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Lipoprotein(a) carries triglyceride species associated with incident cardiovascular disease

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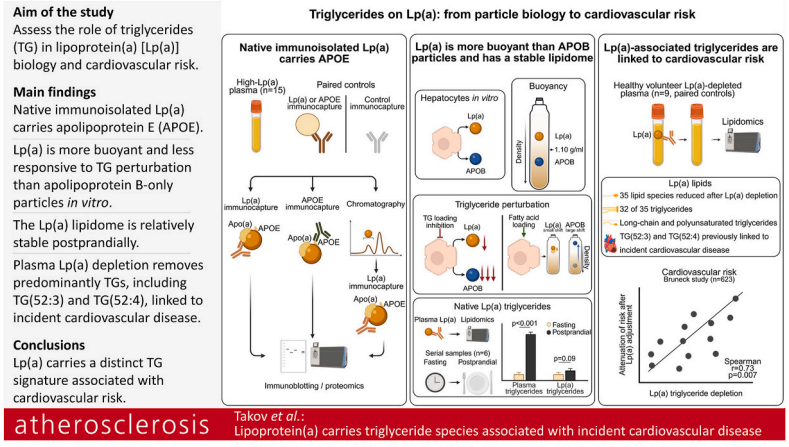
HIGHLIGHTS

- APOE is consistently detected on plasma Lp(a) isolated under mild conditions.
- Lp(a) is more buoyant than APOB-only particles *in vitro*.
- Lp(a) is less responsive to triglyceride perturbation than APOB-only particles *in vitro*.
- Lp(a) lipid cargo remains relatively stable postprandially compared with total plasma lipids.
- Lp(a) carries triglyceride species linked to incident cardiovascular disease.

GRAPHICAL ABSTRACT



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ABSTRACT

Background and aims: Lipoprotein(a) [Lp(a)] is described as a low-density lipoprotein-like particle, but recent work suggests heterogeneity, including triglyceride (TG)-rich features. We investigated whether native Lp(a)

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Apolipoprotein E
Lipidomics
Proteomics
Triglycerides
Residual risk

carries an apolipoprotein E (APOE)-associated TG signature, whether it is remodeled postprandially, and whether Lp(a)-associated TG species are linked to cardiovascular risk.
Methods: Plasma Lp(a) was immunocaptured under native conditions and analyzed by proteomics in patients with high Lp(a) (n = 15) and lipidomics in healthy volunteers (n = 9). Lp(a) produced in HepG2 cells was characterized using immunoprecipitation, density gradient ultracentrifugation, microsomal triglyceride transfer protein inhibition, and fatty acid loading. Matched fasting and postprandial samples assessed Lp(a) lipidome remodeling (n = 6). The relationship between Lp(a)-associated TGs and cardiovascular risk was examined in the community-based Bruneck Study (n = 623).
Results: Direct plasma Lp(a) immunocapture showed APOE enrichment, confirmed by proteomics, immunoblotting, reverse APOE immunocapture, and size-exclusion chromatography followed by Lp(a) immunoprecipitation. *In vitro*, HepG2 cells secreted APOE-containing Lp(a) that was more buoyant than apolipoprotein B (APOB)-only particles and less affected by lomitapide or fatty acid loading. The Lp(a) lipidome was more stable postprandially than plasma lipids. Plasma Lp(a) depletion reduced 35 lipid species, predominantly TGs, including TG(52:3) and TG(52:4), previously linked to incident cardiovascular disease. TG reduction following Lp(a) depletion correlated with attenuation of cardiovascular risk after Lp(a) adjustment in the Bruneck Study, supporting a clinically relevant TG signature of native Lp(a).
Conclusions: Native plasma Lp(a) carries a defined TG signature that is relatively stable postprandially and linked to incident cardiovascular disease.

Nonstandard abbreviations

LC-MS/MS	liquid chromatography-tandem mass spectrometry
IP	immunoprecipitation
IP-MS	immunoprecipitation-mass spectrometry
SEC	size-exclusion chromatography
DGUC	density gradient ultracentrifugation
TG	triglyceride

1. Introduction

Lipoprotein(a) [Lp(a)] is a causal risk factor for atherosclerotic cardiovascular disease (ASCVD), but its distinguishing molecular features and pathophysiology remain incompletely defined [1,2]. Lp(a) is commonly described as a low-density lipoprotein (LDL)-like particle containing apolipoprotein B (APOB) and the characteristic apolipoprotein(a) [Apo(a)]. Yet increasing evidence suggests that Lp(a) is more heterogeneous than this simplified description implies [3]. Apo(a) confers marked size heterogeneity and makes Lp(a) distinct from other APOB-containing lipoproteins [4,5]. The lipid composition of Lp(a) is generally similar to LDL, but important differences are emerging, such as the abundance and functional importance of non-cholesterol lipids [6,7]. Most mechanistic studies to date have focused predominantly on Apo(a) and oxidized phospholipids rather than on broader aspects of the proteome and lipidome of Lp(a) [8–12].

More recently, untargeted lipidomics showed that other lipids on Lp(a) such as diglycerides have proinflammatory effects [6]. Intriguingly, triglycerides (TGs) were shown to be the most significantly enriched lipids in the plasma of individuals with high Lp(a) but their importance for Lp(a) pathophysiology was not investigated [6]. A recent immunocapture assay directly demonstrated the presence of a relatively abundant TG population on Lp(a) [7]. Studies also identified the TG-associated apolipoprotein E (APOE) on Lp(a), especially on TG-rich Lp(a) subpopulations [13,14]. Additionally, levels of another TG-associated apolipoprotein on Lp(a) – apolipoprotein C3 (APOC3) – may serve as a predictor of ASCVD [15]. Together, these observations suggest that Lp(a)-carried TG and TG-associated proteins may have pathophysiological importance.

Several features make Lp(a) difficult to study, including Apo(a) size polymorphism, a restricted species distribution, and lack of convenient model systems [1–3,16]. In addition, standard workflows for purification of Lp(a) often rely on ultracentrifugation with high ionic strength and high speed, which can both disrupt protein–protein and protein–lipid interactions and complicate interpretation of downstream findings [17–21]. This is particularly relevant for exchangeable components, which may be underestimated or redistributed during harsh isolation procedures [17–19].

In the present study, we investigated whether native plasma Lp(a) carries a reproducible APOE-associated TG signature and whether cell-derived Lp(a) shows features consistent with TG-rich lipoprotein assembly. To address this, we combined gentle immunocapture and size-based purification of plasma Lp(a) with mass spectrometry-based proteomics and lipidomics. We then used a hepatoma cell model of Lp(a) secretion to examine its buoyancy, APOE association, and dependence on intracellular TG loading pathways. Finally, we related Lp(a)-associated TGs to incident cardiovascular disease in the prospective, community-based Bruneck Study.

2. Materials and methods

Additional information is provided in the Supplementary material.

2.1. Study cohorts and plasma samples

Plasma samples were obtained from high-Lp(a) patients recruited at the Technische Universität Dresden, Dresden, Germany. Patients were receiving weekly lipid apheresis therapy, provided written informed consent, and were sampled only at pre-apheresis time points under approval of the local Ethics Committee (EK403102014). EDTA plasma from 15 patients was used for direct Lp(a) immunoprecipitation-mass

Table 1
Baseline characteristics for the high-Lp(a) patients. Values are mean (standard deviation) for continuous variables or count (percentage of total) for categorical variables. TC: total cholesterol, LDL-C: low-density lipoprotein cholesterol, HDL-C: high-density lipoprotein cholesterol, TG: triglycerides. SI units of measurement for each continuous variable are shown in the table. Weight-converted concentrations are 141.1 (31.7) mg/dL for TC, 71.5 (29.4) mg/dL for LDL-C, 56.8 (13.9) mg/dL for HDL-C, 123.1 (55.8) mg/dL for TG and 67.3 (32.4) mg/dL for Lp(a). Standard conversion factors were applied: 38.67 for cholesterol, 88.57 for TG and 0.4 for Lp(a).

Proteomics high-Lp(a) patient cohort (n = 15)	
Men (count)	11 (73%)
Women (count)	4 (27%)
Age	59.5 (13.3)
TC (mmol/L)	3.65 (0.82)
LDL-C (mmol/L)	1.85 (0.76)
HDL-C (mmol/L)	1.47 (0.36)
TG (mmol/L)	1.39 (0.63)
Lp(a) (nmol/L)	168.33 (81.08)

spectrometry (IP-MS) with paired control IP, and for orthogonal APOE IP. Baseline characteristics are provided in Table 1. All plasma samples were stored at −70°C, freeze-thaw cycles were minimized, and samples were cleared of debris for 2 min at 500g before downstream analyses. For postprandial lipidomics, paired baseline and 4-h plasma samples from healthy volunteers in the INTERMET study were analyzed (n = 6; test meal: 50 g fat, 85 g carbohydrate, 15 g protein, 850 kcal) [22]. The INTERMET study was registered at ClinicalTrials.gov as NCT03191513, and conducted in accordance with the ethical standards set out in the 1964 Declaration of Helsinki and its later amendments. Total plasma served as the direct matched comparator for immunoisolated Lp(a), allowing assessment of whether Lp(a) remodeling paralleled the overall postprandial plasma lipid response. A colorimetric assay was used to measure TGs in total plasma and chylomicron/VLDL fractions. Lp(a)-depleted plasma lipidomics used nine baseline plasma samples in total, including INTERMET baseline samples (n = 6) and plasma from healthy volunteers (n = 3). The Bruneck Study cohort comprised 623 participants with complete lipidomics, Lp(a), covariate, and outcome data, among whom 83 events occurred during 5356 person-years of follow-up [23].

2.2. Lp(a) IP, size-exclusion chromatography (SEC) and immunoblotting

For Lp(a) or APOE IP, samples were diluted, pre-cleared with naïve Dynabeads, and targets were immunoprecipitated with anti-Apo(a) or control antibodies conjugated to Dynabeads. A second anti-Apo(a) antibody on a different stationary phase (*i.e.* Sepharose) was used for orthogonal validation. High-performance size-exclusion chromatography (SEC) was performed on TSKgel G5000PWXL and G4000PWXL columns connected in series. Lp(a)-rich fractions were pooled, concentrated, and either immunoblotted or subjected to Lp(a) IP. Immunoblotting was performed using antibodies against Apo(a), APOB, APOE, and apolipoprotein A1 (APOA1).

2.3. In vitro Lp(a) secretion model

HepG2 hepatoma cells were engineered to express HiBiT-tagged Apo(a). HiBiT is a split-luciferase peptide tag [24] that enables sensitive luminescence-based quantification of secreted Apo(a)-containing particles [*i.e.* Lp(a)]. Stable cell populations were generated by antibiotic selection and, where indicated, limiting dilution. Assay specificity was confirmed by Apo(a)-directed small interfering RNA (siRNA). Cell-derived Lp(a) was further analyzed by IP, non-ionic density-gradient ultracentrifugation (DGUC), and reductive cleavage with dithiothreitol. Commercial VLDL, LDL, and HDL preparations were used to validate the DGUC workflow. To inhibit intracellular TG loading, cells were treated with lomitapide [25] at the indicated concentrations. The effects of intracellular TG loading on Lp(a) were further studied by treatment with bovine serum albumin (BSA)-conjugated oleate (500 μM) together with the MEK inhibitor U0126 (1 μM) [26].

2.4. MS-based proteomics

Label-free proteomics was performed after tryptic digestion and liquid chromatography-mass spectrometry (LC-MS/MS) on an Orbitrap Fusion Lumos instrument (Thermo Fisher). Proteome Discoverer was used for database searches against a human UniProtKB/Swiss-Prot database with standard contaminant controls, and results were filtered to retain high-confidence proteins.

2.5. MS-based lipidomics

Lipids were extracted using a two-step liquid-liquid chloroform-methanol extraction with spiked-in internal standards (SPLASH LIPIDOMIX, Avanti Polar Lipids), separated by reverse-phase liquid chromatography, and analyzed in positive ion mode on an Orbitrap Exploris 240 instrument (Thermo Fisher). Lipostar2 [27] was used for lipid identification.

2.6. Statistics

Differential expression analyses of proteomics and lipidomics data were performed using the limma package in R. HiBiT assays, immunoblotting, and targeted lipid comparisons were performed with paired t-tests or randomized block ANOVA with Dunnett's post hoc tests where indicated. In the Bruneck Study, Cox proportional hazards models for incident fatal and non-fatal myocardial infarction, ischemic stroke, and sudden cardiac death were adjusted for age, sex, statin use, estimated glomerular filtration rate, high-sensitivity C-reactive protein, alanine transaminase, and diet quality, with additional models further adjusted for Lp(a) categorized as <30, 30–50, or ≥50 mg/dL (molarity-converted: <75, 75-125, or ≥125 nmol/L).

3. Results

3.1. Native plasma Lp(a) reproducibly associates with APOE

To identify proteins enriched on native Lp(a) in an unbiased manner, we first performed direct immunocapture of Lp(a) from high-Lp(a) patient plasma under mild conditions designed to preserve native molecular interactions. To minimize nonspecific carryover, proteins were reported only when they were reproducibly detected in Lp(a) IPs (>1 unique peptide; quantified in at least 13 of 15 samples) and significantly enriched over paired same-aliquot control IPs. This approach efficiently depleted Lp(a) from plasma (Supplementary Fig. 1). In high-Lp(a) patients, Lp(a) IP-MS showed the expected enrichment of Apo(a) and APOB and identified other apolipoproteins and complement/coagulation-related proteins, such as C3, C4A, KNG1, F11, FGA, HRG, and CLU (Fig. 1A, Table 2). These findings are consistent with the wound-healing-related proteome reported by von Zychlinski et al. [28]. Additionally, our label-free nanoLC–MS approach also detected lower-molecular-weight proteins, including neutrophil-associated S100A8 and S100A9 (Table 2). The curated Lp(a)-enriched protein set shown in Table 2 is not an exhaustive catalog of all proteins that may associate with Lp(a) in different biological contexts. Notably, we consistently identified APOE, a TG-rich lipoprotein-associated apolipoprotein, on Lp(a) (Fig. 1A). APOE co-isolation was confirmed using a second anti-Apo(a) antibody, a different stationary phase, and immunoblotting, while reverse APOE pull-down also co-isolated Apo(a) (Fig. 1B).

To further validate that APOE is carried on Lp(a), we used high-performance SEC as an additional purification step before immunocapture. SEC separated Lp(a) from the bulk of APOB- and APOA1-containing particles in plasma (Fig. 1C), and Lp(a) floated at the expected density, suggesting preservation of its integrity (Supplementary Fig. 2A and B). Note that this reflects the fact that most APOB-containing particles in plasma are *bona fide* LDL particles that do not carry Apo(a) and does not indicate a complete absence of APOB in the Lp(a)-rich fraction. Additionally, a proportion of APOE co-eluted with Lp(a) before IP (Fig. 1C). Lp(a) IP-MS of the Lp(a)-rich SEC fractions confirmed consistent APOE detection on Lp(a) (Fig. 1D).

These findings extend prior reports of APOE-positive Lp(a) [13,14,28,29], supporting APOE as a reproducible component of the native Lp(a) proteome rather than an artifact of a single capture workflow.

3.2. In vitro produced Lp(a) carries APOE and is more buoyant than APOB-only particles

To support the plasma findings in a controlled *in vitro* system, HepG2 hepatoma cells were genetically engineered to stably express HiBiT-tagged Apo(a), allowing secreted Apo(a)-containing particles to be quantified in a split-luciferase assay (Fig. 2A and B). Secretome

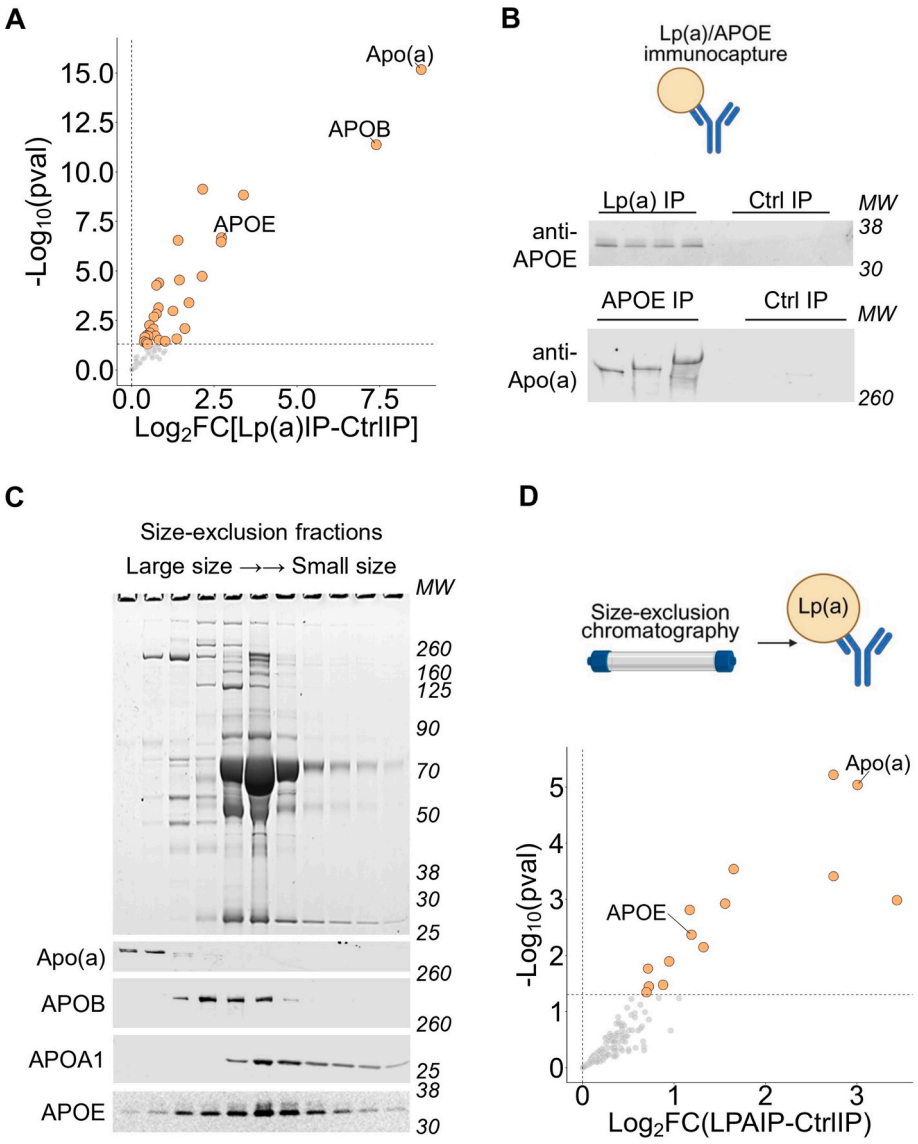

Fig. 1. Native plasma Lp(a) reproducibly associates with APOE. (A) Lp(a) IP-MS in plasma of high-Lp(a) patients (n = 15). Note the enrichment of Apo(a), APOB, and APOE versus paired control IPs (matched plasma pulldowns using isotype control antibodies for each sample). **(B)** Orthogonal validation of APOE association with Lp(a) by Lp(a) IP (n = 4) or APOE IP (n = 3) followed by immunoblotting as indicated. **(C)** SEC of plasma with protein gel and immunoblots for Apo(a), APOB, APOA1, and APOE. Most APOB-containing particles do not carry Apo(a) (i.e. *bona fide* LDL particles), which explains the relative separation of the two signals on immunoblotting. As Western blotting provides a relative readout, APOB appears predominantly in the non-Apo(a)-containing fractions, although a small amount of APOB is still present in the Lp(a)-containing fractions. **(D)** SEC followed by Lp(a) IP-MS confirms that APOE is consistently present on Lp(a) (n = 8). Schematics created in BioRender. Takov, K. (2026) https://BioRender.com/zo8g5o7.

immunoblotting showed clear Lp(a) release in transfected cells without a change in total APOE released compared with vector controls (Fig. 2A). The HiBiT assay performed robustly in secretomes, and signal reduction following Apo(a)-directed siRNA supported its specificity (Fig. 2B). Lp(a) IP from conditioned medium co-isolated APOE, in agreement with the plasma data (Fig. 2C).

To determine whether cell-derived Lp(a) differs from APOB-only particles, secretomes were subjected to non-ionic DGUC calibrated using commercial lipoprotein standards (Supplementary Fig. 2A and B). HepG2 cells are known to secrete dense APOB-containing particles [26], as reflected by a higher density peak than that of commercial LDL particles (peak at ~1.12 g/ml, Fig. 2D, Supplementary Fig. 2A and B). Lp(a) peaked at a lower density than most APOB-only particles, consistent with greater lipid and/or TG content in Lp(a) (Fig. 2D). The density of Lp(a) was similar to the reported range for *in vivo* Lp(a) (peak at ~1.06-1.08 g/ml, Fig. 2D, Supplementary Fig. 2A). Reductive cleavage of Apo(a) away from the lipid core shifted Apo(a) toward higher density (Supplementary Fig. 2C), confirming the integrity of the particle and its similarity to plasma Lp(a). The difference in Lp(a) density compared with APOB-only particles was also reproduced in two independent monoclonal cell populations (Supplementary Fig. 2D).

Together, these data suggest that nascent Lp(a) released by HepG2 cells is distinct from most APOB-only particles and carries features consistent with greater TG enrichment and APOE association.

3.3. *In vitro produced Lp(a) is less responsive to TG perturbation than APOB-only particles*

To test whether intracellular TG loading is required for Lp(a) secretion, cells were treated with lomitapide, an inhibitor of microsomal triglyceride transfer protein. Lomitapide markedly reduced the release of APOB-containing particles in a dose-dependent manner as expected

Table 2
Curated enrichment summary of Lp(a) IP over control IP. Proteins annotated as secreted/extracellular are shown and grouped by function. Log2FC – $\log_2$ fold change in Lp(a) IP versus control IP. Complete proteomics data are available in PRIDE.

Protein	Log_2FC	Mean $\log_2$ abundance	p value
Lipoprotein-/lipid-related proteins			
Apo(a)	8.75	28.09	<0.001
APOB	7.39	28.66	<0.001
APOE	2.73	20.07	<0.001
AZGP1	1.42	20.57	<0.001
APOA2	0.75	20.66	<0.001
CLU	0.45	22.91	0.04
APOA1	0.38	22.53	0.04
Coagulation/complement/wound-healing proteins			
F11	3.39	17.23	<0.001
KNG1	2.16	25.13	<0.001
HRG	2.14	24.91	<0.001
C4A	1.26	18.92	0.001
C3	0.82	19.57	<0.001
FGA	0.67	21.42	0.009
Inflammatory/neutrophil-related proteins			
S100A9	1.62	20.34	0.008
S100A8	1.37	21.70	0.03

(Fig. 3A). Lp(a) release was also lower, but the effect was less pronounced than for APOB (Fig. 3A). This is consistent with earlier work showing that Apo(a) synthesis and secretion are coupled to TG metabolism [25] and suggests differential effects on Lp(a) and APOB-only particles.

To study incorporation of newly synthesized TGs into lipoproteins, cells were loaded with oleate, and the MEK pathway was inhibited to promote secretion of TG-rich APOB particles [26]. APOB-only particles showed a marked shift toward lower density, indicative of TG loading (Fig. 3B). A shift in Lp(a) buoyancy was also apparent, but it was much less pronounced. Thus, while nascent Lp(a) can incorporate newly synthesized TGs, it appeared less responsive to acute TG loading than APOB-only particles released by the same cells.

3.4. The Lp(a) lipidome remains relatively stable during postprandial remodeling

To interrogate the Lp(a) lipidome, immunocaptured plasma Lp(a) from healthy volunteers was subjected to MS-based lipidomics. The lipid classes detected on Lp(a) included cholesteryl esters, TGs, phosphatidylcholines, sphingomyelins, diglycerides, phosphatidylethanolamines, ceramides, lysophosphatidylcholines, glucosylceramides, lysophosphatidylethanolamines, and lactosylceramides. TGs were the second most abundant lipid class after cholesteryl esters, comprising a mean of 27% of total Lp(a) molar lipid content, with a mean molar ratio of TG to cholesterol of 1:3 (Supplementary Fig. 3A). This agrees with early compositional studies identifying cholesteryl esters as the dominant lipid class in Lp(a) [30,31], and extends them by showing that native Lp(a) contains a substantial TG fraction in our immunocapture-based lipidomic analyses.

Plasma TG-rich lipoproteins remodel dynamically after food intake [32]. Prior postprandial studies of Lp(a) mainly examined Lp(a) particle number or broad lipid class responses, rather than generating comprehensive lipidome profiles [14,29,33,34]. To address this, paired fasting and postprandial samples from the INTERMET study [22] were processed together for both total plasma and immunoisolated Lp(a) lipidomics. As expected, the total plasma lipidome showed marked postprandial remodeling, with a pronounced rise in TGs (Fig. 4A; Supplementary Fig. 3B). In contrast, immunoisolated Lp(a) from the

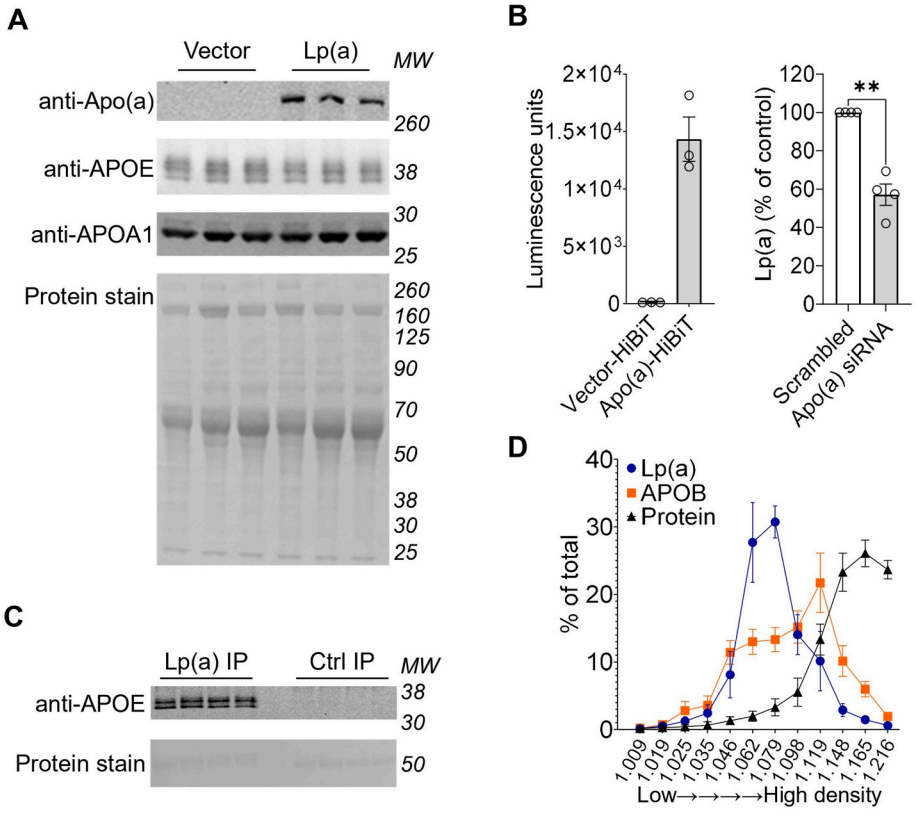

Fig. 2. Hepatoma cell-derived Lp(a) carries APOE and is more buoyant than APOB-only particles. (A) Immunoblotting of secretomes from vector- or Apo(a)-transfected hepatoma cells (n = 3). **(B)** HiBiT-Lp(a) assay validation. Note the robust detection of HiBiT in secretomes of Lp(a)-releasing cells (n = 3) and the reduction in signal by Apo(a)-directed siRNA (n = 4). **p = 0.005; paired, two-tailed t-test. **(C)** Lp(a) IP from hepatoma cell secretomes co-isolates APOE (n = 4). **(D)** DGUC of secretomes (n = 6). Lp(a) quantified by HiBiT assay, APOB by immunoblotting. Note that the Lp(a) peak is at a lower density than the APOB peak.

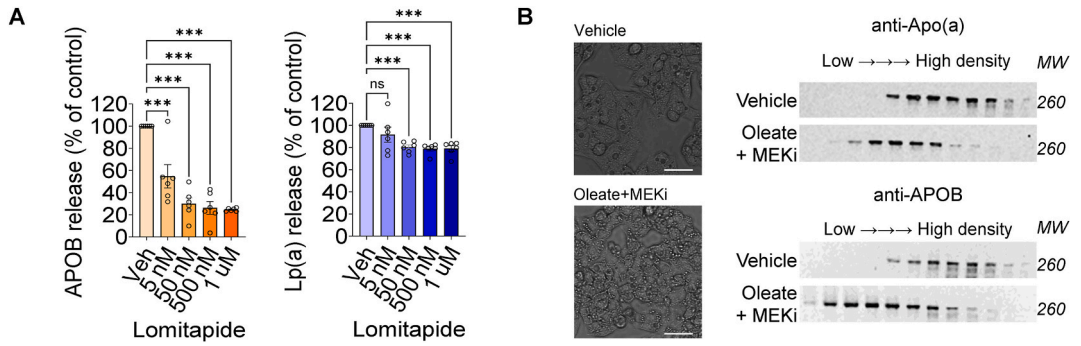

Fig. 3. Lp(a) produced *in vitro* is less responsive to TG perturbation than APOB-only particles. (A) Dose-dependent reduction of APOB (quantified by immunoblotting) or Lp(a) (quantified by HiBiT assay) release by hepatoma cells treated with lomitapide (n = 6). ***p < 0.001, ns: p = 0.14; repeated-measures one-way ANOVA, Dunnett's multiple comparison test. **(B)** Oleate loading with MEK inhibition (MEKi) shifts APOB-only particles toward lower density more profoundly than Lp(a) across density gradients (representative cell images shown on the left; scale bar, 50 μm).

same samples showed a much more stable lipid profile, without a significant TG increase (Fig. 4B). Thus, relative to total plasma lipids, the Lp(a) lipidome is comparatively stable, in keeping with our *in vitro* data.

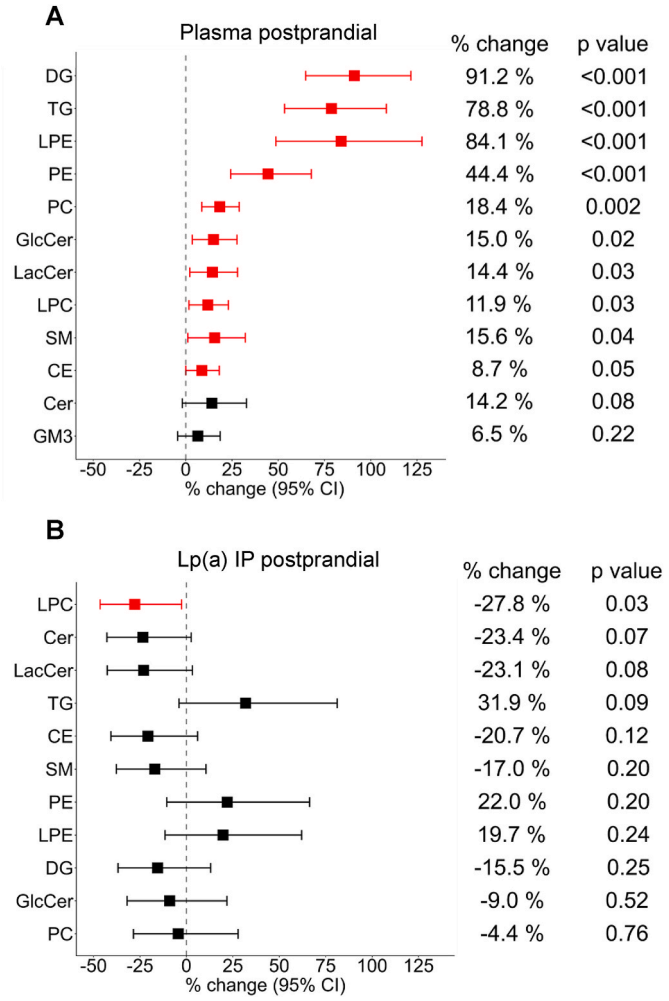

Fig. 4. The Lp(a) lipidome is more stable postprandially than the total plasma lipidome. Lipidomics of plasma **(A)** and paired immunoisolated Lp(a) **(B)** samples from the INTERMET study showing relative postprandial stability of the Lp(a) lipidome compared with plasma (n = 6). CE, cholesteryl esters; Cer, ceramides; DG, diglycerides; GlcCer, glucosylceramides; GM3, ganglioside GM3; LacCer, lactosylceramides; LPC, lysophosphatidylcholines; LPE, lysophosphatidylethanolamines; PC, phosphatidylcholines; PE, phosphatidylethanolamines; SM, sphingomyelins; TG, triglycerides.

Taken together, this suggests that Lp(a)-associated TGs do not simply mirror the overall plasma TG response.

3.5. *Lp(a) carries TG species linked to incident cardiovascular disease*

To determine the fraction of plasma lipids associated with Lp(a), we analyzed Lp(a)-depleted plasma by lipidomics (n = 9; Supplementary Fig. 3C). Lp(a)-depleted plasma was compared with isotype-depleted plasma in paired samples to identify lipids reproducibly removed together with Lp(a), rather than due to nonspecific losses during sample handling. Thirty-five lipid species were significantly reduced after Lp(a) depletion, 32 of which were TGs (Fig. 5A, Table 3, Supplementary Table 1). Among the depleted species were TG(52:3) and TG(52:4), two abundant plasma TG species that were previously associated with incident cardiovascular disease in two prospective cohort studies [23, 35]. Exploratory classification of the Lp(a)-depleted TG species showed enrichment for polyunsaturated TG species and for TGs with 50 or more total acyl carbons (Fig. 5B, Supplementary Table 1; p < 0.001, Fisher's exact test for both). These findings suggest that Lp(a) carries a distinct subset of TGs biased toward common, long-chain, polyunsaturated species.

Finally, using data from the community-based Bruneck Study [23], we calculated hazard ratios for incident cardiovascular disease with and without adjustment for Lp(a) for the 13 overlapping TG species significantly reduced by Lp(a) immunocapture in our study and also measured in the Bruneck Study. The extent of depletion in our data correlated positively with the reduction in hazard ratio after adjustment for Lp(a) (Spearman rho = 0.73, p = 0.007, Fig. 5C). Together, these analyses suggest that Lp(a)-associated TG species may capture risk-related lipid information not reflected by total plasma TG or Lp(a) concentration alone.

4. Discussion

Central strengths of this work are the integration of plasma analyses with a mechanistic secretion model and the use of a gentle immunobased approach to directly capture Lp(a) from plasma. This is particularly important for preserving the native Lp(a) interactome, including more loosely associated exchangeable components and the endogenous lipid cargo of Lp(a). The principal advance of this study is the identification of a reproducible TG signature on native plasma Lp(a) that remains relatively stable postprandially and includes species linked to incident cardiovascular disease. The novelty lies in integrating native-particle preservation, strict contaminant control, molecular lipidomics, postprandial plasma-Lp(a) analysis and prospective cardiovascular-risk data.

We show a strong and reproducible APOE enrichment on immunocaptured native and *in vitro*-produced Lp(a), confirmed by orthogonal

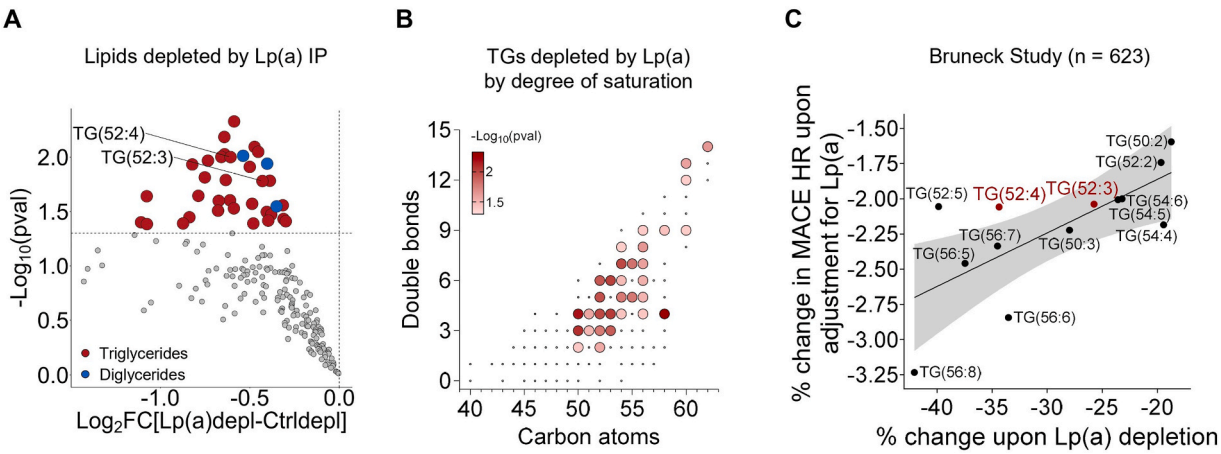

Fig. 5. Lp(a) carries TG species associated with incident cardiovascular disease. (A) Lipid species reduced by Lp(a) depletion of plasma (n = 9). Note the depletion of TG(52:3) and TG(52:4) previously shown to associate with incident cardiovascular disease [23,35]. Control IP: matched plasma pulldowns using isotype control antibodies for each sample. **(B)** TGs depleted by Lp(a) IP by number of acyl carbons and double bonds. Lp(a)-depleted species are shown in larger circles colored according to p values. Note the overrepresentation of polyunsaturated, ≥50 acyl-carbon species ($p < 0.001$, Fisher's exact test). **(C)** Correlation between TG depletion from plasma upon Lp(a) removal and attenuation of hazard ratios (HR) after adjustment for Lp(a) in the community-based Bruneck Study (n = 623). MACE – major adverse cardiovascular events. Note the positive correlation between the extent of TG depletion and the change in MACE HR upon Lp(a) adjustment (Spearman rho = 0.73, 95% confidence interval 0.27-0.91, p = 0.007).

Table 3
Plasma lipids significantly reduced after Lp(a) depletion. Log2FC – $\log_2$ fold change in Lp(a)-depleted versus isotype-depleted plasma. CI – 95% confidence intervals.

Lipid	Log_2FC	CI (lower)	CI (upper)	p value
TG 58:4	−0.59	−0.95	−0.23	0.005
TG 50:4	−0.64	−1.06	−0.23	0.007
TG 50:3	−0.47	−0.79	−0.15	0.008
TG 53:4	−0.45	−0.76	−0.14	0.009
TG 53:6	−0.64	−1.09	−0.20	0.009
DG 18:1_18:2	−0.54	−0.91	−0.16	0.010
TG 54:7	−0.66	−1.12	−0.20	0.010
TG 52:4	−0.61	−1.03	−0.18	0.01
TG 52:5	−0.73	−1.26	−0.21	0.01
DG 16:0_18:2	−0.40	−0.69	−0.11	0.01
TG 52:6	−0.82	−1.41	−0.23	0.01
TG 53:3	−0.50	−0.87	−0.14	0.01
TG 55:7	−0.75	−1.32	−0.18	0.02
TG 55:5	−0.63	−1.12	−0.15	0.02
TG 54:5	−0.39	−0.69	−0.09	0.02
TG 52:3	−0.43	−0.76	−0.10	0.02
TG 56:8	−0.79	−1.44	−0.14	0.02
TG 62:14	−1.08	−1.97	−0.18	0.02
TG 56:7	−0.61	−1.13	−0.09	0.02
TG 60:13	−0.67	−1.24	−0.11	0.03
TG 51:4	−0.49	−0.91	−0.07	0.03
TG 52:2	−0.32	−0.59	−0.04	0.03
DG 18:2_18:2	−0.35	−0.65	−0.05	0.03
TG 56:6	−0.59	−1.11	−0.07	0.03
TG 56:5	−0.68	−1.28	−0.08	0.03
TG 51:3	−0.41	−0.77	−0.04	0.03
TG 54:6	−0.38	−0.73	−0.04	0.03
TG 58:9	−0.84	−1.61	−0.07	0.04
TG 54:4	−0.31	−0.60	−0.02	0.04
TG 56:4	−0.40	−0.77	−0.03	0.04
TG 50:2	−0.30	−0.58	−0.02	0.04
TG 56:9	−1.11	−2.15	−0.06	0.04
TG 60:9	−0.87	−1.70	−0.05	0.04
TG 54:8	−0.48	−0.94	−0.02	0.04
TG 60:12	−1.07	−2.10	−0.05	0.04

immunoprecipitation, reverse APOE pull-down, and SEC coupled to immunoisolation. These data extend earlier reports showing the presence of an APOE-positive Lp(a) subpopulation [13,28,29]. The role of APOE on Lp(a) is not clear but may be linked to the TG cargo of the particle. Previous reports have hypothesized that APOE may be important for Lp(a) clearance due to the association of APOE genetic variants with circulating Lp(a) levels [36,37]. Circumstantial evidence suggests this may be the case, as a buoyant, TG-rich Lp(a) subtype was only found in the circulation of APOE-deficient individuals [38]. Notably, Lp(a) was previously suggested not to be a lipolytic product of other lipoproteins [39]. However, these kinetic studies were performed with exogenously administered traceable particles, which do not exclude the possibility that Lp(a) is produced through the TG-rich lipoprotein route endogenously. Lp(a) interaction with, and internalization through, the VLDL receptor were also documented [40]. Previous studies identified a correlation between Apo(a) and APOE on TG-rich lipoproteins and also suggested that the number of APOE molecules per Lp(a) particle increases with elevated Lp(a) levels [41].

We demonstrated that native plasma Lp(a) contains a higher TG content than previously reported for LDL or Lp(a) [30,42]. The TG:cholesterol molar ratio on Lp(a) of 1:3 observed here is in line with a recent Lp(a) immunocapture assay showing that the TG:cholesterol molecule ratio per Lp(a) particle is 1:2.2 in normotriglyceridemic individuals [7]. This suggests that our observations are not contradictory to prior studies reporting lower TG proportions but may reflect the preservation of native Lp(a) and associated exchangeable components by our gentle isolation strategy. Additionally, this TG finding does not negate the importance of other Lp(a)-carried lipids, such as diglycerides or phospholipids, which we also detect on Lp(a). In fact, Lp(a) pathophysiology has largely been attributed to its oxidized phospholipid cargo and the presence of Apo(a) [8–12]. More recent studies have implicated other lipids, such as diglycerides and lysophosphatidic acid, or lipid modifiers, such as autotaxin, in the proinflammatory effects of Lp(a) [6,43]. In addition to these previously reported mediators, our data point to an additional TG-centered dimension of Lp(a) biology. The enrichment of polyunsaturated and ≥50-carbon TG species among the Lp(a)-depleted lipids adds further context. These species are mostly common plasma TGs, and their preferential depletion suggests that the Lp(a)-associated TG signal represents a biased subset of circulating TGs. One possibility is that this reflects a TG-rich or remnant-like Lp(a) phenotype or selective retention of TG cargo during lipoprotein assembly or remodeling. Our IP is agnostic to the Apo(a) isoform size or Lp(a) subpopulations, so these results do not exclude the possibility that subsets of Lp(a) have different amounts or species of TGs. Since our lipidomics used summed TG carbon and double-bond annotations, chain-resolved analyses will be required to determine the individual

fatty-acyl chains in Lp(a)-associated TGs.

Our findings further support the notion that Lp(a) has distinct features in its assembly and secretion, and a characteristic composition that differentiates it from other APOB-containing particles. Cell-derived Lp(a) floated at a density very similar to its reported *in vivo* plasma density, while most APOB-only particles were lipid-poor and much denser than characteristic VLDL or LDL, as previously reported for hepatoma cultures [26,44]. Lp(a) required intact TG-rich lipoprotein assembly pathways for efficient release, but its buoyancy and secretion were less affected by inhibition of TG loading than those of APOB-only particles. This is consistent with the possibility that Lp(a) is assembled from an APOB-containing precursor that has already entered the TG-rich lipoprotein pathway. This is supported by studies showing intracellular interaction of Apo(a) with APOB, while also suggesting that Lp(a) and VLDL/LDL are produced through distinct pathways [25,45].

TG handling by Lp(a) may also be less dynamic than that of other APOB particles, as seen in our fatty acid loading experiments. *In vitro* cell models do not always capture the full complexity of Lp(a) biology and remodeling in plasma. Nevertheless, the relevance of this model is supported by the consistency of key findings with our postprandial plasma data. Almost all lipid classes, including TGs, increased substantially after a high-fat meal, whereas the Lp(a) lipidome changed much less, with comparatively stable TG levels. Previous studies have reported conflicting data on postprandial changes in Lp(a)-associated lipids, largely at the level of whole lipid classes or particle measures [29,33,34]. By contrast, our lipidomics data show that the Lp(a)-associated TGs are more stable than bulk plasma TGs in matched samples after a defined meal challenge. This relative stability suggests that Lp(a)-associated TGs are less susceptible to rapid postprandial remodeling than TGs on other plasma lipoproteins and may also help explain the poor correlation between total plasma TG and Lp(a) reported previously [46].

Lp(a) capture from plasma reproducibly depleted a discrete set of lipid species, predominantly TGs (32 of the 35 depleted lipid species), including TG(52:3) and TG(52:4), which we previously linked to incident cardiovascular disease [23,35]. They were among the TGs most strongly enriched in the plasma of individuals with high Lp(a) in a previous lipidomics study [6]. TG(52:3) and TG(52:4) were also similarly depleted by Lp(a) capture before and after a meal. Together with the observation of the relative stability of the Lp(a) lipidome, this may be relevant for biomarker development, as specific TGs on Lp(a) may reflect cardiovascular risk even when total plasma TGs fluctuate across different metabolic states. Such biomarkers may refine assessment of residual ASCVD risk associated with Lp(a). This has clinical relevance because Lp(a) is interpreted primarily through particle concentration. A metabolically stable Lp(a)-associated TG signature could help identify patients with qualitatively different Lp(a) particles, refine residual-risk stratification among individuals with elevated Lp(a), or provide a biomarker in studies of Lp(a)-lowering therapies. Such applications will require validation in larger cohorts, but our data support moving beyond total Lp(a) concentration toward particle-resolved omics signatures.

An example of this is the link between particle composition and cardiovascular risk, illustrated by our Lp(a)-depletion data and the Bruneck Study analyses. The correlation between TG species depletion in our study and attenuation of cardiovascular risk in the Bruneck Study after Lp(a) adjustment suggests that a measurable proportion of circulating risk-related TG species may be carried on Lp(a). The enrichment for polyunsaturated TG species may be relevant because polyunsaturated acyl chains are more susceptible to oxidative modification [47,48], potentially providing substrate for generation of oxidized lipids that are implicated in Lp(a) pathophysiology [8,9]. In the context of emerging Lp(a)-lowering drugs [49–51], defining which components of the particle carry clinically-actionable information is increasingly relevant.

This study has several limitations. The sample size is relatively modest, although it is broadly comparable to previous reports [6,28,29,34]. Our study included high-Lp(a) patients rather than a broad population sample. Notably, their basic lipid parameters were not elevated apart from Lp(a) levels (Table 1). A larger sample size and inclusion of reference plasma samples will be required to provide a more comprehensive picture of the Lp(a) lipidome under different metabolic states and across Lp(a) subpopulations. It is important to note that Apo(a) can contain a variable number of Kringle IV Type 2 repeats [4,52], and the samples included in this study were not profiled for Apo(a) isoform size. The antibody used for Lp(a) immunocapture for proteomic and lipidomic analyses recognizes the constant region of Apo(a) and is therefore not expected to introduce systematic isoform bias during Lp(a) isolation. Nevertheless, variation in Kringle IV Type 2 repeat number may influence the Lp(a) protein and lipid interactome, and this remains to be explored in future studies. The postprandial findings relate to one standardized meal and one postprandial time point, whereas meal composition can influence postprandial Lp(a) behavior [34]. Nevertheless, the paired samples and the matched plasma-Lp(a) comparison provide a robust approach. We do not resolve particle/protein-per-particle stoichiometry, which is beyond the scope of our study. Additionally, the mechanisms by which TG species detected on Lp(a) may contribute to cardiovascular risk warrant further investigation, as previously explored in related studies [53]. The Lp(a) isolation workflows provide efficient capture of native particles but do not exclude the possibility of another lipoprotein interacting directly with Lp(a). Our previous MS-based shotgun lipidomics platform used in the Bruneck Study [23] differed from the one used here, resulting in incomplete overlap between the measured TG species.

In summary, our study identifies a reproducible APOE-associated TG signature on native plasma Lp(a) that remains relatively stable during the postprandial phase and includes species linked to cardiovascular risk. This integrated proteomics, lipidomics, and mechanistic framework broadens the view of Lp(a) and highlights Lp(a)-associated TG species as potentially relevant mediators or biomarkers of cardiovascular risk.

Availability of data

The data that support the findings of this study are presented throughout this manuscript or available from the authors upon reasonable request. The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE partner repository with the dataset identifier PXD061363.

Author contributions

Conceptualization: KT, MM. Data curation: KT, SP, RP, BS. Formal analysis: KT, SP, RP. Funding acquisition: MF, MM. Investigation: KT, SP, RP, EE, LEY, LS, BS. Methodology: KT, SP, LS. Resources: SEB, WLH, JW, SK, ST, SRB, MF, MM. Visualization: KT. Writing – original draft: KT. Writing – review and editing: KT, MM.

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Conflict of interest

WLH and SEB have received a grant from Malaysian Health Ministry. SEB provides consultancy and has options in ZOE Ltd. Dr Tsimikas is a co-inventor and receives royalties from patents owned by University of California San Diego (UCSD) and is a co-founder and has an equity interest in Oxitope and Kleanthi Diagnostics and has a dual appointment at UCSD and Ionis Pharmaceuticals. The other authors declare that they have no conflicts of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.atherosclerosis.2026.121866.

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