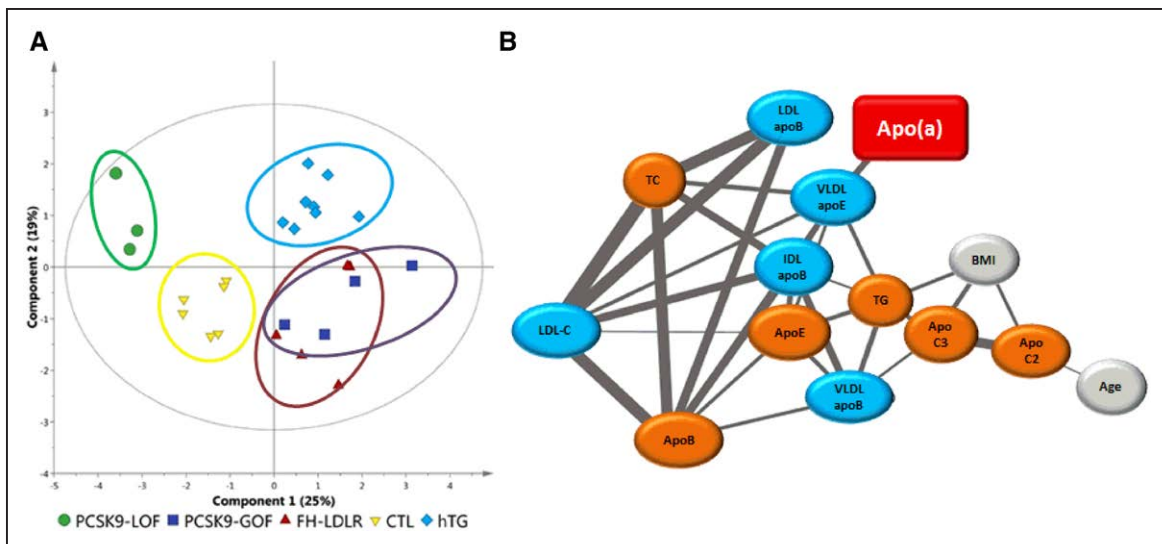


Figure 4. Results of multivariate analyses of lipid and lipoprotein parameters.

A, Comparison of patients' plasma fingerprints by principal component analysis (PCA). **B**, Correlation network obtained for the biochemical parameters. Only positive associations with $r > 0.5$ are presented. ApoE indicates apolipoprotein E; GOF, gain-of-function; and PCSK9, proprotein convertase subtilisin/kexin type 9.



differences in plasma Lp(a) concentrations between apoE genotypes based on the 3 major isoforms of human apoE ($\epsilon 2/\epsilon 3/\epsilon 4$).^{29,30,34,35} We also reported recently that apoE genotypes modulate plasma Lp(a) and apoE concentrations in patients with FH.^{22,30} Here, we showed that total and VLDL apoE concentrations correlated with apo(a) concentrations, irrespective of the apoE genotype, as all patients displayed the E3/E3 phenotype except one (E3/E4). Our observations suggest that apo(a) and therefore Lp(a) are modulated by apoE, with distinct mechanisms involving the apoE genotype and VLDL-apoE concentration.

Interventional studies on plasma Lp(a) levels with different lipid-lowering drugs exhibiting various mechanisms of action (PCSK9 inhibitor,¹⁸ apoB100 antisense oligonucleotide,⁴⁵ CETP inhibitor,^{46,47} ezetimibe,⁴⁸ fibrates,^{48,49} and statins^{37,49–51}) also suggested that plasma Lp(a) reduction is associated with a decrease in the total or VLDL apoE concentration. A decrease in the Lp(a) production rate is the most frequently offered explanation for Lp(a) reduction, irrespective of treatment. In contrast, the mechanisms by which apoE is reduced are more puzzling and involve catabolic or production rates in HDL or VLDL. This uncertainty could be related to the use of different drugs or to the examination of patients with various lipid profiles or apoE genotypes. However, these studies have suggested that apoE concentrations are more critical than apoE kinetics and provide more evidence for the role of apoE in apo(a)-apoB100 binding and modulation. The effect of PCSK9 on Lp(a) could thus be related to its action on apoE metabolism. Changes in VLDL-apoE catabolism through the LDLR and LRP-1 are likely to occur with PCSK9 mutations or inhibitors and could explain the reduction in the plasma

or VLDL apoE concentration and subsequent decrease in the plasma Lp(a) concentration.

This study has some limitations, such as the small numbers of subjects and *PCSK9* mutations related to their low prevalence and to limited access to stable isotope kinetic studies in humans. In addition, only one patient exhibited plasma Lp(a) levels beyond the strong cardiovascular risk threshold of 125 nmol/L (ie, 50 mg/dL). Some methodological aspects have to be also acknowledged. Despite our efforts for isolating Lp(a) by immunoprecipitation, we were not able to detect ²H₃-leucine enrichments for Lp(a)-apoB100 and Lp(a)-apoE, which would be of interest for validating our hypothesis. Another hurdle, unrelated to the MS technology, is the exchange of apoE between lipoproteins and its possible shedding off of the lipoprotein surface by ultracentrifugation, which could lead to biases for the accurate determination of apoE pool sizes in lipoproteins.³⁰ Finally, several protocols can be employed for stable isotope kinetic studies such as fasting versus fed states or bolus versus primed constant infusion of labeled tracer.⁵² The primed constant infusion that we used here presents some disadvantages to estimate kinetics of apolipoproteins with relatively slower turnover rates such as that of Lp(a)-apo(a). Therefore, labeled tracers have tended to be administered as a bolus and the duration of the experimental protocols extended to 96 hours as previously described for Lp(a) kinetics.⁵³ Such differences in experimental protocols lead to contrasted kinetic parameters for Lp(a)-apo(a).

Hence, our study is observational and aimed to generate new hypotheses for Lp(a) metabolism. Our findings need to be investigated further with in vitro or in vivo experiments in patients with elevated Lp(a) plasma

concentrations, and specific interventions involving VLDL-apoE modulation are mandatory to confirm the interaction between apoE and Lp(a) synthesis.

ARTICLE INFORMATION

Received November 17, 2019; accepted January 21, 2020.

Affiliations

From the NUN, INRA, CHU Nantes, UMR 1280, PhAN, IMAD, CRNH-O, France (M. Croyal, K.O., S.B.-C., A.A., M.K.); CRNH-O Mass Spectrometry Core Facility, F-44000 Nantes, France (M. Croyal, K.O., T.M., S.B.-C., A.A., M.K.); Université de La Réunion, INSERM, UMR 1188 Diabète athéromatose Réunion Océan Indien (DéTROI), Plateforme CYROI, Saint-Denis de La Réunion, France (V.B., G.L.); L'institut du thorax, INSERM, CNRS, University of Nantes, France (M. Chétiveaux, C.L.M.); Biogenouest-Corsaire platform, Saint Gilles, France (L.C.); P2R «Production de protéines recombinantes», CRCINA, SFR-Santé, INSERM, CNRS, UNIV Nantes, CHU Nantes, France (K.B.); Pharmacy Department, Nantes University Hospital, France (L.F.); L'institut du thorax, INSERM, CNRS, University of Nantes, CHU Nantes, France (S.H., B.C.); ELSAN, clinique Bretéché, Nantes, France (M.K.); and Nephrology Department, CHU Saint-Pierre, La Réunion, France (E.N.-D.).

Acknowledgments

We thank the staff of the Clinical Investigation Center of the University Hospital in Nantes, especially Dr Yassine Zair and Eliane Hiverneau for their invaluable help with patients and blood collection. We thank Biogenouest, particularly, the Corsaire network of analytical platforms, for their support of this project.

Sources of Funding

This work was funded by an Aspire grant from Pfizer. It was also supported by the French national CHOLEsterol Personalized Innovation (CHOPIN) project, funded by the Agence Nationale de la Recherche (ANR-16-RHUS-0007) and coordinated by the Centre Hospitalo-Universitaire (CHU) of Nantes. V. Blanchard is the recipient of a scholarship from La Région Réunion and the European Union (European Regional Development Fund INTERREG V).

Disclosures

None.

REFERENCES

- Tsimikas S. A test in context: lipoprotein(a): diagnosis, prognosis, controversies, and emerging therapies. *J Am Coll Cardiol*. 2017;69:692–711. doi: 10.1016/j.jacc.2016.11.042
- Tsimikas S, Fazio S, Ferdinand KC, Ginsberg HN, Koschinsky ML, Marcovina SM, Moriarty PM, Rader DJ, Remaley AT, Reyes-Soffer G, et al. NHLBI Working group recommendations to reduce lipoprotein(a)-mediated risk of cardiovascular disease and aortic stenosis. *J Am Coll Cardiol*. 2018;71:177–192. doi: 10.1016/j.jacc.2017.11.014
- Tada H, Kawashiri MA, Yoshida T, Teramoto R, Nohara A, Konno T, Inazu A, Mabuchi H, Yamagishi M, Hayashi K. Lipoprotein(a) in familial hypercholesterolemia with proprotein convertase Subtilisin/Kexin type 9 (PCSK9) Gain-of-Function mutations. *Circ J*. 2016;80:512–518. doi: 10.1253/circj. Cj-15-0999
- Sabatine MS. PCSK9 inhibitors: clinical evidence and implementation. *Nat Rev Cardiol*. 2019;16:155–165. doi: 10.1038/s41569-018-0107-8
- Ray KK, Landmesser U, Leiter LA, Kallend D, Dufour R, Karakas M, Hall T, Troquay RP, Turner T, Visseren FL, et al. Inclisiran in patients at high cardiovascular risk with elevated LDL cholesterol. *N Engl J Med*. 2017;376:1430–1440. doi: 10.1056/NEJMoa1615758
- Raal FJ, Giugliano RP, Sabatine MS, Koren MJ, Blom D, Seidah NG, Honarpour N, Lira A, Xue A, Chiruvolu P, et al. PCSK9 inhibition-mediated reduction in Lp(a) with evolocumab: an analysis of 10 clinical trials and the LDL receptor's role. *J Lipid Res*. 2016;57:1086–1096. doi: 10.1194/jlr.P065334
- Ray KK, Stoekenbroek RM, Kallend D, Leiter LA, Landmesser U, Wright RS, Wijngaard P, Kastelein JJP. Effect of an siRNA therapeutic targeting PCSK9 on atherogenic lipoproteins. *Circulation*. 2018;138:1304–1316. doi: 10.1161/CIRCULATIONAHA.118.034710
- Cao YX, Liu HH, Li S, Li JJ. A meta-analysis of the effect of PCSK9-monoclonal antibodies on circulating lipoprotein (a) levels. *Am J Cardiovasc Drugs*. 2019;19:87–97. doi: 10.1007/s40256-018-0303-2
- Edmiston JB, Brooks N, Tavori H, Minnier J, Duell B, Purnell JO, Kaufman T, Wojcik C, Voros S, Fazio S, et al. Discordant response of low-density lipoprotein cholesterol and lipoprotein(a) levels to monoclonal antibodies targeting proprotein convertase subtilisin/kexin type 9. *J Clin Lipidol*. 2017;11:667–673. doi: 10.1016/j.jacl.2017.03.001
- Enkhaa B, Anuрад E, Zhang W, Yue K, Li CS, Berglund L. The roles of apo(a) size, phenotype, and dominance pattern in PCSK9-inhibition-induced reduction in Lp(a) with alirocumab. *J Lipid Res*. 2017;58:2008–2016. doi: 10.1194/jlr.M078212
- Hopkins PN, Defesche J, Fouchier SW, Bruckert E, Luc G, Cariou B, Sjouke B, Leren TP, Harada-Shiba M, Mabuchi H, et al. Characterization of autosomal dominant hypercholesterolemia caused by PCSK9 gain of function mutations and its specific treatment with alirocumab, a PCSK9 monoclonal antibody. *Circ Cardiovasc Genet*. 2015;8:823–831. doi: 10.1161/CIRCGENETICS.115.001129
- Tsimikas S, Gordts PLSM, Nora C, Yeang C, Witztum JL. Statin therapy increases lipoprotein(a) levels. *Eur Heart J*. 2019. pii: ehz310. doi: 10.1093/eurheartj/ehz310
- Romagnuolo R, Scipione CA, Boffa MB, Marcovina SM, Seidah NG, Koschinsky ML. Lipoprotein(a) catabolism is regulated by proprotein convertase subtilisin/kexin type 9 through the low density lipoprotein receptor. *J Biol Chem*. 2015;290:11649–11662. doi: 10.1074/jbc.M114.611988
- Villard EF, Thedrez A, Blankenstein J, Croyal M, Tran TT, Poirier B, Le Bail JC, Illiano S, Nobécourt E, Krempf M, et al. PCSK9 modulates the secretion but not the cellular uptake of lipoprotein(a) ex vivo: an effect blunted by alirocumab. *JACC Basic Transl Sci*. 2016;1:419–427. doi: 10.1016/j.jacbs.2016.06.006
- Rader DJ, Mann WA, Cain W, Kraft HG, Usher D, Zech LA, Hoeg JM, Davignon J, Lupien P, Grossman M. The low density lipoprotein receptor is not required for normal catabolism of Lp(a) in humans. *J Clin Invest*. 1995;95:1403–1408. doi: 10.1172/JCI117794
- Croyal M, Tran TT, Blanchard RH, Le Bail JC, Villard EF, Poirier B, Aguesse A, Billon-Crossouard S, Ramin-Mangata S, Blanchard V, et al. PCSK9 inhibition with alirocumab reduces lipoprotein(a) levels in nonhuman primates by lowering apolipoprotein(a) production rate. *Clin Sci (Lond)*. 2018;132:1075–1083. doi: 10.1042/CS20180040
- Reyes-Soffer G, Pavlyha M, Ngai C, Thomas T, Holleran S, Ramakrishnan R, Karmaly W, Nandakumar R, Fontanez N, Obunike J, et al. Effects of PCSK9 inhibition with alirocumab on lipoprotein metabolism in healthy humans. *Circulation*. 2017;135:352–362. doi: 10.1161/CIRCULATIONAHA.116.025253
- Watts GF, Chan DC, Somaratne R, Wasserman SM, Scott R, Marcovina SM, Barrett PHR. Controlled study of the effect of proprotein convertase subtilisin-kexin type 9 inhibition with evolocumab on lipoprotein(a) particle kinetics. *Eur Heart J*. 2018;39:2577–2585. doi: 10.1093/eurheartj/ehy122
- Erhart G, Lamina C, Lehtimäki T, Marques-Vidal P, Kähönen M, Vollenweider P, Raitakari OT, Waeber G, Thorand B, Strauch K, et al. Genetic factors explain a major fraction of the 50% lower lipoprotein(a) concentrations in finns. *Arterioscler Thromb Vasc Biol*. 2018;38:1230–1241. doi: 10.1161/ATVBAHA.118.310865
- Moriarty PM, Varvel SA, Gordts PL, McConnell JP, Tsimikas S. Lipoprotein(a) mass levels increase significantly according to APOE genotype: an analysis of 431 239 patients. *Arterioscler Thromb Vasc Biol*. 2017;37:580–588. doi: 10.1161/ATVBAHA.116.308704
- Mack S, Coassin S, Rueedi R, Younsri NA, Seppälä I, Gieger C, Schönherr S, Forer L, Erhart G, Marques-Vidal P, et al.; KORA-Study Group. A genome-wide association meta-analysis on lipoprotein (a) concentrations adjusted for apolipoprotein (a) isoforms. *J Lipid Res*. 2017;58:1834–1844. doi: 10.1194/jlr.M076232
- Blanchard V, Croyal M, Khantalain I, Ramin-Mangata S, Chemello K, Nativel B, Blom DJ, Marais AD, Lambert G. Reduced lipoprotein(a) associated with the apolipoprotein E2 genotype confers cardiovascular protection in familial hypercholesterolemia. *JACC Basic Transl Sci*. 2019;4:425–427. doi: 10.1016/j.jacbs.2019.03.002
- Abifadel M, Varret M, Rabès JP, Allard D, Ouguerram K, Devillers M, Cruaud C, Benjannet S, Wickham L, Erlich D, et al. Mutations in PCSK9 cause autosomal dominant hypercholesterolemia. *Nat Genet*. 2003;34:154–156. doi: 10.1038/ng1161
- Cariou B, Ouguerram K, Zair Y, Guerois R, Langhi C, Kourimate S, Benoit I, Le May C, Gayet C, Belabbas K, et al. PCSK9 dominant negative mutant results in increased LDL catabolic rate and familial hypobetalipoproteinemia. *Arterioscler Thromb Vasc Biol*. 2009;29:2191–2197. doi: 10.1161/ATVBAHA.109.194191
- Ouguerram K, Chetiveaux M, Zair Y, Costet P, Abifadel M, Varret M, Boileau C, Magot T, Krempf M. Apolipoprotein B100 metabolism in autosomal-dominant

- hypercholesterolemia related to mutations in PCSK9. *Arterioscler Thromb Vasc Biol*. 2004;24:1448–1453. doi: 10.1161/01.ATV.0000133684.77013.88
26. Croyal M, Ouguerram K, Passard M, Ferchaud-Roucher V, Chétiveaux M, Billon-Crossouard S, de Gouville AC, Lambert G, Krempf M, Nobécourt E. Effects of Extended-Release nicotinic acid on apolipoprotein (a) kinetics in hypertriglyceridemic patients. *Arterioscler Thromb Vasc Biol*. 2015;35:2042–2047. doi: 10.1161/ATVBAHA.115.305835
 27. Frénais R, Ouguerram K, Maugeais C, Marchini JS, Benlian P, Bard JM, Magot T, Krempf M. Apolipoprotein A-I kinetics in heterozygous familial hypercholesterolemia: a stable isotope study. *J Lipid Res*. 1999;40:1506–1511.
 28. Croyal M, Billon-Crossouard S, Goulitquer S, Aguesse A, León L, Fall F, Chétiveaux M, Moyon T, Blanchard V, Ouguerram K, et al. Stable Isotope Kinetic Study of ApoM (Apolipoprotein M). *Arterioscler Thromb Vasc Biol*. 2018;38:255–261. doi: 10.1161/ATVBAHA.117.310208
 29. Croyal M, Fall F, Ferchaud-Roucher V, Chétiveaux M, Zair Y, Ouguerram K, Krempf M, Nobécourt E. Multiplexed peptide analysis for kinetic measurements of major human apolipoproteins by LC/MS/MS. *J Lipid Res*. 2016;57:509–515. doi: 10.1194/jlr.D064618
 30. Blanchard V, Ramin-Mangata S, Billon-Crossouard S, Aguesse A, Durand M, Chémello K, Nativel B, Flet L, Chétiveaux M, Jacobi D, et al. Kinetics of plasma apolipoprotein E isoforms by LC-MS/MS: a pilot study. *J Lipid Res*. 2018;59:892–900. doi: 10.1194/jlr.P083576
 31. Taskinen MR. Diabetic dyslipidaemia: from basic research to clinical practice. *Diabetologia*. 2003;46:733–749. doi: 10.1007/s00125-003-1111-y
 32. Paige E, Masconi KL, Tsimikas S, Kronenberg F, Santer P, Weger S, Willeit J, Kiechl S, Willeit P. Lipoprotein(a) and incident type-2 diabetes: results from the prospective Bruneck study and a meta-analysis of published literature. *Cardiovasc Diabetol*. 2017;16:38. doi: 10.1186/s12933-017-0520-z
 33. Ko HS, Kim CJ, Ryu WS. Effect of fenofibrate on lipoprotein(a) in hypertriglyceridemic patients: impact of change in triglyceride level and liver function. *J Cardiovasc Pharmacol*. 2005;46:405–411. doi: 10.1097/01.fjc.0000175875.48167.c7
 34. Kettunen J, Demirkan A, Würtz P, Draisma HH, Haller T, Rawal R, Vaarhorst A, Kangas AJ, Lyytikäinen LP, Pirinen M, et al. Genome-wide study for circulating metabolites identifies 62 loci and reveals novel systemic effects of LPA. *Nat Commun*. 2016;7:1122. doi: 10.1038/ncomms11122
 35. Ikwaki K, Cain W, Thomas F, Shamburek R, Zech LA, Usher D, Brewer HB Jr, Rader DJ. Abnormal *in vivo* metabolism of apoB-containing lipoproteins in human apoE deficiency. *J Lipid Res*. 2004;45:1302–1311. doi: 10.1194/jlr.M400020-JLR200
 36. Lambert G, Thedrez A, Croyal M, Ramin-Mangata S, Couret D, Diotel N, Nobécourt-Dupuy E, Krempf M, LeBail JC, Poirier B, et al. The complexity of lipoprotein (a) lowering by PCSK9 monoclonal antibodies. *Clin Sci (Lond)*. 2017;131:261–268. doi: 10.1042/CS20160403
 37. Watts GF, Chan DC, Dent R, Somaratne R, Wasserman SM, Scott R, Burrows S, Barrett PH. Factorial effects of evolocumab and atorvastatin on lipoprotein metabolism. *Circulation*. 2017;135:338–351. doi: 10.1161/CIRCULATIONAHA.116.025080
 38. Hoover-Plow J, Huang M. Lipoprotein(a) metabolism: potential sites for therapeutic targets. *Metabolism*. 2013;62:479–491. doi: 10.1016/j.metabol.2012.07.024
 39. Lamon-Fava S, Diffenderfer MR, Marcovina SM. Lipoprotein(a) metabolism. *Curr Opin Lipidol*. 2014;25:189–193. doi: 10.1097/MOL.0000000000000070
 40. von Zychlinski A, Williams M, McCormick S, Kleffmann T. Absolute quantification of apolipoproteins and associated proteins on human plasma lipoproteins. *J Proteomics*. 2014;106:181–190. doi: 10.1016/j.jprot.2014.04.030
 41. von Zychlinski A, Kleffmann T, Williams MJ, McCormick SP. Proteomics of Lipoprotein(a) identifies a protein complement associated with response to wounding. *J Proteomics*. 2011;74:2881–2891. doi: 10.1016/j.jprot.2011.07.008
 42. Kritharides L, Nordestgaard BG, Tybjaerg-Hansen A, Kamstrup PR, Afzal S. Effect of APOE ε genotype on lipoprotein(a) and the associated risk of myocardial infarction and aortic valve stenosis. *J Clin Endocrinol Metab*. 2017;102:3390–3399. doi: 10.1210/je.2017-01049
 43. de Knijff P, Kaptein A, Boomsma D, Princen HM, Frants RR, Havekes LM. Apolipoprotein E polymorphism affects plasma levels of lipoprotein(a). *Atherosclerosis*. 1991;90:169–174. doi: 10.1016/0021-9150(91)90111-f
 44. Ritter MM, Gewitsch J, Richter WO, Geiss HC, Wildner MW, Schwandt P. Apolipoprotein E polymorphism has no independent effect on plasma levels of lipoprotein(a). *Atherosclerosis*. 1997;131:243–248. doi: 10.1016/s0021-9150(97)06105-4
 45. Nandakumar R, Matveyenko A, Thomas T, Pavlyha M, Ngai C, Holleran S, Ramakrishnan R, Ginsberg HN, Karmally W, Marcovina SM, et al. Effects of mipomersen, an apolipoprotein B100 antisense, on lipoprotein (a) metabolism in healthy subjects. *J Lipid Res*. 2018;59:2397–2402. doi: 10.1194/jlr.P082834
 46. Millar JS, Brousseau ME, Diffenderfer MR, Barrett PH, Welty FK, Cohn JS, Wilson A, Wolfe ML, Nartsupha C, Schaefer PM, et al. Effects of the cholesteryl ester transfer protein inhibitor torcetrapib on VLDL apolipoprotein E metabolism. *J Lipid Res*. 2008;49:543–549. doi: 10.1194/jlr.M700268-JLR200
 47. Millar JS, Lassman ME, Thomas T, Ramakrishnan R, James P, Dunbar RL, deGoma EM, Baer AL, Karmally W, Donovan DS, et al. Effects of CETP inhibition with anacetrapib on metabolism of VLDL-TG and plasma apolipoproteins C-II, C-III, and E. *J Lipid Res*. 2017;58:1214–1220. doi: 10.1194/jlr.M074880
 48. Oikawa S, Yamashita S, Nakaya N, Sasaki J, Kono S; Effect of Fenofibrate and Ezetimibe Combination Treatment on Lipid (EFFECTL) Study Investigators. Efficacy and safety of long-term coadministration of fenofibrate and ezetimibe in patients with combined hyperlipidemia: results of the EFFECTL Study. *J Atheroscler Thromb*. 2017;24:77–94. doi: 10.5551/jat.35626
 49. Ooi EM, Ng TW, Watts GF, Chan DC, Barrett PH. Effect of fenofibrate and atorvastatin on VLDL apoE metabolism in men with the metabolic syndrome. *J Lipid Res*. 2012;53:2443–2449. doi: 10.1194/jlr.P029223
 50. Cohn JS, Tremblay M, Batal R, Jacques H, Veilleux L, Rodriguez C, Barrett PH, Dubreuil D, Roy M, Bernier L, et al. Effect of atorvastatin on plasma apoE metabolism in patients with combined hyperlipidemia. *J Lipid Res*. 2002;43:1464–1471. doi: 10.1194/jlr.m200016-jlr200
 51. Bach-Ngohou K, Ouguerram K, Frénais R, Maugère P, Ripolles-Piquer B, Zair Y, Krempf M, Bard JM. Influence of atorvastatin on apolipoprotein E and AI kinetics in patients with type 2 diabetes. *J Pharmacol Exp Ther*. 2005;315:363–369. doi: 10.1124/jpet.105.085522
 52. Barrett PH, Chan DC, Watts GF. Thematic review series: patient-oriented research. Design and analysis of lipoprotein tracer kinetics studies in humans. *J Lipid Res*. 2006;47:1607–1619. doi: 10.1194/jlr.R600017-JLR200
 53. Ooi EM, Watts GF, Chan DC, Pang J, Tenneti VS, Hamilton SJ, McCormick SP, Marcovina SM, Barrett PH. Effects of extended-release niacin on the postprandial metabolism of Lp(a) and ApoB-100-containing lipoproteins in statin-treated men with type 2 diabetes mellitus. *Arterioscler Thromb Vasc Biol*. 2015;35:2686–2693. doi: 10.1161/ATVBAHA.115.306136