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The effect of hypolipidaemic treatment on oxidized phospholipids of lipoprotein(a)

Amalia Despoina Koutsogianni
Medical Doctor

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ΣΧΟΛΗ ΕΠΙΣΤΗΜΩΝ ΥΓΕΙΑΣ
ΤΜΗΜΑ ΙΑΤΡΙΚΗΣ
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Β΄ ΠΑΘΟΛΟΓΙΚΗ ΚΛΙΝΙΚΗ

ΔΙΔΑΚΤΟΡΙΚΗ ΔΙΑΤΡΙΒΗ

Η επίδραση της υπολιπιδαιμικής αγωγής στα οξειδωμένα φωσφολιπίδια της λιποπρωτεΐνης(α)

Αμαλία Δέσποινα Κουτσογιάννη
Ιατρός

ΙΩΑΝΝΙΝΑ
2026

«Η έγκριση της διδακτορικής διατριβής από την Ιατρική Σχολή του Πανεπιστημίου Ιωαννίνων δεν υποδηλώνει αποδοχή των γνωμών του συγγραφέα» Ν.5343/32 άρθρο 202, παρ.2 (νομική κατοχύρωση του Ιατρικού τμήματος).

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Η Γραμματέας του Τμήματος

Αικατερίνη Λαγού

«Αφιερωμένο στην οικογένειά μου»

ΠΡΟΛΟΓΟΣ

Η παρούσα διδακτορική διατριβή εκπονήθηκε στο Ιατρείο Μελέτης των Διαταραχών του Μεταβολισμού και των Λιπιδίων του Πανεπιστημιακού Γενικού Νοσοκομείου Ιωαννίνων, σε συνεργασία με το Εργαστήριο Βιοχημείας του Τμήματος Χημείας του Πανεπιστημίου Ιωαννίνων. Η διαδρομή που οδήγησε στην ολοκλήρωση αυτής της διατριβής ήταν μακρά, γεμάτη απαιτήσεις, προκλήσεις, αλλά και στιγμές βαθιάς ικανοποίησης. Θα ήθελα, λοιπόν, να εκφράσω την ευγνωμοσύνη μου σε όσους στάθηκαν δίπλα μου με εμπιστοσύνη, γνώση και ουσιαστική παρουσία.

Πρώτα απ' όλα, οφείλω να ευχαριστήσω τον επιβλέποντά μου, Καθηγητή Παθολογίας κ. Ευάγγελο Λυμπερόπουλο, για την αδιάκοπη καθοδήγηση, τις εύστοχες υποδείξεις του και την εμπιστοσύνη που έδειξε στις δυνατότητές μου από τα πρώτα βήματα αυτής της πορείας. Η σταθερή του παρουσία και η υποστήριξή του αποτέλεσαν για μένα σημείο αναφοράς σε όλη τη διάρκεια της διατριβής.

Ευχαριστώ θερμά τα μέλη της τριμελούς συμβουλευτικής επιτροπής. Τον Καθηγητή Παθολογίας κ. Γεώργιο Λιάμη, για τη διαρκή του διαθεσιμότητα, τις ουσιαστικές του παρατηρήσεις και την ανθρώπινη προσέγγισή του. Τον Καθηγητή Βιοχημείας κ. Αλέξανδρο Τσελέπη, τον οποίο ευχαριστώ από καρδιάς για την ευκαιρία που μου έδωσε να εργαστώ στο εργαστήριό του. Η καθημερινή επαφή με το χώρο και την ομάδα του, σε συνδυασμό με την εμπιστοσύνη και την καθοδήγησή του, σημάδεψαν καθοριστικά τη διαμόρφωση αυτής της δουλειάς. Για όλα αυτά, του είμαι βαθιά ευγνώμων.

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τη συγγραφή της διατριβής. Η συνεργασία τους ήταν ανεκτίμητη και χωρίς αυτήν η ολοκλήρωση της διατριβής δεν θα ήταν εφικτή.

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Ιδιαίτερη ευγνωμοσύνη θα ήθελα επίσης να εκφράσω στον φίλο και συνεργάτη μου από τα πρώτα φοιτητικά χρόνια, κ. Πέτρο Σπυρίδωνα Αδαμίδη. Η σταθερή παρουσία του, η υποστήριξή του και η κοινή μας πορεία όλα αυτά τα χρόνια αποτέλεσαν για μένα πηγή δύναμης. Τον ευχαριστώ ειλικρινά για τη φιλία, τη συνεργασία και την πολύτιμη συμβολή του σε αυτή τη διαδρομή.

Τέλος, η βαθύτερη ευγνωμοσύνη μου ανήκει στους γονείς μου, Βάσω και Τάσο, και στα αδέρφια μου, Δανάη και Δημοσθένη. Για την αγάπη τους, την πίστη τους σε εμένα, τη δύναμη και την αδιάκοπη ηθική και πρακτική υποστήριξη που μου προσφέρουν σε κάθε μου βήμα. Χωρίς τη δική τους παρουσία και στήριξη, η πορεία αυτή δεν θα είχε φτάσει μέχρι το τέλος.

Αμαλία Δέσποινα Κουτσογιάννη

Ιωάννινα, Φεβρουάριος 2026

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anti-MDA-mimotope: Autoantibodies to a Malondialdehyde Mimotope

apo(a): Apolipoprotein(a)

apoB-100: Apolipoprotein B-100

apoB-IC: ApoB-100-Containing Immune Complexes

ASCVD: Atherosclerotic Cardiovascular Disease

ASOs: Antisense Oligonucleotides

ATX: Autotaxin

BMI: Body Mass Index

BP: Blood Pressure

CAVS: Calcific Aortic Valve Stenosis

CE: Cholesterol Esters

CETP: Cholesteryl Ester Transfer Protein

CHD: Coronary Heart Disease

CRP: C-Reactive Protein

CVD: Cardiovascular Disease

DAMPs: Damage-Associated Molecular Patterns

DLCN: Dutch Lipid Clinic Network

dsRNA: Double-Stranded RNA

ELISA: Enzyme-Linked Immunosorbent Assay

FH: Familial Hypercholesterolemia

GHD: Growth Hormone Deficiency

HbA1c: Glycosylated hemoglobin

HDL: High-Density Lipoprotein

HDL-C: High-Density Lipoprotein Cholesterol

HeFH: Heterozygous Familial Hypercholesterolemia

HOMA-IR: Homeostatic Model Assessment for Insulin Resistance

HR: Hazard Ratio

IDL: Intermediate-Density Lipoprotein

IL: Interleukin

IQR: Interquartile Range

K: Kringle

LA: Lipoprotein Apheresis

LBSs: Lysine-Binding Sites

LC- MS/MS: Liquid Chromatography–Tandem Mass Spectrometry

LDL: Low-Density Lipoprotein Cholesterol

LDL-C: Low-Density Lipoprotein Cholesterol

LDL-Ccorr: Corrected Low-Density Lipoprotein Cholesterol

LDLR: Low-Density Lipoprotein Receptor

LLT: Lipid-Lowering Treatment

Lp(a): Lipoprotein(a)

Lp-PLA2: Lipoprotein-Associated Phospholipase A2

Lyso-PA: Lysophosphatidic Acid

Lyso-PC: Lysophosphatidylcholine

MACEs: Major Adverse Cardiovascular Events

MANCOVA: Multivariate Analysis of Covariance

MASH: Metabolic Dysfunction-Associated Steatohepatitis

MCP-1: Monocyte Chemoattractant Protein-1

MDA: Malondialdehyde

MDA-apoB: MDA-Modified ApoB-100

MDA-LDL: Malondialdehyde-Modified LDL

MMPs: Matrix Metalloproteinases

MTP: Microsomal Triglyceride Transfer Protein

Non-HDL-C: Non-high-density lipoprotein cholesterol

NPC1L1: Niemann–Pick C1-Like 1

one-way ANOVA: One-Way Analysis of Variance

OxFFAs: Oxidized Non-Esterified Fatty Acids

OxLDL: Oxidized LDL

OxPL-apo(a): OxPLs of Lp(a)

OxPL-apoB: OxPLs of ApoB-100-Containing Lipoproteins

OxPLs: Oxidized Phospholipids

PAI-1: Plasminogen Activator Inhibitor-1

PAPC: 1-Palmitoyl-2-Arachidonoyl-sn-Glycero-3-Phosphocholine

PC: Phosphocholine

PCSK9: Proprotein Convertase Subtilisin/Kexin Type 9

PECPC: 1-Palmitoyl-2-(5,6-Epoxycyclopentenone)-sn-Glycero-3-Phosphorylcholine

PEIPC: 1-Palmitoyl-2-(5,6-Epoxyisoprostane E2)-sn-Glycero-3-Phosphocholine

PGPC: 1-Palmitoyl-2-Glutaroyl-sn-Glycero-3-Phosphocholine

PLD: Phospholipase D

PLG: Plasminogen

POVPC: 1-Palmitoyl-2-(5-Oxovaleroyl)-sn-Glycero-3-Phosphocholine

RISK: RNA-Induced Silencing Complex

ROS: Reactive Oxygen Species

SCORE 2: Systematic Coronary Risk Evaluation 2

SCORE 2 OP: Systematic Coronary Risk Evaluation 2 for Older Persons

SD: Standard Deviation

SERMs: Selective Estrogen Receptor Modulators

siRNA: Small Interfering RNA

SNPs: Single Nucleotide Polymorphisms

SRB1: Scavenger Receptor Class B Member 1

TC: Total Cholesterol

TF: Tissue Factor

TFPI: Tissue Factor Pathway Inhibitor

TGs: Triglycerides

tPA: Tissue-Type Plasminogen Activator

VLDL: Very Low-Density Lipoprotein

CHAPTER 1. LIPOPROTEIN(a)

1.1 INTRODUCTION

Lipoprotein(a) [Lp(a)] was first discovered by the Norwegian physician Kare Berg almost 6 decades ago [1]. Lp(a) has a complex structure that combines a low-density lipoprotein (LDL)-like particle and apolipoprotein(a) [apo(a)], a glycoprotein of variable size. Apo(a) shares a high degree of sequence homology with plasminogen (PLG) (75-94%) but lacks fibrinolytic activity [2]. The structure of Lp(a) led to the suggestion of a possible link between high circulating concentrations of Lp(a) and atherothrombotic risk. After many years of debate, Mendelian randomization studies established a causal role for elevated Lp(a) concentrations in atherosclerotic cardiovascular disease (ASCVD) and, specifically, in coronary heart disease (CHD) and stroke [3]. Lp(a) mediates atherogenesis through mechanisms linked to its LDL and apo(a) components and associated oxidized phospholipids (OxPLs), of which Lp(a) is their major lipoprotein carrier [4]. Elevated Lp(a) plasma concentrations predict the presence and progression of CHD [5]. Lp(a) levels increase following acute coronary syndrome and percutaneous coronary intervention because Lp(a) behaves like a positive acute-phase reactant, increasing in response to inflammation and tissue injury [6]. High Lp(a) levels predict death, myocardial infarction, stroke and need for revascularization in the general population [7, 8]. They also correlate with an amplified risk for peripheral artery disease, abdominal aortic aneurysms and major adverse limb events [9]. Moreover, when coexisting with thrombotic factors, such as fibrinogen, d-dimer, plasmin-antiplasmin complex and factor VIII, elevated Lp(a) levels are associated with even higher risk of ASCVD events [10]. During the COVID-19 pandemic, elevated Lp(a) levels did not seem to increase susceptibility to SARS-CoV-2 infections nor to thromboembolic events, however, SARS-CoV-2 infection may have enforced the association between elevated Lp(a) levels and ischemic heart disease [11].

1.2 STRUCTURE AND METABOLISM OF Lp(a)

The Lp(a) particle consists of an LDL particle with an apo(a) moiety covalently bound to its apolipoprotein B-100 (apoB-100) component [12-15] (Figure 1). Lp(a) exerts pro-inflammatory, pro-thrombotic and pro-atherogenic properties and is an independent cardiovascular risk factor [8, 15, 16]. Lp(a) synthesis occurs almost exclusively in the liver. Approximately 90% of circulating Lp(a) levels are inherited and strongly determined by a single gene, the LPA gene [17]. The LPA gene is evolutionarily derived from and is highly

homologous to the PLG gene [18, 19]. The PLG gene encodes 5 unique kringle (K) domains and an active protease domain that is activated to plasmin by tissue-type plasminogen activator (tPA). The LPA gene, and therefore apo(a), does not contain K I-III, but does contain KIV, KV and a protease domain. This protease domain is catalytically inactive and, thus, cannot be converted to a plasmin-like molecule because of amino acid substitutions at the site of cleavage of PLG activators [20]. Furthermore, because of multiple duplication events during evolution, the LPA gene has accumulated 10 copies of KIV that are each unique in amino acid sequence except for KIV type 2 (KIV2). KIV contains 1 copy of KIV type 1 (KIV1) and KIV types 3-10 (KIV3-10), but variable copies of KIV2, ranging from 1 to >40 on each allele. KIV2 repeats may show variations at the DNA level between copies, but these variations do not change the resulting amino acid sequence; therefore, all KIV2-encoded protein domains are identical [20]. Consequently, the different repeats of KIV2 in apo(a) account for the various size polymorphisms of this apolipoprotein.

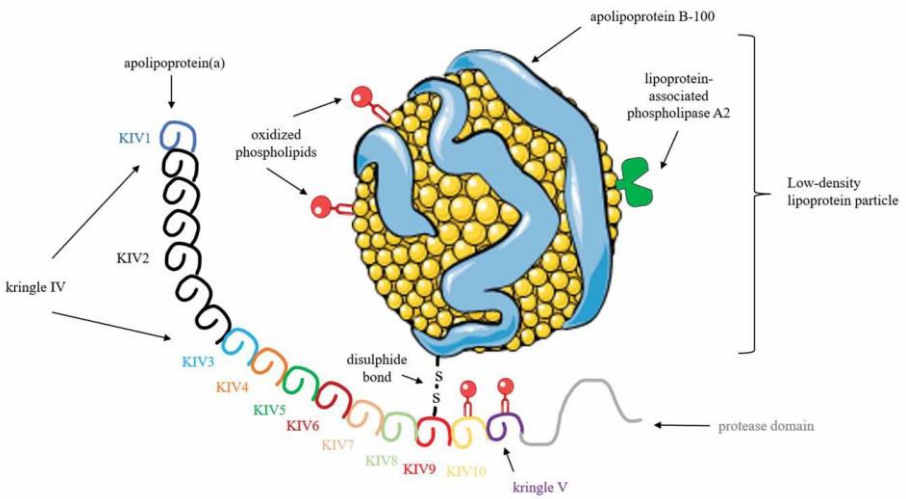

Figure 1. Structure of lipoprotein(a). Lipoprotein(a) [Lp(a)] consists of a low-density lipoprotein-like particle, in which the apolipoprotein B-100 is linked by a single disulphide bond to a glycoprotein, known as apolipoprotein(a). Apolipoprotein(a) contains 10 copies of kringle IV (KIV), KV and a catalytically inactive protease domain. KIV contains 1 copy of KIV1 and KIV3-10, but variable copies of KIV2, ranging from 1 to >40 on each allele. Up to 90% of all oxidized phospholipids found in human lipoproteins are carried on Lp(a) and subjected to degradation by lipoprotein-associated phospholipase A2. Adopted from Koutsogianni et al., [21].

Besides from the apo(a) isoform size, however, other genetic variants have an impact on Lp(a) levels for a given isoform. Functional single nucleotide polymorphisms (SNPs) within the LPA KIV2-encoding region (+4733G>A, +4925G>A, R21X, rs41272114, rs3798220) cooperate in determining Lp(a) variance and induce low Lp(a) concentrations [22-25]. This moderate but lifelong genetic Lp(a) reduction translates to noticeable CHD risk reduction,

[22, 24, 25]. KIV type 9 (KIV9) contains an unpaired cysteine residue, which is attached by a disulfide bridge to a cysteine residue of apoB-100, located near the binding site of LDL to its receptor [26]. Specific functionalities relevant to the assembly and molecular pathology of Lp(a) are attributable to lysine-binding sites (LBSs), which are present in some of the KIV domains of apo(a). The KIV type 10 (KIV10) domain in apo(a) has a LSB of comparable affinity to that in PLG KIV, while the KIV types 5-8 (KIV5-8) each contain a weak LBS [4]. The strong LBS in KIV10 is thought to mediate binding of apo(a)-Lp(a) to fibrin, cell surface receptors and extracellular matrix proteins [4]. On the other hand, those in KIV5-KIV8 participate in the non-covalent interactions with apoB-100 that precede disulfide bond formation [27, 28]. Importantly, KIV10 also contains the site to which a proinflammatory OxPL is covalently attached [4].

Due to the high structural heterogenicity of Lp(a), it remains a challenge to standardize a biochemical quantification for clinical practice. Since now, none of the available commercial assays for Lp(a) quantification is completely isoform insensitive. The only method (gold standard) that is independent of apo(a) size polymorphism uses a monoclonal antibody that does not interact with epitopes in KIV2 but does interact with an epitope in KIV9. Antibodies binding to epitopes in the KIV2 region will generate multiple antibody-antigen complexes dependent on the size of apo(a). However, this ELISA method may be affected by protein conformational diversity [29]. The use of Liquid Chromatography - Tandem Mass Spectrometry (LC- MS/MS) analysis seems to be independent of apo(a) size polymorphism by targeting specific quantification peptides not present in the KIV2 region. Indeed, the results obtained by this method are in excellent agreement with those of the current gold standard ELISA [29]. Despite all the attempts to harmonize commercial assays, the conversion between mg/dL of Lp(a) mass and nmol/L of apo(a) is not accurate and should be avoided in clinical practice [29].

Plasma Lp(a) levels vary widely among individuals and are largely determined by their apo(a) size [20]. There is an inverse relationship between the number of KIV2 repeats of apo(a) and the level of Lp(a) in plasma reflecting apo(a) isoform–dependent biosynthetic efficiency: large apo(a) isoforms (high KIV2 copy number) are less efficiently processed and secreted (more intracellular retention/degradation), leading to lower Lp(a) production rates, whereas small isoforms are secreted more efficiently and yield higher plasma Lp(a) [30]. Unlike other traditional lipoproteins, lifestyle changes have little impact on Lp(a) levels [16, 31]. Lp(a) concentrations may vary with ethnicity and gender [32, 33] (Figure 2). Furthermore, Lp(a) levels reach near-adult concentrations by ~5 years of age and remain relatively stable

throughout life except in states of major liver, renal, or inflammatory disease [20, 30]. Thus, Lp(a) levels need only be measured once, unless a secondary cause is suspected or specific treatment is instituted [33].

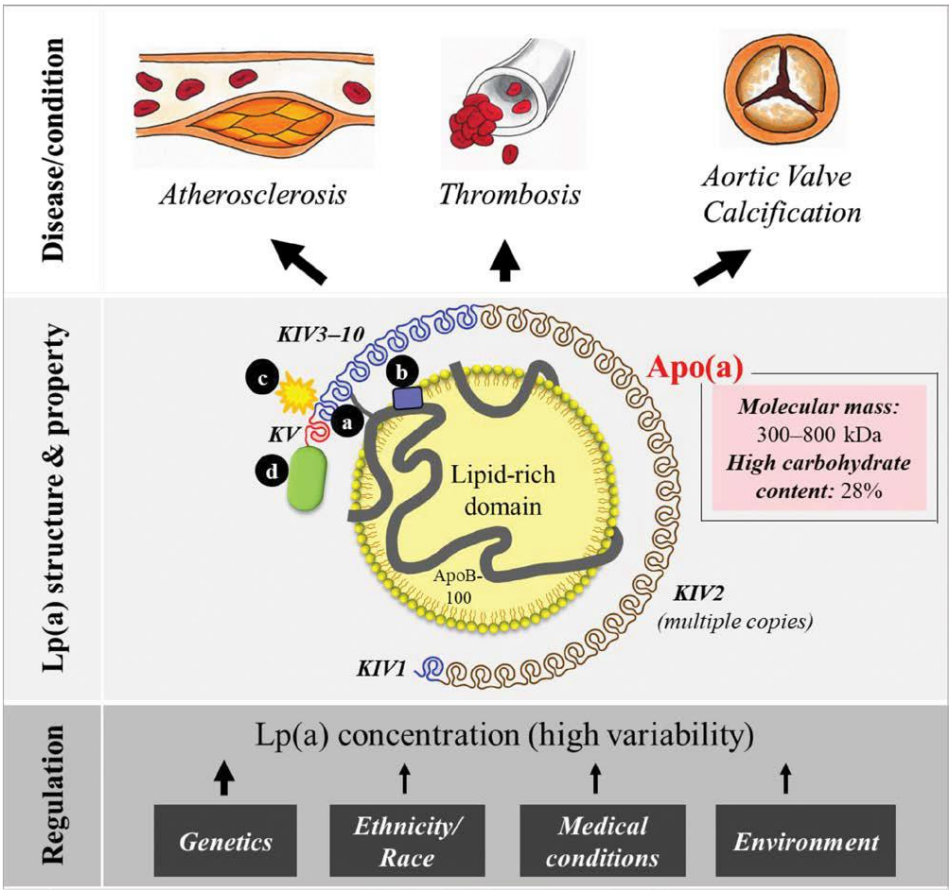

Figure 2. Factors affecting variation in lipoprotein(a) concentration. The lipoprotein(a) concentration is heterogeneous and, to a major extent, controlled by genetics, inversely related to the copy number variation in the LPA gene. Other factors such as ethnicity and race as well as medical and environmental conditions also play a role in lipoprotein(a) regulation. Modified from Reyes-Soffer et al., [30].

Unfortunately, little is known about the dominant sites and processes accountable for the removal of Lp(a) from circulation. Scientists debate between liver and kidneys as the dominant clearance site (Figure 3). Spleen and muscles may also play a modest role in the clearance process [17]. Multiple receptors for Lp(a) have been identified, with the best evidence available for the LDL receptor (LDLR) [34, 35], various PLG receptors [36] and scavenger receptor class B member 1 (SRB1) [37].

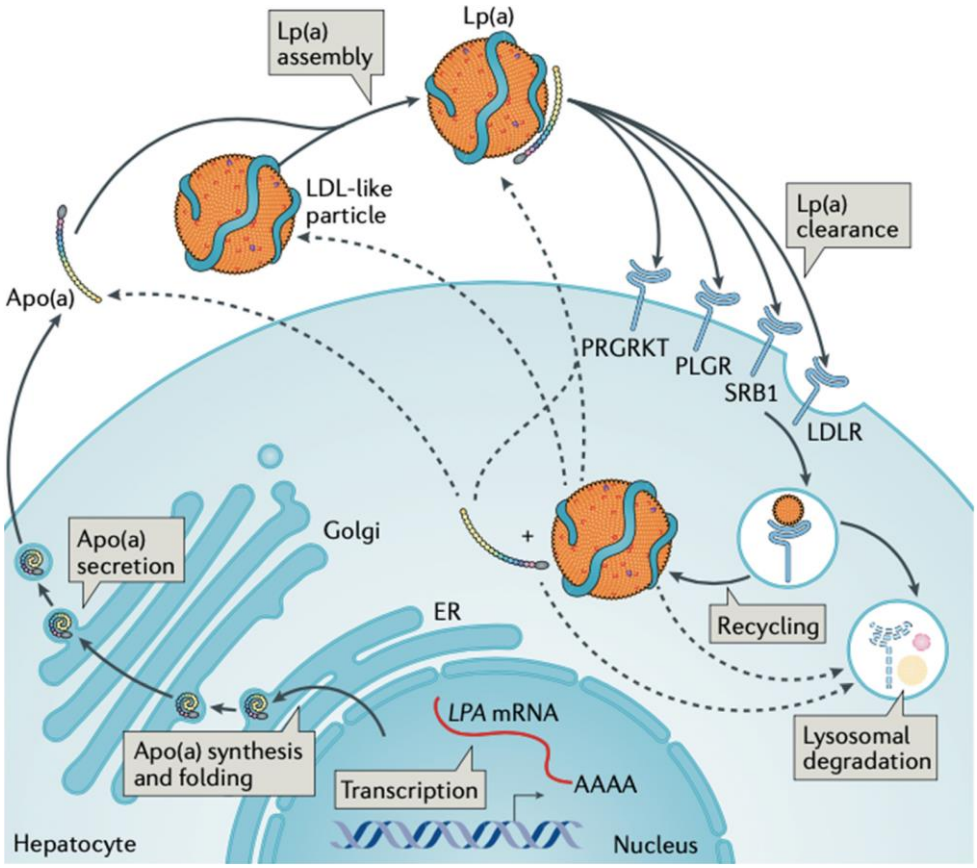

Figure 3. Regulation of lipoprotein(a) biosynthesis and catabolism. Circulating lipoprotein(a) is predominantly of liver origin. Apolipoprotein(a) production is regulated at the levels of transcription and protein folding and processing in the endoplasmic reticulum (ER) and possibly other compartments of the secretory system, including the Golgi apparatus and endosomal compartments. Lipoprotein(a) assembly occurs either intracellularly or on the cell surface. Lipoprotein(a) holoparticle clearance seems to be mediated by different putative classes of receptors. LDLR, low-density lipoprotein receptor; PLGR, plasminogen receptors; SRB1, scavenger receptor class B member 1. Modified from Boffa & Koschinsky, [38].

Risk from high Lp(a) increases slightly at levels of 30 mg/dL (62 nmol/L) to 50 mg/dL (105 nmol/L) and becomes clinically relevant above 50 mg/dL (105 nmol/L), with higher levels associated with a greater cardiovascular risk [39]. The 2025 Focused Update of the 2019 ESC/EAS Guidelines for the management of dyslipidaemias recommends that Lp(a) levels above 50 mg/dL (105 nmol/L) should be considered in all adults as a cardiovascular risk-enhancing factor, with higher Lp(a) levels associated with greater in risk (Class of recommendation: IIa, Level of Evidence of: B) [39] (Figure 4). Therefore, Lp(a) measurement should be considered at least once in every adult's lifetime [39], either at the first lipid profile or at the next one if lipid profiles have previously been performed. Screening is particularly relevant in younger patients with familial hypercholesterolemia (FH) or premature ASCVD and no other identifiable risk factors, or a family history of premature ASCVD or high Lp(a) levels, or in individuals at moderate risk or around treatment decision thresholds to improve risk classification.

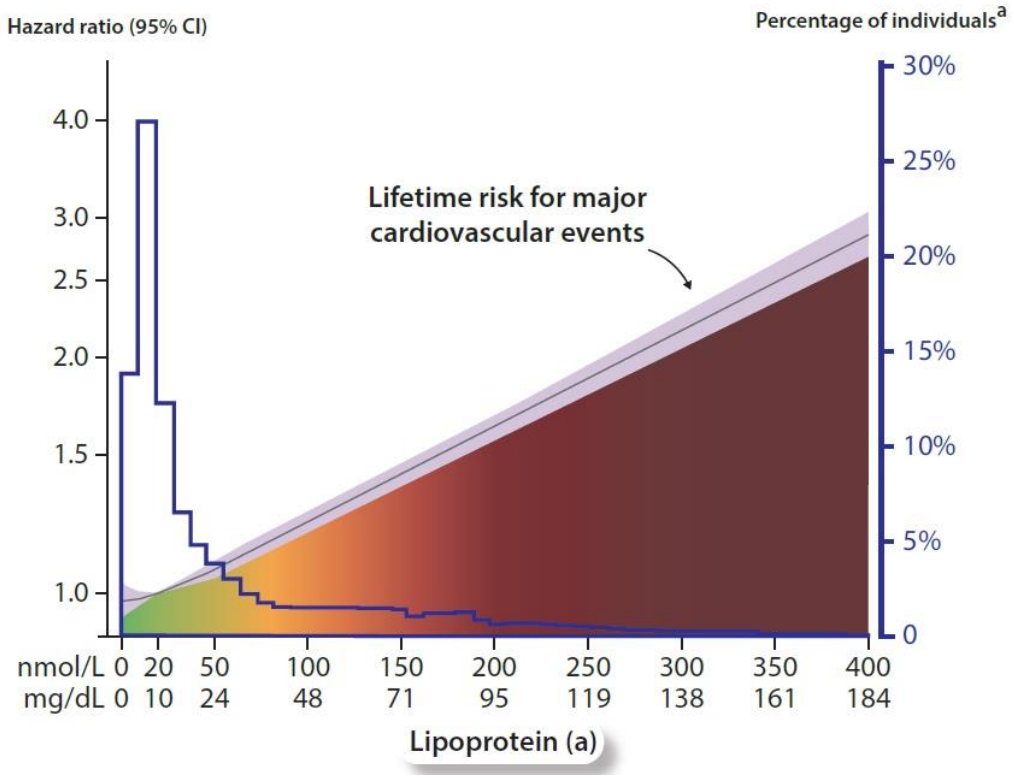

Figure 4. Association between lipoprotein(a) levels and lifetime risk of major cardiovascular events. CI, confidence interval; Lp(a), lipoprotein (a); CV, cardiovascular. The risk of major CV events starts to slightly increase in individuals with Lp(a) levels >30 mg/dL, and the increase in risk becomes more pronounced in individuals with Lp(a) levels ≥50 mg/dL. The grey lines indicate the smoothed adjusted hazard ratio and 95% confidence interval for lifetime risk for major CV events for a given Lp(a) concentration relative to the median Lp(a) in the population. Modified from Mach et al., [39].

Lp(a) levels may increase after menopause and a second measurement is reasonable, particularly if the pre-menopausal levels were borderline [33, 40]. On the contrary, low levels of Lp(a) could be associated with increased risk of diabetes or bleeding [41]. One hypothesis is that the increased risk for diabetes results from the harmful trapping of large apo(a) isoforms in hepatocytes or other cells, and thus not from the low levels per se [41]. This intracellular accumulation may impair cellular function or metabolic signaling, thereby contributing to glucose dysregulation and increased diabetes risk [41]. However, it remains possible that patients with low Lp(a) levels could have increased risk for diabetes because of some unidentified genetic or acquired predisposing cofactor that acts synergistically with low Lp(a) [41]. Supporting this idea, the risk of type 2 diabetes is higher in homozygous carriers of mutations that cause Lp(a) deficiency as well as in individuals with low genetically imputed Lp(a), independent of body mass index (BMI) [42, 43].

1.3 PHYSIOLOGICAL FUNCTIONS OF Lp(a)

The physiological role of Lp(a) in humans is still not fully elucidated. Individuals with extremely low levels of plasma Lp(a) present with no disease or deficiency syndromes [44]. In a large, contemporary, general population cohort low levels of Lp(a) and corresponding LPA genotypes were not associated with any major disease groups, including cancer/cancer subtypes [45] and infections [46]; however, very low Lp(a) levels have been associated with increased risk of type 2 diabetes [42, 47, 48]. Observationally and genetically low levels of Lp(a) are associated with decreased risk of myocardial infarction, aortic valve stenosis, and ischemic stroke [41]. In addition, evidence shows that Lp(a) plays a significant role in inhibiting angiogenesis and tumor growth [45]. Lp(a) due to its homology with PLG may function as an anti-neoplasmic protein. It may decrease the activation of proteases, which are mandatory for the activation of matrix metalloproteinases (MMPs) and the subsequent activation of angiogenesis [44]. Moreover, early investigators have purported a positive role of Lp(a) in wound healing and tissue repair, by limiting bleeding at sites of injury and delivering cholesterol for cell replenishment [44, 49]. Indeed, Lp(a) accumulates in endothelial injuries, binds to several components of the vessel wall and sub-endothelial matrix, stimulates chemotactic activation of monocytes/macrophages and modulates angiogenesis. Contemporary human data do not support, however, such a role. In a recent clinical cohort, higher Lp(a) independently predicted slower wound healing after endovascular therapy for chronic limb-threatening ischemia, suggesting that elevated Lp(a) may impair rather than facilitate tissue repair [50]. Any physiologic “benefit” remains unproven and possible context-dependent [14]. In addition, a meta-analysis including 4 prospective cohorts (n = 74,575 subjects), the risk of incident type 2 diabetes was significantly higher in the first two quintiles of Lp(a) concentration -mean Lp(a) 7.5 nmol/L (≈ 3 mg/dL) and 17.5 nmol/L (≈ 7 mg/dL), respectively- compared with quintile 5 (155 nmol/L; ≈ 63 mg/dL): quintile 1 hazard ratio (HR): 1.28 (95% CI: 1.14-1.43); quintile 2 HR: 1.14 (95% CI: 1.01-1.28) [51]. This finding was replicated recently in a very large case-control study, with a greater risk of type 2 diabetes in the 10% of subjects with Lp(a) <3.5 nmol/L (≈ 0.014 g/L) (odds ratio: 1.44; $p < 0.0001$) [52]. Whilst the mechanisms underlying this association are unknown, a causal risk has been hypothesized [47], as the risk of type 2 diabetes is higher in homozygous carriers of an LPA mutation responsible for Lp(a) deficiency (odds ratio: 1.45; $p = 0.022$) and in subjects with low genetically imputed Lp(a) molar concentrations (<3.5 nmol/L) (odds ratio: 1.16; $p = 0.0012$), independent of BMI [42, 43]. Regardeless of the complicated associations and potential mechanistic effects of Lp(a) on

the development of type 2 diabetes, current evidence tend to favor that the anti-atherogenic benefits of lowering Lp(a) shall override the paradoxical negative impact on the new-onset type 2 diabetes [48].

As previously described, apo(a) isoforms share substantial structural and functional homology with PLG, the principal component of the fibrinolytic pathway, which is converted to plasmin for fibrinolysis [4]. This homology allows apo(a) to compete with PLG for fibrin affinity sites [38]. Additionally, Lp(a) attenuates PLG activation to plasmin by the tPA in the presence of fibrin [53]. Thus, Lp(a) seems to inhibit fibrinolysis. However, there is little evidence to support such a role in vivo. Many studies have reported that Lp(a) levels may increase under certain conditions, such as chronic kidney disease, myocardial infarction, inflammatory bowel disease, and certain infections [52]. It is hypothesized that Lp(a) acts as a positive acute-phase reactant [4]. This is why inflammatory status should always be considered when interpreting plasma Lp(a) concentrations [23]. In contrast, Lp(a) may behave as a negative acute-phase reactant in cases of serious burns and severe infections, such as visceral leishmaniasis or sepsis [53, 54].

OxPLs play a fundamental role in the early stages of atherosclerosis [55]. It has been suggested that a normal physiologic role of Lp(a) may be to bind and transport proinflammatory OxPLs [56]. Because of the high content of lipoprotein-associated phospholipase A2 (Lp-PLA2), which hydrolyzes OxPLs, Lp(a) may also mediate their clearance [4, 47]. This occurs through the formation of a covalent bond between the KIV10 and KV of the apo(a) fragment of Lp(a) and OxPLs [55].

1.4 THE PATHOGENICITY OF Lp(a)

Convincing evidence has emerged from pathophysiological, epidemiological, and genetic studies on the causality of high serum Lp(a) levels as a potent risk factor for CHD, ischemic stroke, peripheral artery disease, chronic kidney disease, heart failure, venous thromboembolism, calcific aortic valve stenosis (CAVS), as well as retinopathy in patients with diabetes [16, 57-62]. Evidence from experimental, observational, and genetic studies show that increased Lp(a) is associated with increased risk for CHD, ischemic stroke, peripheral artery disease, heart failure, CAVS, mitral valve stenosis, and possibly retinopathy in patients with diabetes [63-65] (Figure 5). On the contrary, elevated Lp(a) concentrations do not appear to be a risk factor for venous thromboembolism [33, 66], whereas very low Lp(a) levels have been linked to an increased risk of incident type 2 diabetes [42].

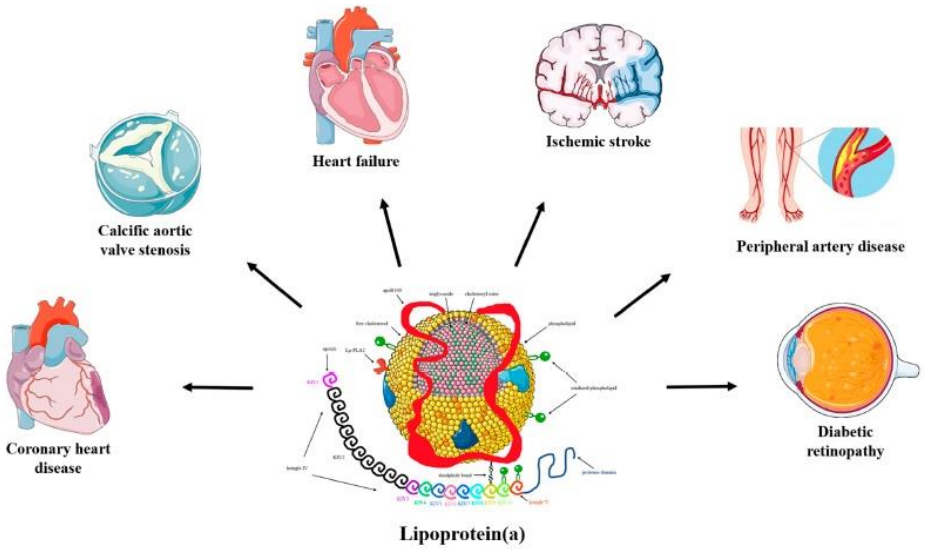

Figure 5. The pathogenicity of lipoprotein(a). Convincing evidence has emerged from pathophysiological, epidemiological, and genetic studies on the causality of high serum lipoprotein(a) (Lp(a)) levels as a potent risk factor for coronary heart disease (CHD), ischemic stroke, peripheral artery disease, heart failure, calcific aortic valve stenosis (CAVS), mitral valve stenosis, and retinopathy in patients with diabetes. Mendelian randomization and genome-wide association studies support the role of Lp(a) as an independent cardiovascular risk factor. Adopted from Koutsogianni et al., [67].

Cardiovascular risk assessment with Lp(a) at middle-age may include direct Lp(a) measurement or an LPA genetic risk score, comprising 43 variants at the LPA gene [68]. LPA genetic risk score is able to discriminate people with the hyper-Lp(a) phenotype (i.e., high Lp(a) levels) and high risk for coronary artery disease even among closely related family members [68]. Hyper-Lp(a) phenotype frequently co-exists with other risk factors for cardiovascular disease (CVD), such as FH or apolipoprotein E4-allele [69-71]. Indeed, hyper-Lp(a) phenotype is a frequent finding in FH subjects, especially in the presence of CHD [69-71].

Individuals with heterozygous familial hypercholesterolaemia (HeFH) have almost 2.5-fold increased Lp(a) levels compared with controls [72]. It is suggested that Lp(a) has a binding affinity to LDLR but less than LDL due to apo(a) mask. Thus, its clearance may be decreased in HeFH patients [73]. Also, Lp(a) levels have been proposed as a marker of restenosis after percutaneous transluminal coronary angioplasty, saphenous vein bypass graft atherosclerosis and accelerated coronary atherosclerosis of cardiac transplantation [74].

Moreover, various inflammatory conditions, such as rheumatoid arthritis, systemic lupus erythematosus, acquired immunodeficiency syndrome and pulmonary arterial hypertension are associated with high Lp(a) levels [46]. Particularly, in cases of rheumatoid arthritis interleukin-6 (IL-6) receptor blockade by tocilizumab reverses high Lp(a) levels [75, 76]. This

suggests that IL-6 may have a potential role in regulating Lp(a) plasma levels [46]. Differences in Lp(a) levels have also been observed in pregnancy [77]. Apart from inflammation, Lp(a) correlates inversely with bile acid levels in plasma in patients with biliary obstructions. It has been revealed that the activation of the Farnesoid X Receptor by bile acids suppresses LPA mRNA transcription and therefore decreases plasma Lp(a) levels [78].

Regarding pathophysiological mechanisms, Lp(a) may act via its LDL-like component [79]. Indeed, in some patients, a substantial fraction of LDL-cholesterol (LDL-C) may be transported by Lp(a) particles rather than the archetypical LDL particle [80]. The LDL-like component of Lp(a) undergoes different types of modification after entry into the vessel wall [79]. The atherogenic actions of LDL in the arterial tissue include the formation of macrophage-derived foam cells upon phagocytic uptake of aggregated LDL particles, or LDL in which lipid and/or protein components have undergone covalent modification, triggering uptake by scavenger receptors [81]. Also, they include the release of bioactive pro-inflammatory lipids exerting both local and systemic actions, the formation of extracellular lipid deposits, and induction of an adaptive immune response with the activation of antigen-specific T-cell responses and antibodies [81]. Additionally, LDL particles induce an innate immune response in the arterial wall, that involves damage associated molecular patterns (DAMPs). In turn, these DAMPs promote recruitment of immuno-inflammatory cells, such as monocyte-macrophages, neutrophils, lymphocytes, and dendritic cells, leading to local and potentially chronic inflammation. Cell death by apoptosis or necrosis can then be induced, thereby contributing to necrotic core formation [81] (Figure 6).

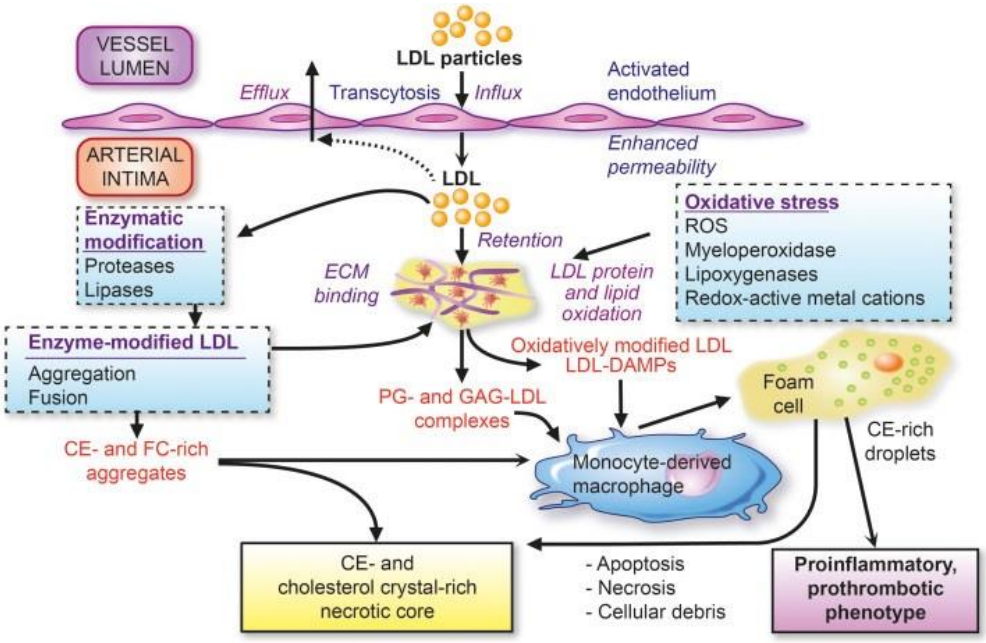

Figure 6. Low-density lipoprotein (LDL) as the primary driver of atherogenesis.
Key features of the influx and retention of LDL in the arterial intima, with ensuing pathways of modification leading to (i) extracellular cholesterol accumulation and (ii) formation of cholesterylester droplet engorged macrophage foam cells with transformation to a ninflammatory and prothrombotic phenotype. Both of these major pathways favour formation of the plaque necrotic core containing cellular and extracellular debris and LDL-cholesterol-derived cholesterol crystals. CE, cholesteryl ester; DAMPs, damage-associated molecular patterns; ECM, extracellular matrix; FC, free cholesterol; GAG, glycosaminoglycans; PG, proteoglycans; ROS, reactive oxygen species. Modified from Boren et al., [81].

Moreover, Lp(a) may act via the antifibrinolytic/prothrombotic effects of apo(a). Apo(a) is highly homologous to PLG and its binding to PLG binding sites prevents interaction between PLG and tPA. If tPA cannot cleave PLG to plasmin, then fibrin clots cannot be dissolved [38]. Lp(a) also increases the production and activity of tissue plasminogen activator inhibitor-1 (PAI-1) [82]. Nonetheless, it is a competitor with both PLG and tPA for binding sites on fibrin, which subsequently promote a thrombotic state by preventing plasmin-mediated clot lysis. Indeed, smaller apo(a) isoforms display higher affinity binding to fibrin [82]. Thrombosis may be augmented by Lp(a) binding and then inhibiting tissue factor pathway inhibitor (TFPI), which is a potent inhibitor of the tissue factor mediated coagulation cascade [59]. Also, Lp(a) prolongs fibrinolysis and activates platelets via PAR1/CD36 pathways [83] (Figure 7).

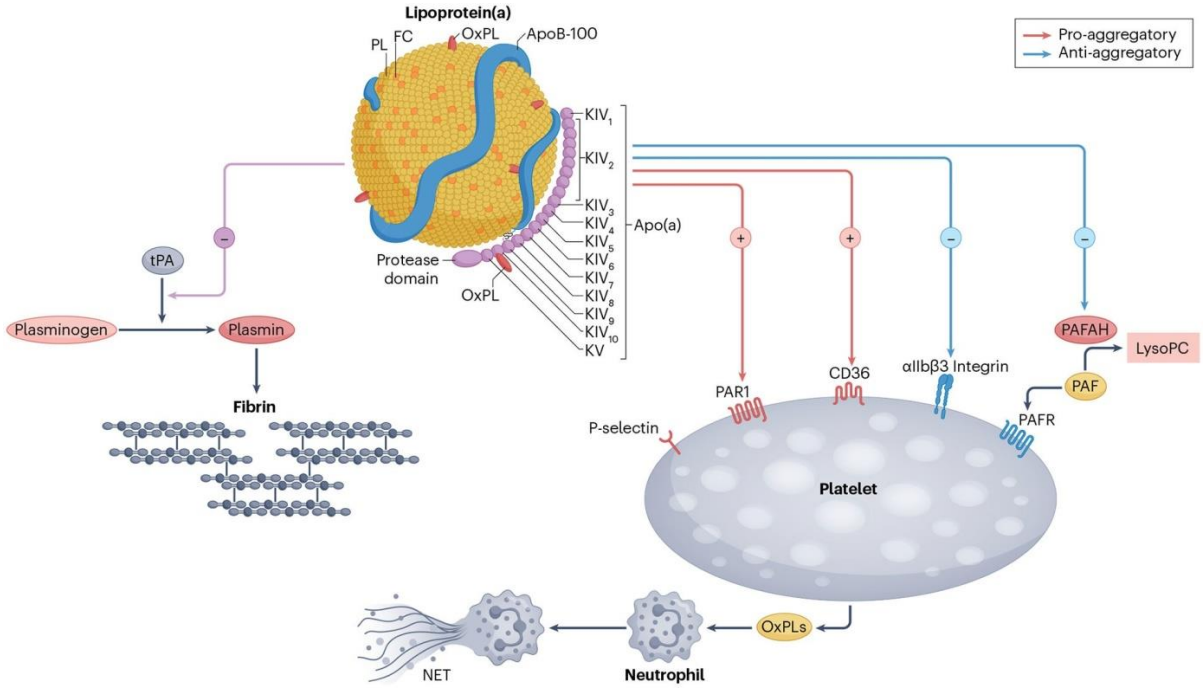

Figure 7. Potential lipoprotein(a) interactions with platelets and the coagulation system. Lipoprotein(a) [Lp(a)] can prolong fibrinolysis by inhibiting tissue plasminogen activator (tPA)-mediated activation of plasminogen and conversion to plasmin. Lp(a) also binds to several platelet receptors that can influence platelet activity. The apolipoprotein(a) (apo(a)) component of Lp(a) can bind to proteinase-activated receptor 1 (PAR1) and oxidized phospholipids (OxPLs) on Lp(a) might theoretically bind to CD36 receptors, both of which activate platelets and have pro-aggregatory effects. OxPLs released from platelets promote platelet–neutrophil interactions and neutrophil extracellular trap (NET) formation. By contrast, Lp(a) might prevent the activation of platelets by inhibiting the binding of fibrinogen to αIIbβ3 integrin (also known as GPIIb/IIIa) and by preventing the generation of platelet-activating factor (PAF) by PAF acetylhydrolase (PAFAH). ApoB, apolipoprotein B; FC, free cholesterol; KIV, Kringle IV; KV, Kringle V; LysoPC, lysophosphatidylcholine; PAFR, PAF receptor; PL, phospholipid. Modified from Bhatia et al., [83].

Lp(a) is susceptible to oxidative stress, leading to the formation of pro-inflammatory and pro-atherogenic OxPLs, found on apoB-100, apo(a), and its lipid phase [31]. OxPLs induce proinflammatory signaling in endothelial cells, smooth muscle cells, monocytes, macrophages, dendritic cells, and platelets [31]. They also mediate plaque destabilizing processes, and as they are present in higher quantities (70-fold) in plaque than in plasma, are capable of stimulating proinflammatory genes leading to vascular inflammation [4]. Additionally, OxPLs are pro-apoptotic in high concentrations, accumulate in atherosclerotic lesions and play an important role in atherosclerosis [84-87]. High Lp(a) and OxPLs of Lp(a) levels, through their proinflammatory and procalcific activity on valvular interstitial cells, are causally associated with increased valve calcification in elderly patients with advanced aortic stenosis, a faster hemodynamic progression of aortic stenosis, and increased risk of aortic valve replacement and death [88, 89]. OxPL plasma levels correlate strongly with high Lp(a) levels and small apo(a) isoforms, or with the presence of LPA SNPs rs3798220 and rs10455872, which are also correlated with high Lp(a) levels [90]. This association of OxPLs

with small Lp(a) particles may at least partially explain their enhanced atherogenicity and association with higher ASCVD risk as compared with large ones [91].

Nevertheless, up to 90% of all OxPLs found in human lipoproteins are carried on Lp(a), which is not necessarily oxidized. Thus, OxPLs may impart additional and potent proinflammatory properties to Lp(a) and play a key role in Lp(a) functionality [4, 56, 92]. OxPLs are degraded into lysophosphatidylcholine (lyso-PC) and oxidized free fatty acids, which also manifest proinflammatory and proatherogenic effects. Lp-PLA2, among other lipoproteins, is associated with Lp(a) [91, 93]. However, the Lp-PLA2 associated with small apo(a) isoforms has a lower catalytic efficiency compared with the Lp-PLA2 associated with larger apo(a) isoforms. This could be another factor that favors the sequestration of plasma OxPLs on small apo(a) isoforms, and therefore the strong correlation between small apo(a) isoforms and high OxPL levels in plasma [91].

Moreover, Lp(a) may serve as a carrier of monocyte chemo-attractant protein-1 (MCP-1). MCP-1 is a major chemokine involved in the development of atherosclerosis via monocyte recruitment to the vascular wall [94]. Lp(a) in plasma may serve as a carrier for MCP-1, and OxPLs are major determinants of the MCP-1 binding [94]. Once Lp(a) has entered the arterial intima with its associated MCP-1, it may subsequently enhance the trafficking of monocytes to the vascular wall, and thereby exacerbate lesion progression [94, 95]. Furthermore, Lp(a) promotes the adhesion and transendothelial migration of monocytes, through the interaction of apo(a) with the b2-integrin Mac-1 [96, 97].

Last but not least, autotaxin (ATX) is another important molecule associated with Lp(a). ATX, preferentially transported by Lp(a), catalyzes the hydrolysis of lyso-PC into lysophosphatidic acid (lyso-PA) [98]. As mentioned previously, lyso-PC is formed by hydrolysis of OxPLs, mediated by the Lp(a)-associated Lp-PLA2 [91]. Lyso-PA stimulates complex intracellular signaling pathways. As a result, it generates various cellular responses, such as inflammatory cytokine release, monocyte attraction and adhesion, abnormal endothelial cell behavior, endothelial permeability, and LDL uptake for plaque formation, and participates in different pathophysiological conditions such as inflammation, atherogenesis and CAVS [98, 99].

All these pathophysiological mechanisms of Lp(a), including proatherogenic, proinflammatory and antifibrinolytic ones, probably contribute to cardiovascular risk in all age groups [100]. However, it is proposed that specific properties may predominate and manifest clinically in different age groups, with antifibrinolytic effects mainly in children, proinflammatory effects in young adults, and proatherogenic effects in the elderly [100].

1.5 EFFECT OF HYPOLIPIDEMIC TREATMENT INTERVENTIONS ON Lp(a)

Lp(a) levels above 50 mg/dL (105 nmol/L) should be considered in all adults as a cardiovascular risk-enhancing factor, with higher Lp(a) levels associated with a greater in risk [39]. Importantly, about 20% of the general population have Lp(a) >50 mg/dL [33].

Elevated Lp(a) improves myocardial infarction risk prediction beyond conventional risk factors as Lp(a) concentrations >47 mg/dL reclassified 23% of first myocardial infarction events correctly, while no events were reclassified incorrectly, in a cohort of 8,720 individuals [101]. Whether lowering Lp(a) reduces the risk of ASCVD and CAVS progression has yet to be shown; the extent of Lp(a) required for clinical benefit is also not known [39]. In the absence of specific Lp(a)-lowering therapies, early risk factor management and more intensive LDL-C and other risk factor lowering is reasonable, considering both absolute cardiovascular risk and Lp(a) levels [33]. Lipoprotein apheresis (LA) is effective in reducing Lp(a) levels [102]. Statins [15] may raise Lp(a) levels by approximately 10-30%. On the other hand, fibrates [103], and most hormones (except growth hormone) [104, 105] may reduce Lp(a) levels. Niacin [106], proprotein convertase subtilisin kexin 9 (PCSK9) [107-109] and cholesteryl transfer protein (CETP) inhibitors [110], aspirin [104], antibodies to IL-6 [104], nutraceuticals [111-114], L-carnitine [115] and ezetimibe [116], may decrease Lp(a) levels. Vitamin C [117] and bile acid sequestrants [118] have a neutral effect on plasma Lp(a) levels.

1.5.1 Effects of Lipid-Modifying Interventions on Lp(a) Levels

1.5.1.1 Lipoprotein Apheresis (LA)

The most effective clinically available intervention for Lp(a)-lowering is LA [102]. Lp(a) levels are reduced by 70-80% acutely after treatment, but rebound elevations between apheresis sessions, which are typically weekly, biweekly, or less frequently, result in a mean interval Lp(a) reduction of 25-40% [102]. LA is infrequently used worldwide, except for Germany, and long-term studies in patients with high Lp(a) undergoing LA suggest that this therapy may reduce 5-year cardiovascular risk by up to 86% [119]. However, LA remains a semi-invasive, time-consuming, and expensive therapy with variable adherence [120]. The Hellenic consensus panel on the clinical use of LA proposes that LA treatment still has a role in individuals with hypercholesterolemia, including HeFH for the secondary prevention of ASCVD on maximum-tolerated lipid-lowering treatment (LLT) and Lp(a) levels >100 mg/dL (>238 nmol/L) [121]. Furthermore, LA may be implemented in individuals with severely elevated Lp(a) [>180 mg/dL (>430 nmol/L)] for the primary prevention of ASCVD if they

have LDL-C >190 mg/dL on maximum LLT as well as in individuals with diabetes and Lp(a) >180 mg/dL (>430 nmol/L) with (a) ASCVD, (b) chronic kidney disease stage 4 or 5, or (c) 24-urine albumin >300 mg [121].

1.5.1.2 Statins

The effect of statin treatment on Lp(a) levels remains an area of controversy. Clinical trials of statin therapy have shown mixed results. A large meta-analysis of 6 trials, including 5,256 patients randomized to receive various statins or placebo, indicated that most statins may increase Lp(a) by an average of 8-24%, although significant heterogeneity has been reported [104]. However, another meta-analysis of 39 trials, including 24,448 patients randomized to receive various statins or placebo, indicated a non-significant 0.1% increase in Lp(a) in the statin group, with no significant differences among statins or statin intensities [122]. Nonetheless, statin therapy has demonstrated a clinical benefit in patients with elevated Lp(a) in both primary and secondary prevention [123-124]. A meta-analysis of 7 randomized controlled trials, including 29,069 patients with high Lp(a) levels and a history of cardiovascular event, concluded that those with Lp(a) levels >50 mg/dL, at baseline or on treatment with statins, have significantly higher risk of ASCVD as compared with Lp(a) <30 mg/dL, independent of other conventional risk factors [123]. The data from a meta-analysis suggest that patients with raised concentrations of Lp(a) (>50 mg/dL), representing about 25% of those with ASCVD, are at substantial residual risk, despite statin treatment [123]. The mechanisms by which statins influence Lp(a) levels remain unclear [104]. Statins elevate the expression of LPA mRNAs as well as the synthesis and secretion of the apo(a) protein in HepG2 cells, which may result in an increase in Lp(a) levels [104]. Moreover, statins activate the expression of PCSK9 genes and increase PCSK9 levels, which then may enhance Lp(a) production [125-126].

1.5.1.3 Niacin

Niacin decreases Lp(a) in a dose-dependent manner by approximately 30-40% [127], but only by 18% in those with the highest Lp(a) levels [128]. The effect of niacin is likely due to a decreased apo(a) production rate [129]. Importantly, studies with cardiovascular outcomes showed no benefit of adding niacin to statins [130, 131]. Moreover, niacin use is limited by side effects, such as flushing, gastrointestinal discomfort, and new-onset diabetes [130, 131].

1.5.1.4 Ezetimibe

Ezetimibe reduces LDL-C levels by limiting cholesterol intestinal absorption via the inhibition of Niemann-Pick C1-Like1 (NPC1L1) protein [132]. A meta-analysis of 10 randomized placebo-controlled clinical trials involving a total of 5,188 (3,020 ezetimibe and 2,168 control) showed that ezetimibe therapy had no effect on plasma Lp(a) concentrations [-2.59%, (95% CI: -8.26; 3.08), p = 0.370]. In addition, subgroup analysis revealed no significant alteration in plasma Lp(a) levels either in trials comparing ezetimibe versus placebo [-4.64%, (95% CI: -11.53; 2.25), p = 0.187] or in trials comparing ezetimibe plus a statin versus statin alone [-1.04%, (95% CI: -6.34, 4.26), p = 0.700].

1.5.1.5. PCSK9 Inhibitors

PCSK9 monoclonal antibodies, i.e., alirocumab and evolocumab, reduce Lp(a) levels by approximately 20-30% [133, 134]. In the two major PCSK9 inhibitor trials, the FOURIER trial with evolocumab, and the ODYSSEY OUTCOMES trial with alirocumab, both PCSK9 inhibitors were associated with an approximately 25% reduction in the risk of major adverse cardiovascular events (MACEs) [108, 109, 135, 136]. Secondary analyses of these trials indicated that one of the main factors determining the risk for MACEs was Lp(a) level [43]. In the FOURIER trial patients in the evolocumab arm with Lp(a) >50 mg/dL experienced a larger absolute reduction in the risk of MACE compared to those with lower Lp(a) levels (-2.4% vs -1.4%, respectively) [135]. Similarly, in a sub-analysis of the ODYSSEY OUTCOMES trial, the absolute reduction in the risk of MACE was 3.7% in patients with Lp(a) >60 mg/dL compared to 0.5% in patients with basal Lp(a) <7 mg/dL [137]. In this trial, 25% of the observed MACE reduction was suggested to be the consequence of 20 mg/dL reduction in Lp(a) levels by alirocumab in patients with baseline Lp(a) >60 mg/dL. A very recent meta-analysis of 62 trials comparing PCSK9 inhibitors mainly to placebo estimated a -6.37 mg/dL (95% CI -7.26; -5.47) mean Lp(a) reduction (corresponding to a ~29% mean reduction) [138] (Figure 8).

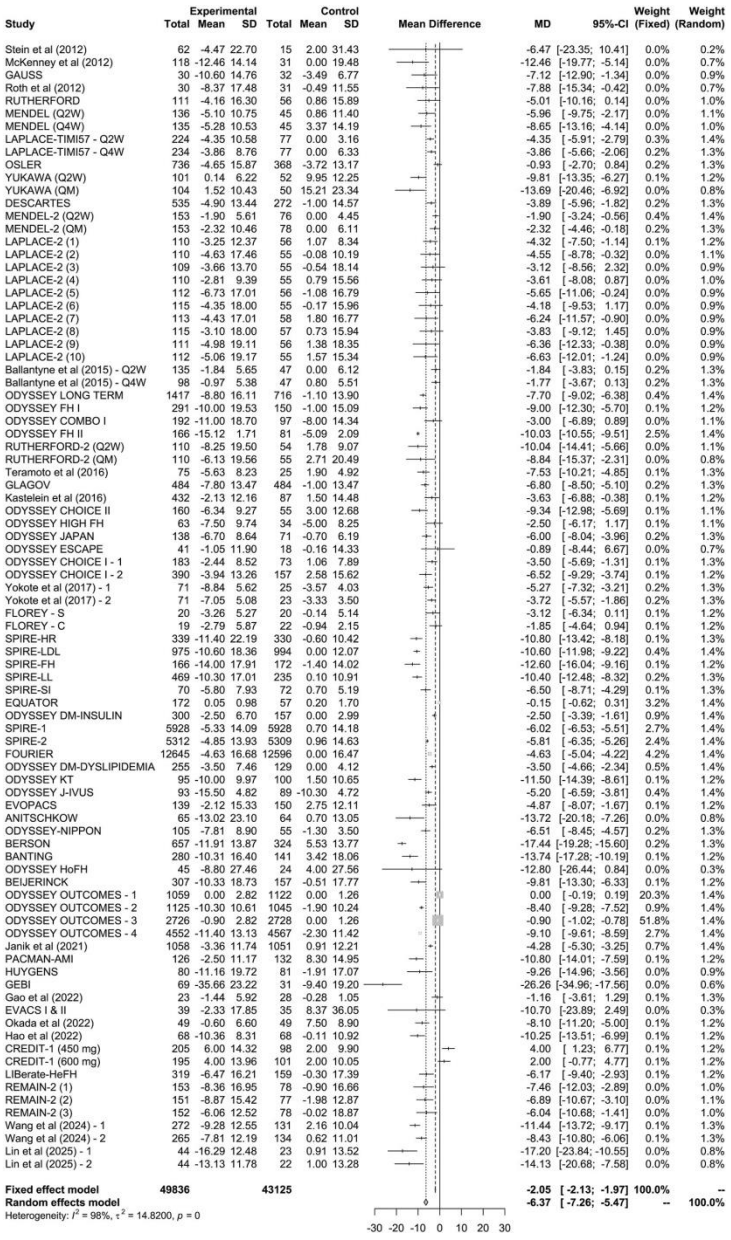

Figure 8. Forest plots indicating the impact of PCSK9 inhibitors on lipoprotein(a) levels. The trials are sorted by published year. The pooled estimate and 95% confidence intervals are shown in absolute mean differences (mg/dL). CI: confidence interval; MD: mean difference; SD: standard deviation. Modified from Xie et al., [138].

The exact mechanism by which PCSK9 inhibitors reduce Lp(a) levels remains unclear [139]. Current hypotheses include increased clearance of Lp(a) particles via the LDLR, increased clearance of Lp(a) via other receptors (the LDL receptor-related protein 1, the cluster of differentiation 36 receptor, toll-like receptor 2, SRB1, and PLG receptors), as well as a reduction in apo(a) production, secretion, and/or assembly [139].

Inclisiran, a small interfering RNA (siRNA) targeting intracellular PCSK9, has been shown to reduce Lp(a) levels by approximately 20% [110]. More recently a meta-analysis of 4 RCT's

with inclisiran revealed a mean decrease of -4.76 mg/dL (95% CI -5.83; -3.69) corresponding to a -22% reduction [138].

1.5.1.6 Fibrates

A very recent meta-analysis of 7 trials revealed that fibrates did not affect Lp(a) concentration [-0.61 mg/dL (95%CI -1.42; 0.20), -2.30% from mean Lp(a) value of 26.55 mg/dL at baseline] [138].

1.5.1.7 Lomitapide

Lomitapide inhibits the microsomal triglyceride transfer protein (MTP), thus halting the assembly/lipidation of VLDL and consequently LDL [140]. Lomitapide, was effective in reducing Lp(a) levels up to 17% in one study [140].

1.5.1.8 Cholesteryl-Ester Transfer Protein (CETP) Inhibitors

The most potent inhibitors of the CETP [which is involved in the exchange of cholesterol esters (CE) and triglycerides between the apoB-100-containing particles and the high-density lipoproteins (HDL)] are also capable of reducing Lp(a) levels [141]. This is the case for obicetrapib which could lower Lp(a) up to 56% [142]. Obicetrapib seems to reduce not only Lp(a), but also LDL-C (~30% at the dose of 10 mg), even in HeFH [143, 144]. CETP mediates the transfer of CEs by bridging the apoB-100-containing lipoprotein and the HDL with its N-terminal and C-terminal domains, which penetrate the phospholipid monolayer of both lipoproteins [138]. Whether the content of OxPLs or the number of K repeats interferes with the ability of CETP to connect the lipoproteins and regulate the exchange of CEs is unknown. The mean Lp(a) reduction across 17 trials on CETP was estimated at -6.77 mg/dL (95% CI -8.67; -4.88) [138] (Figure 8). Recent trials with obicetrapib either as monotherapy at 5 or 10 mg/day [142], monotherapy at 10 mg/day [143] or in combination with ezetimibe [144] revealed mean reductions of -10.4 mg/dL (95% CI -15.7; -5.1), -5.7 mg/dL (95% CI -7.1; -4.4) and -15.0 mg/dL (95% CI -20.2; -9.8) [138] (Figure 9).

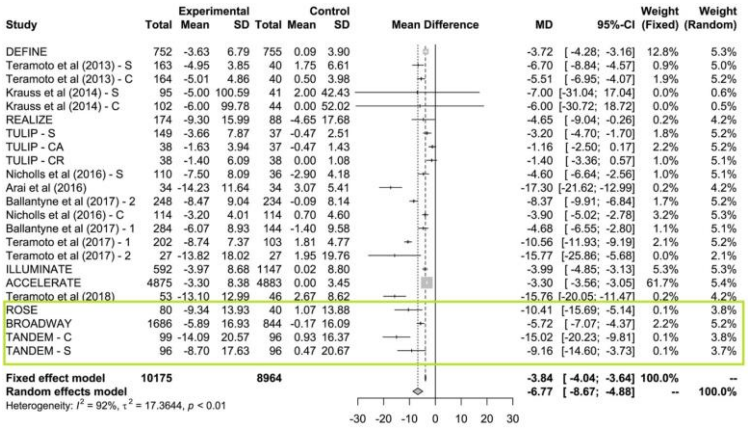

Figure 9. Forest plots indicating the impact of CETP inhibitors on lipoprotein(a) levels. The trials are sorted by published year. The pooled estimate and 95% confidence intervals are shown in absolute mean differences (mg/dL). CI: confidence interval; MD: mean difference; SD: standard deviation. Modified from Xie et al., [138].

1.5.1.9 Bempedoic Acid

Bempedoic acid is a small oral molecule that inhibits cholesterol synthesis by blocking the action of ATP citrate lyase, a cytosolic enzyme, upstream of 3-hydroxy-3-methylglutaryl coenzyme A reductase. The CLEAR Outcomes trial was a double-blind, randomized controlled trial targeting patients who were statin-intolerant (those unable or unwilling to take statins owing to side effects). These patients had ASCVD or were at high risk for ASCVD and received either 180 mg/day bempedoic acid or placebo. The baseline mean LDL-C level was 139.0 mg/dL in both groups. At 6 months, the bempedoic acid group showed a greater reduction in LDL-C compared to the placebo group by 29.2 mg/dL, resulting in a relative reduction difference of 21.1%, however Lp(a) did not change [0% [-1.58; 1.58]. Over a follow-up period of 40.6 months, the incidence of the primary composite endpoint, which included cardiovascular death, non-fatal myocardial infarction, non-fatal stroke, and coronary revascularization, was significantly lower in the bempedoic acid group (11.7% vs. 13.3%; HR 0.87, 95% CI 0.79-0.96, p = 0.004). Similarly a recent meta-analysis also revealed no effect of bempedoic acid on Lp(a) levels [0.01 mg/dL (95% CI -1.36 to 1.39)] [138].

1.5.1.10 Bile Acid Sequestrants

Bile acid sequestrants (colesevalem, cholestyramine, and colestipol) interrupt the enterohepatic circulation of bile acids [145]. Thus, they increase the conversion of cholesterol into bile acids and upregulate LDLR. These drugs have not been shown to affect Lp(a) levels [145].

1.5.1.11 Antisense Oligonucleotides (ASOs)

ASOs are short DNA single-stranded modified oligonucleotides, which directly target complementary mRNA sequences causing their degradation by RNase-H [146]. ASOs are internalized either via clathrin- or caveolin-dependent endocytic pathways, or via nonconventional endocytic pathways (including micropinocytosis), depending on their design. Once inside the cell, they traffic from early-to late endosomes, to lysosomes, and, escaping from these organelles, they reach the cytosol [147]. Pelacarsen (formerly IONIS-APO(a)-LRX, AKCEA-APO(a)-LRX, TQJ230) is a second-generation ASO that binds to its target complementary RNA sequence via base pairing, thereby leading to the degradation of the apo(a) mRNA strand and reduced Lp(a) production [148] (Figure 10). To limit the possibility to target sequences that are shared between apo(a) and PLG, pelacarsen has been designated to bind to the exon 24:25 splice site of the mature human apo(a) mRNA, at position 3901-3920 with the unique sequence (-CTTGTTCTGCTCCGTTGGTG-) that is absent in the mRNA of PLG; this is a region where no polymorphisms in the DNA sequence have been reported, hereby being able to target all the human isoforms of apo(a) [149].

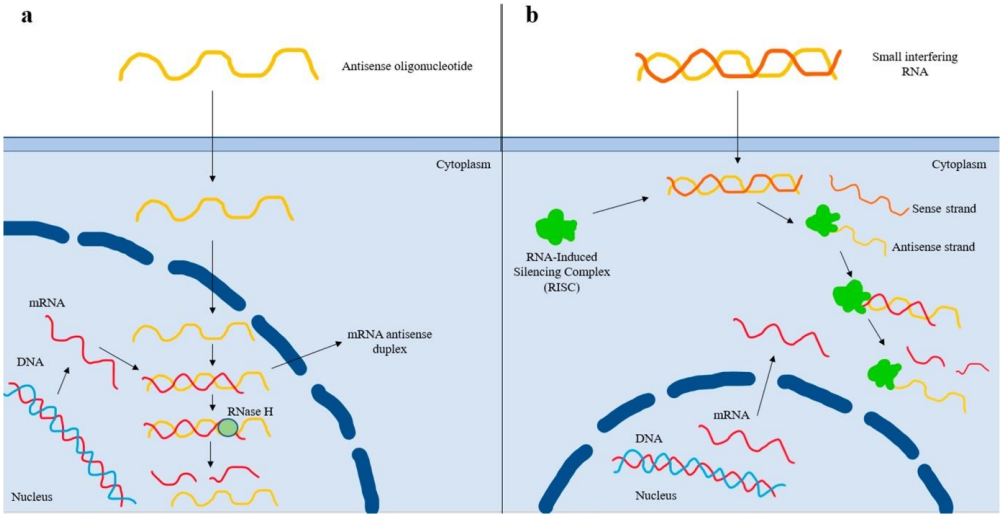

Figure 10. Mechanism of action of antisense oligonucleotide and small interfering RNA-based therapies. (a) After entering the nucleus, the antisense oligonucleotide (ASO) binds to the complementary sequence of the targeted mRNA. The resulting mRNA antisense duplex is recognized by RNase H, which cleaves the mRNA and, thus, prevents protein translation. (b) After the small interfering RNA (siRNA) enters the cell, it is recognized by the RNA-Induced Silencing Complex (RISC), which removes the sense strand. The resulting complex binds to the complementary mRNA sequence and degrades it, thus preventing protein translation. The antisense strand bound to the RISC forms a recyclable, stable complex, and, as a result, its action against target mRNA strands is long-lasting. Thereby, less-frequent dosing of siRNAs is needed compared to that of ASOs. Modified from Koutsogianni et al., [67].

Clinical trials demonstrated the efficacy of pelacarsen in reducing Lp(a) levels. In phase 1, a dose-escalating study in healthy volunteers with elevated Lp(a) levels, a dose dependent reduction of 40-78%, together with a decrease in the content of OxPLs carried by Lp(a) was reported [150]. Phase 2 studies in subjects with ASCVD and elevated Lp(a) plasma levels tested a single or multiple-administrations, with a dose-escalating regimen, followed by a 4-month wash-out phase, to assess the kinetics of Lp(a) [148]. In these studies, Lp(a) was reduced by 60-70% in the single dose regimen and by 66-92% in the multiple dose regimen group. Administration of pelacarsen resulted in dose-dependent decreases in Lp(a) levels, with mean percent decreases of 35% at a dose of 20 mg every 4 weeks, 56% at 40 mg every 4 weeks, 58% at 20 mg every 2 weeks, 72% at 60 mg every 4 weeks, and 80% at 20 mg every week, as compared with 6% with placebo (Figure 11). Regarding the safety profile there were no significant differences between any pelacarsen dose and placebo with respect to platelet counts, liver and renal measures, or influenza-like symptoms. The most common adverse events were injection-site reactions. Of note, the levels of Lp(a) remained almost undetectable for up to 3 months after the last dose and interestingly, while plasma clearance of pelacarsen is estimated of around 4 hours, tissue half-life was shown to be approximately 3 weeks, indicating that the compound can accumulate in some tissues [151]. It should be mentioned that, along with the dramatic reduction of Lp(a), LDL-C and apoB-100 levels were significantly decreased [149]. The latter observation suggests the possibility that apoB-100 particles that do not any longer carry apo(a) are catabolized faster or, if the LDLR plays a role in Lp(a) catabolism, that there is a decreased competition of LDL versus Lp(a) for the hepatic LDLR.

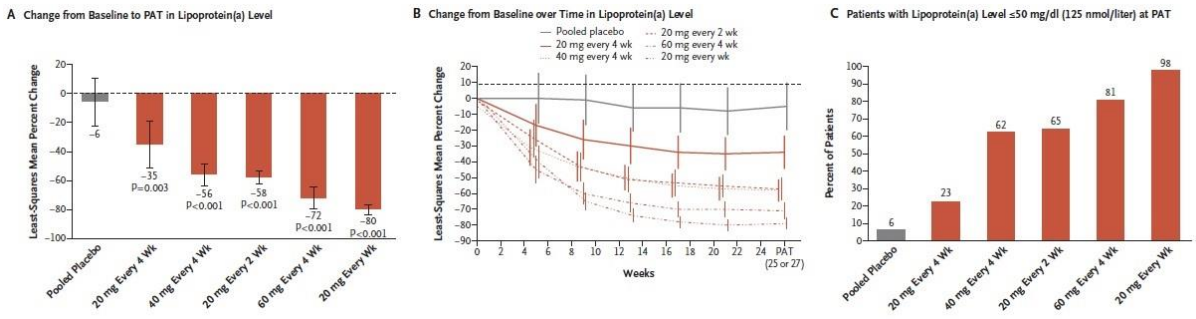

Figure 11. Effect of Pelacarsen on lipoprotein(a) level. (A) least-squares mean percent changes from baseline to the primary analysis time point (PAT) (i.e., 6 months of exposure [at week 25 in the groups that received monthly doses and at week 27 in the groups that received more frequent doses]). I bars denote 95% confidence intervals. (B) least-squares mean percent changes from baseline in lipoprotein(a) over time. Error bars denote 95% confidence intervals. (C) percent of patients with lipoprotein(a) levels of less than 50 mg per deciliter (125 nmol per liter) in each group at the primary analysis time point. Modified from Tsimikas et al., [104].

1.5.1.12 Small interfering RNA (siRNA) agents

siRNAs are double-stranded RNA molecules that dissociate once inside the cell, where the antisense strand is inserted into the RNA-Induced Silencing Complex (RISC) [152]. The antisense strand binds to its homologous target mRNA sequence, leading to its degradation [152]. The antisense strand bound to the RISC forms a recyclable, stable complex, and, as a result, its action against target mRNA is long-lasting [152]. Thereby less-frequent dosing of siRNAs is needed compared to that of ASOs [152].

1. Olpasiran, is a Gal-NAc siRNA, designed to target apo(a). While pelacarsen requires monthly injections, olpasiran is being evaluated as a 3-monthly injection. In a phase I trial, olpasiran was administered as a single subcutaneous dose of 3, 9, 30, 75 or 225 mg to 64 subjects with Lp(a) >70 nmol/L and to a subgroup of subjects with Lp(a) ≥200 nmol/L [153]. Lp(a) was reduced by 71-98%, in a dose-dependent manner and Lp(a) levels remained below baseline values for up to 225 days. Overall, olpasiran was well tolerated, without relevant concerns regarding safety. The phase 2 study, the olpasiran trial of Cardiovascular Events And lipoproteiN(a) reduction-DOSE finding study (OCEAN(a)-DOSE) enrolled 281 subjects with prior CVDs and Lp(a) levels ≥150 nmol/L and demonstrated that subcutaneous injections of 10, 75, 225 mg olpasiran for 4 times every 12 weeks lowered Lp(a) in a dose dependent manner (-97% at the maximal titration) compared to placebo [152]. At 36 weeks, olpasiran substantially reduced Lp(a) levels in a dose-dependent manner, resulting in placebo-adjusted mean percent changes of 70.5% with the 10 mg dose, 97.4% with the 75 mg dose, 101.1% with the 225 mg dose administered every 12 weeks, and 100.5% with the 225 mg dose administered every 24 weeks [152]. The overall incidence of adverse events was similar across groups, with the most common olpasiran-related adverse events being injection site reactions [152]. The Phase III trial [OCEAN(a) Outcomes Trial] is currently ongoing and will test whether olpasiran also reduces the incidence of MACEs over 4 years of follow-up in patients in secondary prevention for ASCVD and at least one risk factor. OCEAN(a) Outcomes Trial has recruited patients with high Lp(a) levels (Lp(a) ≥200 nmol/L).

2. Zerlasiran [SLN360 (NCT04606602)] is another GalNAc-conjugated siRNA against the apo(a) mRNA, which reduced circulating Lp(a) by up to 95% in cynomolgus monkeys [154]. A phase 1 trial of SLN360 showed a dose-dependent Lp(a) reduction of up to 98% in 32 patients with elevated Lp(a) levels and no known CVD [155] (Figure 12).

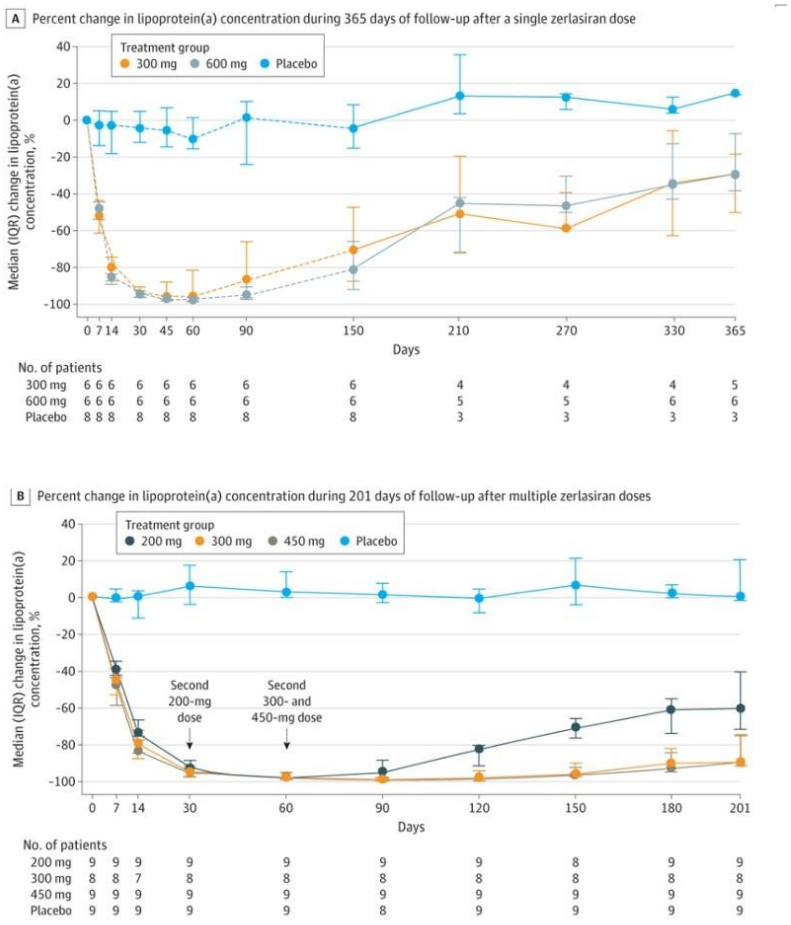

Figure 12. Percent change in lipoprotein(a) serum concentrations following zerlasiran administration in phase I trial. Modified from Nissen et al., [155].

Most patients treated with SLN360 experienced mild to moderate injection site reactions [155]. In the phase II clinical trial of zerlasiran participants were randomized to receive a subcutaneous dose of placebo every 16 weeks for 3 doses (n = 23) or every 24 weeks for 2 doses (n = 24) or zerlasiran 450 mg every 24 weeks for 2 doses (n = 45), 300 mg every 16 weeks for 3 doses (n = 42), or 300 mg every 24 weeks for 2 doses (n = 44) [156]. The primary outcome was the time-averaged percent change in Lp(a) concentration from baseline to 36 weeks, with follow-up to 60 weeks.The results of the phase II zerlasiran trial indicated that compared with the pooled placebo group, median (IQR) percent change in Lp(a) concentration at week 36 was -94.5% (-97.3% to -84.2%) for the 450 mg every 24 weeks group, -96.4% (-97.7% to -92.3%) for the 300 mg every 16 weeks group, and -90.0% (-93.7% to -81.3%) for the 300 mg every 24 weeks group [156]. The most common treatment-related adverse effects were injection site reactions, with mild pain occurring in 2.3-7.1% of participants in the first day following drug administration (Figure 13).

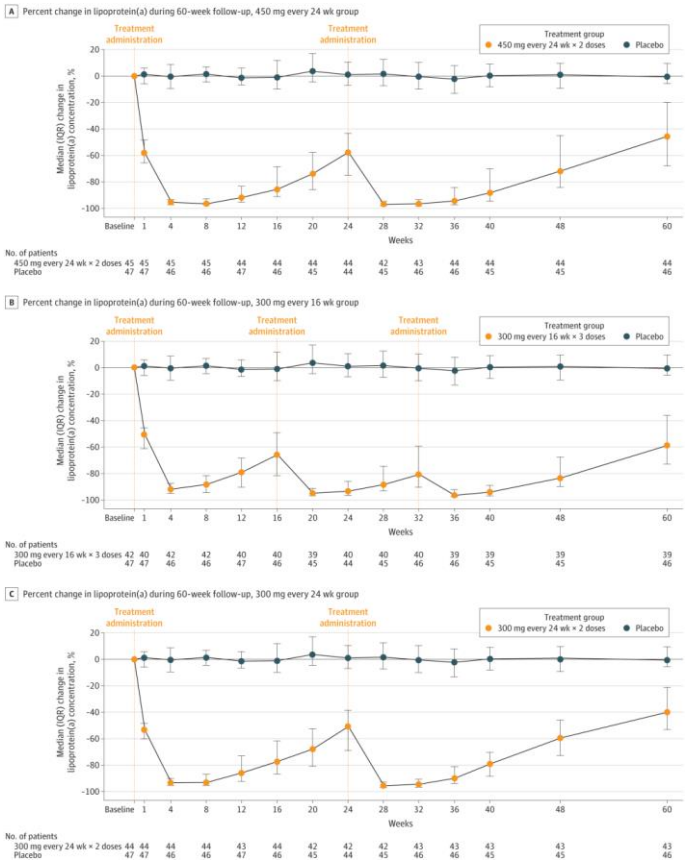

Figure 13. Percent change of lipoprotein(a) serum concentrations following zerlasiran administration in phase II trial. Modified from Nissen et al., [156].

3. Lepodisiran (LY3819469) is a Gal-NAc siRNA with the potential to maintain Lp(a) reduced up to 1 year after the injection, by virtue of its longer nucleotide length and the double-stranded RNA (dsRNA) design. Indeed, a single subcutaneous administration of lepodisiran at the dosage of 608 mg in subjects with baseline Lp(a) levels >30 mg/dL, led to a 97% reduction of Lp(a) on day 29, which persisted after 48 weeks of follow-up [157]. Participants were randomized to receive placebo or a single dose of lepodisiran (4 mg, 12 mg, 32 mg, 96 mg, 304 mg, or 608 mg) administered subcutaneously. The primary outcome was the safety and tolerability of the single ascending doses of lepodisiran. The secondary outcomes included plasma levels of lepodisiran for 168 days after dose administration and changes in Lp(a) serum concentrations through a maximum follow-up of 336 days (48 weeks) (Figure 14). The plasma concentrations of lepodisiran reached peak levels within 10.5 hours and were undetectable by 48 hours. The maximal median change in Lp(a) concentration was -5% (IQR, -16% to 11%) in the placebo group, -41% (IQR, -47% to -20%) in the 4 mg of lepodisiran group, -59% (IQR, -66% to -53%) in the 12 mg dose group, -76% (IQR, -76% to -75%) in the 32 mg dose group, -90% (IQR, -94% to -85%) in the 96 mg dose group, -96% (IQR, -98% to -95%) in the 304 mg dose group, and -97% (IQR, -98% to -96%) in the 608

mg dose group [157]. Currently the ongoing phase 3 cardiovascular outcomes randomized, double-blind, placebo-controlled trial of lepodisiran [ACCLAIM-Lp(a)] is testing whether lepodisiran reduces ASCVD events in adults with elevated Lp(a) who have established ASCVD or are at high risk for a first event.

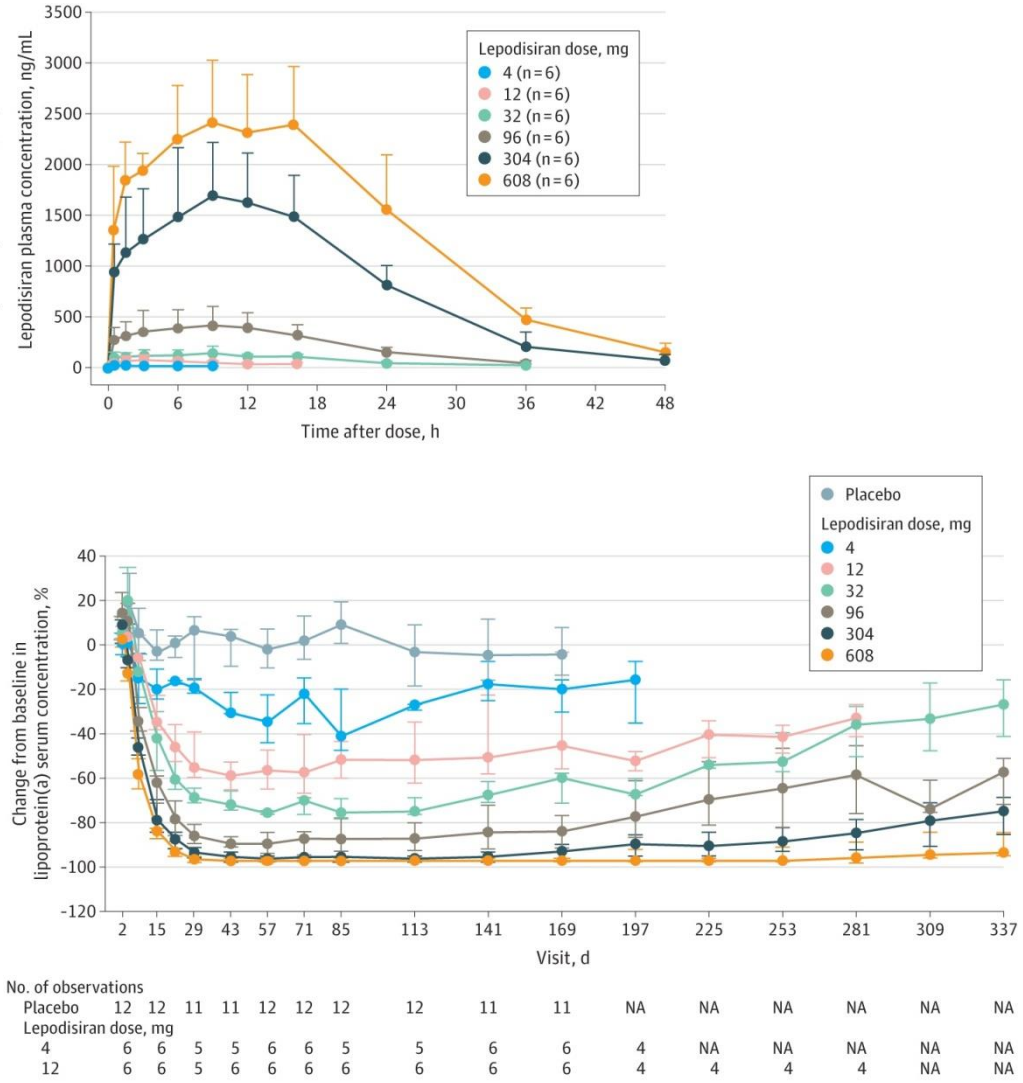

Figure 14. Upper panel: Plasma concentrations of lepodisiran from dose administration to 48 hours post-injection; Lower panel: percentage change in levels of lipoprotein(a) from baseline to days 336 (48 weeks) post-administration. Modified from Nissen et al., [157].

4. Muvalaplin is an orally administered molecule blocking the interaction of apo(a) with apoB-100 and, therefore, the assembly of Lp(a). A phase I study, enrolling healthy individuals and subjects with Lp(a) >30 mg/dL, revealed a significant Lp(a) lowering effect of muvalaplin. Muvalaplin reduced Lp(a) up to 65% following daily administration for 14 days compared to placebo [158]. The cross-interaction between muvalaplin and PLG cannot be ruled-out; however, the activity of PLG was only marginally reduced at the maximal dose of

500 mg (-14%). In the phase II placebo-controlled, randomized, double-blind muvalaplin trial 233 participants with Lp(a) concentrations of 175 nmol/L or greater with ASCVD, diabetes, or FH at 43 sites in Asia, Europe, Australia, Brazil, and the United States were enrolled [159]. Participants were randomized to receive orally administered muvalaplin at dosages of 10 mg/day (n = 34), 60 mg/day (n = 64), or 240 mg/day (n = 68) or placebo (n = 67) for 12 weeks. The primary end point was the placebo-adjusted percentage change from baseline in Lp(a) molar concentration at week 1, whilst secondary end points included the percentage change in apoB-100 and high-sensitivity CRP [159]. Muvalaplin resulted in placebo-adjusted reductions in Lp(a) of 47.6% (95% CI, 35.1%-57.7%), 81.7% (95% CI, 78.1%-84.6%), and 85.8% (95% CI, 83.1%-88.0%) for the 10 mg/day, 60 mg/day, and 240 mg/day dosages, respectively (Figure 15). Dose-dependent reductions in apoB-100 were observed at 8.9% (95% CI, -2.2% to 18.8%), 13.1% (95% CI, 4.4%-20.9%), and 16.1% (95% CI, 7.8%-23.7%) at 10 mg/day, 60 mg/day, and 240 mg/day, respectively. No change in high-sensitivity CRP was observed.

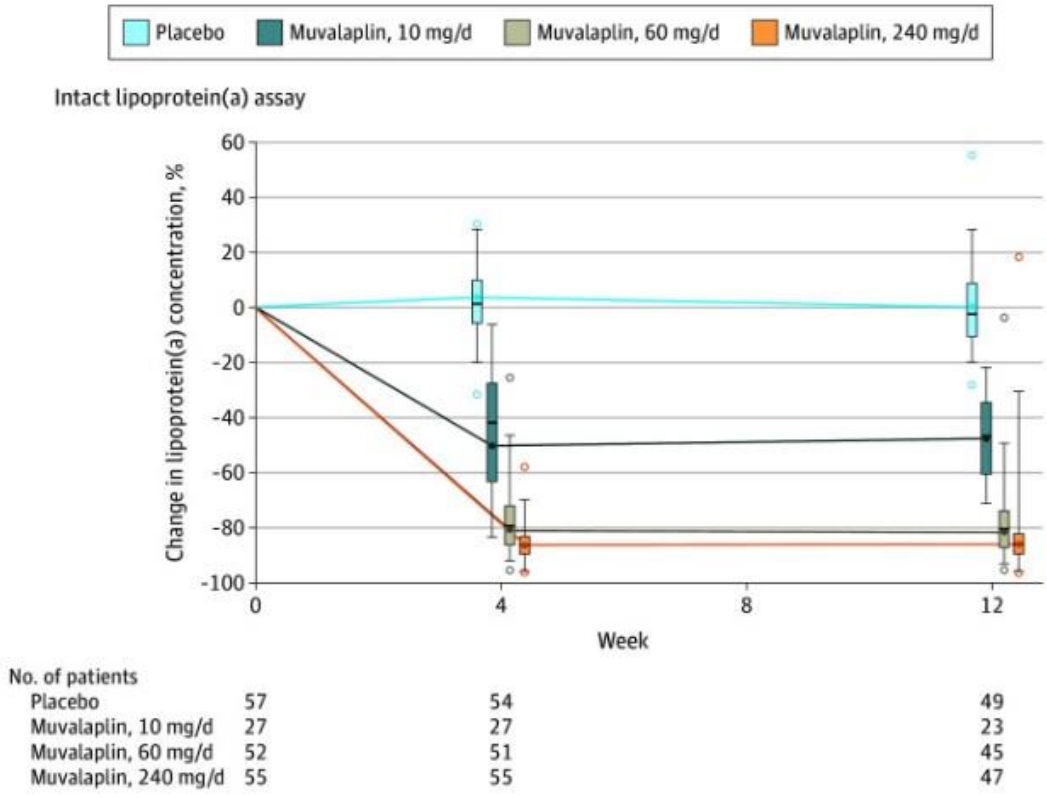

Figure 15 Percentage change in lipoprotein(a) concentration in the phase II muvalaplin. Modified from Nicholls et al., [159].

1.5.2 Effects of other medications on Lp(a) levels

1.5.2.1 Sex Hormone Therapies

Estrogen and its analogs reduce the transcription of the LPA gene [160]. While endogenous sex hormones do not substantially affect Lp(a) levels, postmenopausal hormone replacement therapy can lower Lp(a) levels by approximately 20-25% [161]. A systematic review and meta-analysis of 10 clinical trials, which included 2049 postmenopausal women, A systematic review and meta-analysis of 10 clinical trials, which included 2049 postmenopausal women showed that treatment with selective estrogen receptor modulators (SERMs) significantly reduced Lp(a) levels by 5.9% [162]. However, adverse effects (e.g., breast cancer, stroke, and thrombosis) may outweigh potential cardiovascular benefits [163]. Although previous data suggested that testosterone treatment may lower Lp(a) levels [164], randomized control trials did not confirm this effect [165, 166]. Tamoxifen administration in patients with breast cancer has been found to reduce Lp(a) levels by approximately 40% [167].

1.5.2.2. Thyroid Hormone Therapies

Lp(a) levels are decreased in hyperthyroidism and increased in hypothyroidism [168]. Thyromimetic agents (e.g., eprotirome) have been shown to reduce Lp(a) levels [168]. However, these agents were discontinued because of significant adverse effects, such as elevations in liver enzymes and cartilage side effects [168]. Liver-selective thyromimetic agents, such as the thyroid hormone receptor agonist eprotirome and resmetirom (MGL-3196), a liver-directed, orally active thyroid hormone receptor beta agonist, FDA-approved for the treatment of metabolic dysfunction-associated steatohepatitis (MASH), reduced Lp(a) levels (by 32.3% and 37.9%, respectively) without extrahepatic adverse events [169, 170]. The phase III trial of resmetirom demonstrated reductions in Lp(a) levels by 27-38% compared to placebo after 52 weeks at the oral dose of 80 or 100 mg [171]. Of note in the same study, LDL-C was reduced up to 20% (mean baseline value 115 mg/dL) and apoB-100 up to 22% (mean baseline value 101.1 mg/dL). These findings suggest that the effect is not solely due to increased apoB-100 particle catabolism; however, the precise cellular mechanisms responsible for the reduction in Lp(a) remain to be elucidated. In a recent systematic review and meta-analysis, treatment of overt hyperthyroidism with thyroidectomy, antithyroid drugs, or radioactive iodine increased Lp(a) levels by 20-25%, whereas the treatment of overt and subclinical hypothyroidism with levothyroxine decreased Lp(a) levels by 5-20% [168].

1.5.2.3. Growth Hormone Replacement Therapy

A prospective observational study in men with growth hormone defieciency (GHD) documented a small but significant rise in Lp(a) (from a mean of 27.4 to 34.3 nmol/L after growth hormone replacement), a finding suggesting non-genetic Lp(a) influences [172]. The increase in Lp(a) levels induced by growth hormone replacement therapy did not correlate with thyroid hormone levels [172]. However, long-term studies with growth hormone replacement therapy in GHD are warranted.

1.5.2.4 Aspirin

Available data regarding the effects of aspirin on Lp(a) concentrations and any potent clinical implications are scarce. In a post hoc analysis of the Women's Health Study, a randomized primary prevention trial, 25,131 healthy White women with Lp(a) >65 mg/dL received aspirin (100 mg every other day) or placebo [173]. Aspirin treatment was not associated with any significant reduction of ASCVD events over a 10-year period [173]. In a subgroup analysis, though, carriers of the rs3798220 variant, which is associated with high circulating Lp(a) levels, with a baseline median Lp(a) 80 mg/dL, significantly benefitted from aspirin treatment (HR 0.44; 95% CI: 0.20-0.94, p = 0.03) [173]. This may imply that the benefit of aspirin treatment depends on the Lp(a) levels, but this warrants validation in further studies [33]. Thus, individuals with high Lp(a) levels might be considered for aspirin therapy if they have other indications (high ASCVD risk and low bleeding risk) [33, 174]. A recent analysis showed that participants with Lp(a) >50 mg/dL had a higher burden of ASCVD risk factors, more frequent aspirin use (61.7% vs 55.3%, p = 0.02), and higher rate of incident CHD events (13.7% vs 8.9%, p < 0.01) compared with those with Lp(a) ≤50 mg/dL [83]. Aspirin was associated with a significant reduction in CHD events among those with elevated Lp(a) [HR, 0.54 (95% CI, 0.32-0.94); p = 0.03]. Those with Lp(a) >50 mg/dL and aspirin use had similar CHD risk as those with Lp(a) ≤50 mg/dL regardless of aspirin use. The authors concluded that aspirin use was associated with a significantly lower risk for CHD events in participants with Lp(a) >50 mg/dL at primary prevention [83, 174].

1.5.2.5 Anti-Inflammatory and anti-human immunodeficiency virus (HIV) agents

Tocilizumab, a monoclonal antibody targeting IL-6, reduced Lp(a) levels by approximately 30-40% when administered in patients with rheumatoid arthritis [175]. The LPA gene

promoter contains functional IL-6-responsive elements; thus, the effect of tocilizumab may be attributed to its inhibitory action in this region of the gene [176]. On the contrary, protease inhibitors and antiretroviral therapy have been associated with increased Lp(a) levels when administered in patients with HIV with baseline Lp(a) levels >20-30 mg/dL [177].

1.5.3 Effects of dietary intervention and physical activity on Lp(a) levels

Lifestyle changes, including low-fat diets, have no significant effect on Lp(a) levels [178, 179]. Some, but not all, studies reported that isocaloric replacement of dietary saturated fats with carbohydrates or unsaturated fats increased Lp(a) levels by approximately 8-20% [179-181]. On the contrary, low-carbohydrate, high-saturated fat diets and diets enriched with walnuts or pecans decreased Lp(a) levels by 15% and 6-15%, respectively [179, 182]. Other studies have investigated the effect of dietary supplements (L-carnitine and coenzyme Q10) and specific foods (coffee, tea, and alcoholic beverages, especially red wine) on Lp(a) levels and have shown modest decreases of 10-30% with these interventions [115, 178]. Moreover, vitamin C, in supplementary (1 g/day) or pharmacologic doses (4.5 g/day), has a neutral effect on plasma Lp(a) levels as shown in a meta-analysis [117]. Most studies suggest that physical activity has no or minimal impact on Lp(a) levels, although there are conflicting data, particularly among younger or populations with diabetes in whom physical fitness correlated inversely with Lp(a) levels and decreased ASCVD risk [183, 184].

1.5.4 Effect of bariatric surgery on Lp(a) levels

A prospective observational study of 59 patients with severe obesity (BMI 48 kg/m^2) undergoing metabolic surgery (31 underwent Rouxen- Y gastric bypass, 19 sleeve gastrectomy, and 9 omega loop bypass) demonstrated a significant increase in Lp(a) levels at 12 months [from 10.2 (range 3.8-31.9) to 16.9 (range 4.9-38.6) mg/dL, $p = 0.002$] [185]. These results may suggest an increased synthetic liver capacity, leading to enhanced production and secretion of Lp(a) and, thus, to higher plasma levels [185].

Table 1 summarizes the effects of non-genetic factors on Lp(a) levels. Several lines of evidence suggest that large absolute reductions in Lp(a) concentrations of ~100 mg/dL are needed to achieve meaningful reductions in ASCVD risk [33, 47]. Therefore, only those drugs that are specifically designed for Lp(a)-lowering could be expected to have beneficial effects on ASCVD outcomes.

Table 1. Agents modifying lipoprotein(a) concentrations and their possible mechanisms of action

Lp(a)-Lowering Therapy	Lp(a) effect	Mechanism of possible Lp(a) effect
Lipoprotein apheresis	Acute decrement of 70-75%, but regular apheresis can translate to mean Lp(a) reduction between 25-40%.	Direct physical removal of circulating particles, independent of hepatic synthesis or clearance pathways.
Statins	Most statins may increase Lp(a) on average 8-24%, although significant heterogeneity in response is present.	i. Elevate the expression of LPA mRNA [15]. ii. Elevate the synthesis and secretion of apo(a) protein in HepG2 cells [15]. iii. Activate the expression of PCSK9 genes and increase PCSK9 levels [125].
Niacin	Potential Lp(a) lowering effect between 20-30%, but use is limited by side effects.	Decreases apo(a) production rate [129].
Ezetimibe	As monotherapy provides a modest 7% reduction in Lp(a) levels.	May influence Lp(a) production via anti-inflammatory activity [103].
PCSK9 inhibitors	Mean reduction of Lp(a) levels between 14-30% (evolocumab, alirocumab). Mean reduction of Lp(a) levels between 15%-25% (inclisiran).	i. Increased clearance of Lp(a) particles via the LDLR [186]. ii. Increased clearance of Lp(a) via other receptors [187]. iii. Reduce apo(a) production, secretion, and/or assembly [188].
Lomitapide	Mean reduction of 17%.	Decreases the number of apoB-100-containing lipoprotein particles secreted into the bloodstream, including Lp(a) particles [189].
CETP inhibitors	25-40% reduction.	Mediate the transfer of cholesteryl esters from HDL to apoB-100-containing particles in exchange for triglycerides [136, 142, 190]. ~30% reduction in Lp(a) [144, 159].
Bempedoic acid	~0.1% mean reduction in Lp(a) levels.	Inhibition of ATP citrate lyase, high-sensitivity C-reactive protein (hsCRP) and low-density lipoprotein cholesterol (LDL-C) [138].
Antisense oligonucleotides	Lp(a) reductions up to 92% (IONIS-Apo(a)LRX (pelacarsen, TQJ230).	Bind to its target complementary RNA sequence via base pairing, thereby leading to degradation of the apo(a) mRNA strand and reduced Lp(a) production [148].
Small interfering RNAs	Observed maximum reduction of > 90% [Olpasiran (AMG890)]. 98% in 32 patients (phase 1 trial) (Zerlasiran) [155]. 97% reduction of Lp(a) on day 29 (Lepodisiran) [157].	Double-stranded RNA molecules that dissociate once inside the cell, and the antisense strand is inserted into the RISC. The antisense strand binds to its homologous target mRNA sequence, leading to its degradation. The antisense strand bound to the RISC forms a recyclable, stable complex, and, as a result, its action against target mRNA strands can be repeated [152].
Muvalaplin	Up to 65% reduction following daily administration for 14 days compared to placebo [158].	Reduction in Lp(a) assembly.

CHAPTER 2. OXIDIZED PHOSPHOLIPIDS AND LIPOPROTEIN(a)

2.1 OXIDATIVE MODIFICATION OF LIPOPROTEINS

Oxidative stress and inflammation lead to the oxidative modification of phospholipids in cellular membranes and lipoproteins, and thus generation of OxPLs. OxPLs play important role in a variety of diseases, including atherosclerosis, and exert either proinflammatory or anti-inflammatory effects [191, 192]. Lp(a) is susceptible to oxidative stress, leading to the formation of proinflammatory and proatherogenic OxPLs [15]. Lp(a) mediates atherogenesis through mechanisms linked to its associated OxPLs, as Lp(a) is their major lipoprotein carrier [92]. Elevated Lp(a) plasma concentrations and OxPLs associated with Lp(a) [OxPLs of apoB-100-containing lipoproteins (OxPL-apoB) and OxPLs of Lp(a) (OxPL-apo(a)] predict the presence and progression of coronary, femoral and carotid artery disease [15, 57, 59]. Furthermore, they are increased following acute coronary syndrome and percutaneous coronary intervention as well as predict death, myocardial infarction, stroke and the need for revascularization [57]. They are also correlated with an amplified risk of aortic valve stenosis and peripheral artery disease [59]. Notably, there are currently no available treatments for potent reduction in high Lp(a) or OxPL-apoB / OxPL-apo(a). In this section, we present an overview on OxPLs, focusing mainly on the OxPLs associated with Lp(a) and their biological role, relationship with cardiovascular risk and the effect of current and future medical interventions.

2.2 STRUCTURE AND FORMATION OF OxPLs

Under conditions of oxidative stress or inflammation, different biomolecules, including proteins, lipids and nucleic acids, can be oxidatively modified. Phospholipids in cellular membranes and lipoproteins are among such biomolecules. This oxidative modification is observed when there is an imbalance between the reactive oxygen species (ROS) generation and the protective capacity of cellular antioxidant mechanisms [191, 192]. The most important bioactive phospholipids in oxidized LDL (OxLDL) are generated by oxidation of the sn-2 arachidonoyl-phospholipids [91]. Structurally, OxPLs derived from 1-palmitoyl-2-arachidonoyl-sn-glycero-3-phosphocholine (PAPC) can be separated into two main classes: the first class generated by the oxidative fragmentation of sn-2 fatty acids. 1-palmitoyl-2-(5,6-epoxyisoprostane E2)-sn-glycero-3 phosphocholine (PEIPC) and the esterified epoxycyclopentenone 1-palmitoyl-2-(5,6-epoxycyclopentenone)-sn-glycero- 3-phosphorylcholine (PECPC). Isoprostanes are a unique series of prostaglandin-like

compounds that are generated from arachidonic acid peroxidation [92]. Unstable phospholipid hydroperoxides are initially formed during phospholipid peroxidation and subsequently are degraded into smaller fragments named as “fragmented OxPLs” (Figure 16). The most important fragmented OxPLs are 1-palmitoyl-2-(5-oxovaleroyl)-sn-glycero-3-phosphocholine (POVPC) and 1-palmitoyl-2-glutaroylsn-glycero-3-phosphocholine (PGPC). Lp-PLA2 could detoxify OxPLs in the circulation and the product lyso-PC is carried by albumin in plasma [91]. Lyso-PC induces the formation of atherosclerotic plaques, and it is related to inflammatory factors such as IL-1β and IL-6 [92]. The concentration of Lyso-PC in plasma correlates with vascular damage and heart rate. Different acyl chain dependent mechanisms were involved in vascular relaxation [193]. Lyso-PC has pro-inflammatory effects on monocytes, endothelial cells, and macrophages. Lyso-PC may also increase ROS production and have a number of potentially atherogenic effects, such as monocytes chemoattraction and an increased expression of adhesion molecules [93]. Another bioactive lipid is Lyso-phosphatidic acid (Lyso-PA), which is produced by the hydrolytic action of Lyso-PC by phospholipase D (PLD). Lyso-PA as well as Lyso-PC promote inflammation. Except for the acyl chains in the sn-2 position glycerol structure of OxPLs, the phosphocholine (PC)-containing OxPLs could regulate endothelial cell and macrophage function [193]. PC headgroup of OxPLs could bind to CD36, a class B scavenger that mediates the recognition and uptake of OxLDL by macrophages [93].

2.3 PREFERENTIAL ASSOCIATION OF OxPLs WITH Lp(a) IN HUMAN PLASMA

Most of circulating OxPLs are associated with Lp(a) with only small amounts found on other apoB-100 particles (eg LDL) and HDL [193]. OxPLs are also associated with PLG, favouring its fibrinolytic action [10]. The levels of OxPLs are measured in a chemiluminescent immunoassay using the murine monoclonal antibody MB47, which binds human apoB-100 in plasma [92]. Then, the natural murine IgM monoclonal antibody, E06, that recognizes the PC head group on oxidized but not on native phospholipids is added to determine the amount of OxPLs present on apoB-100 [194-196]. Therefore, OxPL plasma levels, mainly associated with Lp(a), are expressed as OxPL-apo(a) and OxPL-apoB. OxPLs can be formed on oxLDL, apoptotic cells, oxidized cell membranes and sites of inflammation [55]. Afterwards, OxPLs are released into the circulation from these sources and are preferentially transferred to Lp(a) [83, 197].

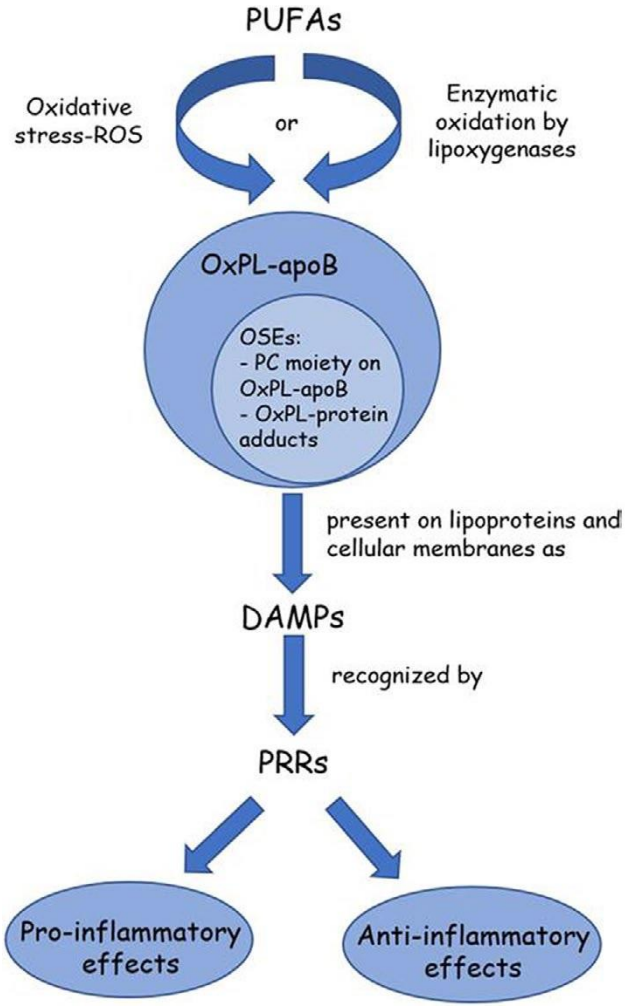

Figure 16. Formation of oxidized phospholipids. OxPLs generally arise from the oxidation of the PUFAs of phospholipids, which are usually esterified at the sn-2 position of phospholipids. PUFAs are highly susceptible to radical-mediated oxidation. Their oxidation is initiated either enzymatically by lipoxygenases or ROS under conditions of oxidative stress and propagates via the classical mechanism of lipid peroxidation chain reaction. The number of oxidation products from each PUFA is likely to be at least 50, and effects of all these products are unknown. During the oxidative modification of phospholipids, different OSEs are formed, including the PC moiety revealed on OxPLs, as well as different types of OxPL-protein adducts that are generated during lipid peroxidation. Different OxPLs and OSEs are present on cellular membranes and lipoproteins as DAMPs, which are recognized by a set of PRRs, and a wide variety of proinflammatory and anti-inflammatory effects is generated. PUFAs, polyunsaturated fatty acid residues; ROS, reactive oxygen species; OxPL-apoB, OxPLs of Lp(a); OSEs, oxidation-specific epitopes; PC, phosphorylcholine; OxPL, oxidized phospholipid; DAMPs, danger-associated molecular patterns; PRRs, pattern-recognition receptors. Modified from Rader & Bajaj [55].

Up to 90% of all OxPLs found in human lipoproteins are carried on Lp(a), which is not necessarily oxidized. Thus, OxPLs may impart additional and potent proinflammatory properties to Lp(a) and play a key role in Lp(a) functionality [193] (Figure 17). An interesting observation has been made in patients undergoing percutaneous coronary intervention [198]. Before percutaneous coronary intervention, most OxPLs in plasma were associated with Lp(a). Immediately after percutaneous coronary intervention, an acute increase was noted in

both plasma OxPL-apoB and Lp(a). However, only 50% of OxPLs were associated with Lp(a), whereas the remaining were present on non-Lp(a) apoB-100 particles. However, by 6 h, >90% of OxPLs were again present on Lp(a), suggesting their transfer and selective binding on Lp(a) [198]. This observation strongly supports the physiological function of Lp(a) in preferentially binding OxPLs compared with other apoB-100-containing particles and suggests a transfer of OxPLs to Lp(a).

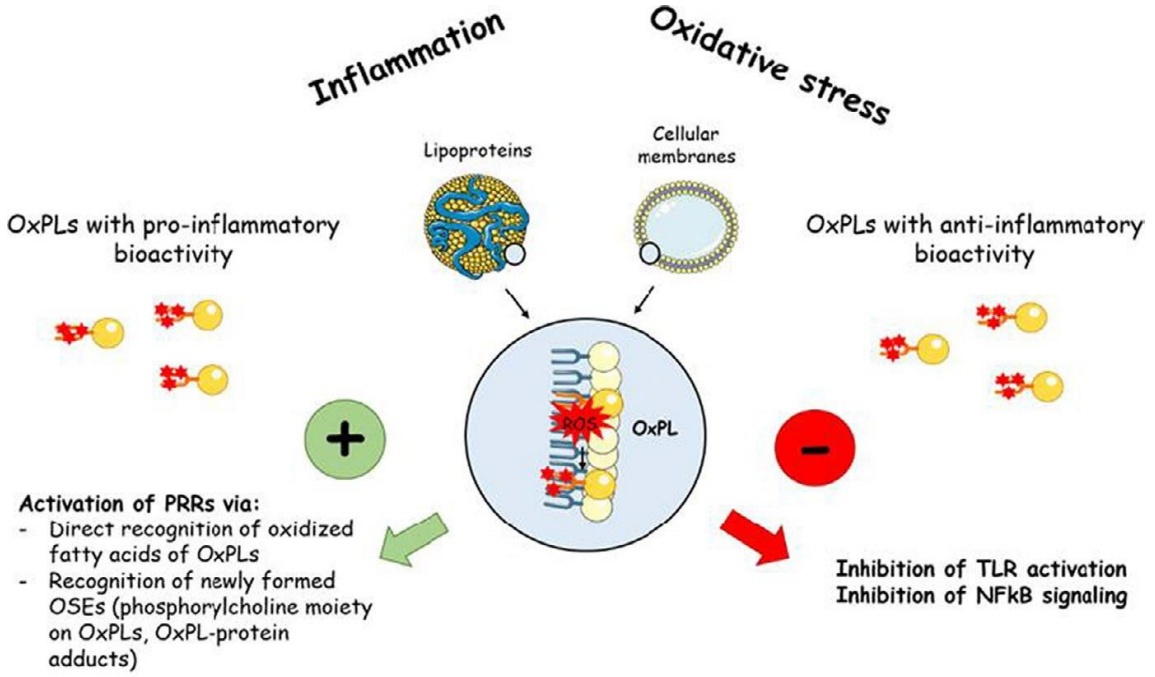

Figure 17. Bioactivity of oxidized phospholipids. Under conditions of oxidative stress or during inflammation, phospholipids in cellular membranes or lipoproteins can be oxidatively modified. This oxidative modification is observed when there is an imbalance between the ROS generation and the protective capacity of cellular antioxidant mechanisms. During the oxidative modification of phospholipids, different OSEs are formed. OSEs include the phosphorylcholine moiety revealed on OxPLs, as well as different types of OxPL-protein adducts that are generated during lipid peroxidation. OxPL, oxidized phospholipid; ROS, reactive oxygen species; PRRs, pattern-recognition receptors; OSEs, oxidation-specific epitopes; TLR, Toll-like receptors; NFkB, nuclear factor kappa-light-chain- enhancer of activated B cells. Modified from Koutsogianni et al., [21].

Currently available evidence suggests that the majority of the OxPLs present on Lp(a) are probably transferred to Lp(a) rather than being generated exclusively in situ by oxidation of the lipids of the LDL-like component of Lp(a) [55, 193]. Studies of the production of recombinant apo(a) constructs in cell culture, which generates large numbers of apoptotic cells, showed that these purified apo(a) constructs already contain substantial amounts of covalently attached OxPLs before any exposure of the apo(a) to environmental stimuli [193]. It is postulated that hepatocytes from patients with MASH, which contain copious amounts of OxPLs [199], might transfer their OxPLs to newly synthesized and assembled Lp(a), which,

when secreted, will carry the OxPLs into the circulation. Patients with rheumatic diseases often have elevated plasma Lp(a) levels and potentially also higher plasma OxPL levels, as has been shown in those with lupus erythematosus [176]. Another hypothesis is that OxPLs are generated at sites of inflammation and are subsequently transferred to Lp(a) particles, which will further increase the risk of ASCVD. Presence of OxPLs on triglyceride-rich lipoproteins has not been reported to date.

OxPL-apoB is not a measure of plasma OxLDL, because OxPLs can be present on Lp(a), LDL, intermediate-density lipoprotein (IDL) and very low-density lipoprotein (VLDL). By design, the OxPL-apoB assay detects OxPLs on all apoB-100-containing lipoproteins -with Lp(a) being the predominant carrier- rather than quantifying a discrete pool of fully OxLDL particles [55]. Moreover, the current view is that highly/fully OxLDL is not expected to persist in plasma because it is rapidly cleared via scavenger-receptor pathways; most oxidation occurs on vessel-wall-retained LDL [200]. A further complication is assay specificity. The widely used E6 antibody-based ELISAs (marketed "OxLDL" assays) recognize aldehyde-modified apoB-100 epitopes [e.g., malondialdehyde-modified LDL (MDA-LDL), copper-oxLDL] and can cross-react with native LDL, limiting their specificity for "OxLDL" [201, 202]. In classic spiking experiments, adding native LDL to plasma artificially increases the measured "OxLDL" signal, demonstrating lack of specificity (e.g., from 88 U/L to 141/399 U/L after adding 5-15 ng native LDL), while adding OxLDL raises the readout to a similar extent -a pattern inconsistent with a specific OxLDL measurement [203].

2.4 Lp-PLA2: ASSOCIATION BETWEEN Lp(a) AND OxPLs OF Lp(a)

Once OxPLs are on Lp(a), they are accessible to endogenous Lp-PLA$_2$ activity on the particle -functionally facilitated by the apoB-100 moiety of Lp(a) [4, 93] (Figure 18). Lp-PLA$_2$ on apoB-100-containing lipoproteins is heavily glycosylated and can be oxidatively modified in vitro, which diminishes catalytic activity [93]. Across equimolar protein concentrations, Lp(a)-Lp-PLA$_2$ mass is ~1.5-7-fold higher than LDL-bound Lp-PLA$_2$, while catalytic activity is lower and varies by apo(a) isoform -implying that apo(a) modulates the enzyme's performance on Lp(a) without serving as the binding site itself [93, 204]. Small-isoform Lp(a) tends to show lower Lp(a)-Lp-PLA$_2$ catalytic efficiency, a feature proposed to favor

retention/accumulation of OxPLs on these particles and to help explain the strong correlation between small apo(a) isoforms and higher OxPL-apoB levels in plasma [93].

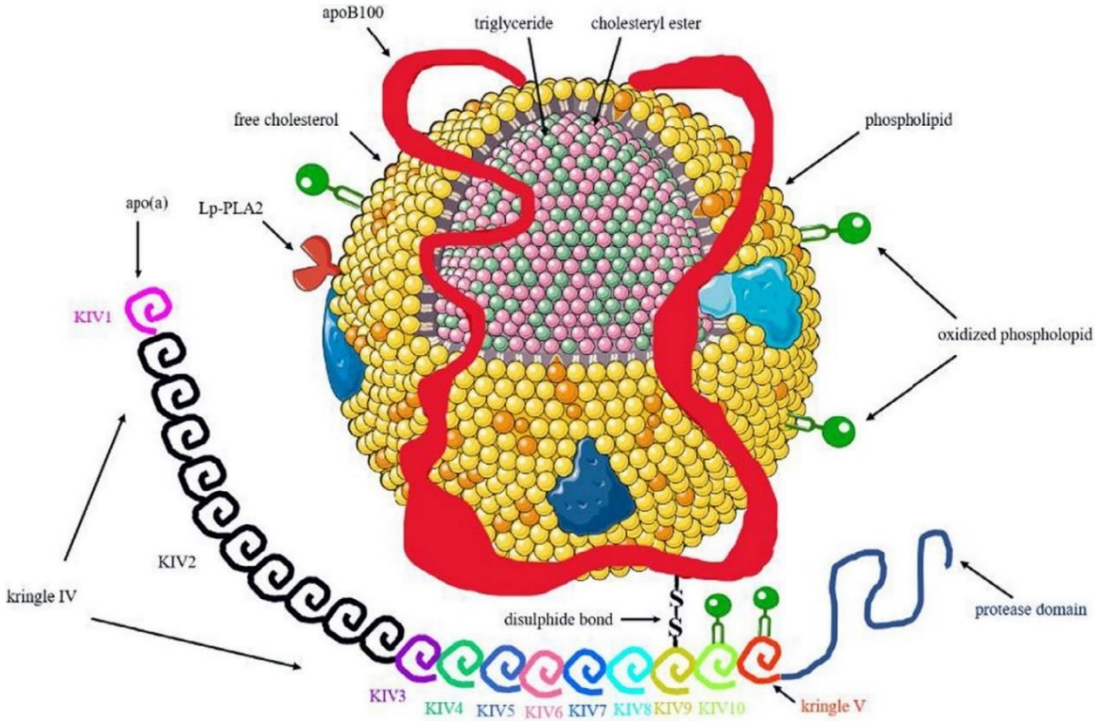

Figure 18. Attachment of Lp-PLA2 on lipoprotein(a). Up to 90% of all oxidized phospholipids found in human lipoproteins are carried on lipoprotein(a) and subjected to degradation by lipoprotein-associated phospholipase A2. Modified from Koutsogianni et al., [21].

Hydrolysis of OxPLs by Lp-PLA$_2$ generates oxidized non-esterified fatty acids (OxFFAs) and lyso-PC, which can transfer from Lp(a) to albumin and retain bioactivity in the circulation [17, 92]. Thus, while Lp(a) may act as a scavenger/transport vehicle for OxPLs, its net effect depends on particle concentration, isoform, and local redox conditions; at high Lp(a) levels, arterial wall deposition under oxidative stress likely amplifies atherogenesis [4, 55]. Early observational data suggest patients with coronary artery disease can exhibit lower Lp(a)-Lp-PLA$_2$ activity than controls, consistent with enzyme oxidative inactivation and substrate accumulation on Lp(a) [93]. Clinically, plasma Lp-PLA$_2$ (as a biomarker/target) has not translated into outcome benefit: large randomized clinical trials of the Lp-PLA$_2$ inhibitor darapladib (STABILITY and SOLID-TIMI 52) were neutral for cardiovascular events [205, 206]. The primary end point occurred in 769 of 7924 patients (9.7%) in the darapladib group and in 819 of 7904 patients (10.4%) in the placebo group (HR in the darapladib group, 0.94; 95% CI, 0.85 to 1.03; p = 0.20) and in 903 of 6504 participants in the darapladib group and 910 of 6522 participants in the placebo group [16.3% vs 15.6% at 3 years; HR, 1.00 (95% CI, 0.91-1.09) for the STABILITY trial and SOLID-TIMI 52 clinical trials respectively] (Figure

19). Notably, both trials quantified total-plasma or LDL-associated Lp-PLA$_2$ but did not specifically measure Lp(a)-bound Lp-PLA$_2$, so any isoform-dependent Lp(a) effects remain unresolved [55].

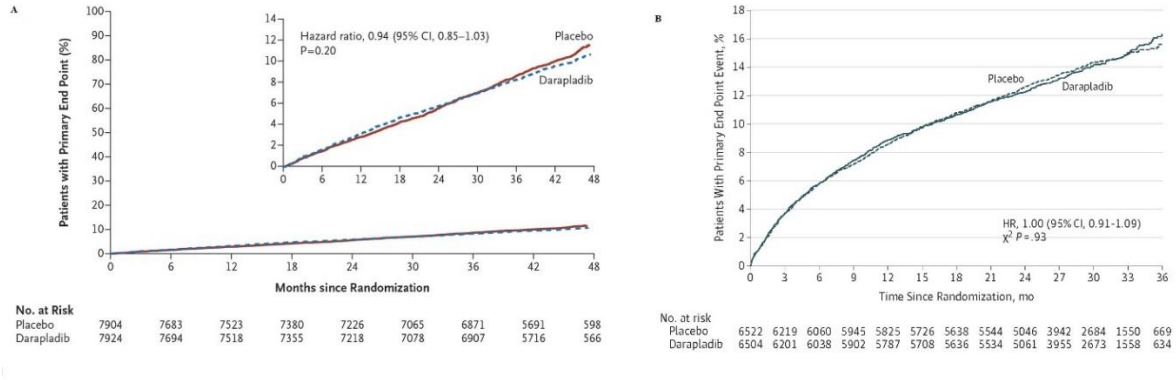

Figure 19. Kaplan-Meier curves for the primary end point of death from cardiovascular causes, myocardial infarction, or stroke for the STABILITY (A) and SOLID-TIMI 52 (B) clinical trials. Modified from White et al., [205] and O'Donoghue et al., [206].

2.5 RELATIONSHIP OF Lp(a)-ASSOCIATED OxPLs WITH CARDIOVASCULAR RISK

Several studies have shown that plasma levels of OxPL-apoB can be used to predict the presence of anatomical atherosclerotic stenoses in multiple vascular beds, improve the prediction of the risk of first and recurrent myocardial infarction, ischemic stroke and peripheral artery disease in primary prevention settings and in patients with pre-existing CVD, and optimize patient risk stratification [15, 194, 198, 207-211]. Elevated OxPL-apoB levels have also been associated with both early and late CAVS, its rapid progression, and the need for aortic valve surgery [86, 88, 89, 212-215].

From an epidemiological and clinical perspective, these studies reveal a range of weak to strong correlations between plasma Lp(a) and OxPL-apoB measurements that vary by baseline demographic characteristics, race and ethnicity, apo(a) isoform distribution and acuity of CVD presentation. For example, in the Dallas Heart study, individuals with small apo(a) isoforms tended to have a narrower range of Lp(a) levels but a significantly larger spread in plasma OxPL-apoB levels [210, 216]. By contrast, individuals with large apo(a) isoforms tended to have a large spread of plasma Lp(a) levels but a much narrower range of plasma OxPL-apoB levels within the low range (Figure 20).

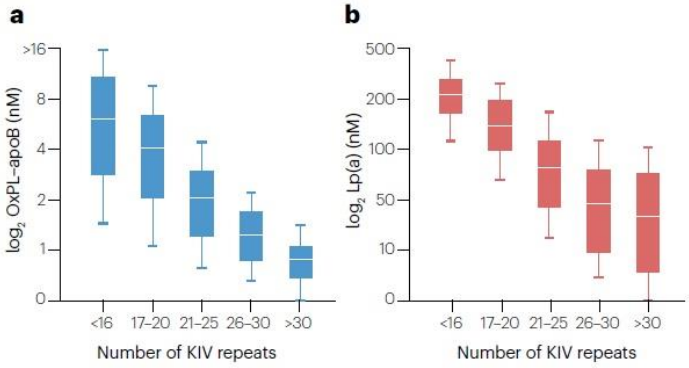

Figure 20. Relationship of OxPL-apoB and lipoprotein(a) levels according to size of the major apolipoprotein(a) isoform. Small apo(a) isoforms are associated with elevated OxPL-apoB and Lp(a) levels; however, at the extremes small apo(a) isoforms are associated with a wide range of OxPL-apoB concentrations but narrow range of Lp(a) concentrations, and large apo(a) isoforms is associated with low OxPL-apoB levels but a larger range of Lp(a) levels. Modified from the Dallas Heart study [210, 216].

This observed variability in the correlations between plasma Lp(a) and OxPL-apoB measurements has implications for both the underlying pathophysiological relationships and the observed differences in the predictive value of these measurements. For example, Lp(a) and OxPL-apoB levels represent distinct pathophysiological phenomena. The level of Lp(a) in plasma is primarily (~90%) genetically determined by the LPA gene, with minor influences from the LDLR, APOE, CETP and APOH genes [217]. Given that the LPA promoter also contains an IL-6 response element, LPA expression can be regulated by conditions that increase IL-6 levels [218]. Sex hormones, growth hormone and dietary fat also affect Lp(a) levels [219, 220]. By contrast, OxPL-apoB encompass genetic, environmental and dietary factors that increase inflammation and oxidation of lipoproteins [55]. Because Lp(a) is a preferential carrier of OxPL, OxPL-apoB can summate both the genetics of Lp(a) and the extent of lipoprotein oxidation, thereby conferring a 'two-hit' effect on cardiovascular risk. For this reason, in the studies in which participants had elevated oxidative and/or inflammatory stress, such as those with acute coronary syndromes or undergoing percutaneous coronary intervention, plasma Lp(a) and OxPL-apoB levels showed lower correlations than in individuals with lower oxidative and inflammatory stress [209, 221]. Although both markers can predict CVD events, OxPL-apoB is usually a numerically stronger [197] and/or independent predictor of CVD risk even after adjustment for Lp(a) levels [85, 104]. When both OxPL-apoB and Lp(a) levels are elevated, the risk of CVD seems to be higher than when either marker alone is elevated [222]. The relationship between elevated plasma OxPL-apoB levels and anatomical CVD and its downstream clinical manifestations is depicted in Figure 21.

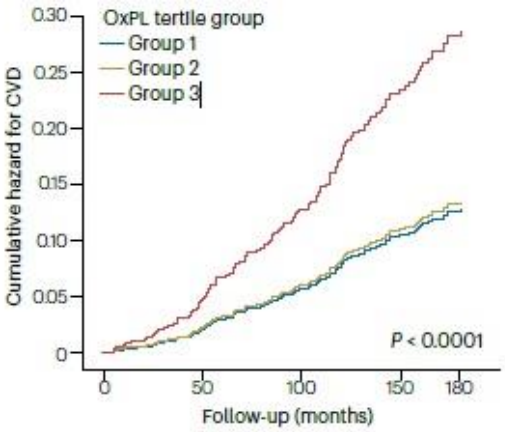

Figure 21. Association of OxPL-apoB with anatomical cardiovascular events. Modified from Tsimikas &Witztum, [92].

Taken together, the findings indicate that elevated OxPL-apoB levels are associated with endothelial dysfunction, coronary, carotid and peripheral artery disease, and calcific aortic valve disease [56, 83, 223]. For example, in patients undergoing LA, plasma OxPL-apoB levels showed a significant inverse correlation with myocardial perfusion, but no correlation was noted for other blood lipid variables [224].

The association between elevated OxPL-apoB levels and coronary artery disease as well as peripheral artery disease has been documented [55, 197]. In the classic MAYO study, plasma OxPL-apoB levels showed a strong and graded association with the presence and extent of coronary artery disease (defined as >50% stenosis of the luminal diameter) [209]. The multivariable-adjusted association between OxPL-apoB level and coronary artery disease was independent of all clinical and lipid measures, except Lp(a). However, among patients aged ≤60 years, OxPL-apoB remained an independent predictor of coronary artry disease, whereas Lp(a) did not [209]. Among patients aged ≤60 years, the odds ratio for coronary artery disease for OxPL-apoB level in the highest quartile was 3.12 ($p < 0.001$), and 16.8 ($p < 0.001$) if the patient had concomitant hypercholesterolemia [209]. These findings were replicated ~20 years later in the CASABLANCA study in a contemporary cohort of 1098 patients undergoing coronary angiography [197]. The authors used a convenience cohort of 1098 participants undergoing coronary angiography (93% were white, 71% were male, and 51% had coronary artery disease at baseline). All 3 measures were strongly intercorrelated (Figure 22 A & B). Importantly, during a median follow-up of 4.2 years, Lp(a), OxPL-apoB and OxPL-apo(a) were all strongly associated with incident MACE outcomes [odds ratio 1.08 (95% CI 1.03-1.14), 1.15 (95% CI 1.05-1.26) and 1.07 (95% CI 1.01-1.14) for Lp(a), OxPL-

apoB level and OxPL-apo(a), respetively] [197] (Figure 22 C). Moreover, while prior studies have shown a significant association between OxPL-apoB and CVD risk, in most studies, this association was dependent on Lp(a) levels [222, 225]. Furhermore, when the authors adjusted for the OxPLs, the association of Lp(a) with MACE outcomes was substantially attenuated, suggesting the importance of OxPL itself to CVD. These findings are consistent with prior work asserting the association of Lp(a) with risk for atherosclerotic disease and cardiovascular events [226] and it expands on prior evidence [25] by demonstrating the association of OxPLs with these outcomes in a statin-treated population with relatively low LDL-C levels (baseline 83.4 mg/dL).

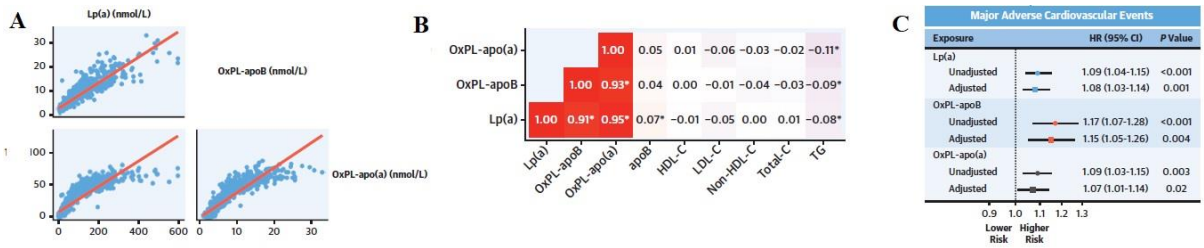

Figure 22. Interrealtionships between lipoprotein(a), OxPL-apo(a) and OxPL-apoB and their association with both prevalent and incident cardiovascular disease. Modified from Gilliland et al., [197].

The MAYO and CASABLANCA studies are similar in that both enrolled a patient population undergoing clinically indicated coronary angiography and revascularization if required, and were different in that only a minority of patients in the MAYO study were receiving statin therapy (baseline LDL-C level 124 mg/dL), whereas most of the participants in the CASABLANCA study were receiving statins (baseline LDL-C level 83 mg/dL). Additionally, the medical, percutaneous and surgical care of patients undergoing coronary angiography has been improved since the MAYO study. Despite these improvements, elevated OxPL-apoB levels remained strong predictors of both anatomical coronary artery disease and recurrent MACE in the CASABLANCA study, suggesting that targeting OxPLs might provide additional cardiovascular benefit in these patients [55].

The Dallas Heart Study is a multi-ethnic, probability-based sample of the Dallas County population in which Black individuals were systematically oversampled so that the final cohort consisted of 50% Black persons [216]. A total of 3481 individuals from three racial or ethnic groups (Black, white and Hispanic) were followed up for 9.5 years. In the entire cohort, Cox regression analysis with multivariable adjustment revealed that quartile 4 of OxPL-apoB levels was associated with a HR for time to MACE of 1.89 (95% CI 1.26-2.84; p = 0.003) versus quartile 1 [216]. Addition of the major apo(a) isoform size and three LPA

SNPs linked to elevated Lp(a) levels in plasma to these models attenuated the risk of MACE associated with high OxPL-apoB or Lp(a) levels, but this remained significant for both parameters [216]. These findings were generally consistent among specific ethnic groups. Others have also shown that the two most common LPA SNPs associated with elevated plasma Lp(a) levels, rs37898220 and rs10455982, have been associated with elevated plasma OxPL-apoB levels [89, 227]. A meta-analysis of OxPL-apoB and the risk of coronary artery disease including 8 studies and a total of 2578 patients with coronary artery disease and 14,008 control individuals demonstrated that those in the top fifth of OxPL-apoB levels had an approximately 2-fold higher relative risk of coronary artery disease than individuals in the bottom fifth (relative risk 2.11, 95% CI 1.78-2.52) [222].

The role of OxPL-apoB measurement in secondary prevention of CVD was assessed in a random sample of 1503 participants from the TNT study [194]. Levels of OxPL-apoB at randomization predicted the risk of subsequent MACE, with a HR of 1.21 (95% CI 1.04-1.41; $p = 0.018$) per doubling of OxPL-apoB concentration and HR of 1.69 (95% CI 1.14-2.49; $p = 0.01$) for tertile 3 versus tertile 1 of OxPL-apoB level after multivariate adjustment [194]. Tertile 3 of OxPL-apoB concentration was associated with a higher risk of MACE than tertile 1 in the group receiving atorvastatin 10 mg (HR 2.08, 95% CI 1.20-3.61; $p = 0.01$), but not in the group receiving atorvastatin 80 mg (HR 1.40, 95% CI 0.80-2.46; $p = 0.24$). These data suggest that elevated OxPL-apoB levels predict secondary MACE in patients with stable coronary artery disease, a risk that was mitigated by atorvastatin 80 mg. These observations were further confirmed in the SPARCL study with 4385 patients with previous stroke or transient ischemic attack at baseline but no previous coronary artery disease [207]. In this study, elevated OxPL-apoB levels were associated with increased risk of first major coronary events (HR 4.0; $p < 0.0001$) and any cardiovascular event (HR 4.4; $p < 0.0001$) over the 5 years of follow-up [207].

2.6 EFFECT OF HYPOLIPIDEMIC TREATMENT INTERVENTIONS ON OxPL-apoB/OxPL-apo(a)

The most effective intervention in reducing plasma OxPLs levels, ranked from highest to lowest reductions, include the ASO pelacarsen [15], LA [228], bariatric surgery [185] and niacin therapy [229]; these therapies are associated with mean decreases of 17-88% (Figure 23).

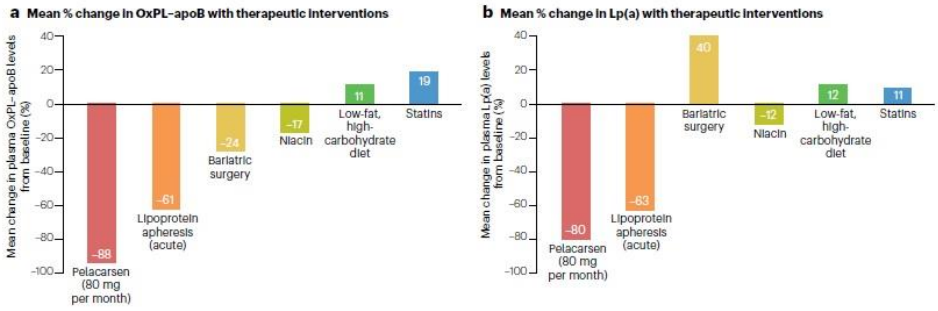

Figure 23. Effect of therapeutic interventions on plasma levels of OxPL-apoB and lipoprotein(a). Mean percentage change in the plasma levels of oxidized phospholipid (OxPL)-apolipoprotein B (apoB) (a) and lipoprotein(a). Modified from Tsimikas & Witztum, [92].

2.6.1 ASOs (Pelacarsen)

In prior studies of pelacarsen, it has been observed that not only does it lower Lp(a) but it also lowers OxPL-apo(a) and OxPL-apoB by a consistent effect (~70%-90%) across all studies [15, 148, 226]. In a phase IIb trial [15], the mean percentage reduction in Lp(a) level was 80% with the highest dose of pelacarsen (20 mg weekly) versus 6% reduction in the placebo group (placebo-corrected reduction of 74%). However, pelacarsen had a more potent effect on OxPL-apoB levels, with an 88% reduction in the pelacarsen group versus 14% increase in the placebo group (placebo-corrected reduction of 102%) [15]. Plasma OxPL-apo(a) levels were reduced by 70% in the pelacarsen group and 20% in the placebo group (placebo-corrected reduction of 50%) (Figure 24).

Measure	APO(a)-L_{Rx}					Pooled Placebo (N=47)
	20 mg Every 4 Wk (N=48)	40 mg Every 4 Wk (N=48)	20 mg Every 2 Wk (N=48)	60 mg Every 4 Wk (N=47)	20 mg Every Wk (N=48)	
Lipoprotein(a) — nmol/liter	−95.9±94.4	−116.9±71.7	−130.3±66.1	−149.5±67.4	−187.8±80.3	−15.2±34.6
Lipoprotein(a) — mg/dl	−38.4±7.7	−46.8±28.7	−52.1±26.4	−59.8±27.0	−75.1±32.1	−6.1±13.8
OxPL-apoB — nmol/liter	−8.0±10.3	−11.3±11.0	−12.2±7.9	−14.9±10.3	−20.1±8.5	3.7±8.1
OxPL-apo(a) — nmol/liter	−16.8±14.3	−24.5±20.1	−25.9±17.2	−33.3±16.8	−41.6±16.5	−12.3±14.7
LDL cholesterol — mg/dl	−5.6±27.4	−13.5±30.1	−13.2±19.8	−8.2±17.3	−16.4±14.8	−1.2±17.8
Apolipoprotein B — mg/dl	−2.2±17.4	−8.3±18.2	−6.3±11.6	−3.9±13.5	−10.9±10.9	0.6±12.0
Total cholesterol — mg/dl	−3.9±32.1	−11.6±32.1	−11.6±24.4	−3.9±23.2	−11.6±20.9	−3.9±21.3
HDL cholesterol — mg/dl	0.0±6.2	0.0±9.7	3.7±8.9	3.7±11.6	3.7±10.1	0.0±6.6
Triglycerides — mg/dl	−8.9±32.8	−8.9±31.0	0.0±52.3	0.0±50.5	−8.9±41.6	0.0±51.4
hsCRP — mg/liter	−0.9±4.24	−0.7±4.24	−0.3±2.84	−0.5±2.22	−0.1±6.30	−0.8±5.13

Figure 24. Effect of pelacarsen on oxidized phospholipids. Data are absolute change from baseline at 6 months of Exposure. Modified from Tsimikas et al., [15].

2.6.2 Lipoprotein apheresis

A study in patients with FH and varying levels of Lp(a) showed that LA induced acute reductions in OxPL-apoB levels by 60-70%, in parallel with reductions in Lp(a) levels [228].

This finding is not surprising given that LA removes all apoB-100-containing lipoproteins. In another study in patients undergoing LA who also underwent intensive coronary blood flow measurements before and after LA, the baseline OxPL-apoB level was an inverse predictor ($r = -0.75$) of the change in myocardial perfusion in contrast to other lipid parameters, including Lp(a) [224] (Figure 25). In a prospective observational study in patients with multiple recurrent CVD events who had controlled LDL-C, patients who underwent LA to reduce elevated Lp(a) levels had a reduction of 80-90% in CVD events compared with event rates before LA [119]. LA in patients with refractory angina, 60% of whom had elevated Lp(a) levels, improved coronary blood flow (as measured by MRI) and reduced the frequency of angina episodes; however, OxPL-apoB levels were not measured in this study [230].

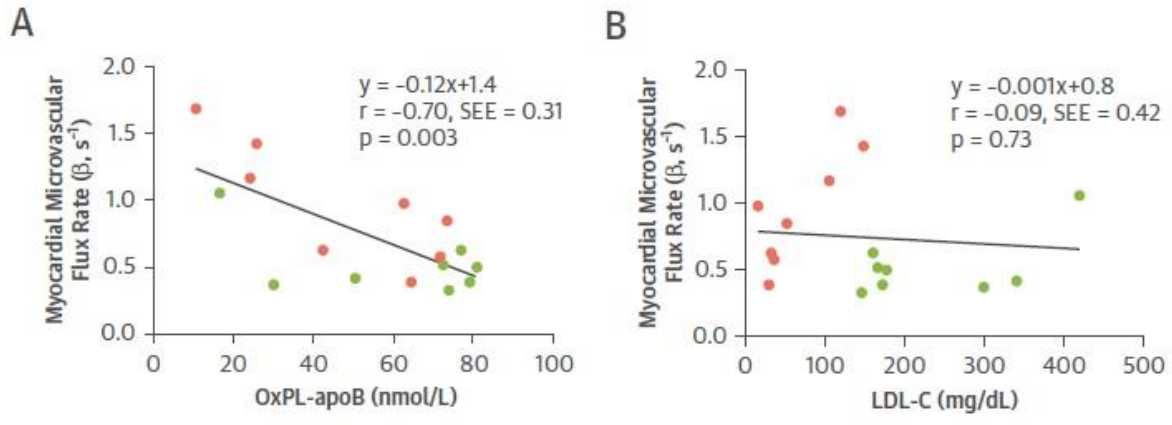

Figure 25. OxPL-apoB as predictor of the change in myocardial perfusion. Correlations are shown for (A) OxPL on apo B-100 lipoproteins and (B) LDL cholesterol with myocardial perfusion on myocardial contrast echocardiography expressed as the microvascular blood flux rate (b). Data are combined for pre-apheresis (green) and post-apheresis (pink) stages. Modified from Wu et al., [224].

Taken together, the above studies showing that pelacarsen and LA significantly lower OxPL-apoB and OxPL-apo(a) levels suggest that the presence of OxPL on Lp(a) might, in part, mediate its detrimental effects. As a clinical corollary, the binding, inactivation and neutralization of OxPLs -for example, with an E06-like antibody-might be an alternative approach to reduce the Lp(a)-mediated risk of CVD. The observed effects of the above interventions on Lp(a) are concordant with most of the studies that assessed the effect of therapeutic interventions aimed at lowering lipid levels on plasma OxPL-apoB levels. These studies showed a modest-to-substantial concordance in the direction of changes in OxPL-apoB and Lp(a) levels. One exception is a study of bariatric surgery associated with potent reductions in weight and BMI (from 48 to 33 kg/m^2 at 12 months) [185]. Mean OxPL-apoB levels decreased 12 months after surgery by 25% (from 5.0 to 3.8 nM; $p = 0.001$), whereas

Lp(a) increased by 40% (from 10.2 to 16.9 mg/dL; $p = 0.002$) (Figure 26). The reductions in OxPL-apoB levels were accompanied by reductions in several other biomarkers of oxidized lipoproteins (IgG and IgM MDA-LDL autoantibodies and apoB-100-immune complexes). The interpretation of these findings was that weight loss resulted in improved hepatocyte function, leading to lower oxidative stress and thus a reduction in OxPL-apoB levels. Furthermore, the marked weight loss likely improved hepatocyte metabolic function, thereby increasing hepatocyte synthetic capacity and leading to enhanced Lp(a) production, with levels returning to or exceeding those observed before bariatric surgery. This explanation is consistent with data suggesting that progressive liver disease results in a reduction in Lp(a) levels [231]. Future studies might further address this aspect by measuring total OxPLs levels to assess the global effects on plasma OxPLs of weight loss interventions in patients with liver disease.

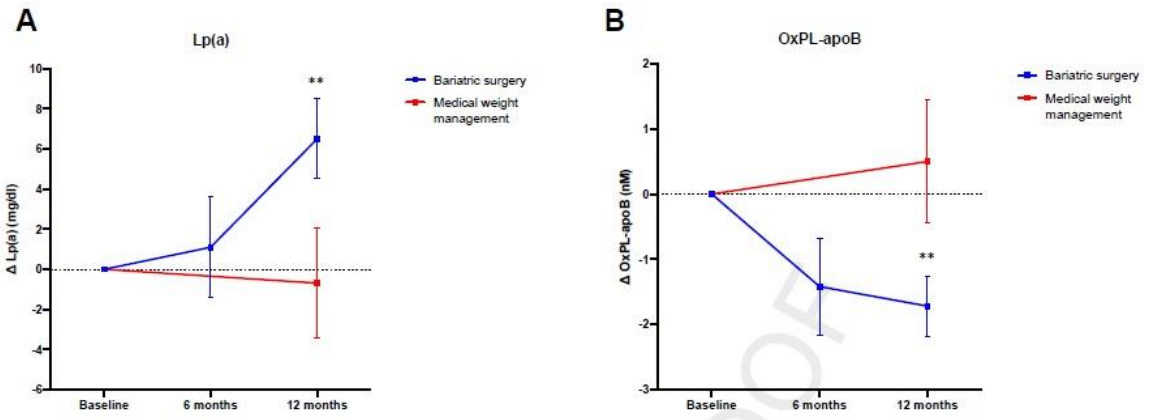

Figure 26. Absolute changes in A) lipoprotein(a) and B) OxPL-apoB from baseline to 6 months and 12 months after bariatric surgery and medical weight management. Modified from Ho et al., [185].

2.6.3. Low-fat, high-carbohydrate dietary interventions

Interestingly, low-fat, high-carbohydrate dietary interventions [232] and statin therapy tend to raise both OxPL-apoB [15, 229] and Lp(a) [15] levels. At that time, the increase in OxPL-apoB levels was difficult to interpret in view of the beneficial effects of both low-fat diets and statins on CVD events. However, these effects became clearer when newer studies showed that a low-fat diet and statin treatment increased the plasma levels of Lp(a), which is the main lipoprotein carrier of OxPLs [192].

2.6.4 Statins

An analysis of 13 distinct cohorts of patients receiving statin therapy for the first time, with follow-up measurements of plasma OxPL-apoB and Lp(a) levels, show mean pooled estimates of 19% and 10% change, respectively (Figure 27). Of the 13 cohorts, 12 showed increases in OxPL-apoB levels after statin therapy initiation, with a mean increase of 19% [86, 194, 207, 208, 233-236]. Lp(a) measurements were available in 10 cohorts, and 7 of these showed increases in Lp(a) levels after statin therapy initiation, with a mean increase of 10% [86, 198, 233-236].

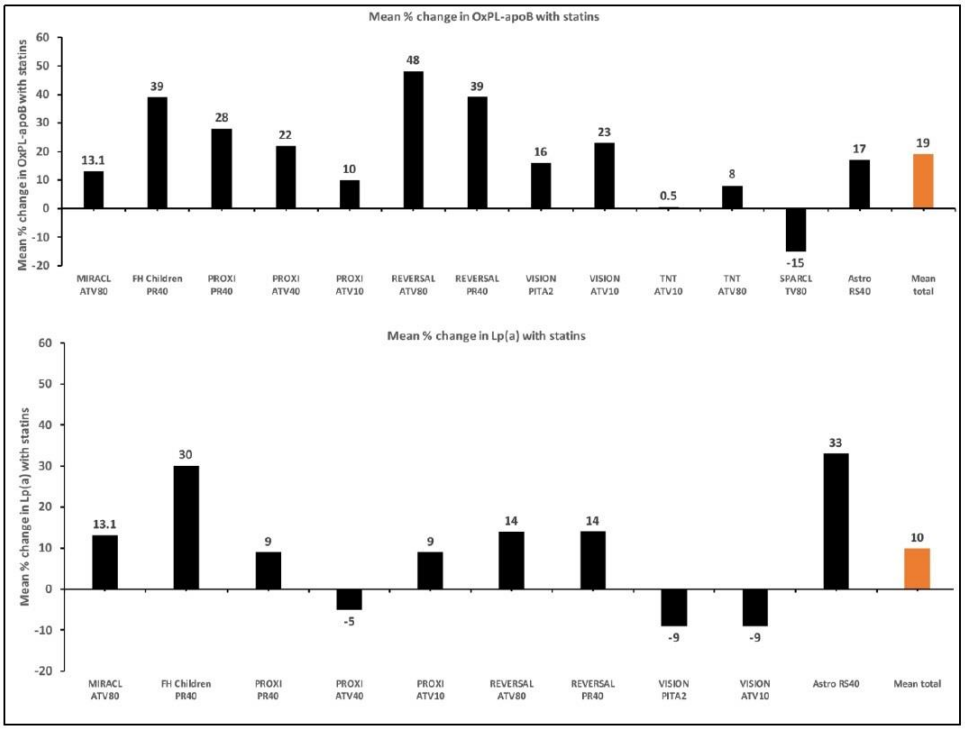

Figure 27. Mean percentage change from baseline in oxidized phospholipids and lipoprotein(a) in response to first-time statin therapy. The figure displays three distinct cohorts of patients receiving statin therapy for the first time, with follow-up measurements of plasma OxPL-apoB and lipoprotein(a) (Lp(a)) levels using the same methodology in all studies. In the vast majority of the studies, an increase in OxPL-apoB is present, with an overall mean increase of 19%. Lp(a) measurement was available in 10 cohorts and 7 of the 10 showed an increase in Lp(a) levels, with a mean increase of 10%. ApoB, apolipoprotein B; OxPL, oxidized phospholipid. Modified from Tsimikas & Witztum, [92].

In a previous, patient-level meta-analysis (n = 5256) of studies using the same Lp(a) assay, the mean percentage change from baseline ranged from 8.5% to 19.6% in the statin groups and -0.4% to -2.3% in the placebo groups [15] (Figure 28). The mechanism underlying the increase in Lp(a) with statin therapy is not known, but preliminary data suggest that the increase might be attributable to an elevation in PCSK9 protein level, which then may induce a higher production rate of apo(a) [15, 237]. The source of enriched OxPLs in Lp(a) is also not known but might reflect changes in fatty acid substrates and oxidative stress in the liver,

given that no evidence is available to date to indicate that the OxPL-apoB measure reflects the oxidation of lipids on Lp(a) itself [238].

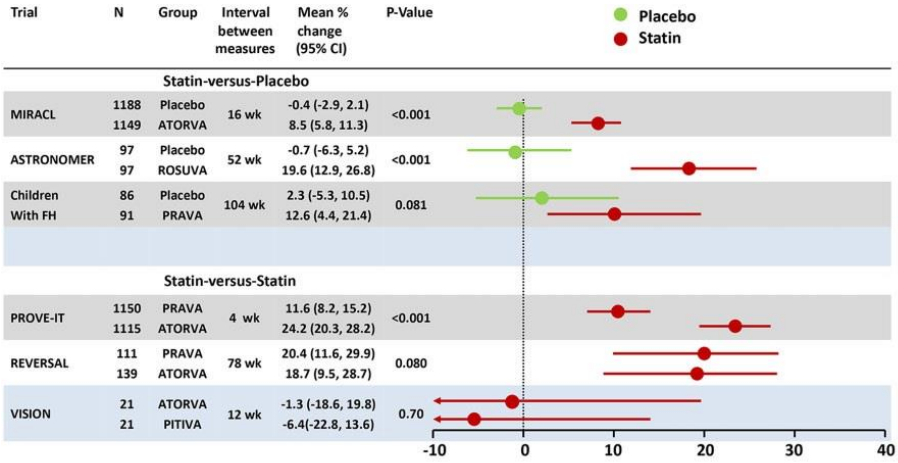

Figure 28. The forest plot shows the mean percent changes (95% confidence interval) in lipoprotein(a) values in the placebo and statin groups in individual trials. Modified from Tsimikas et al., [15].

Despite these potentially unfavourable changes in Lp(a) and OxPL-apoB levels with statins, patients with elevated Lp(a) levels do benefit from statin therapy. However, these patients tend to derive less benefit from statins than individuals with lower Lp(a) levels and with similar achieved LDL-C levels, as suggested in multiple studies, including the 4S study [239] and the PCSK9 inhibitor studies [135, 136]. However, a retrospective study of consecutive patients undergoing percutaneous coronary intervention showed that plasma Lp(a) levels increased by 19% after initiation of statin therapy, and patient with the highest increase (>10 mg/dL) had the highest rates of MACE at 3 years, suggesting that the effect might not be benign or neutral [240]. This is an area of active interest and requires further mechanistic and clinical studies.

2.6.5 siRNA agents

OxPL-mediated risk of CVD might be addressed by therapeutic use of oxidation-specific antibodies [196], and OxPLs specifically present on Lp(a) might be reduced by novel RNA therapeutics targeting Lp(a) production and assembly [15, 152]. The study by O'Donoghue et al was a randomized, double-blind, placebo-controlled, dose-finding trial involving patients with established ASCVD and a Lp(a) concentration of more than 150 nmol/L. Participants were randomly assigned to receive one of 4 doses of olpasiran (10 mg every 12 weeks, 75 mg every 12 weeks, 225 mg every 12 weeks, or 225 mg every 24 weeks) or matching placebo,

administered subcutaneously. The primary end point was the percent change in the Lp(a) concentration from baseline to week 36. At 36 weeks, the Lp(a) concentration increased by a mean of 3.6% in the placebo group, whereas olpasiran therapy led to a significant reduction of Lp(a) concentration in a dose-dependent manner, resulting in placebo-adjusted mean percent changes of -70.5% with the 10 mg dose, -97.4% with the 75 mg dose, -101.1% with the 225 mg dose administered every 12 weeks, and -100.5% with the 225 mg dose administered every 24 weeks ($p < 0.001$ for all comparisons with baseline) (Figure 29). Interestingly an off-treatment effect follow-up reported that participants receiving doses ≥75 mg Q12W sustained a ∼40% to 50% reduction of Lp(a) levels close to 1 year after the last dose [241]. Subsequently, the results of phase 2 dose-ranging trial of olpasiran in 281 individuals with ASCVD and elevated Lp(a) levels demonstrated a significant and sustained reduction in OxPL-apoB [242]. Specifically, the placebo-adjusted mean percentage change in OxPL-apoB from baseline to week 36 was -51.6% (95% CI, -64.9% to -38.2%), -89.7% (95% CI, -103.0% to -76.4%), -92.3% (95% CI, -105.6% to -78.9%) and -93.7% (95% CI, -107.1% to -80.3%) for the 10 mg Q12W, 75 mg Q12W, 225 mg Q12W and 225 mg Q24W dose, respectively (all $p < 0.001$). In addition, there was a strong correlation between percentage reduction in Lp(a) and OxPL-apoB for patients treated with olpasiran ($r = 0.79$; $p < 0.001$).

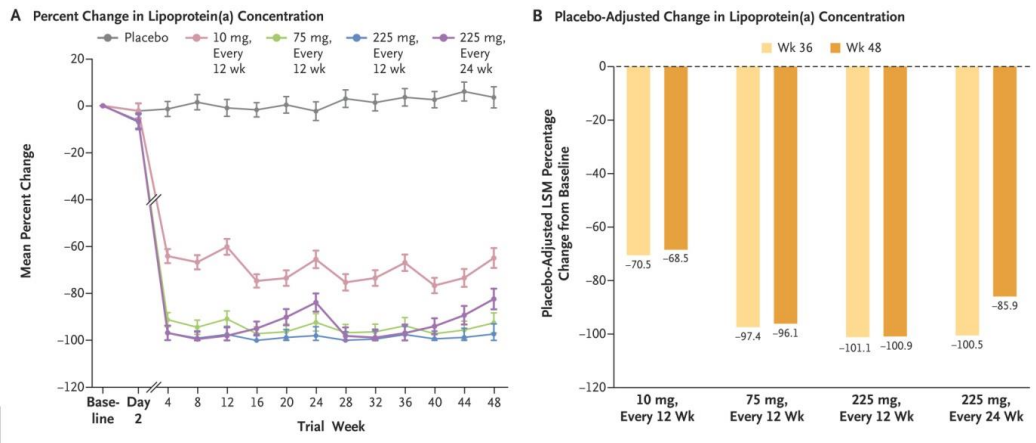

Figure 29. Percent change in lipoprotein(a) concentration and the placebo-adjusted mean percent change from baseline in the lipoprotein(a) concentration with olpasiran at weeks 36 and 48. Modified from O'Donoghue et al., [241].

Lepodisiran, another short interfering RNA directed at hepatic synthesis of apo(a), was tested in a single ascending-dose trial conducted at 5 clinical research sites in the US and Singapore [157]. The trial enrolled 48 adults without CVD and with Lp(a) serum concentrations of 75 nmol/L or greater (≥30 mg/dL). This phase 1 study reported that lepodisiran was well tolerated and produced dose-dependent, long-duration reductions in serum Lp(a)

concentrations; however, neither OxPL-apoB nor OxPL-apo(a) were included as study outcomes. In a subsequent study lepodisiran reduced mean serum concentrations of Lp(a) from 60 to 180 days after administration, but no data on OxPL-apoB/OxPL-apo(a) were reported [243]. Similarly, zerlarisan reduced Lp(a) in phase I [155] and phase II trials [156], but effects on OxPL-apoB/OxPL-apo(a) were not reported. Finally, muvalaplin reduced Lp(a) up to 65% following daily administration for 14 days phase I trial [158] and by ~45-85% in phase II trial [159], but again effects on OxPL-apoB/OxPL-apo(a) were not mentioned.

Gaps within this research field include the limited data regarding OxPL-apoB and therapeutic interventions, including the effects of the Mediterranean, plant-based and ketogenic diets as well as the effects of drugs including antioxidants, ezetimibe, bempedoic acid, ω-3 fatty acids, glucagon-like peptide 1 receptor agonists, CETP and PCSK9 inhibitors. PCSK9 inhibitors resulted in no significant difference in the mean per cent change in OxPL-apoB in both the ANITSCHKOW and EQUATOR studies [244], but this finding requires confirmation in larger studies. There are no studies addressing the impact of PCSK9 inhibitors on OxPL-apo(a). Monotherapy with extended-release niacin lowers OxPL-apoB (by 17%), as demonstrated in one study where OxPL-apoB and Lp(a) were determined in 591 patients at baseline and after 24 weeks [229]. In the same study the combination of ezetimibe, simvastatin and niacin increased OxPL-apoB by 26% [229].

Table 2 presents the various therapeutic interventions and their effect on plasma OxPL-apoB/OxPL-apo(a) and Lp(a) levels.

Table 2. Agents modifying OxPL-apoB/OxPL-apo(a) concentrations and their possible mechanisms of action

Lp(a)-Lowering Therapy	Plasma Lp(a)	Plasma OxPL-apoB/OxPL-apo(a)
Lipoprotein apheresis	25-40% reduction [119].	50-75% reduction [224, 228].
Statins	7-26% increase (mean 10.6%).	8-48% increase [86, 194, 207, 208, 233-236].
Low-saturated-fat diets	7-79% increase (mean 19.4%).	12-54% increase (mean 21.3%) [15, 150].
Bariatric surgery	~11.3% increase.	~2% reduction [185].
Niacin	20-30% reduction.	~17% reduction [229].
PCSK9 inhibitors	~25-30% reduction.	Neutral effect [135, 136, 244].
CETP inhibitors	~40% reduction.	Unknown effect.
ASO targeting apo(a) (pelacarsen)	Reduction up to 92.4%.	Reduction up to 72% [15, 150].
AMG 890 (olpasiran)	Reduction of > 90% [15; 152].	Reduction of ~50-90% across dose range [242].
Lepodisiran	Reductions ~40-95% [157, 243].	Unknown effect [157, 243].
Zerlasiran	Reductions ~95% [155, 156].	Unknown effect [155,156].

Muvalaplin	Reductions 45-85% [158, 159].	Unknown effect [158,159].

CHAPTER 3. PLASMINOGEN, LIPOPROTEIN(a) AND OXIDIZED PHOSPHOLIPIDS

3.1 Lp(a) AND FIBRINOLYSIS

While much in vitro data point to a role for Lp(a) in inhibiting fibrinolysis, in vivo findings [38] and a recent translational ex vivo study [245] generally cast doubt on this theory. Physiological hemostasis is initiated on tissue factor (TF)-bearing cells, and results in a small amount of thrombin that mediates the amplification phase which produces the active coagulation factors and active cofactors required for the propagation phase (Figure 27) [4]. This phase, occurring on the negatively charged surface of activated platelets, produces the large amounts of thrombin required for formation of a stable fibrin clot. Clotting can also be initiated by the contact pathway; this pathway may be particularly important in atherothrombosis where the ruptured atherosclerotic plaque could provide the necessary surface [4]. Lp(a) and apo(a) can promote TF expression on monocytes and can bind to and TFPI and may also promote platelet activation and aggregation [193, 246] (Figure 30).

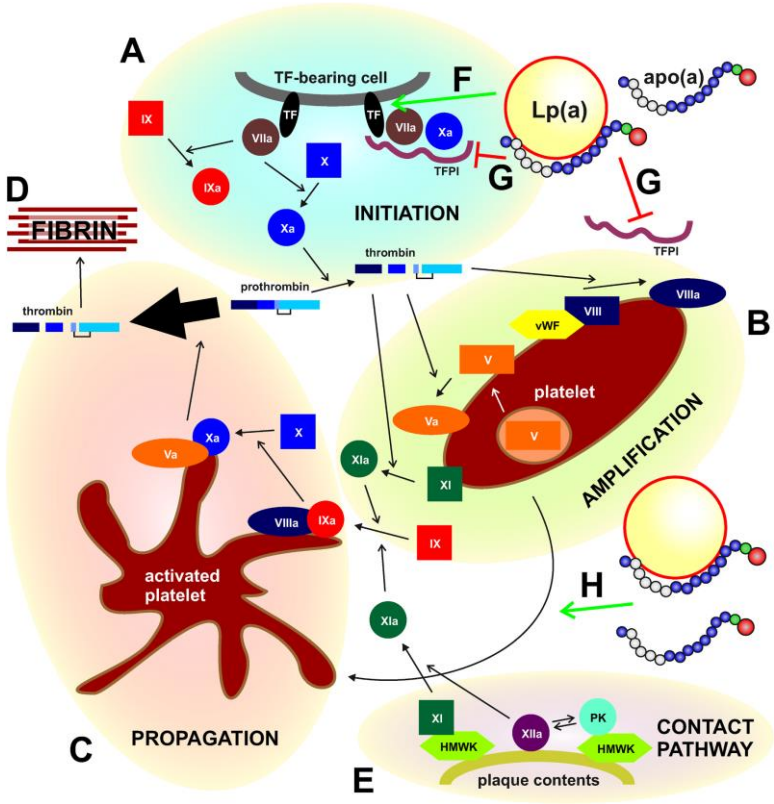

Figure 30. Interactions of lipoprotein(a) and apolipoprotein(a) with the coagulation system. HMWK: high molecular weight kininogen; PK: prekallikrein. Modified from Boffa, [4].

3.2 IMPAIRED FIBRINOLYSIS IN DYSLIPIDEMIA

Dyslipidemia promotes the progression of atherosclerosis, whereas impaired fibrinolysis can contribute to thrombosis upon atherosclerotic plaque rupture. Atherosclerotic plaque forms as lipids build up in the intima layer of the subendothelial matrix. Lipoproteins ionically bind to the negatively charged proteoglycans of the extracellular matrix on the arterial wall [247]. When this occurs, lipoproteins may become oxidized [248] and then engulfed by macrophages, which then become lipid-laden foam cells that exacerbate atherogenesis. When the plaque becomes overloaded with lipids, it becomes more vulnerable to rupture. When the rupture occurs, the exposed subendothelium triggers platelet activation and aggregation [249], and coagulation follows, which leads to a thrombus formation. When the fibrinolytic mechanism is impaired, the undissolved thrombus worsens the narrowed atherosclerotic artery to occlude, resulting in tissue ischemia. The coupling of dyslipidemia and impaired fibrinolysis contributes to atherosclerosis and severe thrombotic cardiovascular outcomes.

A recent review summarizes the correlations between tPA activity, PAI-1 antigen levels, atherogenic apoB-100-containing lipoproteins, and serum or plasma lipid levels [248]. Overall, the authors concluded that high tPA activity is associated with low triglyceride levels and apoB-100-containing lipoprotein levels. Patients with low tPA activity have the highest levels of serum cholesterol and triglyceride, while high serum triglyceride levels are associated with high PAI-1 antigen levels. Also, high Lp(a) levels are associated with high PAI-1 activity and inhibition by Lp(a) is dependent on the interaction of apo(a) with fibrin [248].

Apo(a) on Lp(a) can bind to fibrin through its lysinebinding site on its Kringle 4 domain [250]. The interaction between apo(a) and fibrin becomes competitive for PLG/fibrin binding, which halts the conversion of PLG to plasmin, thus impairing the fibrinolysis mechanism that follows this reaction [25, 193, 250]. Lp(a) also inhibits fibrinolysis by inhibiting the conversion from glutamine-PLG to lysine-PLG, which is a better substrate for tPA [246].

Some evidence for an adaptive role of Lp(a) in inhibiting clot lysis (or promoting hemostasis) comes from a Mendelian randomization study which found that elevated Lp(a) and genotypes associated with elevated Lp(a) decreased risk of major bleeding in the brain and airways [251]. The major focus regarding the prothrombotic effects of Lp(a) has been on inhibition of PLG activation in the context of the fibrin clot and thus inhibition of clot lysis. However, Lp(a) can also inhibit plasmin generation on the surface of endothelial cells, monocytes/macrophages, and smooth muscle cells [252]. The reduction in plasmin generation could impact both the development of atherosclerosis as well as the clearance of arterial

thrombi [25, 193]. Others have speculated that elevated Lp(a) may predispose to worse clinical outcomes in patients with COVID-19 [253]. This is based on the potential antifibrinolytic and pro-inflammatory activity of Lp(a) and the dramatically enhanced occurrence of venous and arterial thrombosis in these patients, as well as the presence of airway fibrin deposition in COVID-19-related acute respiratory distress syndrome [254]. Notably, Lp(a) concentrations could be acutely raised in COVID-19 by the action of IL-6 [253].

Three studies have taken the first steps to examine these possibilities. Di Maio and coworkers examined the UK BioBank and found that elevated Lp(a) was causally associated with increased risk for ischemic heart disease in both population controls (n = 435,104) and SARS-CoV-2 positive patients [23]. However, the risk related to elevated Lp(a) appeared to be heightened in COVID-19 patients. On the other hand, elevated Lp(a) did not influence the risk for VTE in either population [23]. Lippi et al., [255] studied 50 COVID-19 patients and 30 matched controls who had respiratory symptoms but were COVID-19-negative. Lp(a) concentration was not elevated in the COVID-19 patients but was significantly associated with severity of COVID-19 illness, including acute kidney failure stage and admission disease severity. Additionally, a report from Nurmohamed and coworkers studied 219 COVID-19 patients admitted to hospital, with 146 having at least 4 serial blood samples available for measurement of Lp(a) and inflammatory markers [256]. Together, these studies suggest that while Lp(a) may not consistently rise during acute COVID-19 infection, elevated baseline Lp(a) could contribute to increased cardiovascular risk in affected patients. However, the precise role of Lp(a) in COVID-19 pathophysiology remains uncertain and warrants further investigation.

3.3 BENEFICIAL EFFECTS OF LIPID-LOWERING THERAPIES ON FIBRINOLYSIS

Lipid-modifying therapies aim to reduce both cholesterol and triglyceride levels associated with apoB-100-lipoproteins [257]. These therapies also show improving fibrinolytic capacity (Table 3) [258-265].

Table 3. Summary of lipid-lowering therapies that improve fibrinolysis in humans

Medication	Mechanism	Effect on blood lipids	Effect on fibrinolysis
Statins	Inhibit β-hydroxy β methylglutaryl-CoA reductase activity, resulting in increased SERBP2 and LDLR gene expression.	Mostly lower LDL cholesterol.	Decrease mean clot lysis time, increase blood tPA, decrease plasmin inhibitor and TAFI activity [258]; Increase clot permeability [259].
Ezetimibe	Increases flow-mediated dilation (FMD), resulting in improved endothelial function.	Mostly lower LDL cholesterol.	Proinflammatory LPS stimulation and platelet–endothelium interactions increased endothelial uPAR expression, which was significantly attenuated by pre-incubation with ezetimibe (100-1000 ng/ml) [266].
Bembedoic acid	Inhibits ATP-citrate lyase (ACLY) that converts citrate (exported from mitochondria) to acetyl-CoA.	Mostly lower LDL cholesterol.	Reduced lipid accumulation in the aortic wall, suggesting a protective effect on endothelial integrity [267].
PCSK9 inhibitors	Increase hepatic LDLR levels by inhibiting PCSK9-mediated LDLR endocytosis and degradation.	Mostly lower LDL cholesterol.	Reduce venous thromboembolism risk and Lp(a) levels [260].
Fibrates	Activate peroxisome proliferator-activated receptor alpha (PPARα), resulting in reduced VLDL production and increased LPL activity.	Mostly lower plasma triglyceride.	Decrease plasma PAI-1 and increase tPA release [261]; Increases clot permeability and decreases clot lysis time [259].
Niacin	Decrease HDL clearance rate.	Mostly increases HDL cholesterol.	Decrease PAI-1 [Brown et al., 1995] and Lp(a) levels [263].

Statin use in the acute phase of stroke after thrombolysis may improve both short-term (7 days after onset of stroke) and long-term (3 months after onset of stroke) neurologic function [268]. In patients with coronary artery disease, statin use increases plasma fibrinolytic potential [258]. Specifically, following statin treatment, the mean clot lysis time decreased, tPA levels increased, and plasmin inhibitor levels and TAFI activity decreased [258] (Figure 31). Statins also increase clot permeability [259].

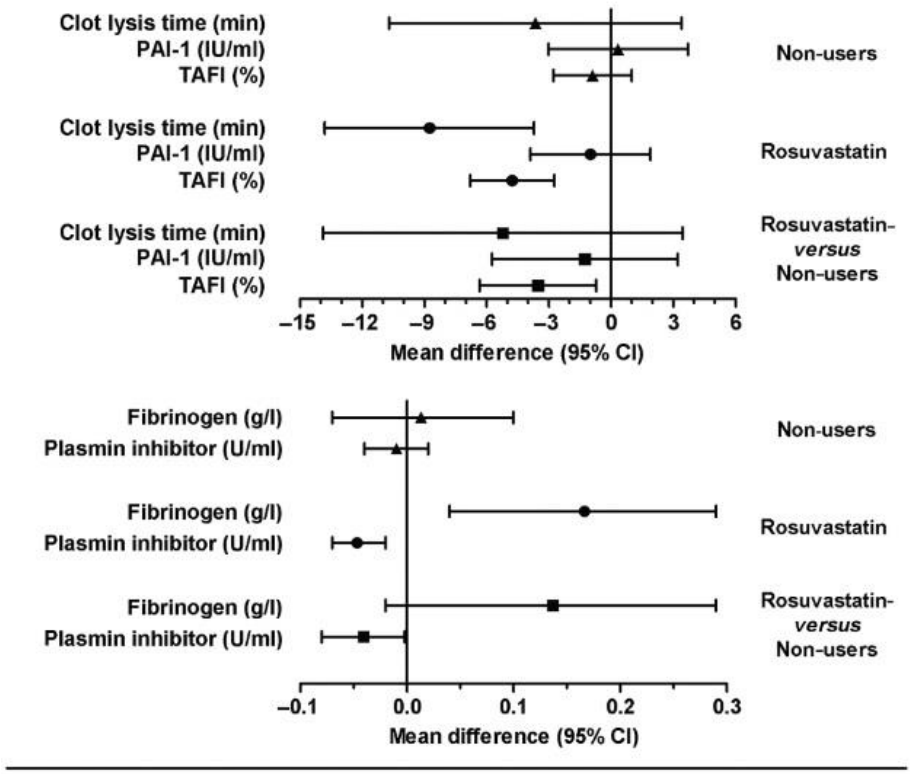

Figure 31. Effects of rosuvastatin on measures of fibrinolysis. Modified from Schol Gelok et al., 2020.

PCSK9 binds to the cell surface of LDLR and reduces LDLR by increasing its intracellular lysosomal degradation [264]. Treatment with PCSK9 inhibitors leads to increased cell surface LDLR [269]. Interestingly, the use of PCSK9 inhibitors has been shown to reduce venous thromboembolism risk. In the FOURIER study of a population with high cardiovascular risk, evolocumab reduced the HR for venous thromboembolism events and plasma Lp(a) levels beyond one year [270] (Figure 32). Subgroup analyses showed that evolocumab reduced Lp(a) by 33 nmol/L and risk of venous thromboembolism by 48% in patients with higher baseline Lp(a) levels, whereas evolocumab reduced Lp(a) by only 7 nmol/L and had no effect on venous thromboembolism risk in patients with lower baseline Lp(a) levels. Modeled as a continuous variable, there was a significant interaction between baseline Lp(a) concentration and venous thromboembolism risk reduction, suggesting that Lp(a) reduction may be an important mediator of the protective effect of PCSK9 inhibition on venous thromboembolism. Because Lp(a) inhibits fibrinolysis by inhibiting PLG activation, increasing fibrin density, and enhancing platelet activation [2]. Future studies should investigate the correlation of Lp(a), fibrin density, and platelet activity with venous thromboembolism incidence.

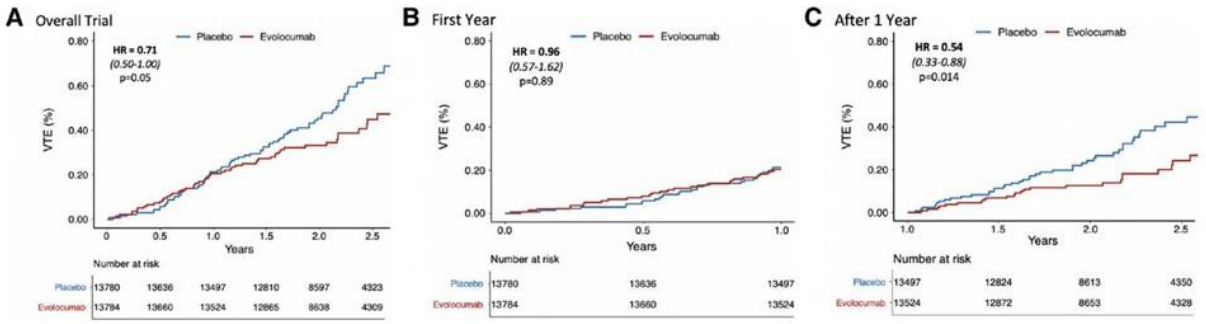

Figure 32. Effect of evolocumab on the reduction in venous thromboembolism. A, A 29% reduction in the overall FOURIER trial (HR, 0.71 [95% CI, 0.50-1.00]; p = 0.05) is demonstrated. B, No difference in VTE during the first year (HR, 0.96 [95% CI, 0.57-1.62]; p = 0.89) is shown. C, A landmark analysis highlighting the 46% reduction in VTE beyond 1 year (HR, 0.54 [95% CI, 0.33-0.88]; p = 0.014). FOURIER indicates Further Cardiovascular Outcomes Research with PCSK9 Inhibition in Subjects With Elevated Risk; HR, hazard ratio; and VTE, venous thromboembolism.

Therapeutic intervention to decrease triglyceride and increase HDL cholesterol levels with a goal of lowering atherosclerosis risk also decreases clot lysis time and increases tPA release, suggesting increased fibrinolysis [259, 261, 265, 271, 272, 274]. Fibrates, which are peroxisome proliferator-activated receptor alpha (PPARα) activators, led to decreased plasma PAI-1 level and increased tPA release upon venous occlusion stimulation [261]. Fibrates also increase clot permeability and decrease clot lysis time [259]. Niacin, a type of vitamin B that increases HDL cholesterol [275], attenuates the synthesis of PAI-1 [262] and lowers Lp(a) [263].

Recombinant tPA has been approved as a thrombolytic therapy in thrombotic CVD, including ischemic stroke and myocardial infarction [276]. Interestingly, human studies showed decreased total cholesterol and LDL-C after use of recombinant tPA in patients with ischemic stroke [277]. Also, tPA infusion therapy leads to decreased plasma apoB-100 and Lp(a) levels in patients with unstable angina [278].

CHAPTER 4. AIM AND EXPERIMENTAL DESIGN

4.1 BACKGROUND AND AIM

As discussed above in detail, over the past few years several studies have focused on Lp(a), a lipid molecule with atherogenic, thrombogenic and inflammatory properties [26]. Elevated Lp(a), mostly genetically determined by the LPA gene locus, has been identified as an important cardiovascular risk factor as well as a risk factor for CAVS, with a prevalence rate of ~20% in the general population [31]. Currently, there are no medications specifically approved for Lp(a) lowering [15, 21, 67]. In this context, current guidelines recommend optimal control of other cardiovascular risk factors 33]. In terms of cholesterol management, statins remain the cornerstone of lipid-lowering management, but they slightly increase Lp(a) levels [190, 124]. Although most lipid-modifying agents do not significantly alter Lp(a) concentration [17, 21, 67], PCSK9 inhibitors have been shown to reduce Lp(a) levels [35, 133, 134].

ASCVD remains the leading cause of mortality worldwide despite intensive LLT [279]. OxPLs contribute to atherosclerosis by promoting inflammation and oxidative modification of lipoproteins, while PLG plays a central role in fibrinolysis that can be modified by OxPLs, potentially affecting thrombotic risk [21]. Lp(a), structurally similar to LDL, is strongly linked to ASCVD [21]. Apo(a) shares homology with PLG, potentially interfering with fibrinolysis [21]. Importantly, Lp(a) is the preferential lipoprotein carrier of OxPLs in the bloodstream [21].

OxPLs, arising from oxidative modifications of phospholipids, are involved in inflammation, immunity, and lipid metabolism [21]. They primarily associate with apo(a) [OxPL-apo(a)] and apoB-100 [OxPL-apoB], a key component of various lipoproteins, including Lp(a) [91, 193]. OxPLs accumulate in atherosclerotic lesions and impact vascular endothelial cells, immune cells, and coagulation, influencing the pathogenesis of atherosclerosis and thrombosis [21]. Increased levels of OxPL-apoB and OxPL-apo(a) are associated with higher risks of cardiovascular events [92].

PLG is the precursor to plasmin, responsible for fibrinolysis, and exhibits pleiotropic functions in extracellular matrix remodeling, cell migration, and inflammatory signaling [280]. OxPLs are also bound covalently to PLG, and when they are enzymatically removed are associated with longer time to fibrinolysis [281]. Increased levels of OxPL-PLG are associated with lower risk of acute thrombotic events [10, 280].

The effects of currently used lipid-lowering medications on OxPL levels remain unclear. Statins show mixed effects on OxPL-apoB [282, 229] with limited data on OxPL-apo(a) [282]. Ezetimibe's effect on OxPL-apoB and OxPL-apo(a) is not well studied. PCSK9 inhibitors have a neutral effect on OxPL-apoB [135, 136, 244, 134], but data for any effect on OxPL-apo(a) are lacking. Novel Lp(a)-lowering agents, pelacarsen and olpasiran, has shown significant reductions in OxPL-apoB and OxPL-apo(a) levels [150, 242], but for other novel pharmacological agents (Lepodisiran, Zerlasiran, muvalaplin) the effect on OxPL-apoB/OxPL-apo(a) levels remains unknown [15, 152, 155-159].

Atherosclerosis is characterized by chronic inflammation and oxidative modification of lipoproteins, particularly LDL [283]. Among oxidative modifications, malondialdehyde (MDA) is a highly reactive byproduct of lipid peroxidation formed during the oxidative degradation of polyunsaturated fatty acids, reacting with peptides including apoB-100 to form MDA-modified apoB-100 (MDA-apoB) adducts or neoepitopes [284]. These MDA-apoB adducts are recognized as non-self by the immune system, thereby triggering humoral immune responses, including the production of IgG and IgM autoantibodies against MDA-modified epitopes [285]. MDA-modified apoB is considered an important biomarker of oxidative stress, inflammation, endothelial dysfunction, immunogenicity and atherogenicity in ASCVD [286-288]. Autoantibodies generated by these oxidative modifications can be measured as autoantibodies targeting apoB-100-containing immune complexes (IgG and IgM apoB-IC), as well as autoantibodies targeting MDA-modified LDL, which can be detected using an MDA-peptide mimotope mimicking oxidation-specific epitopes on apoB-100 (IgG and IgM anti-MDA-mimotope) [289]. Elevated IgG autoantibodies against MDA-apoB, especially against MDA-modified peptide epitopes such as p45 and p210, have been linked to incident cardiovascular events and plaque instability [288, 290].

Elevated Lp(a) concentrations are associated with increased oxidative burden and immune activation, which may predispose to enhanced apoB-100 modification and autoantibody generation [85, 238, 281]. Lp(a) carries a disproportionately high content of OxPLs, particularly on its apo(a) moiety, which colocalizes with apoB-100 on the LDL-like particle [85, 238, 281]. OxPLs on Lp(a) serve as potent epitopes for immune recognition, leading to the generation of circulating IgG and IgM autoantibodies targeting oxidation-specific epitopes, including MDA-apoB and mimotopes of oxLDL [85, 238, 281]. These immunogenic features may contribute to the proatherogenic and proinflammatory properties of Lp(a) [85, 235, 281].

Although high-intensity statins, ezetimibe and PCSK9 inhibitors are effective in lowering LDL-C and apoB-100 concentrations [39] their effect on oxidized forms of apoB-100 and immune responses remain less well understood. To our knowledge, no study has evaluated how these treatments impact IgG and IgM autoantibodies against apoB-IC or anti-MDA-mimotope, either in the general population or in individuals with elevated Lp(a) concentrations.

Taken together, these considerations highlight the need to examine lipid-lowering therapy in patients with elevated Lp(a) at clinical, biochemical, and immunologic levels. In this context, the aim of this thesis was to evaluate the effect of hypolipidemic therapies on patients with elevated levels of Lp(a). Specifically, the thesis aimed to evaluate: (i) the effect of hypolipidemic therapies on the lipidemic profile of patients with elevated levels of Lp(a) (≥75 nmol/L); (ii) the impact of high-intensity statins, add-on ezetimibe and add-on PCSK9 inhibitors on OxPL-apoB, OxPL-apo(a) and OxPL-PLG levels in individuals with elevated Lp(a) concentrations; and (iii) the effect of high-intensity statin treatment, add-on ezetimibe and add-on PCSK9 inhibitor therapy on IgG and IgM autoantibodies to apoB-IC as well as IgG and IgM anti-MDA-mimotope.

4.2 RESEARCH DESIGN

The present study included consecutive adult patients with elevated Lp(a) levels and dyslipidemia attending the Outpatient Lipid Clinic of the University Hospital of Ioannina. Patients were non-randomly allocated to three treatment groups according to the national guidelines for the management of dyslipidemias [291]. The 10-year total cardiovascular risk was estimated using the Systematic Coronary Risk Evaluation (SCORE) 2 model in apparently healthy subjects aged 40-69 years and the SCORE 2 old persons (OP) model in those aged ≥70 years [292] These models are recommended for the implementation of primary prevention strategies and have been integrated into the European Society of Cardiology prevention guidelines [39].

According to these guidelines, patients were allocated to one of the following treatment strategies: high-intensity statin monotherapy, add-on ezetimibe on stable high-intensity statin treatment, or add-on PCSK9 inhibitor (evolocumab or alirocumab) on stable high-intensity statin treatment plus ezetimibe (Figure 33). Patients receiving add-on ezetimibe or PCSK9 inhibitor had been on high-intensity statin treatment or high-intensity statin treatment plus ezetimibe, respectively, for at least three months prior to treatment escalation. High-intensity

statin treatment was defined as rosuvastatin 20-40 mg or atorvastatin 40-80 mg [39]. Follow-up duration was three months.

All study participants were of Caucasian origin. The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki and was approved by the Ethics Committee of the University General Hospital of Ioannina, Greece (896/21-12-2020). Written informed consent was obtained from all participants.

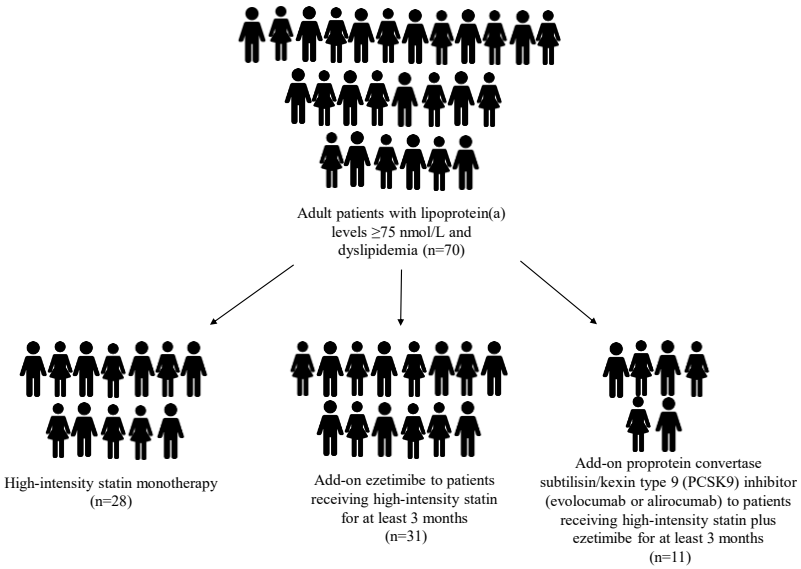

Figure 33. Study design and allocation groups.

4.3 METHODS

4.3.1 Sample

The study population consisted of consecutive adult patients with elevated Lp(a) levels (≥30 mg/dL or ≥75 nmol/L) according to the Hospital Biochemical Laboratory) and dyslipidemia. A complete assessment of demographic and clinical characteristics was performed at baseline and at three months following initiation or modification of lipid-lowering treatment. Recorded variables included sex, age, smoking history, concomitant diseases with emphasis on established ASCVD, including coronary artery disease, stroke, peripheral artery disease, and carotid stenosis ≥50%, as well as CAVS and cardiometabolic risk factors. BMI and blood pressure (BP) were recorded for all participants.

Office BP measurements were performed according to ESC/ESH guidelines using a validated upper-arm cuff BP measurement device and appropriate cuff size [293] Height and weight

were measured using validated and calibrated instruments, and BMI was calculated as weight (kg) divided by height squared (m²). HeFH was defined according to the Dutch Lipid Clinic Network (DLCN) criteria [294].

4.3.2 Measurements

4.3.2.1 Routine laboratory assessments

Venous blood samples were obtained in the morning after overnight fasting of at least 8-12 hours, using sterile Vacutainer-SST II advance tubes (Becton-Dickinson, Plymouth, UK) and potassium EDTA tubes, as appropriate. Tubes were refrigerated immediately after collection, centrifuged at 4 °C within 40 minutes, and analyzed within two hours or stored at −80 °C until analysis. Plasma and serum samples were subjected to no more than two freeze-thaw cycles.

Routine laboratory measurements included fasting plasma glucose, insulin, uric acid, creatinine, and a complete lipid profile comprising total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), LDL-C, apoB-100, and Lp(a). Serum TC and TG were determined enzymatically using the Olympus AU600 Clinical Chemistry Analyzer (Olympus Diagnostica, Hamburg, Germany). HDL-C was measured by a direct assay (Olympus Diagnostica, Hamburg, Germany). LDL-C was calculated using the Friedewald formula when TG levels were <400 mg/dL (4.5 mmol/L). Renal function was estimated using the CKD-EPI equation based on creatinine values traceable to isotope dilution mass spectrometry [295].

Insulin resistance was estimated using the Homeostatic Model Assessment for Insulin Resistance (HOMA-IR), calculated as fasting plasma glucose (mg/dL) × insulin (mU/L) / 405 [296]. Common reference levels for HOMA-IR range from 0.7 to 2.2. Lp(a)-cholesterol content was estimated as 30% of Lp(a) mass and subtracted from LDL-C to derive corrected LDL-C [LDL-Ccorr = LDL-C − 0.3 × Lp(a)] [297]. Concomitant therapies were also recorded.

4.3.2.2 Measurement of Lp(a)

Lp(a) plasma concentrations were measured using a validated, isoform-independent enzyme-linked immunosorbent assay (ELISA) traced to WHO/IFCC reference material SRM 2B, ensuring accurate quantification irrespective of apo(a) isoform size [298]. The assay employed the LPA4 monoclonal antibody to capture plasma Lp(a) and a second monoclonal

antibody (LPA-KIV9) targeting a unique antigenic site on kringle IV type 9. The analytical range of the assay was 0.27-1.402 nmol/L and met strict criteria for accuracy, linearity, recovery, and precision.

4.3.2.3 Measurement of oxidized phospholipids

OxPL-apoB and OxPL-apo(a) reflect the relative enrichment of OxPLs on normalized amounts of apoB-100-containing lipoproteins or Lp(a), respectively, captured on microtiter plates, independent of total plasma concentrations. These assays therefore reflect the OxPL-carrying capacity per lipoprotein particle. Although Lp(a) is the major carrier of OxPLs, measuring OxPL-apoB provides additional information on OxPL content across other apoB-100-containing lipoproteins, including LDL and very low-density lipoprotein (VLDL).

Both the OxPL-apoB and the OxPL-apo(a) ELISA assays utilize a two-antibody system consisting of a capture antibody specific to the lipoprotein of interest and a detection antibody that targets OxPL. For the OxPL-apoB assay, the capture antibody is recombinant murine monoclonal IgG MB47, which binds an epitope on human apoB-100, ensuring selective binding to all apoB-100-containing particles. For the OxPL-apo(a) assay, the capture antibody is recombinant murine monoclonal IgG antibody LPA4, which recognizes the 14-amino acid peptide TRNYCRNPDAEIRP present on apo(a) KIV 5, KIV 7, and KIV 8 and the partial sequence NYCRNPDA present on KIV 2, enabling specific capture of Lp(a). In both assays, OxPL are detected using the well-characterized biotinylated murine IgM monoclonal antibody E06, which binds to phosphocholine-containing oxidized but not native phospholipids, thereby ensuring specificity for oxidatively modified phospholipids.

The assays were replicated at the Medpace Reference Laboratories based on the predicate methods developed at the University of California, San Diego (UCSD). MB47, LPA4, and E06 monoclonal antibodies were produced under controlled conditions from hybridoma-derived and recombinant expression systems (GenScript). Lot-to-lot comparability and functional equivalence were verified prior to assay implementation using standardized quality-control materials within the CLIA laboratory.

For both the OxPL-apoB and the OxPL-apo(a) chemiluminescent ELISAs, 96-well microtiter plates (Eppendorf) were coated overnight at 4^{o}C with 40 µl per well of either MB47 (5 µg/ml for OxPL-apoB) or LPA4 [5 µg/ml for OxPLapo(a)] in Dulbecco's phosphate-buffered saline (pH 7.0). Plates were then blocked with 1% bovine serum albumin (BSA; Sigma-Aldrich) in Tris-buffered saline (TBS) for 1 h at room temperature. Cartoons of the methodologies for the OxPL-apoB and OxPL-apo(a) assays are shown in Figure 34. Serum or EDTA plasma

samples are diluted 1:50 for OxPL-apoB and OxPL-apo(a) in TBS containing 0.025 mM Butylated hydroxytoluene (BHT), 1% BSA, 0.27 mM EDTA, and 0.02% sodium azide. Samples are added in duplicate (40 µl/well) and incubated for 1 h at room temperature. Following three washes with TBS-EDTA, biotinylated E06 (1 µg/ml) is added to each well and incubated for an additional hour. After washing, NeutrAvidin-conjugated alkaline phosphatase (AP; Thermo Fisher Scientific) with activators (1 M MgCl 2, 0.1 mM ZnCl 2) is applied for 1 h, followed by detection using 25 µl/well of Lumi-Phos 530 chemiluminescent substrate (Lumigen Inc). Plates are read using a BioTek Synergy H1 plate reader (Agilent Technologies), and relative light units per 100 ms (RLU) are recorded. Assay calibration curves are generated using serial dilutions of a pooled high-OxPL serum reference material (Medpace Reference Laboratories) whose values are referenced to known quantities of PC-BSA, and results are interpolated using a 4-parameter logistic fit. The OxPL-apoB and OxPL-apo(a) assays were implemented at the Medpace Reference Laboratories and the analytical validation was performed in accordance with the Clinical Laboratory Improvement Amendments (CLIA) and College of American Pathologists (CAP) accreditation requirements. Testing procedures were based on the Clinical and Laboratory Standard Institute (CLSI) method evaluation standard. Acceptability criteria for quantitation limits, as well as storage and freeze/thaw stability, were based on the 2022 FDA M10 Bioanalytical Method Validation and Study Samples (FDA M10 BMVSS) guidance. Validation parameters included within-run precision, total imprecision, linearity, analytical measuring range (AMR), lower and upper limits of quantification (LLOQ, ULOQ), spike and recovery, dilutability, accuracy, matrix equivalency, and reference ranges in normolipidemic individuals [299]. Results were expressed as nmol/L of phosphocholine equivalents.

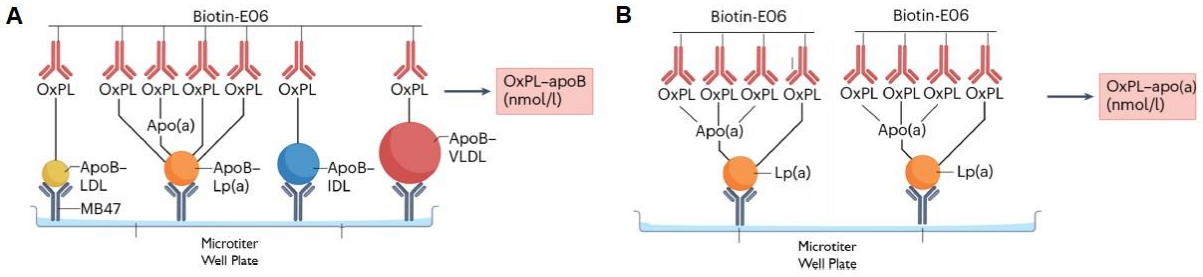

Figure 34. Methodologies for the OxPL-apoB and OxPL-apo(a) assays. (A) To quantify OxPL-apoB, the MB47 antibody is plated overnight at 5 µg/ml to bind apoB-100 on the microtiter plate; the excess material is then washed off, and the plasma sample is added at a 1:50 dilution to allow apoB-100 to bind to the immobilized MB47. Because MB47 recognizes all apoB-100-containing particles with similar specificity, the apoB-100 captured on the plate will reflect the proportion of apoB-100-containing particles that are present in the plasma sample. For example, if Lp(a) levels are elevated, a higher proportion of apoB-100 from Lp(a) will be captured on the microtiter well plate. Biotin-modified E06 (1 µg/ml) is then added to the plate to bind to the OxPLs present on apoB-100-containing lipoproteins, and streptavidin modified with alkaline phosphatase is added to

bind to the biotin–E06 in a 1:1 ratio. A chemiluminescent substrate for alkaline phosphatase (Lumi-Phos) is then added to generate light, which is directly proportional to the amount of phosphocholine-containing OxPLs present in the microtiter well plate and reported as RLU emitted per 100 ms. RLUs are then converted to nanomoles per liter using a standard curve of phosphocholine (PC) equivalents; (B) To quantify OxPL-apo(a), the LPA4 antibody is plated overnight at 5 μg/ml to bind apo(a) on the microtiter plate; the excess material is then washed off, and the plasma sample is added at a 1:50 dilution to allow Lp(a)/apo(a) to bind to the immobilized LPA4. Biotin-modified E06 (1 μg/ml) is then added to the plate to bind to the OxPLs present on Lp(a)/apo(a)-containing lipoproteins, and streptavidin modified with alkaline phosphatase is added to bind to the biotin–E06 in a 1:1 ratio. A chemiluminescent substrate for alkaline phosphatase (Lumi-Phos) is then added to generate light, which is directly proportional to the amount of PC-containing OxPLs present in the microtiter well plate and reported as RLU emitted per 100 ms. RLUs are then converted to nanomoles per liter using a standard curve of PC equivalents. Modified from Marcovina et al., 2026.

4.3.2.4 Measurement of oxidation-specific autoantibodies

Plasma IgG and IgM autoantibodies against apoB-IC and MDA-mimotope peptides were measured using validated chemiluminescent ELISAs adapted from previously published protocols [198, 208, 209]. Microtiter plates were coated either with monoclonal antibodies against apoB-100 (MB47) to capture circulating apoB-100-containing lipoproteins or with synthetic MDA-mimotope peptides. Diluted plasma samples were added in duplicate and incubated for two hours at room temperature. Internal controls consisting of high and low standard plasma samples were included on each microtiter plate to detect potential variations between plates. Each sample was assayed in triplicate, and data are expressed as relative light units in 100ms. The intra-assay coefficients of variation for all assays were 6% to 10%. For oxidation-specific autoantibody detection: Plasma titers of IgG and IgM antibodies against MDA-modified LDL were measured using defined plasma dilutions (e.g., 1:200 for MDA epitopes), based on previously validated oxidation-specific epitope immunoassays.

Bound antibodies were detected using alkaline phosphatase-conjugated goat anti-human IgG or IgM secondary antibodies, followed by chemiluminescent development with Lumi-Phos 530. Relative light units were measured using a BioTek SYNERGY HTX multi-mode luminometer. Results were expressed in arbitrary units relative to reference plasma samples. Internal high- and low-standard plasma controls were included on each plate. Intra-assay and inter-assay coefficients of variation were <10% and <15%, respectively.

4.3.3 Statistical analysis

Continuous variables were tested for normality using the Kolmogorov-Smirnov test. Data are presented as mean ± standard deviation (SD) for normally distributed variables and as median [interquartile range (IQR)] for non-normally distributed variables. Categorical variables are presented as frequency counts and percentages. Comparisons between categorical variables were performed using the chi-square (χ^2) test. Comparisons between baseline and post-

treatment measurements were performed using paired statistical tests (paired t-test or Wilcoxon signed-rank test, as appropriate) within each group. The independent samples t-test and Mann-Whitney U test were used for the comparison of continuous numeric values between 2 groups, as appropriate. One-way analysis of variance (one-way ANOVA) and Kruskal-Wallis test were used to assess differences in continuous variables among two or more groups, as appropriate. Multivariate analysis of covariance (MANCOVA) was used to compare continuous variables between two or more groups, adjusting for the baseline value of the variable of interest. In analyses examining OxPLs and immune-related biomarkers, MANCOVA models were additionally adjusted for key covariates, including age, sex, LDL-C, Lp(a), and ASCVD status. Correlation analyses were performed using Pearson's correlation coefficient to assess associations between Lp(a), LDL-C, oxidized phospholipid biomarkers [OxPL-apoB and OxPL-apo(a)], apoB-100, and IgG apoB-IC at baseline and follow-up, as well as between changes observed over time. Correlation analyses were conducted for the entire cohort and separately for each treatment group. All statistical tests were two-tailed, and statistical significance was defined as $p < 0.05$. Statistical analyses were performed using SPSS software (SPSS Statistics for Windows, Version 28.0; IBM Corp., Armonk, New York, NY, USA).

CHAPTER 5. RESULTS

5.1 SAMPLE

In total, 70 subjects were included. Mean age was 51 ± 15 years, 40% of participants were male, 39% were diagnosed with HeFH, and 16% had established ASCVD. Overall, 36%, 33%, and 15% of participants were classified as being at very high, high, and moderate cardiovascular risk, respectively (Table 4).

Table 4. Study participants baseline characteristics.

	High-intensitystatin monotherapy	Add-on ezetimibe to statin	Add-on PCSK9 inhibitor to statin and ezetimibe	Total sample
Number of patients (N)	28	31	11	70
Gender Male	32%	42%	55%	40%
Age (years)	45 (±16)	54 (±13)	55 (±14)	51 (±15)
Smoking				
Never smoker	64%	58%	60%	61%
Former smoker	11%	16%	30%	16%
Current smoker	25%	26%	10%	23%
Cardiovascular Risk Categories				
Low risk	26%	11%	9%	17%
Intermediate risk	22%	15%	0	15%
High risk	39%	37%	9%	33%
Very high risk	13%	37%	82%	36%
Arterial hypertension	11%	36%*	36%	26%
Type 2 diabetes	4%	23%*	20%	15%
Heterozygous familial hypercholesterolemia	21%	32%	100%*#	39%
Coronary heart disease	0	7%	64%*#	13%
Stroke	0	3%	9%	3%
Carotid stenosis	4%	0	9%	3%
Peripheral artery disease	0	0	18%*#	3%
Atherosclerotic cardiovascular disease	4%	10%	100%*#	16%
Calcific aortic valve stenosis	0	0	9%	1%
Chronic kidney disease	0	0	18%*#	3%

Data are presented as N (%). Parametric variables are presented as mean (±SD) and non-parametric as median (IQR). *$p < 0.05$ for the comparison with high-intensity statin monotherapy; #$p < 0.05$ for comparison with high-intensity statin plus ezetimibe. Abbreviations: LDL-C; Low-density lipoprotein cholesterol, PCSK9; proprotein convertase subtilisin/kexin type 9.

Regarding treatment allocation, 28 naïve patients received high-intensity statin monotherapy, 31 patients on stable high-intensity statin treatment received add-on ezetimibe, and 11 patients on stable high-intensity statin treatment plus ezetimibe received add-on PCSK9 inhibitor therapy. In the statin monotherapy group, all patients received rosuvastatin (median

dose 20 mg). In the statin plus ezetimibe group, 77% were on rosuvastatin (median dose 20 mg) and 23% on atorvastatin (median dose 40 mg). In the PCSK9 inhibitor group, 55% were on rosuvastatin (median dose 40 mg) and 45% on atorvastatin (median dose 40 mg).

Patients who received add-on ezetimibe ± PCSK9 inhibitor had significantly increased prevalence of arterial hypertension and type 2 diabetes compared with patients on high-intensity statin monotherapy ($p = 0.026$ and $p = 0.033$, respectively). In addition, patients on triple therapy had significantly increased prevalence of HeFH, ASCVD and chronic kidney disease compared with the other 2 groups ($p = 0.000$, $p = 0.000$ and $p = 0.021$, respectively, for the comparison with the high-intensity statin monotherapy group and $p = 0.000$, $p = 0.000$ and $p = 0.015$, respectively, for the comparison with the add-on ezetimibe group). All therapeutic interventions were well tolerated, and no adverse events were reported during the study.

5.2 LIPIDEMIC AND GLYCEMIC PROFILE

All interventions were associated with significant reductions of apoB-100, TC, LDL-C and non-high-density lipoprotein cholesterol (non-HDL-C), but only PCSK9 inhibitors were associated with significant decreases of Lp(a) (Table 5 and Figures 35-39). Indeed, PCSK9 inhibitors were associated with the highest reduction in LDL-C (add-on PCSK9 inhibitor: -55% vs add-on ezetimibe: -22% vs high-intensity statin: -43%, $p < 0.05$) and Lp(a) levels (add-on PCSK9 inhibitor: -43.1% vs add-on ezetimibe: +15.3% vs high-intensity statin: +5.5%, $p < 0.05$). The same was noted for the change in LDL-Ccorr across 3 groups (Table 5). TGs and HDL-C were reduced only in the high-intensity statin group (Table 5 and Figure 40 and 41).

Table 5. Effect of lipid-lowering drugs on the lipidemic profile of patients with elevated lipoprotein(a)

	Baseline Visit	After 3 Months	Absolute Change within each Group	Within group % change
Total Cholesterol, mg/dL, mean (SD)				
High-intensity statin (n=28)	256 (±38)	177 (±35)	-79 (±51) ‡	-31% ‡
Add-on ezetimibe to statin (n=31)	176 (±23) *	151 (±23) *	-25 (±20) ‡	-14% ‡
Add-on PCSK9 inhibitor to statin and ezetimibe (n=11)	217 (±52) *#	138 (±43) *	-78 (±30) ‡*#	-36% ‡*#
Triglycerides, mg/dL, median (IQR)				
High-intensity statin (n=28)	97 (57;263)	78 (45;371)	-21 (-140;284) ‡	-20% ‡
Add-on ezetimibe to statin	105 (44;198)	81 (45;254)	-8 (-98;74)	-23%

(n=31)				
Add-on PCSK9 inhibitor to statin and ezetimibe (n=11)	96 (72-270)	79 (44-363)	-20 (-119 to 93)	-18%
High-density Lipoprotein Cholesterol, mg/dL, mean (SD)				
High-intensity statin (n=28)	60 (±15)	58 (±13)	-3 (±7) ‡	-3% ‡
Add-on ezetimibe to statin (n=31)	54 (±16)	54 (±14)	-1 (±7)	0
Add-on PCSK9 inhibitor to statin and ezetimibe (n=11)	52 (±12)	51 (±15)	0	-2%
Non-high-density Lipoprotein Cholesterol, mg/dL, mean (SD)				
High-intensity statin (n=28)	196 (±38)	119 (±31)	-76 (±49) ‡	-39% ‡
Add-on ezetimibe to statin (n=31)	122 (±20) *	97 (±19)	-25 (±20) ‡*	-20% ‡*
Add-on PCSK9 inhibitor to statin and ezetimibe (n=11)	165 (±47) *#	87 (±36) *	-78 (±39) ‡#	-47% ‡#
Low-density Lipoprotein Cholesterol, mg/dL, mean (SD)				
High-intensity statin (n=28)	174 (±34)	99 (±27)	-75 (±44) ‡	-43% ‡
Add-on ezetimibe to statin (n=31)	101 (±19) *	79 (±15) *	-22 (±19) ‡	-22% ‡
Add-on PCSK9 inhibitor to statin and ezetimibe (n=11)	140 (±45) *#	63 (±30) #*	-78 (±37) ‡*#	-55% ‡*#
Low-density Lipoprotein Cholesterol corrected for Lipoprotein(a) Cholesterol, mg/dL, mean (SD)				
High-intensity statin (n=28)	153 (±36)	78 (±30)	-77 (±46) ‡	-50% ‡
Add-on ezetimibe to statin (n=31)	81 (±20) *	56 (±20) *	-25 (±20) ‡*	-31% ‡*
Add-on PCSK9 inhibitor to statin and ezetimibe (n=11)	113 (±54) *#	42 (±34) *	-71 (±38) ‡#	-63% ‡#
Apolipoprotein B-100, mg/dL, mean (SD)				
High-intensity statin (n=28)	102 (±27)	70 (±18)	-32 (±28) ‡	-31% ‡
Add-on ezetimibe to statin (n=31)	85 (±26) *	65 (±13)	-20 (±20) ‡	-24% ‡
Add-on PCSK9 inhibitor to statin and ezetimibe (n=11)	107 (±22) #	59 (±19)	-47 (±28) ‡*	-45% ‡*
Lipoprotein(a), nmol/L, median (IQR)				
High-intensity statin (n=28)	131.4 (100.4;171.9)	138.7 (111.0;195.5)	17.9 (-111.4;124.2)	+5.5%
Add-on ezetimibe to statin n=31)	128.5 (75.0;184.8)	148.2 (106.6;179.9)	11.3 (-84.3;129.7)	+15.3%
Add-on PCSK9 inhibitor to statin and ezetimibe (n=11)	139.6 (75.0;278.9)	84.8 (39.5;210.9)	-44.9 (-249.6;46.3)	-43.1 ‡

Parametric variables are presented as mean (±SD) and non-parametric as median (IQR). ‡$p < 0.05$ for the comparison within each group; *$p < 0.05$ for the comparison with patients treated with a high-intensity statin; #$p < 0.05$ for comparison with patients treated with ezetimibe plus high-intensity statin. Abbreviations: PCSK9; proprotein convertase subtilisin/kexin type 9.

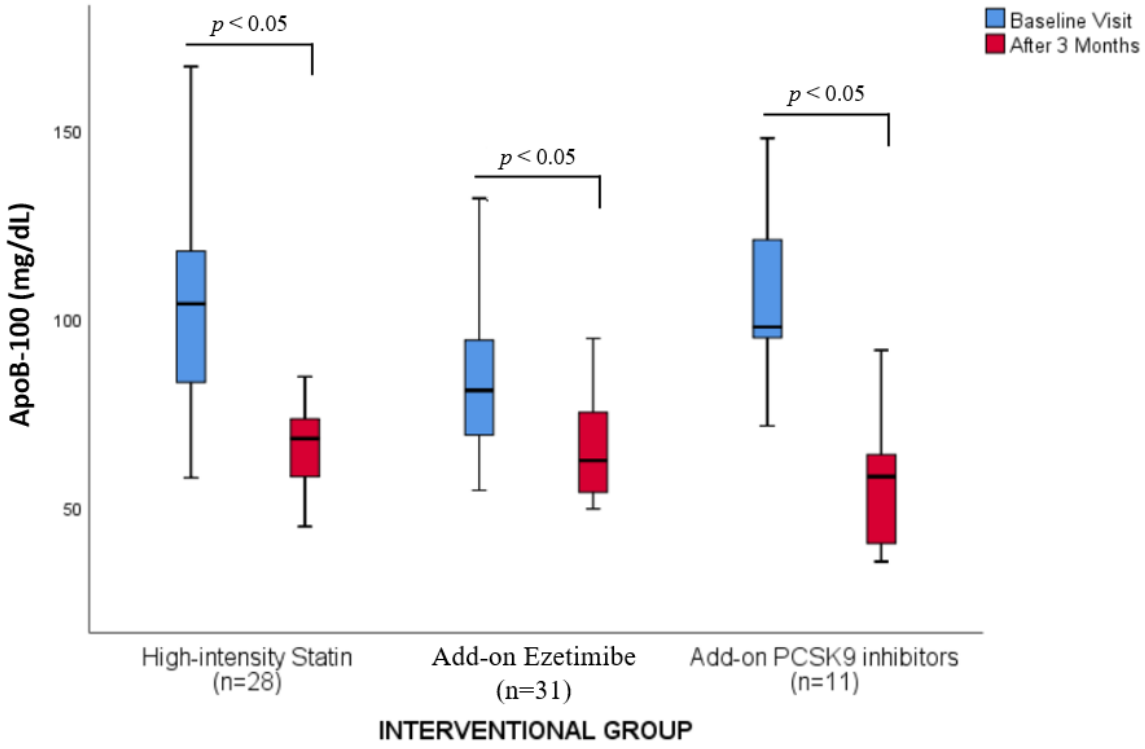

Figure 35. Effect of lipid-lowering treatment on the levels [mean (SD)] of apolipoprotein B-100 (apoB-100).

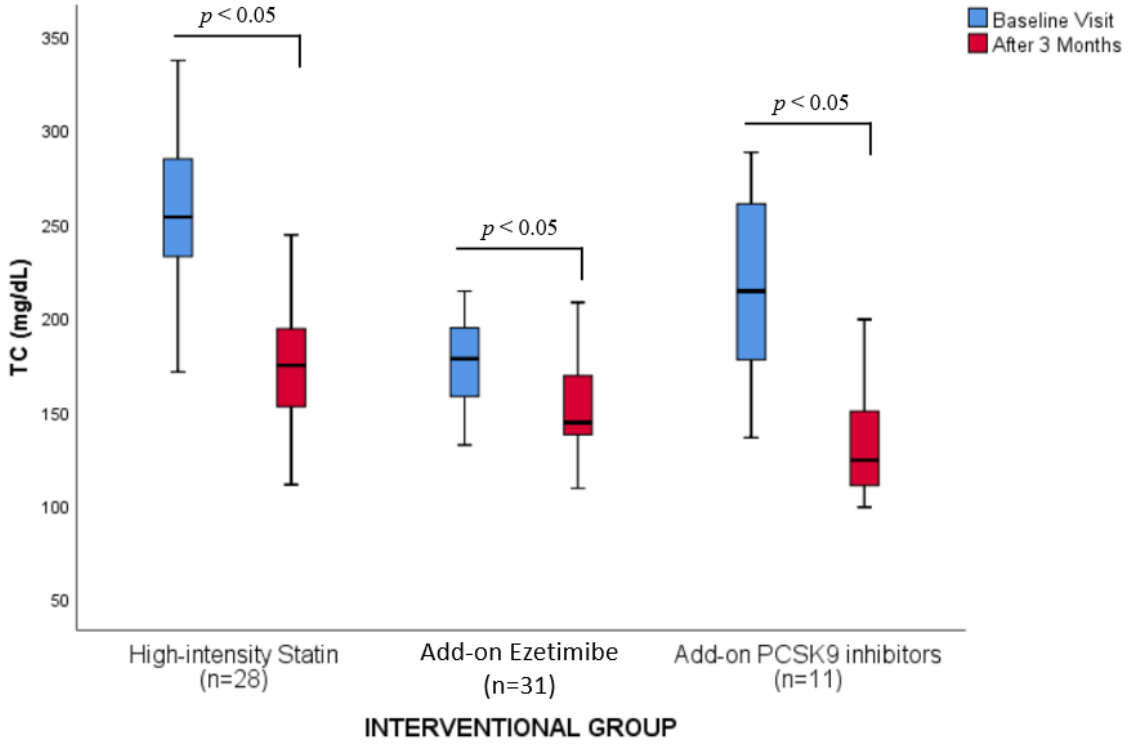

Figure 36. Effect of lipid-lowering treatment on the levels [mean (SD)] of total cholesterol (TC).

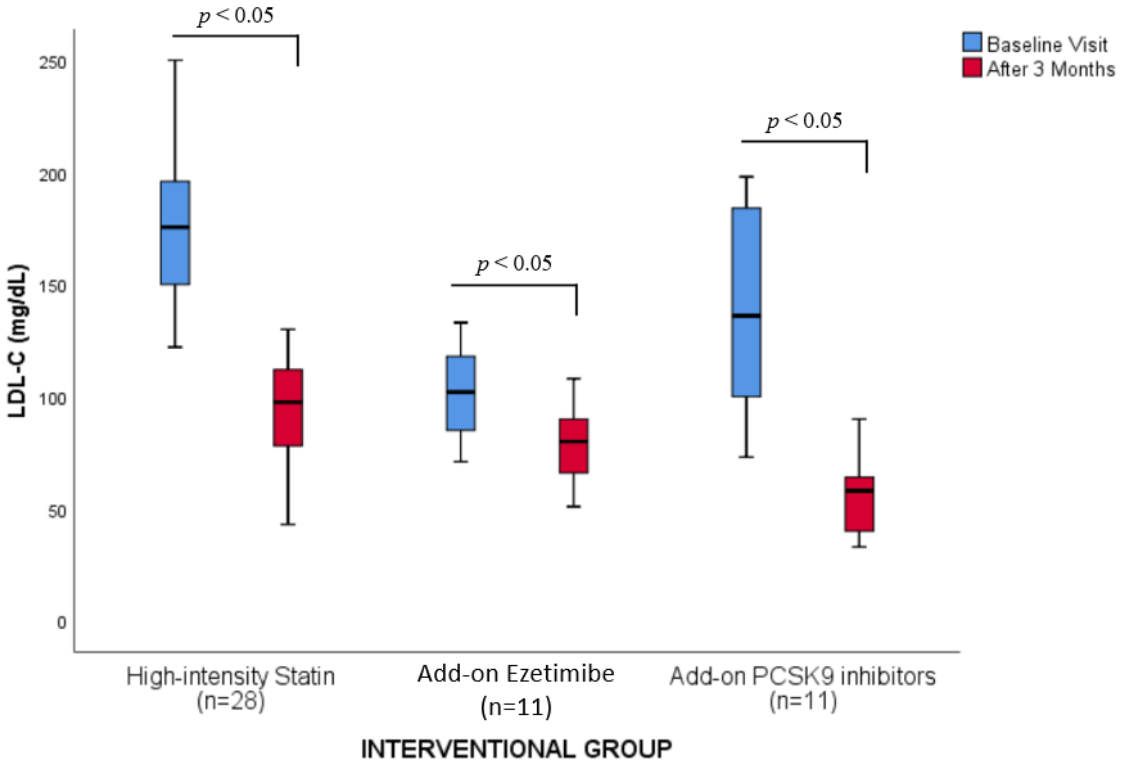

Figure 37. Effect of lipid-lowering treatment on the levels [mean (SD)] of low-density lipoprotein cholesterol (LDL-C).

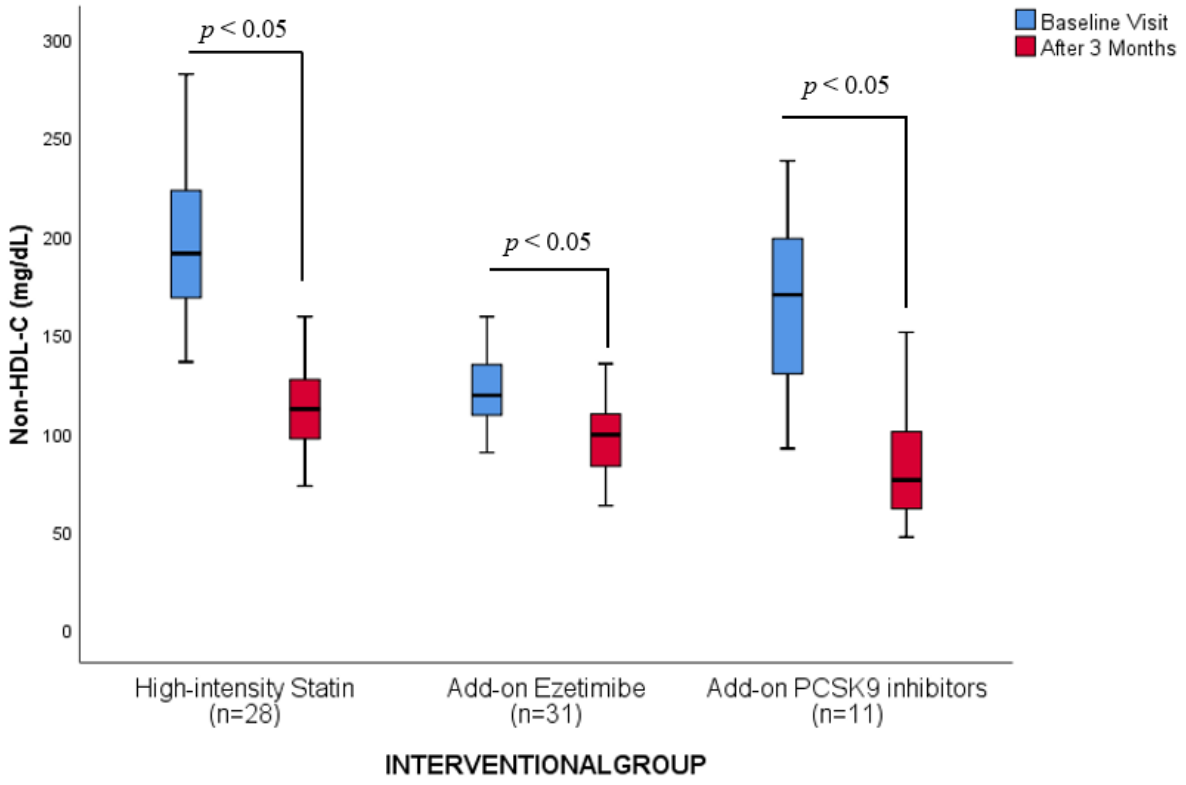

Figure 38. Effect of lipid-lowering treatment on the levels [mean (SD)] of non-high-density lipoprotein cholesterol (non-HDL-C).

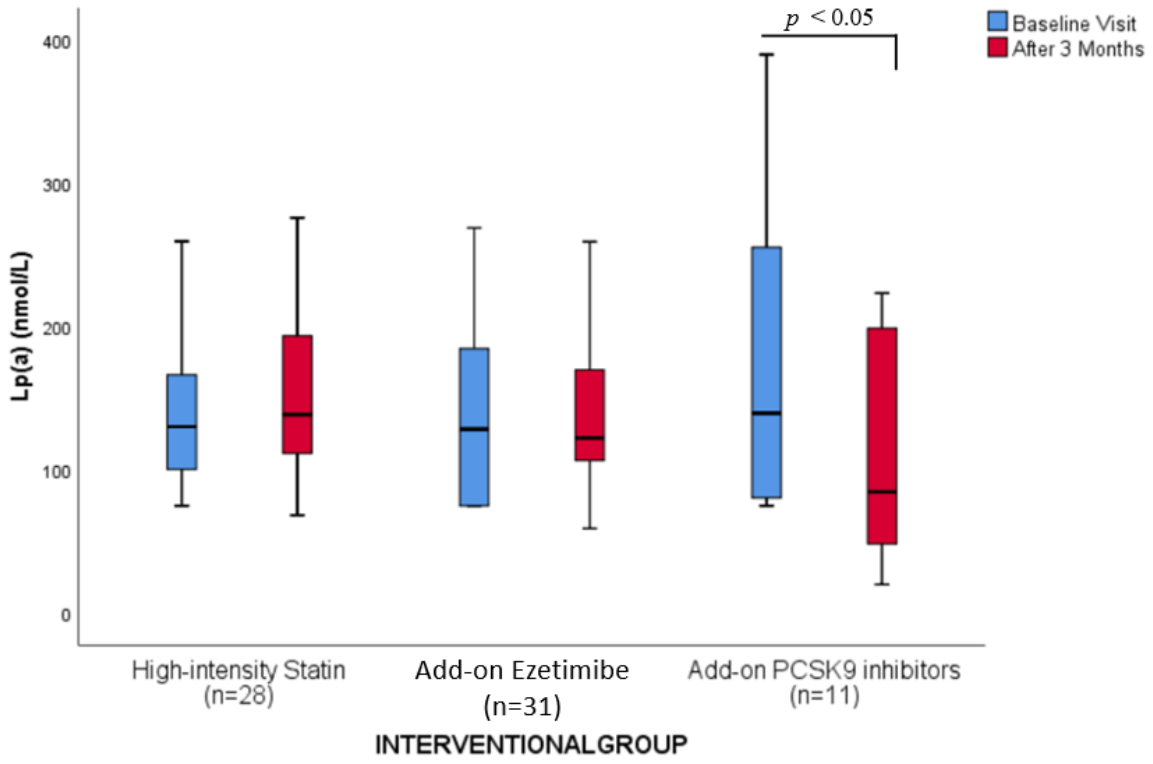

Figure 39. Effect of lipid-lowering treatment on the levels [median (IQR)] of lipoprotein(a) [Lp(a)].

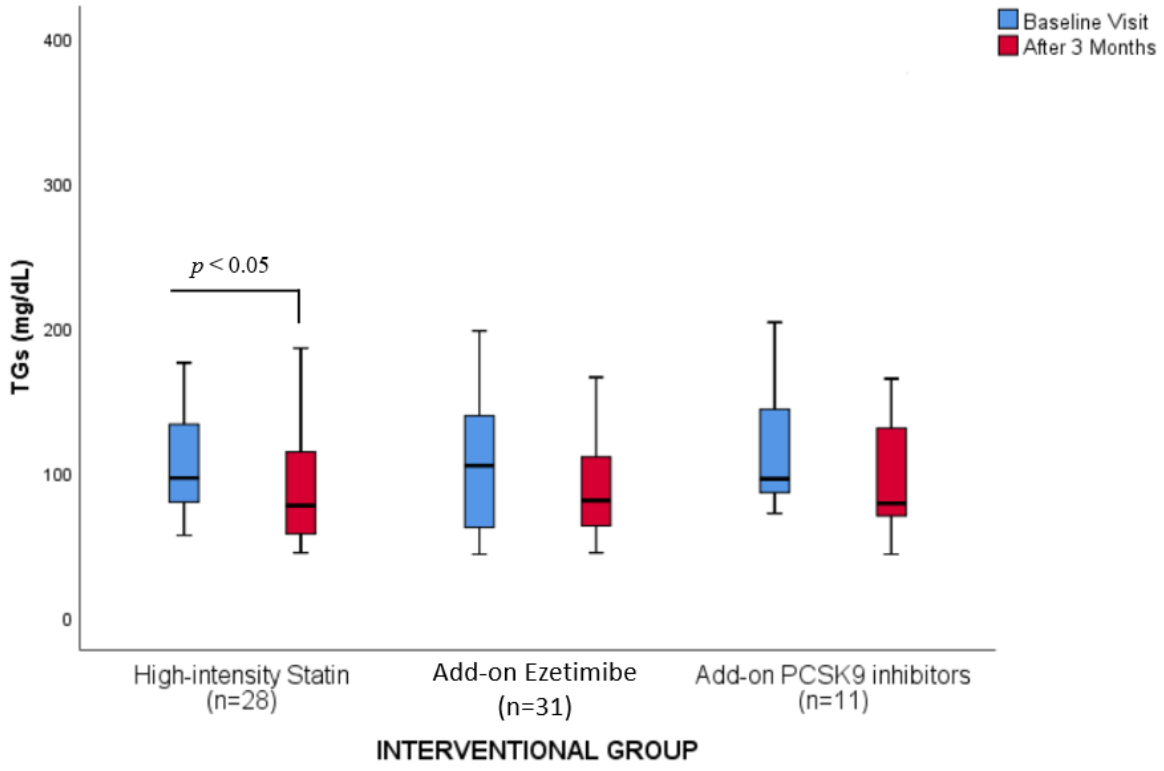

Figure 40. Effect of lipid-lowering treatment on the levels [median (IQR)] of triglycerides (TGs).

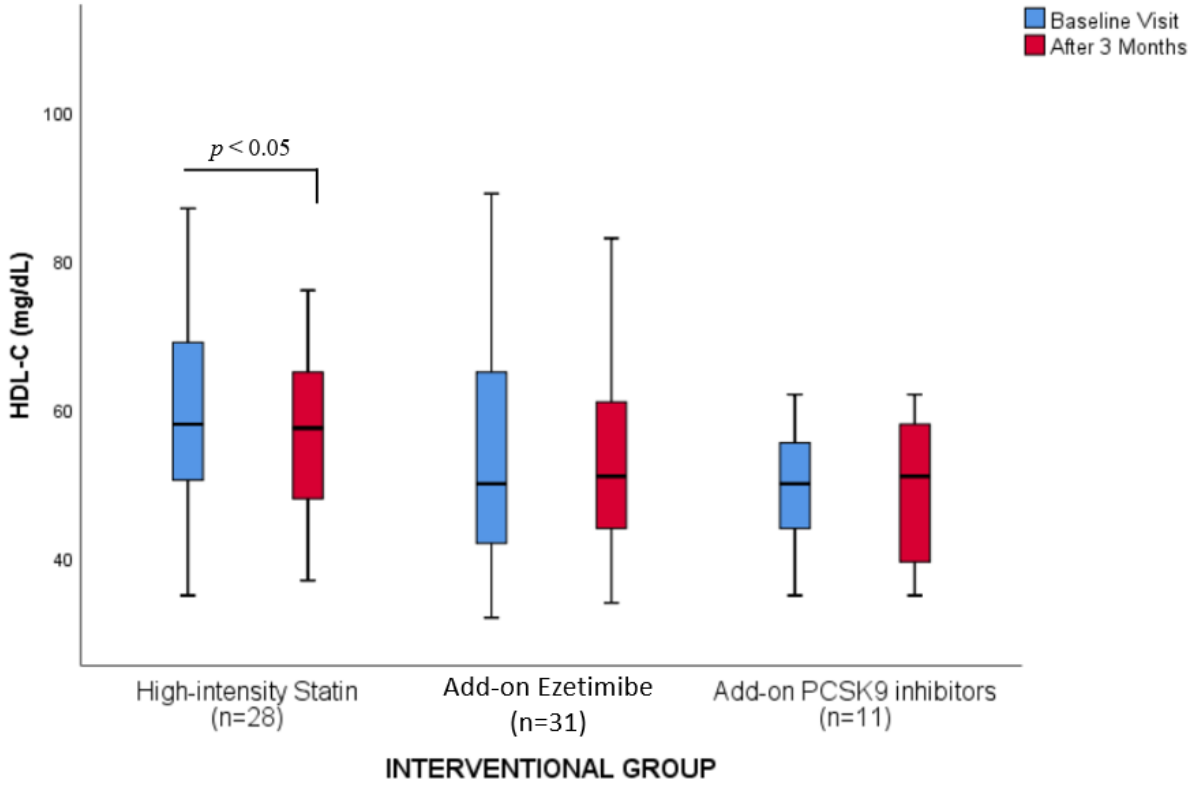

Figure 41. Effect of lipid-lowering treatment on the levels [mean (SD)] of high-density lipoprotein cholesterol (HDL-C).

Patients in the add-on ezetimibe to statin group and the add-on PCSK9 inhibitor to statin and ezetimibe group achieved the highest rates of LDL-C target attainment (Table 6).

Table 6. Effect of lipid-lowering drugs on achieving low-density lipoprotein cholesterol (LDL-C) target.

	LDL-C target achieved
High-intesity statin (n=28)	36% (10/28)
Add-on ezetimibe to statin (n=31)	45% (14/31)
Add-on PCSK9 inhibitor to statin and ezetimibe (n=11)	45% (5/11)

Abbreviations: LDL-C; Low-density lipoprotein cholesterol, PCSK9; proprotein convertase subtilisin/kexin type 9.

Among study participants without diabetes (n=60), high-intensity statin treatment and the addition of ezetimibe to high-intensity statin had no significant effect on glycemic profile (Table 7). Similar results were noted for PCSK9 inhibitors, although a non-significant trend towards a decrease in HOMA-IR index by 19% was noticed (Table 7).

Table 7. Effect of lipid-lowering drugs on the glycemic profile of patients with elevated lipoprotein(a).

	Baseline Visit	After 3 Months	Absolute Change within each Group	% Change within each group
Glucose, mg/dL, mean (SD)				
High-intesity statin (n=28)	90 (±8)	91 (±12)	1 (±11)	1%
Add-on ezetimibe to statin (n=31)	97 (±11) *	98 (±10) *	0	0
Add-on PCSK9 inhibitor to statin and ezetimibe (n=11)	91 (±6)	90 (±10)	-1 (±11)	-1%
Insulin, µU/mL, median (IQR)				
High-intesity statin (n=28)	7.2 (2.8-151.4)	7.3 (3.3-123.3)	0.4 (-28.1 to 18.4)	6%
Add-on ezetimibe to statin (n=31)	6.7 (2.8-42.0)	8.3 (2.9-30.3)	0.1 (-21.8 to 18.8)	1%
Add-on PCSK9 inhibitor to statin and ezetimibe (n=11)	10.8 (3.1-55.9)	9 (2.4-37.1)	-1.8 (-27.5 to 6.4)	-17%
HOMA Insulin Resistance Index, median (IQR)				
High-intesity statin (n=28)	1.7 (0.4-38.1)	1.6 (0.6-42.6)	-0.1 (-1.5 to 4.5)	-6%
Add-on ezetimibe to statin (n=31)	1.7 (0.7-10.0)	2.1 (0.7-7.6)	0	0
Add-on PCSK9 inhibitor to statin and ezetimibe (n=11)	2.6 (0.7-12.0)	2 (0.5-9.0)	-0.5 (-6.3 to -0.6)	-19%

Parametric variables are presented as mean (±SD) and non-parametric as median (IQR). *$p < 0.05$ for the comparison with patients treated with a high-intensity statin. Abbreviations: PCSK9; proprotein convertase subtilisin/kexin type 9.

5.3 OxPL-apoB, OxPL-apo(a) AND OxPL-PLG

Both high-intensity statins and add-on ezetimibe significantly increased OxPL-apoB by 2.9% and 28.7% (Table 8 and Figure 42), respectively, as well as OxPL-apo(a) by 100.8% and 40.1% (Table 8 and Figure 43), respectively. Add-on PCSK9 inhibitors resulted in a numerical decrease of OxPL-apoB by 12.5% (p = NS) and a numerical increase of OxPL-apo(a) by 12% (p = NS) (Table 8 and Figures 42 and 43).

Table 8. Effect of lipid-lowering drugs on oxidized phospholipids and plasminogen levels.

	Baseline Visit	After 3 Months	% Change within each group
OxPL-apoB, nmol/L, median (IQR)			
High-intensity statin (n=28)	27.9 (19.7;33.0)	28.7 (21.2;49.2)	+2.9% ǂ
Add-on ezetimibe to statin (n=31)	27.5 (21.3;37.8)	35.4 (19.8;57.9)	+28.7% ǂ
Add-on PCSK9 inhibitor to statin and ezetimibe (n=11)	24.8 (16.7;45.4)	21.7 (5.7;55.3)	-12.5%

OxPL-apo(a), nmol/L, median (IQR)			
High-intensity statin (n=28)	11.9 (9.7;15.6)	23.9 (13.4;33.1)	+100.8% ǂ
Add-on ezetimibe to statin (n=31)	14.2 (11.6;20.0)	19.9 (13.8;34.5)	+40.1% ǂ
Add-on PCSK9 inhibitor to statin and ezetimibe (n=11)	15.0 (9.4;20.1)	16.8 (10.5;39.8)	+12.0%
OxPL-PLG, nmol/L, median (IQR) §			
High-intensity statin (n=28)	257.9 (224.4;343.2)	252.2 (175.5;294.6)	-2.2% ǂ
Add-on ezetimibe to statin (n=31)	303.3 (237.9;373.8)	246.4 (202.3;333.3)	-18.8% ǂ
Add-on PCSK9 inhibitor to statin and ezetimibe (n=11)	283.6 (170.4;380.2)	268.8 (194.9;299.6)	-5.2%
Total Plasminogen, mg/dL, median (IQR)			
High-intensity statin (n=28)	10.1 (7.5;12.6)	10.3 (7.9;13.3)	+2.0%
Add-on ezetimibe to statin (n=31)	10.7 (9.1;14.0)	11.2 (7.6;12.5)	+4.7%
Add-on PCSK9 inhibitor to statin and ezetimibe (n=11)	11.3 (8.5;14.3)	12.0 (9.7;13.6)	+6.2%

ǂ$p < 0.05$ for the comparison within each group; *$p < 0.05$ for the comparison with patients treated with high-intensity statin; #$p < 0.05$ for the comparison with patients treated with high-intensity statin plus ezetimibe; § p values were calculated after excluding 4 outliers. Abbreviations: PCSK9; proprotein convertase subtilisin/kexin type 9, OxPL-apoB; oxidized phospholipids on apolipoprotein B-100, OxPL-apo(a); oxidized phospholipids on apolipoprotein(a), OxPL-PLG; oxidized phospholipids on plasminogen.

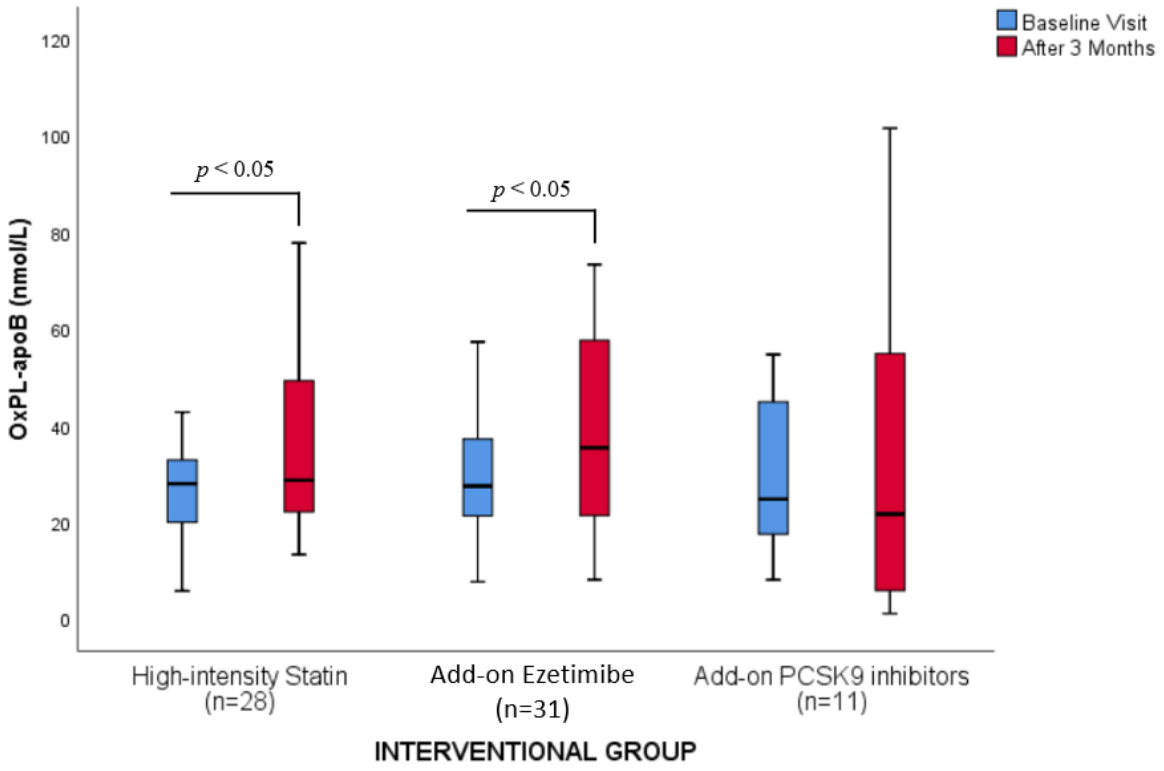

Figure 42. Effect of lipid-lowering treatment on the levels [median (IQR)] of oxidized phospholipids on apolipoprotein B-100 (OxPL-apoB).

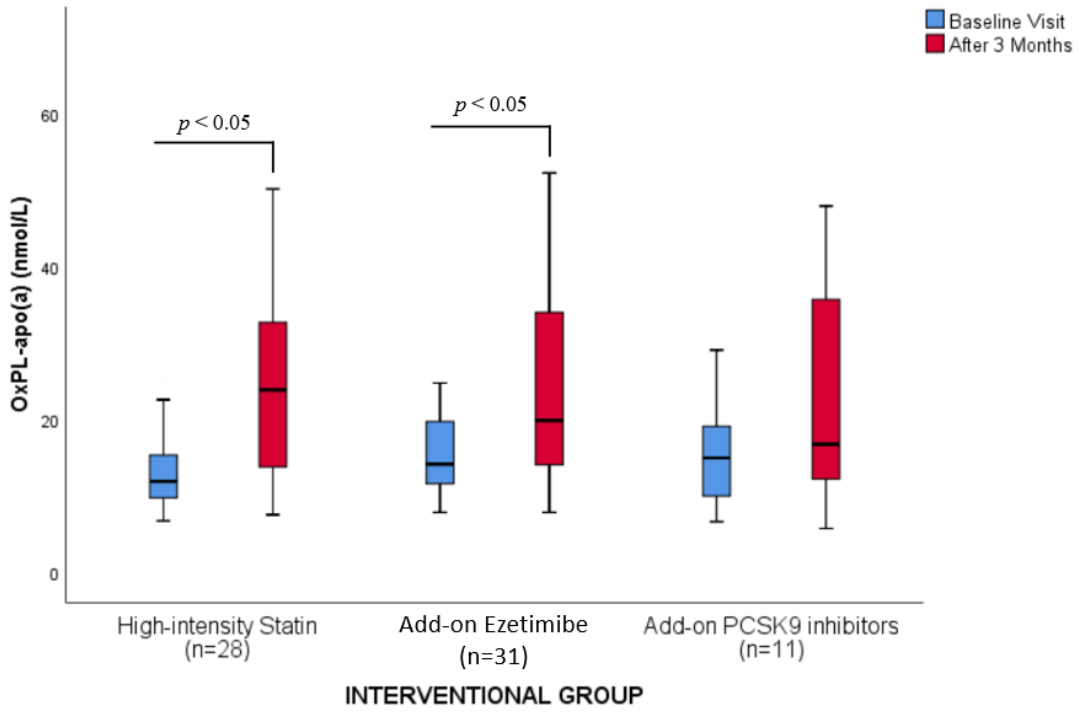

Figure 43. Effect of lipid-lowering treatment on the levels [median (IQR)] of oxidized phospholipids on apolipoprotein(a) levels [OxPL-apo(a)].

In the total population, correlation analyses at baseline revealed a significant association between Lp(a) and OxPL-apo(a) (r = 0.667, p < 0.001, Figure 44) as well as between Lp(a) and OxPL-apoB (r = 0.695, p < 0.001) (Figure 45), while LDL-C was not significantly correlated with OxPL-apoB (r = –0.198, p = 0.109) (Figure 46).

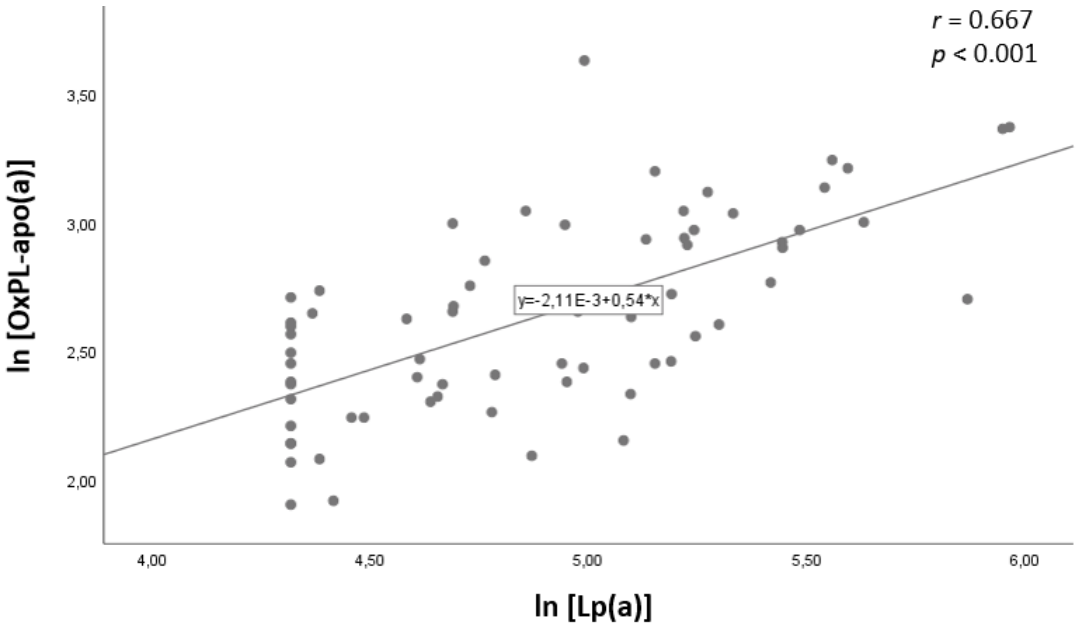

Figure 44. Correlation between lipoprotein(a) and oxidized phospholipids on apolipoprotein(a) [OxPL-apo(a)] levels at baseline for the whole sample.

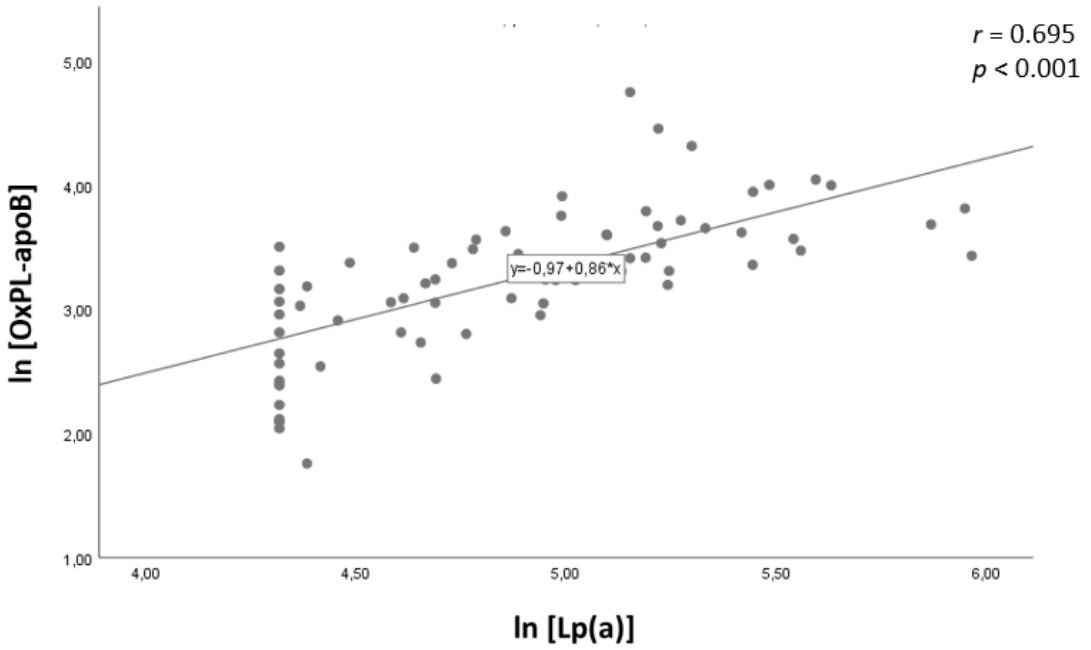

Figure 45. Correlation between lipoprotein(a) and oxidized phospholipids on apolipoprotein B-100 [OxPL-apoB] levels at baseline for the whole sample.

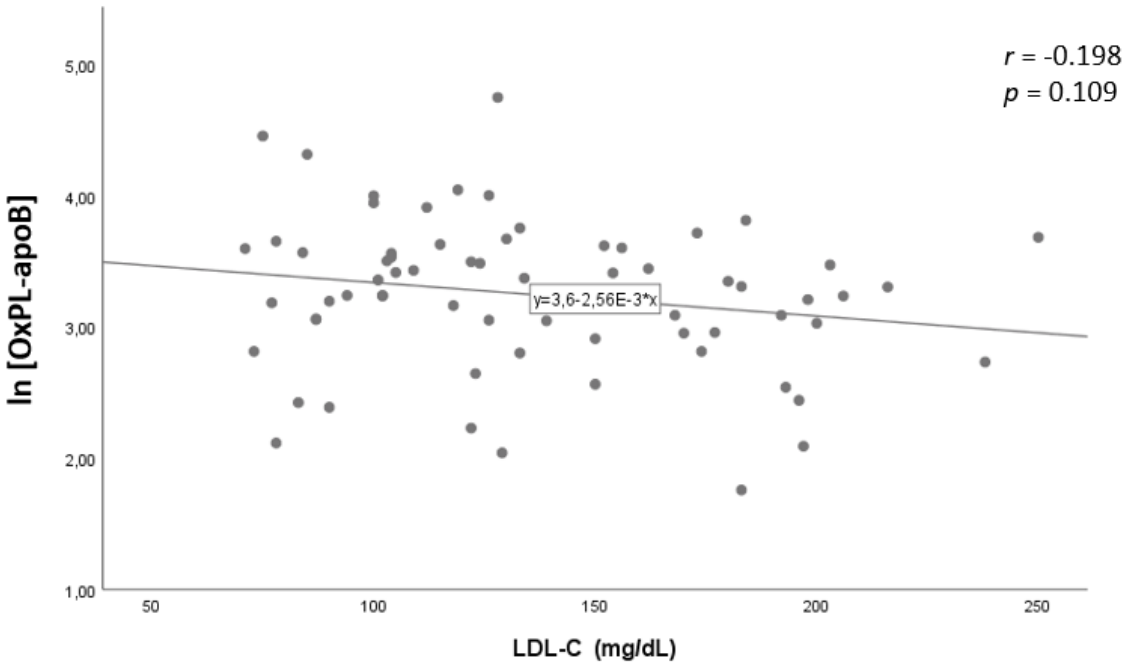

Figure 46. Correlation between low-density lipoprotein cholesterol (LDL-C) and oxidized phospholipids on apolipoprotein B-100 [OxPL-apoB] levels at baseline for the whole sample.

Similar patterns were observed at 3 months for the whole sample, with Lp(a) showing significant correlations with both OxPL-apo(a) (r = 0.696, p < 0.001) (Figure 47) and OxPL-apoB (r = 0.861, p < 0.001) (Figure 48), while LDL-C was not significantly associated with OxPL-apoB (r = 0.114, p = 0.355) (Figure 49).

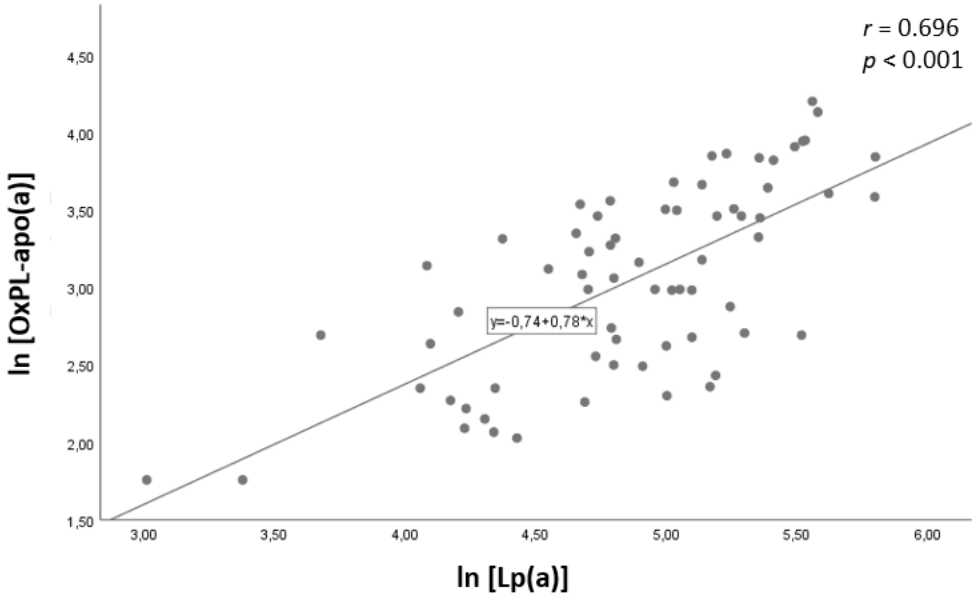

Figure 47. Correlation between lipoprotein(a) and oxidized phospholipids on apolipoprotein(a) [OxPL-apo(a)] levels at 3 months for the whole sample.

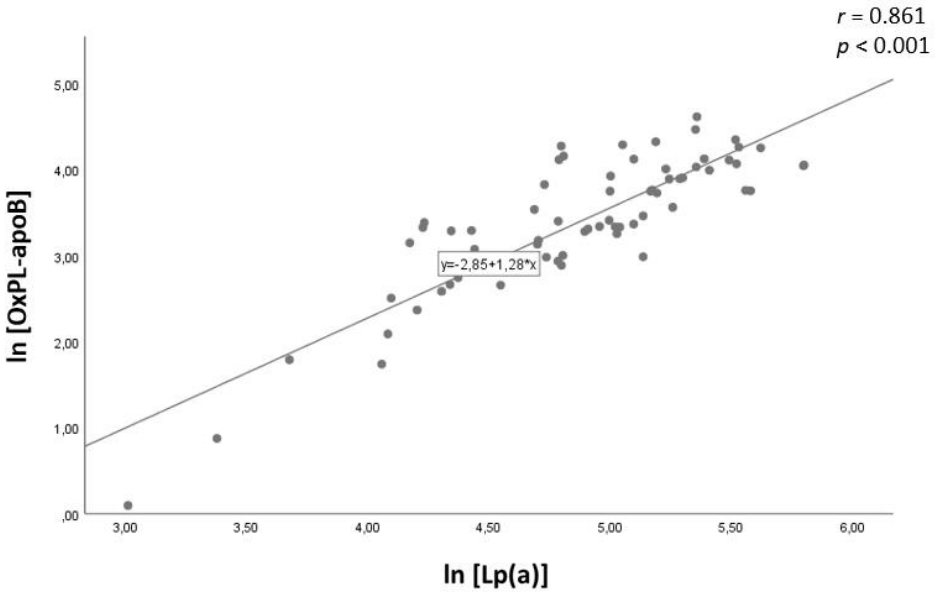

Figure 48. Correlation between lipoprotein(a) and oxidized phospholipids on apolipoprotein B-100 [OxPL-apoB] levels at 3 months for the whole sample.

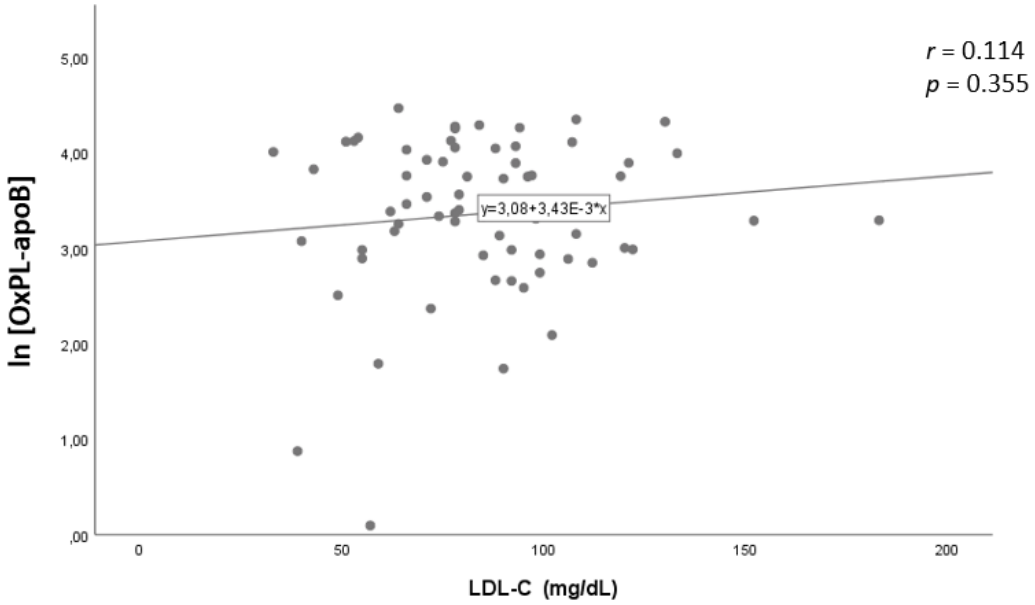

Figure 49. Correlation between low density lipoprotein cholesterol (LDL-C) and oxidized phospholipids on apolipoprotein B-100 [OxPL-apoB] levels at 3 months for the whole sample.

Correlation analyses based on changes from baseline to follow-up for the whole sample showed no significant associations between changes in OxPL-apoB and LDL-C (r = –0.007, p = 0.958) (Figure 50), whereas a significant correlation was observed between changes in OxPL-apoB and Lp(a) (r = 0.646, p < 0.001) (Figure 51) or OxPL-apo(a) and Lp(a) (r = 0.520, p < 0.001) (Figure 52).

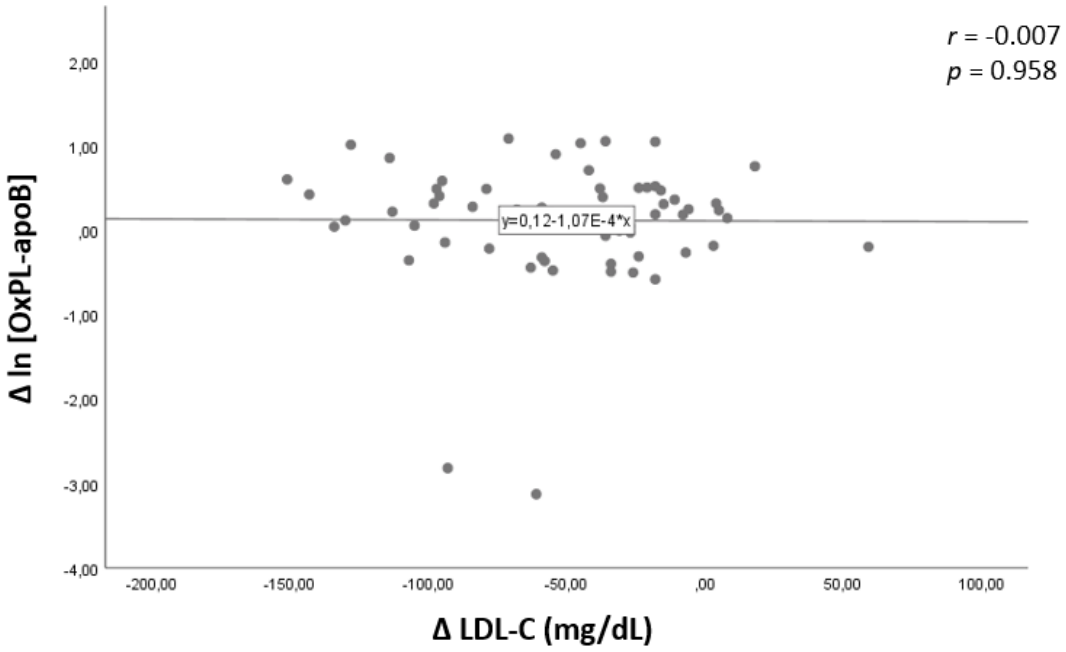

Figure 50. Correlation between changes in oxidized phospholipids on apolipoprotein B-100 [OxPL-apoB] and low-density lipoprotein cholesterol (LDL-C) levels for the whole sample.

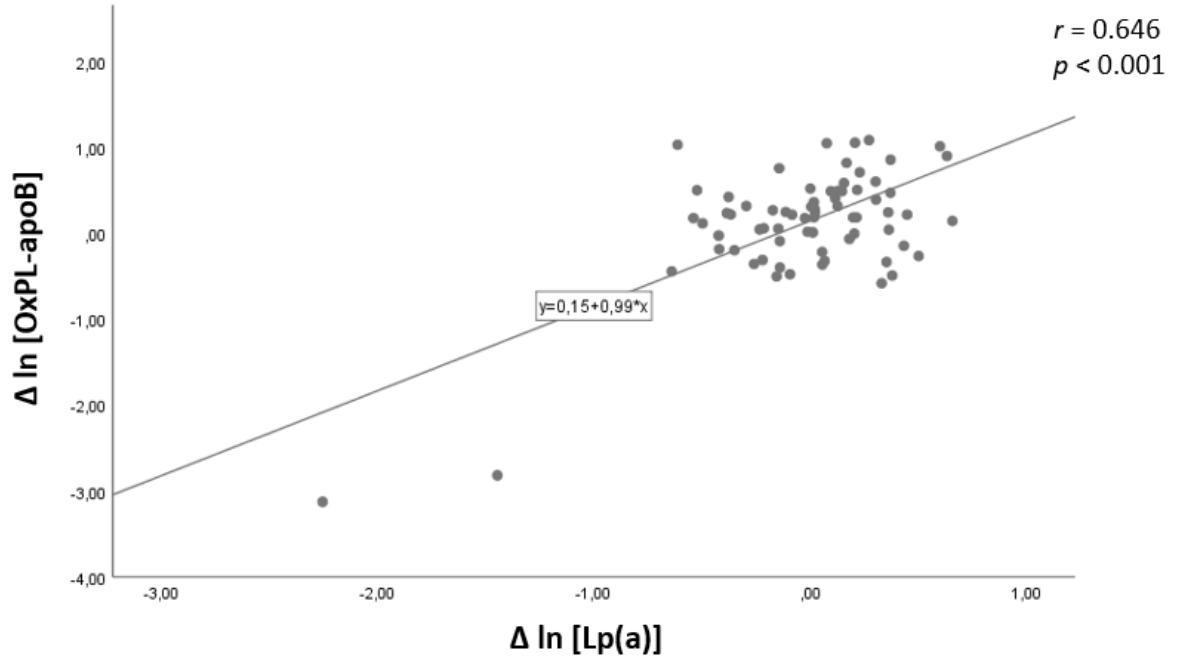

Figure 51. Correlation between changes in oxidized phospholipids on apolipoprotein B-100 [OxPL-apoB] and lipoprotein(a) levels for the whole sample.

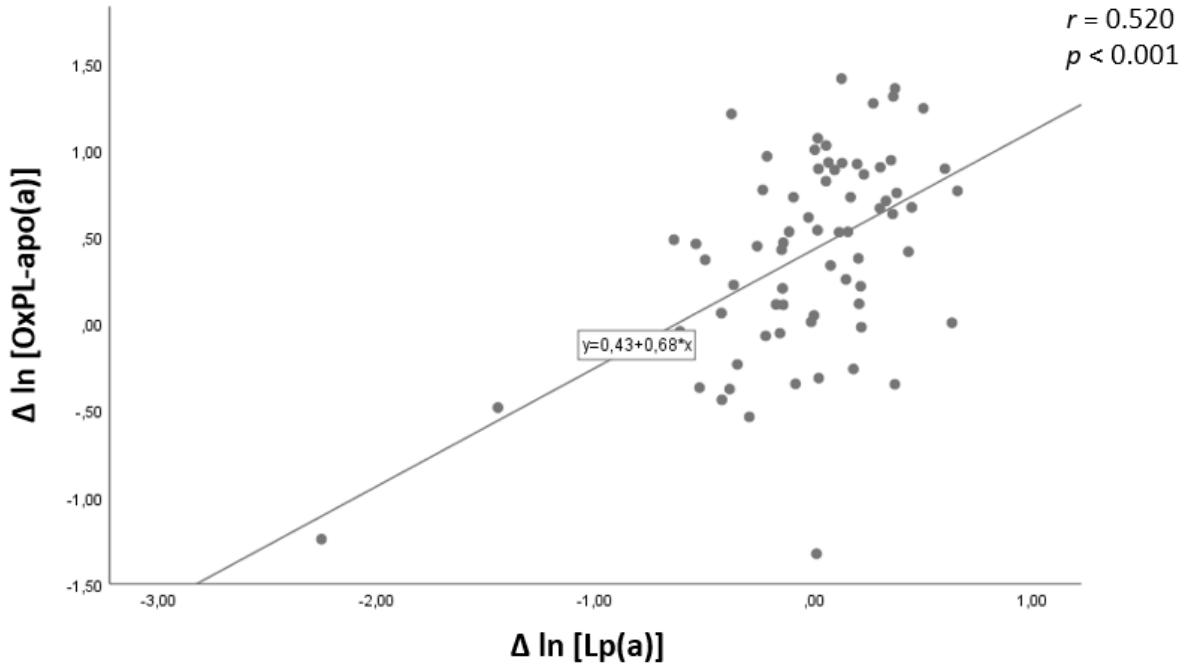

Figure 52. Correlation between changes in oxidized phospholipids on apolipoprotein(a) [OxPL-apo(a)] and lipoprotein(a) levels for the whole sample.

For the high-intensity statin group correlation analyses at baseline revealed a significant association between Lp(a) and OxPL-apo(a) (r = 0.602, p = 0.001) (Figure 53) as well as between Lp(a) and OxPL-apoB (r = 0.694, p < 0.001) (Figure 54), while LDL-C was not significantly correlated with OxPL-apoB (r = –0.299, p = 0.13) (Figure 55).

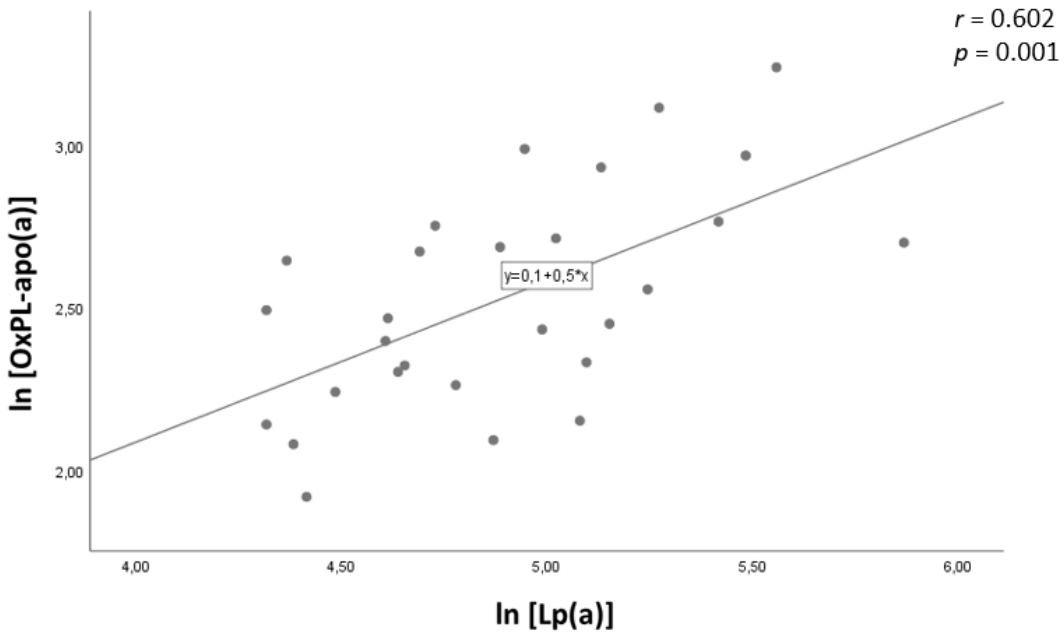

Figure 53. Correlation between lipoprotein(a) and oxidized phospholipids on apolipoprotein(a) [OxPL-apo(a)] levels at baseline for the high-intensity statin group.

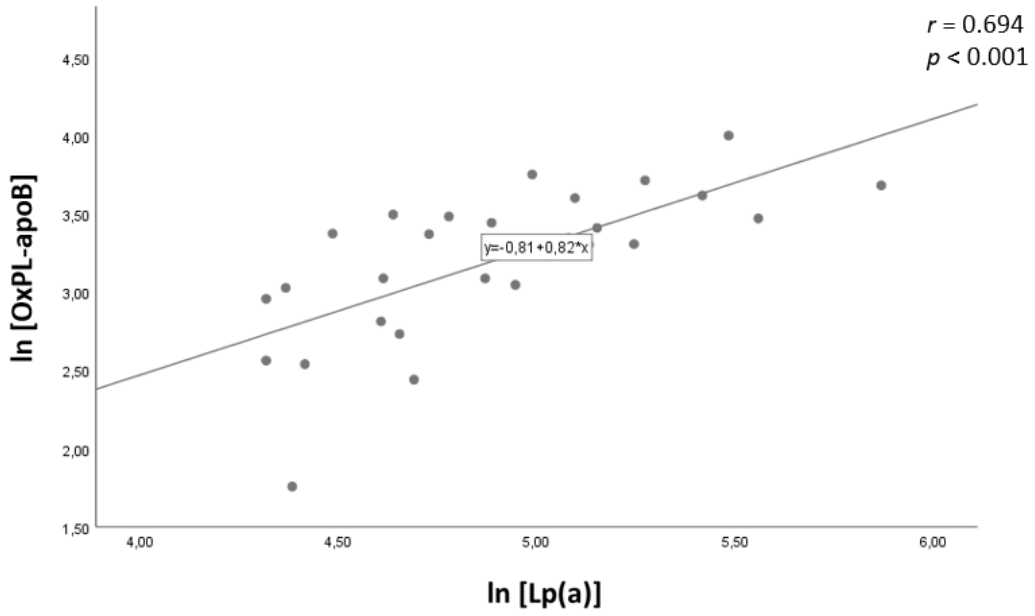

Figure 54. Correlation between lipoprotein(a) and oxidized phospholipids on apolipoprotein-apoB [OxPL-apoB] levels at baseline for the high-intensity statin group.

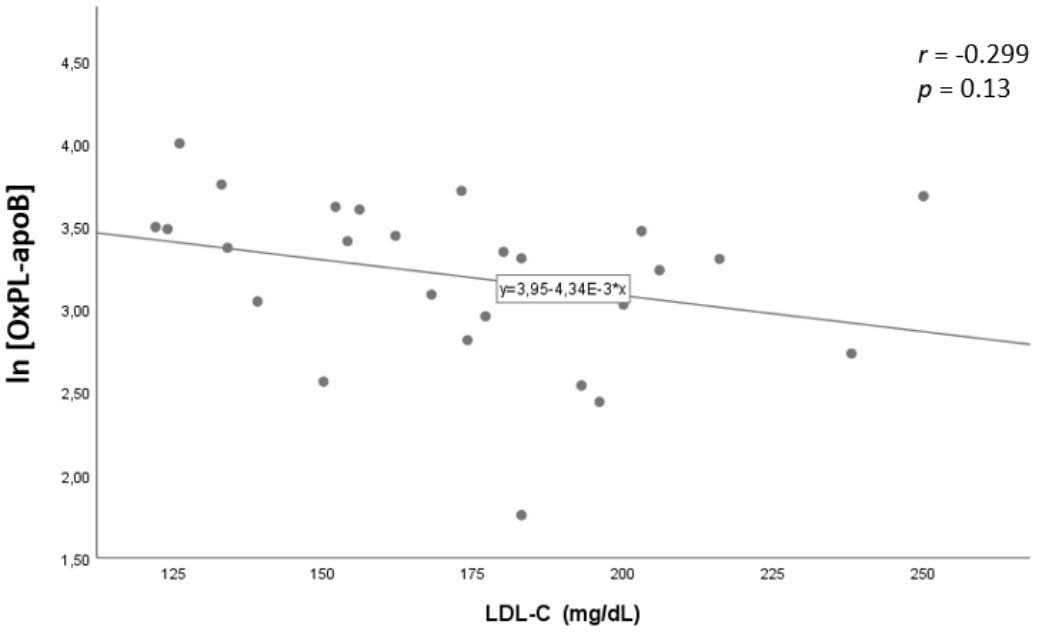

Figure 55. Correlation between low-density lipoprotein cholesterol (LDL-C) and oxidized phospholipids on apolipoprotein B-100 [OxPL-apoB] levels at baseline for the high-intensity statin group.

Similar patterns were observed at 3 months for the high-intensity statin group, with Lp(a) showing significant correlations with both OxPL-apo(a) (r = 0.573, p = 0.001) (Figure 56) and OxPL-apoB (r = 0.763, p < 0.001) (Figure 57), and LDL-C not significantly associated with OxPL-apoB (r = -0.019, p = 0.554) (Figure 58).

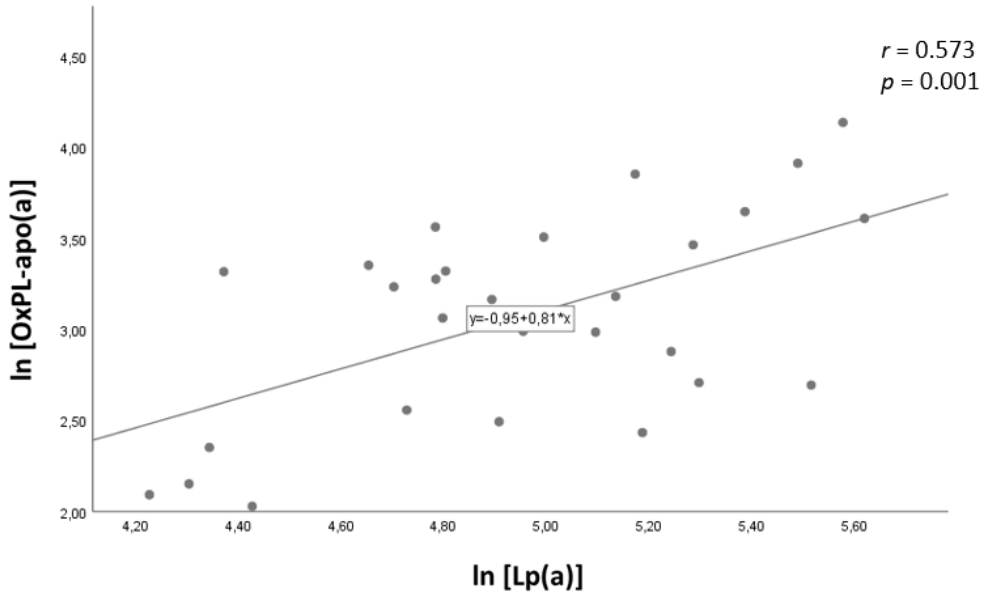

Figure 56. Correlation between lipoprotein(a) and oxidized phospholipids on apolipoprotein(a) [OxPL-apo(a)] levels at 3 months for the high-intensity statin group.

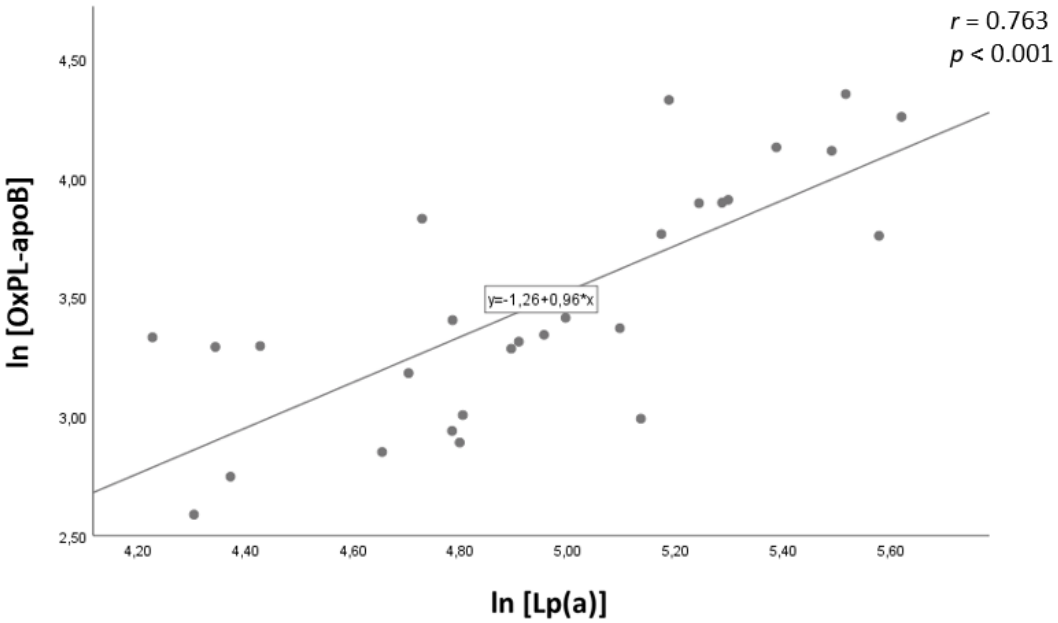

Figure 57. Correlation between lipoprotein(a) and oxidized phospholipids on apolipoprotein-apoB [OxPL-apoB] levels at 3 months for the high-intensity statin group.

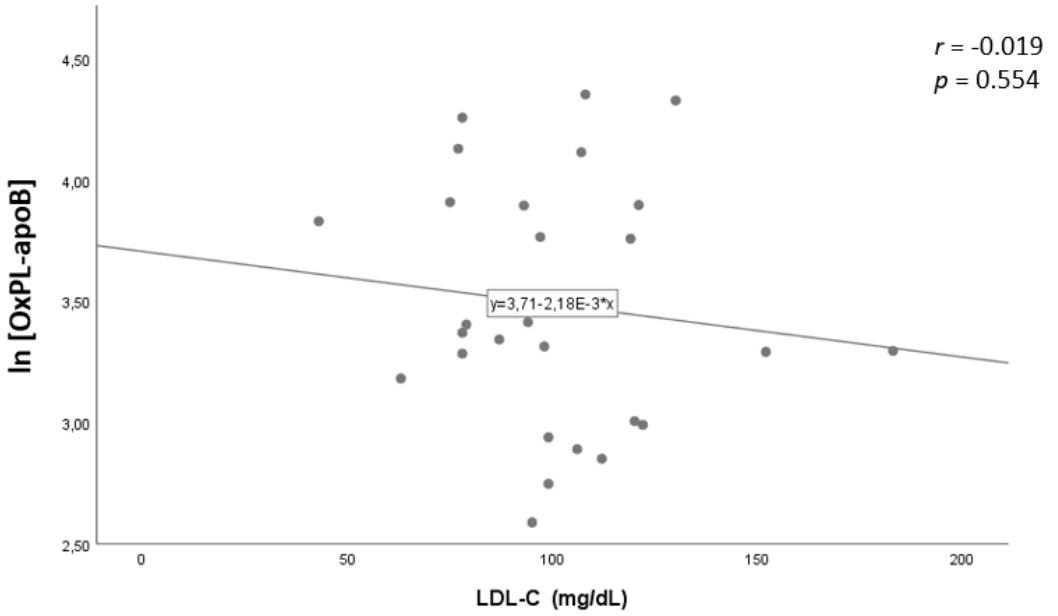

Figure 58. Correlation between low-density lipoprotein cholesterol (LDL-C) and oxidized phospholipids on apolipoprotein B-100 [OxPL-apoB] levels at 3 months for the high-intensity statin group.

Correlation analyses based on changes from baseline to follow-up for the high-intensity statin group showed no significant associations between changes in OxPL-apoB and LDL-C (r = –0.375, p = 0.059) (Figure 59) or OxPL-apo(a) and Lp(a) (r = 0.366, p = 0.055) (Figure 60), whereas a significant correlation was observed between changes in OxPL-apoB and Lp(a) (r = 0.483, p = 0.009) (Figure 61).

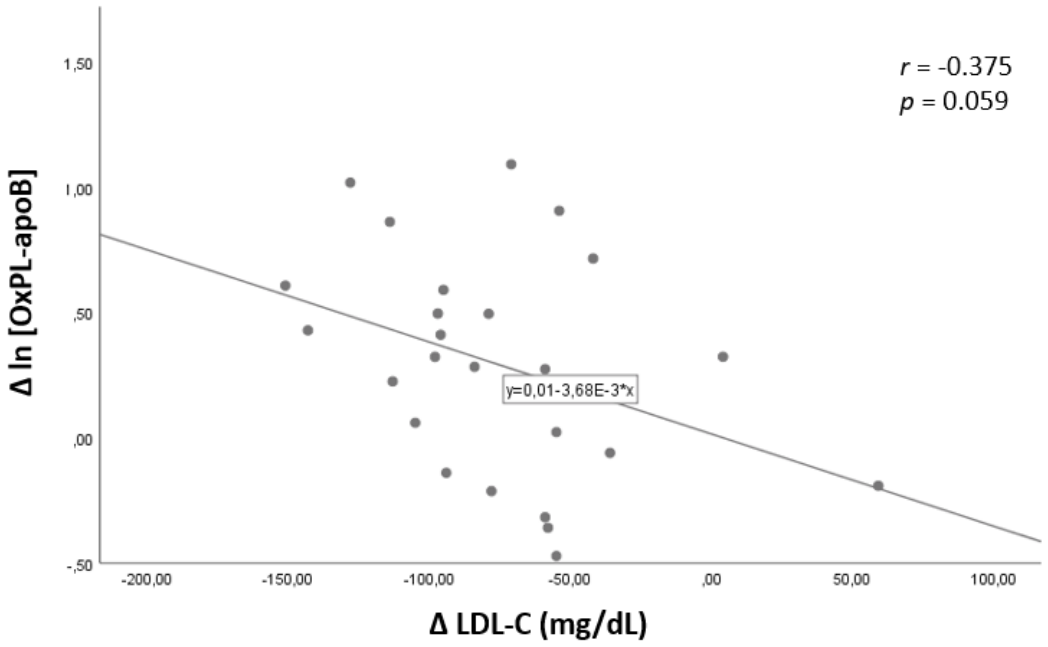

Figure 59. Correlation between changes in oxidized phospholipids on apolipoprotein B-100 [OxPL-apoB] and low-density lipoprotein cholesterol (LDL-C) levels for the high-intensity statin group.

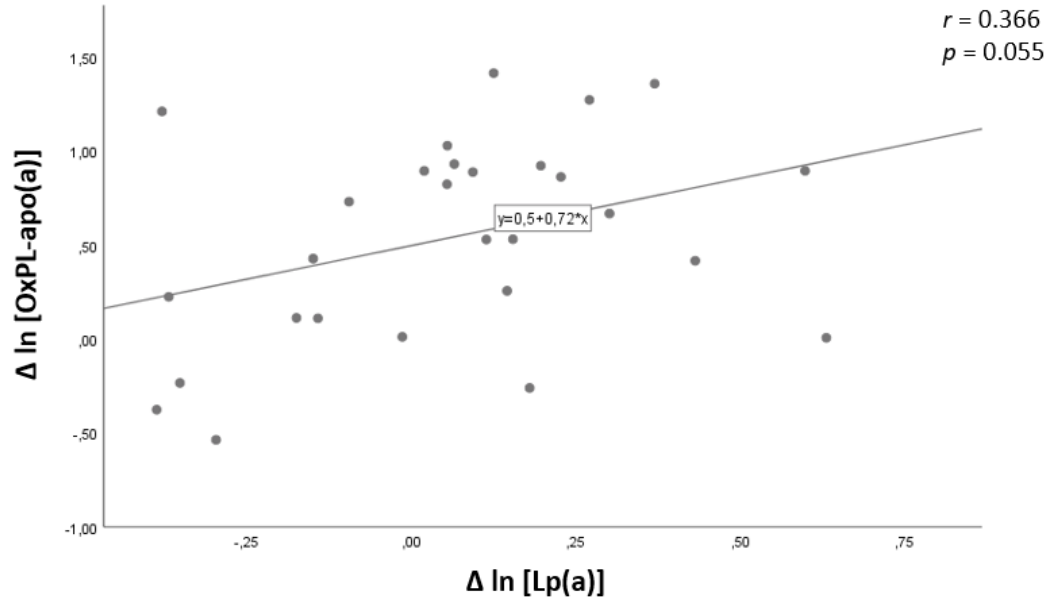

Figure 60. Correlation between changes in oxidized phospholipids on apolipoprotein(a) [OxPL-apo(a)] and lipoprotein(a) levels for the high-intensity statin group.

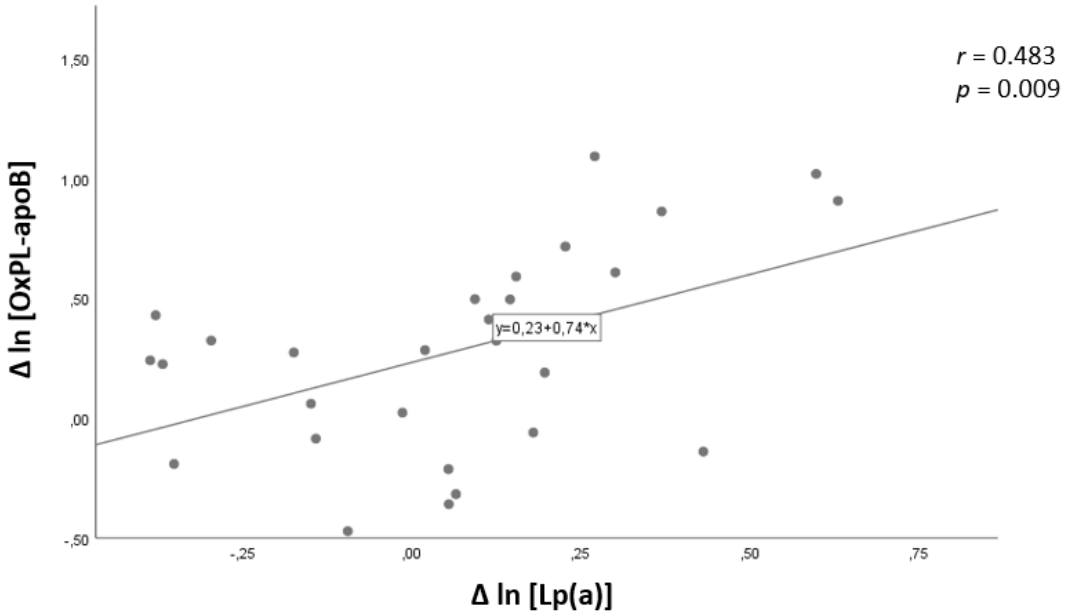

Figure 61. Correlation between changes in oxidized phospholipids on apolipoprotein-apoB [OxPL-apoB] and lipoprotein(a) levels for the high-intensity statin group.

For the add-on ezetimibe group correlation analyses at baseline revealed a significant association between Lp(a) and OxPL-apo(a) (r = 0.651, p < 0.001, Figure 62) as well as between Lp(a) and OxPL-apoB (r = 0.686, p < 0.001) (Figure 63), while LDL-C was not significantly correlated with OxPL-apoB (r = –0.003, p = 0.987) (Figure 64).

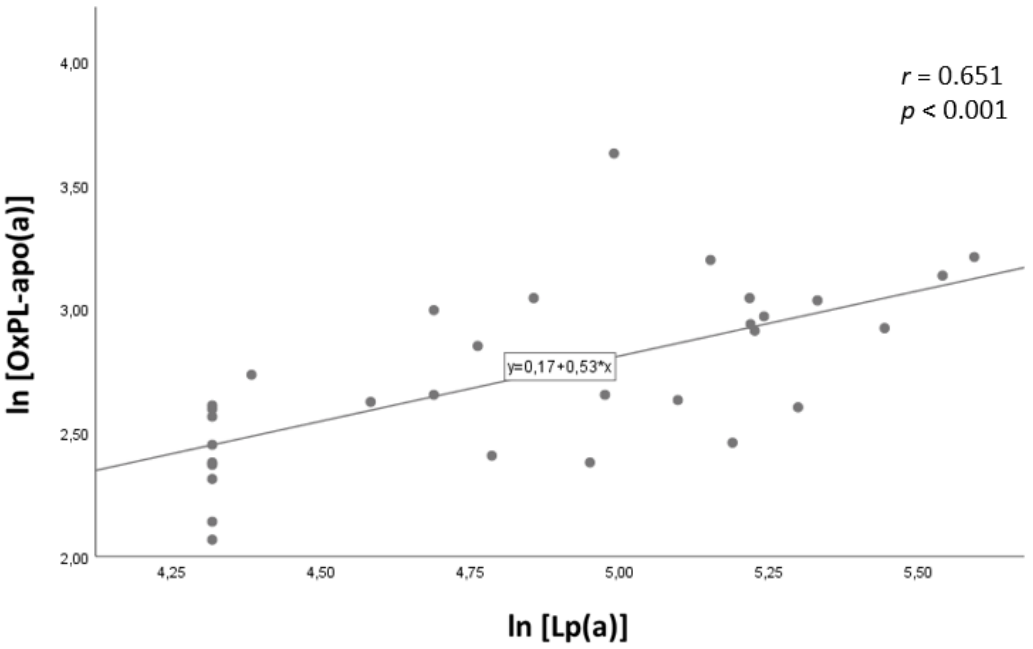

Figure 62. Correlation between lipoprotein(a) and oxidized phospholipids on apolipoprotein(a) [OxPL-apo(a)] levels at baseline for the add-on ezetimibe group.

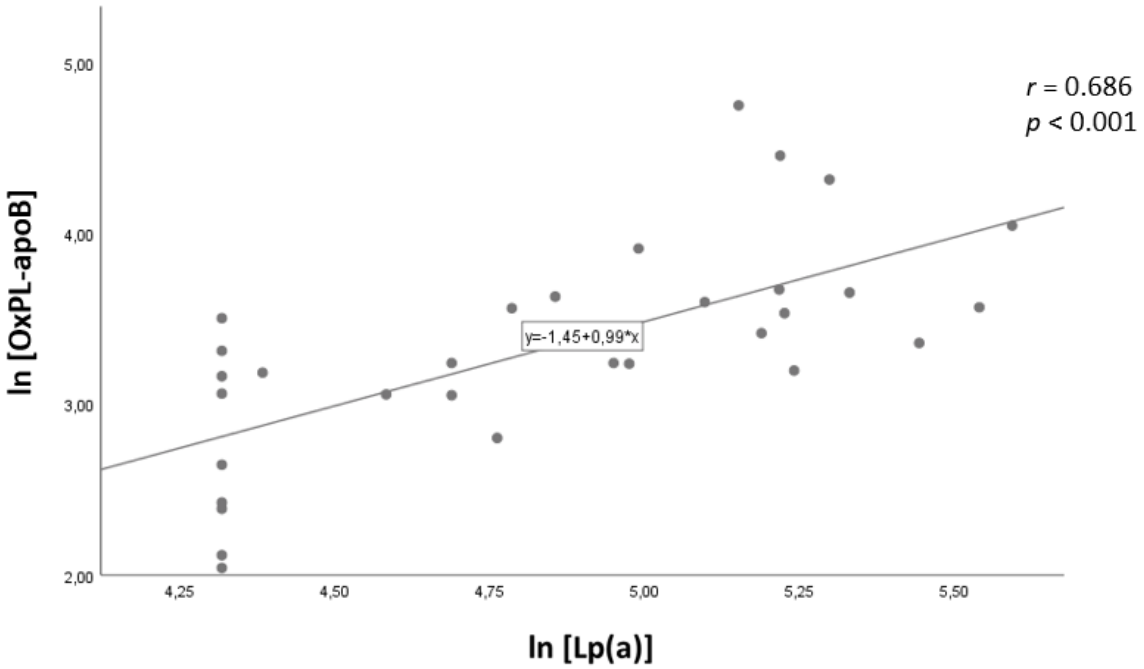

Figure 63. Correlation between lipoprotein(a) and oxidized phospholipids on apolipoprotein B-100 [OxPL-apoB] levels at baseline for the add-on ezetimibe group.

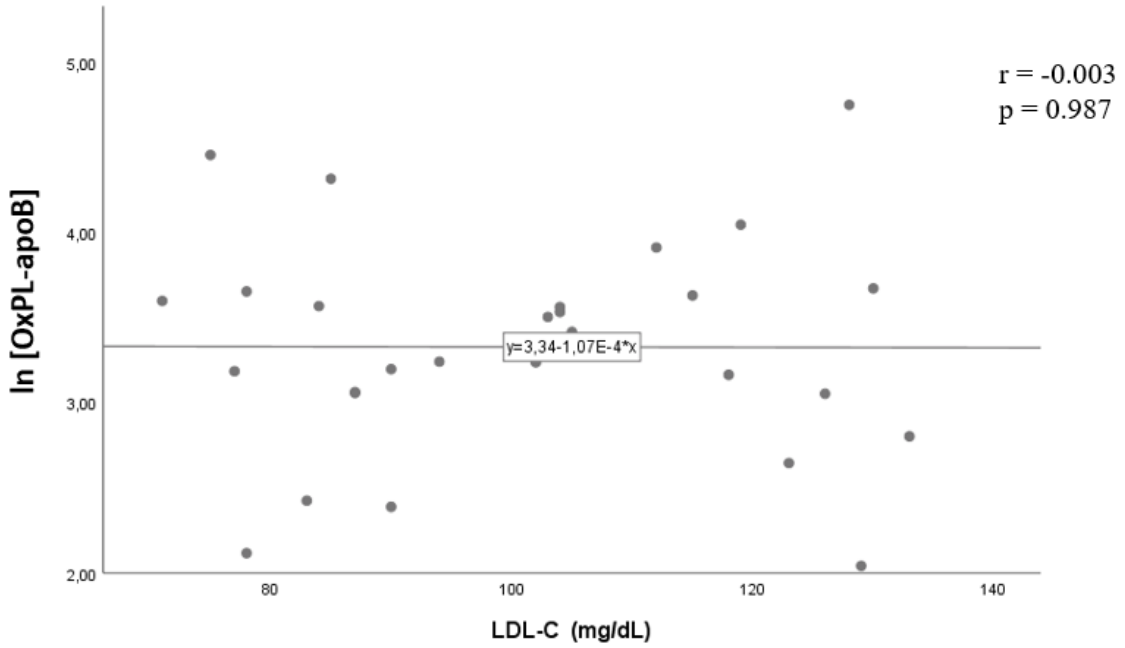

Figure 64. Correlation between low-density lipoprotein cholesterol (LDL-C) and oxidized phospholipids on apolipoprotein B-100 [OxPL-apoB] levels at baseline for the add-on ezetimibe group.

Similar patterns were observed at 3 months for the add-on ezetimibe group, with Lp(a) showing significant correlations with both OxPL-apo(a) (r = 0.655, p < 0.001) (Figure 65) and OxPL-apoB (r = 0.724, p < 0.001) (Figure 66), and LDL-C not significantly associated with OxPL-apoB (r = -0.241, p = 0.191) (Figure 67).

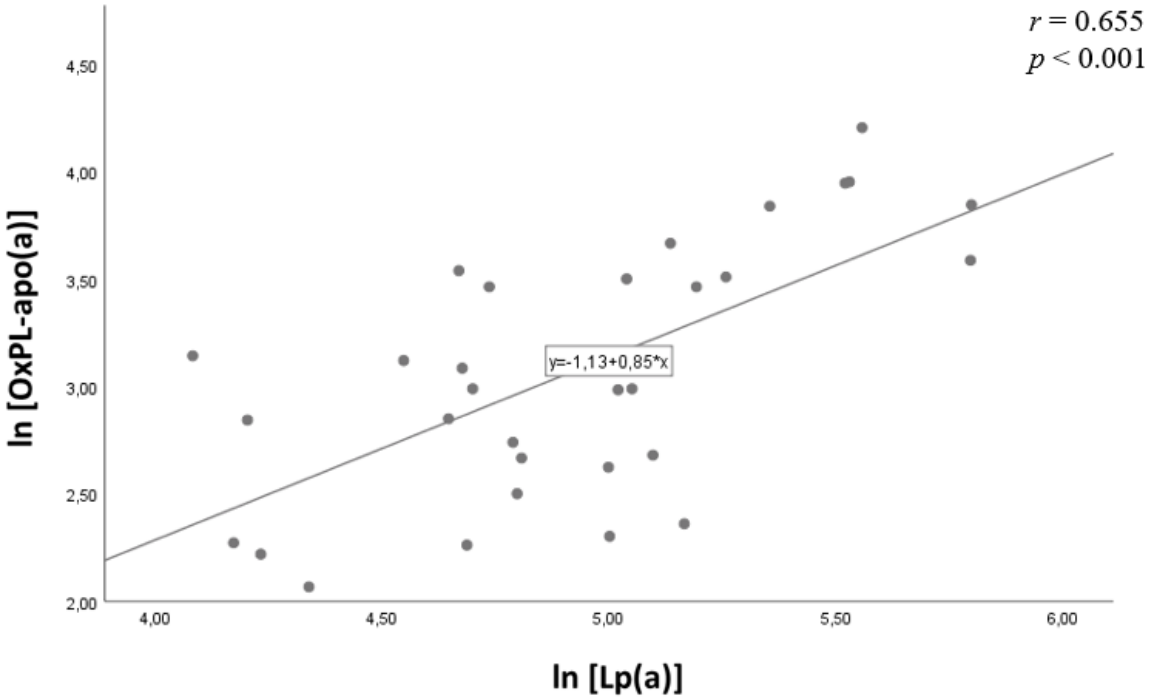

Figure 65. Correlation between lipoprotein(a) and oxidized phospholipids on apolipoprotein(a) [OxPL-apo(a)] levels at 3 months for the add-on ezetimibe group.

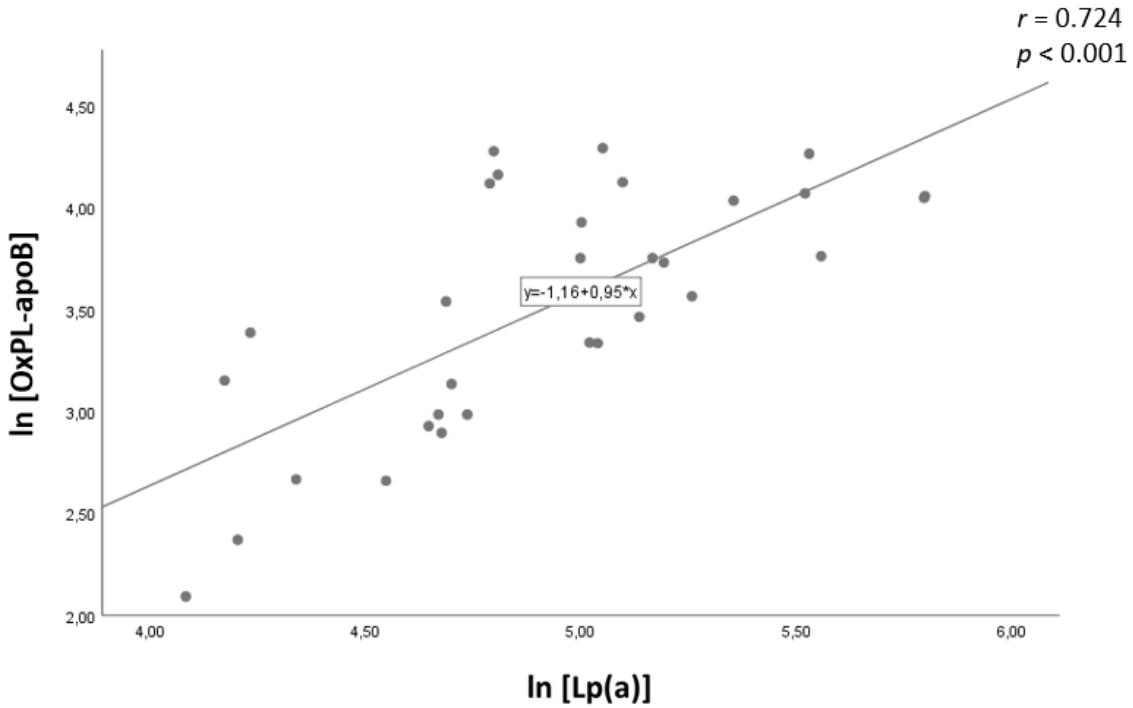

Figure 66. Correlation between lipoprotein(a) and oxidized phospholipids on apolipoprotein B-100 [OxPL-apoB] levels at 3 months for the add-on ezetimibe group.

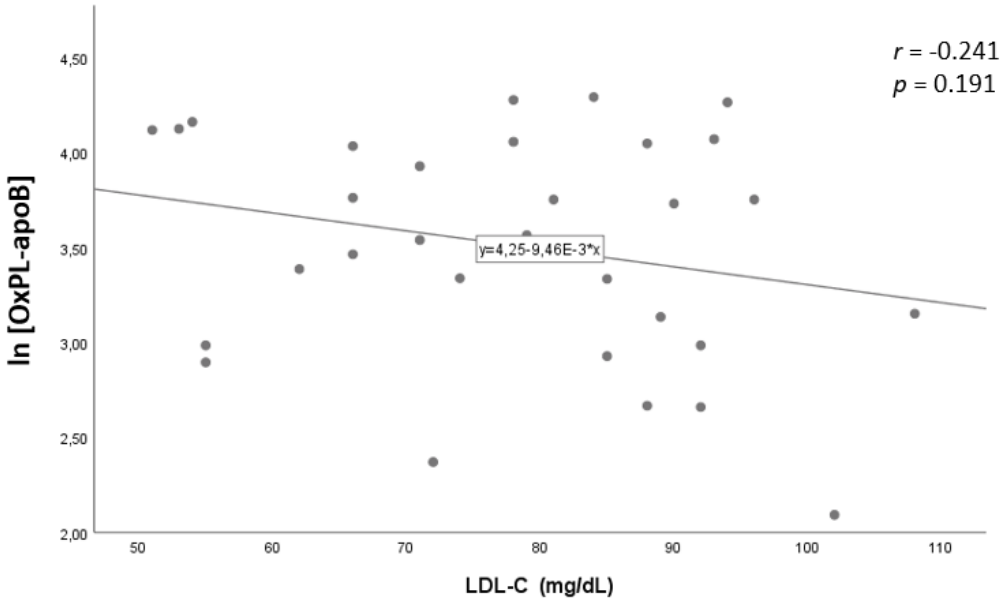

Figure 67. Correlation between low-density lipoprotein cholesterol (LDL-C) and oxidized phospholipids on apolipoprotein B-100 [OxPL-apoB] levels at 3 months for the add-on ezetimibe group.

Correlation analyses based on changes from baseline to follow-up for the add-on ezetimibe group showed no significant associations between changes in OxPL-apoB and LDL-C (r = –0.068, p = 0.722) (Figure 68) or Lp(a) (r = -0.057, p = 0.762) (Figure 69), whereas a significant correlation was observed between changes in OxPL-apo(a) and Lp(a) (r = 0.440, p = 0.013) (Figure 70).

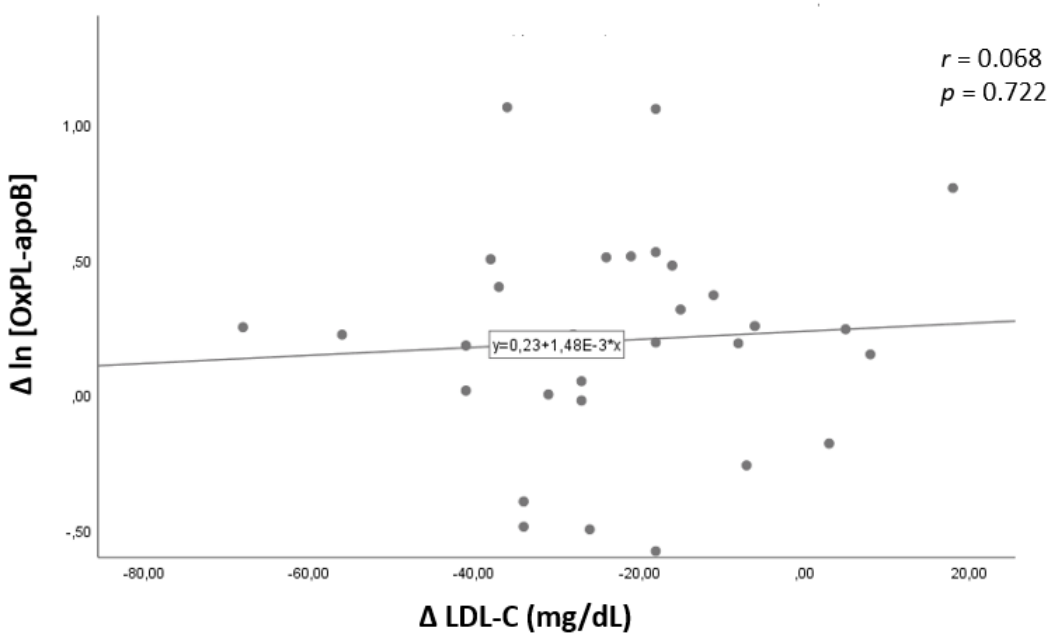

Figure 68. Correlation between changes in oxidized phospholipids on apolipoprotein B-100 [OxPL-apoB] and low-density lipoprotein cholesterol (LDL-C) levels for the add-on ezetimibe group.

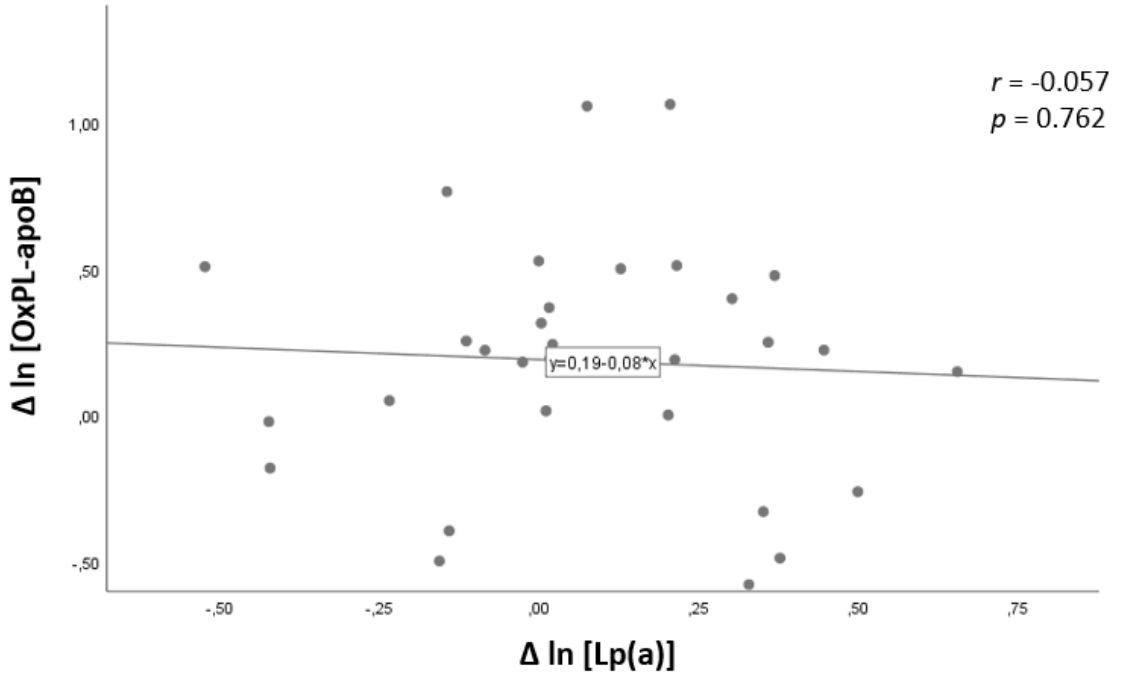

Figure 69. Correlation between changes in oxidized phospholipids on apolipoprotein B-100 [OxPL-apoB] and lipoprotein(a) levels for the add-on ezetimibe group.

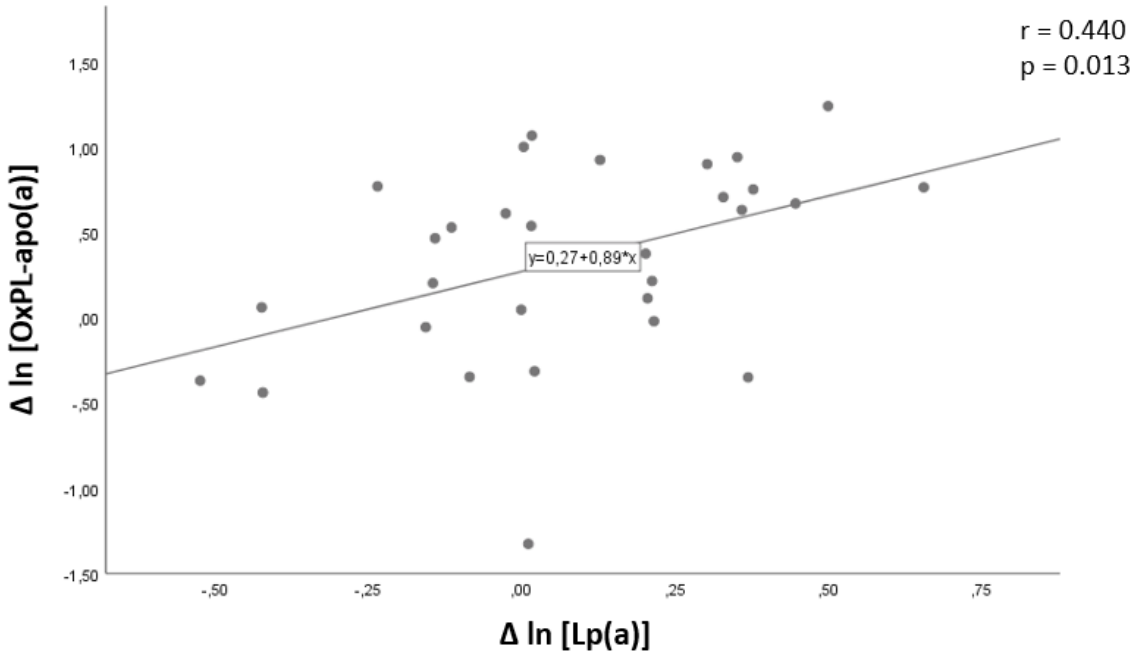

Figure 70. Correlation between changes in oxidized phospholipids on apolipoprotein(a) [OxPL-apo(a)] and lipoprotein(a) levels for the add-on ezetimibe group.

For the add-on PCSK9 inhibitor group correlation analyses at baseline revealed a significant association between Lp(a) and OxPL-apo(a) (r = 0.907, p < 0.001, Figure 71) as well as between Lp(a) and OxPL-apoB (r = 0.828, p = 0.002) (Figure 72), while LDL-C was not significantly correlated with OxPL-apoB (r = –0.261, p = 0.467) (Figure 73).

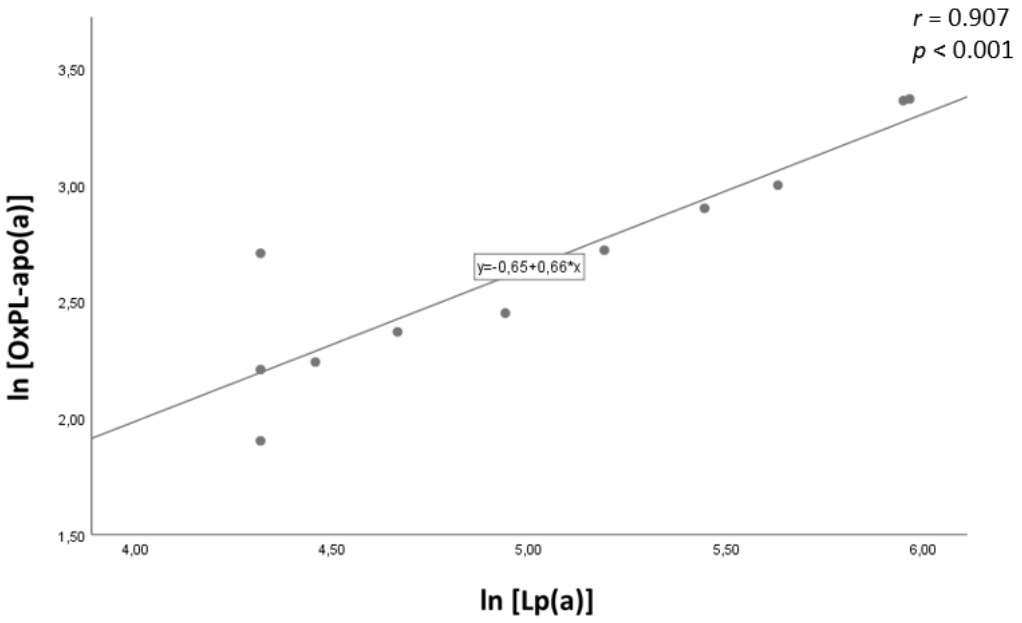

Figure 71. Correlation between lipoprotein(a) and oxidized phospholipids on apolipoprotein(a) [OxPL-apo(a)] levels at baseline for the add-on PCSK9 inhibitor group.

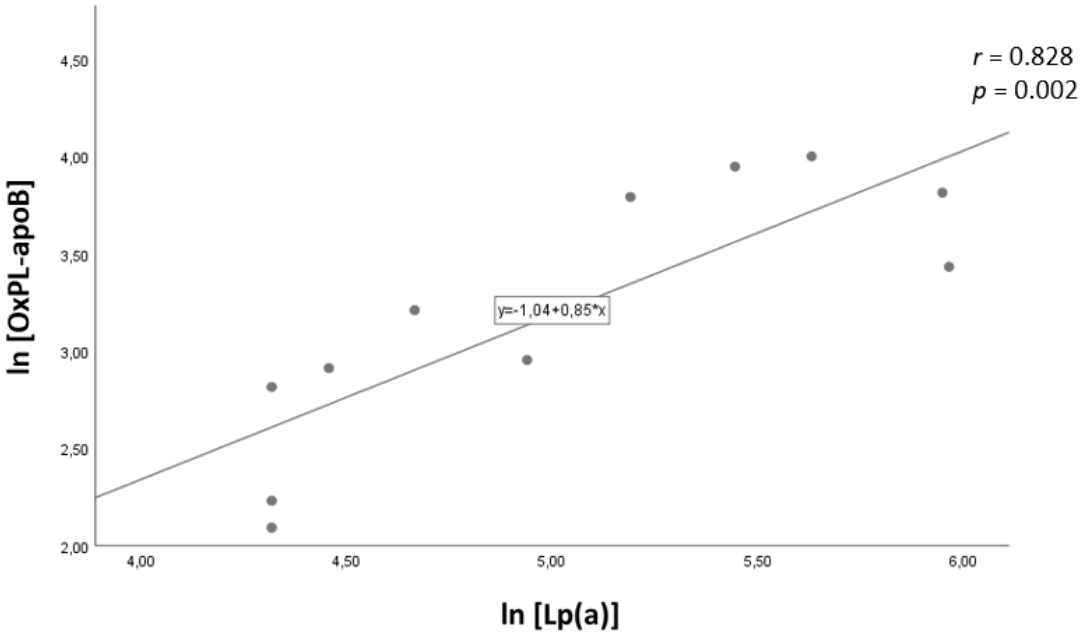

Figure 72. Correlation between lipoprotein(a) and oxidized phospholipids on apolipoprotein B-100 [OxPL-apoB] levels at baseline for the add-on PCSK9 inhibitor group.

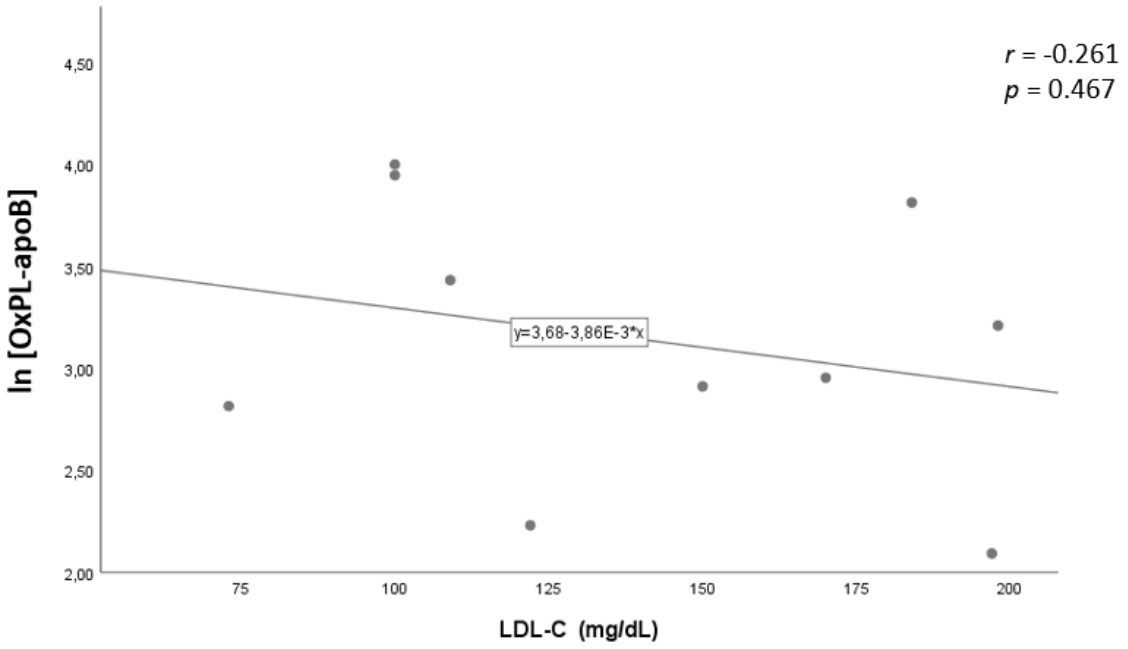

Figure 73. Correlation between low-density lipoprotein (LDL-C) and oxidized phospholipids on apolipoprotein B-100 [OxPL-apoB] levels at baseline for the add-on PCSK9 inhibitor group.

Similar patterns were observed at 3 months for the add-on PCSK9 inhibitor group, with Lp(a) showing significant correlations with both OxPL-apo(a) (r = 0.942, p < 0.001) (Figure 74) and OxPL-apoB (r = 0.976, p < 0.001) (Figure 75), whilst LDL-C was not significantly associated with OxPL-apoB (r = 0.220, p = 0.987) (Figure 76).

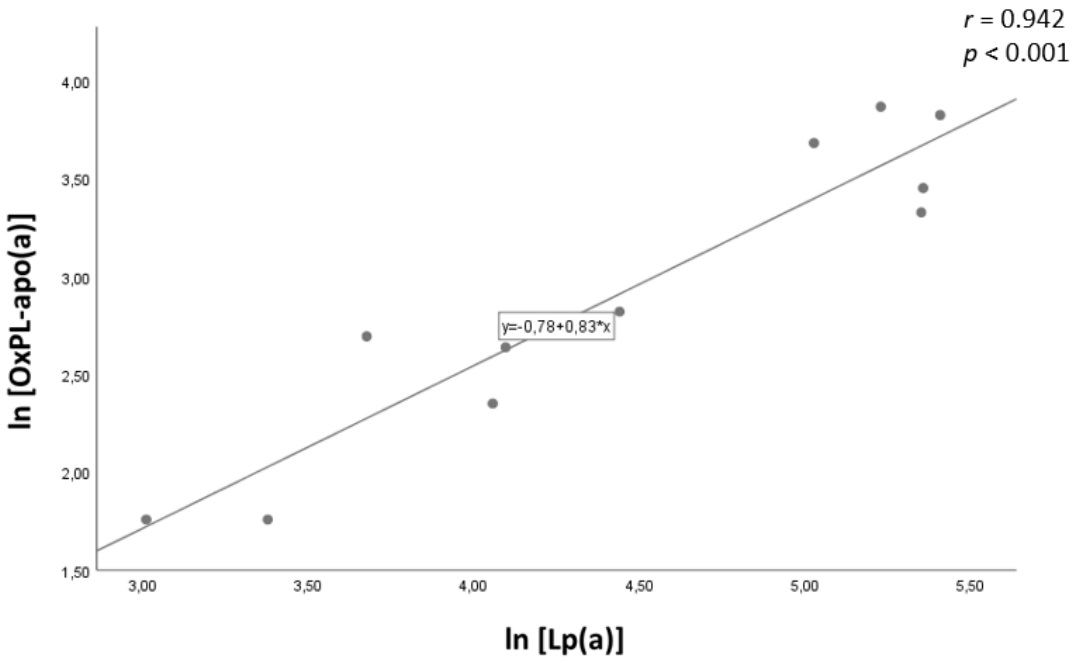

Figure 74. Correlation between lipoprotein(a) and oxidized phospholipids on apolipoprotein(a) [OxPL-apo(a)] levels at 3 months for the add-on PCSK9 inhibitor group.

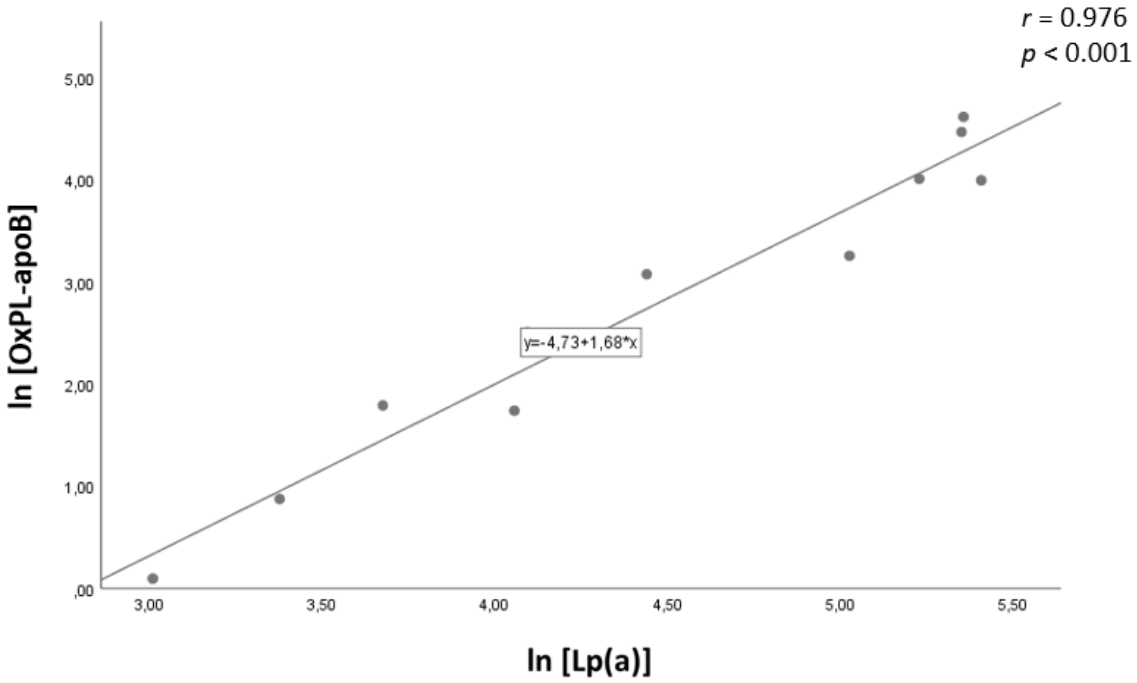

Figure 75. Correlation between lipoprotein(a) and oxidized phospholipids on apolipoprotein B-100 [OxPL-apoB] levels at 3 months for the add-on PCSK9 inhibitor group.

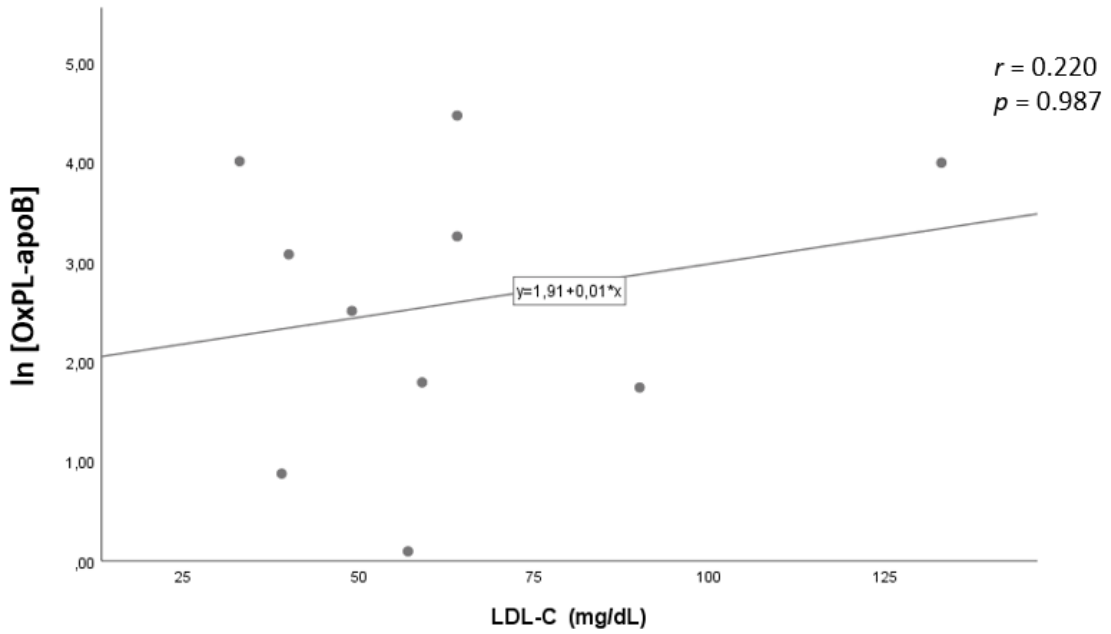

Figure 76. Correlation between low-density lipoprotein cholesterol (LDL-C) and oxidized phospholipids on apolipoprotein B-100 [OxPL-apoB] levels at 3 months for the add-on PCSK9 inhibitor group.

Correlation analyses based on changes from baseline to follow-up for the add-on PCSK9 inhibitor group showed no significant associations between changes in OxPL-apoB and LDL-C ($r = 0.022$, $p = 0.952$) (Figure 77), whereas a significant correlation was observed between

changes in OxPL-apo(a) and Lp(a) (r = 0.912, p < 0.001) (Figure 78) or between changes in OxPL-apoB and Lp(a) (r = 0.841, p = 0.001) (Figure 79).

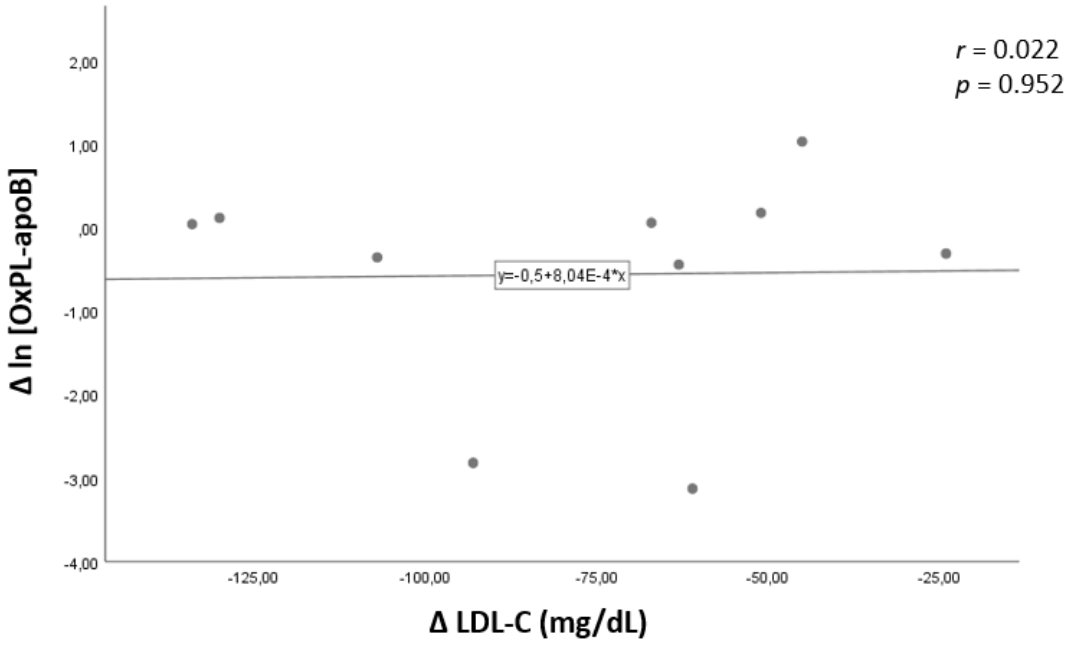

Figure 77. Correlation between changes in oxidized phospholipids on apolipoprotein B-100 [OxPL-apoB] and low-density lipoprotein cholesterol (LDL-C) levels for the add-on PCSK9 inhibitor group.

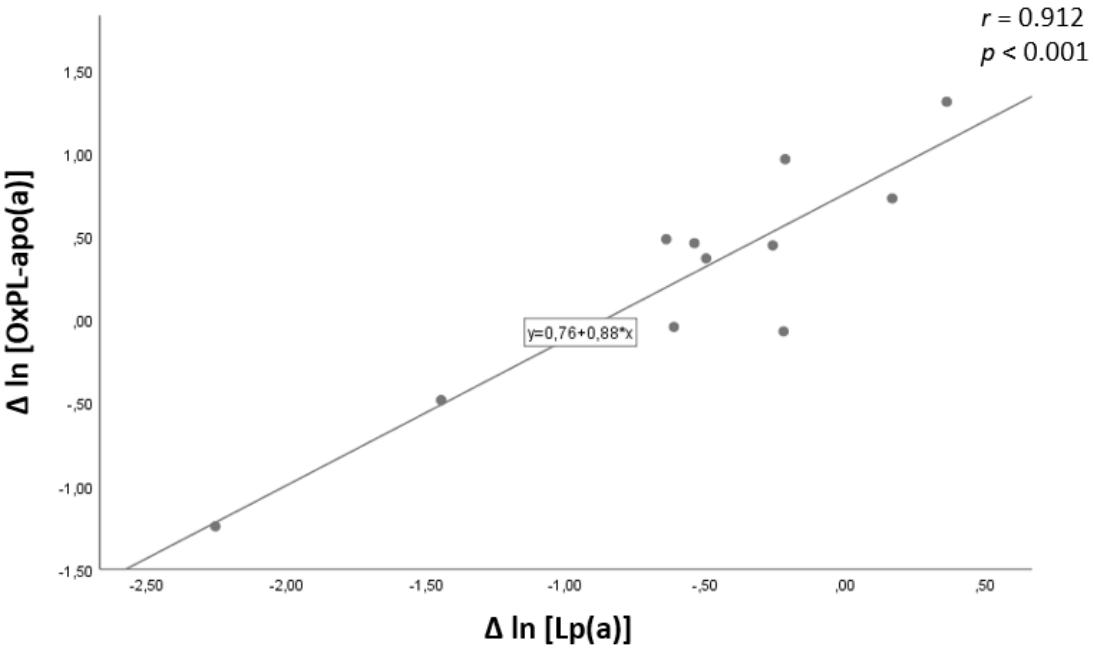

Figure 78. Correlation between changes in oxidized phospholipids on apolipoprotein(a) [OxPL-apo(a)] and lipoprotein(a) levels for the add-on PCSK9 inhibitor group.

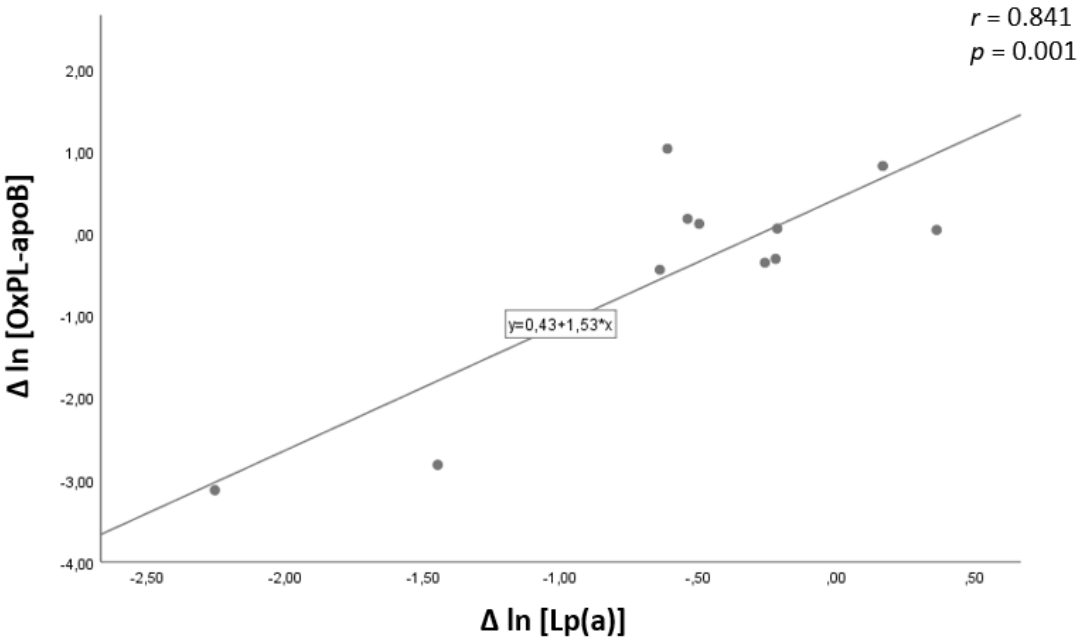

Figure 79. Correlation between changes in oxidized phospholipids in apolipoprotein B-100 [OxPL-apoB] and lipoprotein(a) levels for the add-on PCSK9 inhibitor group.

Both high-intensity statins and add-on ezetimibe significantly decreased OxPL-PLG by 2.2% and 18.8% (Table 8 and Figure 80), respectively, whereas no significant changes in PLG were noted (Table 8 and Figure 81). Add-on PCSK9 inhibitors resulted in no significant changes in OxPL-PLG and PLG (Table 8, Figure 80 and 81).

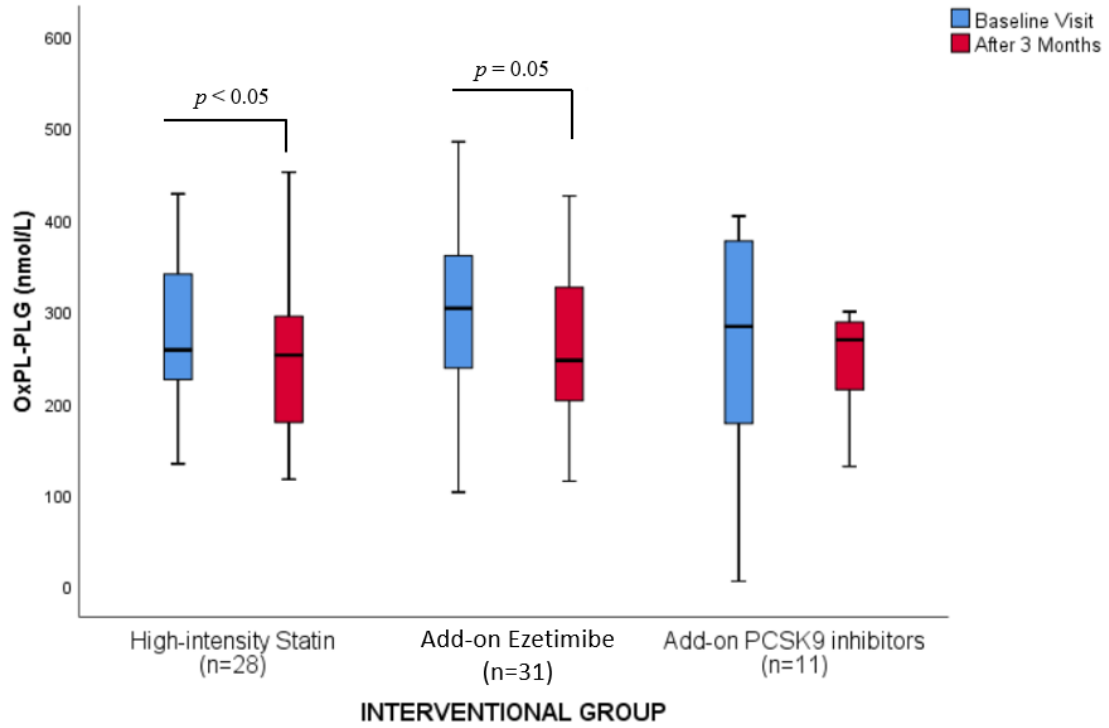

Figure 80. Effect of lipid-lowering treatment on the levels [median (IQR)] of oxidized phospholipids on plasminogen (OxPL-PLG).

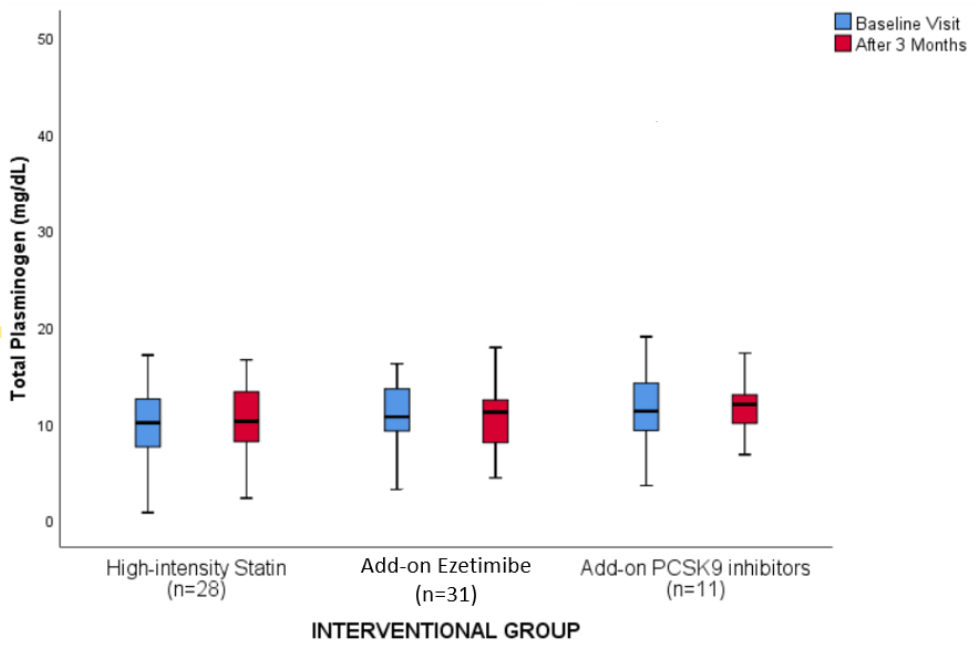

Figure 81. Effect of lipid-lowering treatment on the levels [median (IQR)] of plasminogen (PLG).

5.4 IgG/ IgM apoB-IC AND IgG/IgM anti-MDA-MIMOTOPE

Baseline values and follow-up measurements for these biomarkers are detailed in Table 9. Significant decreases in IgG apoB-IC levels of 18.3%, 17.5%, and 25.5% were noted in all groups, respectively (all $p < 0.05$) (Figure 82). On the contrary, IgM apoB-IC, IgG and IgM anti-MDA-mimotope levels did not significantly change for any treatment group (Figures 83-85, respectively).

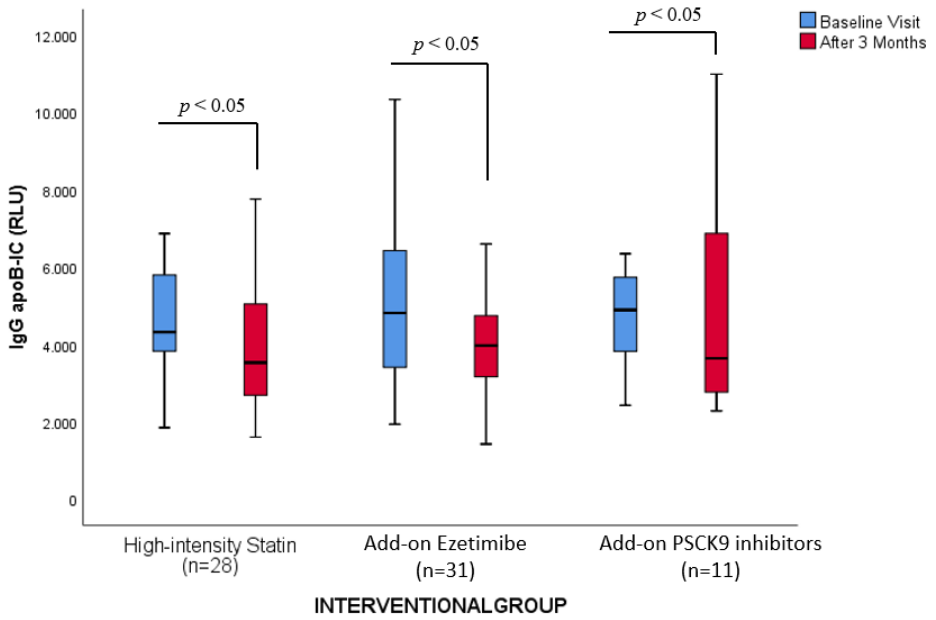

Figure 82. Effect of lipid-lowering treatment on the levels [median (IQR)] of IgG apoB-immune complexes (IgG apoB-IC).

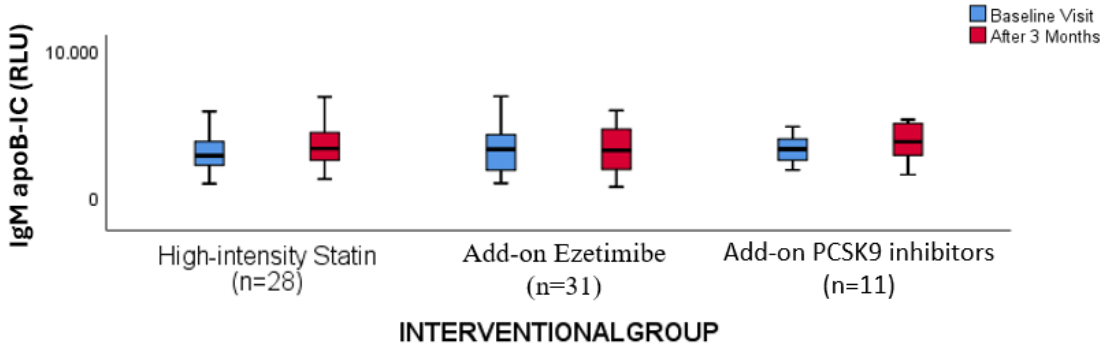

Figure 83. Effect of lipid-lowering treatment on the levels [median (IQR)] of IgM apoB-immune complexes (IgM apoB-IC).

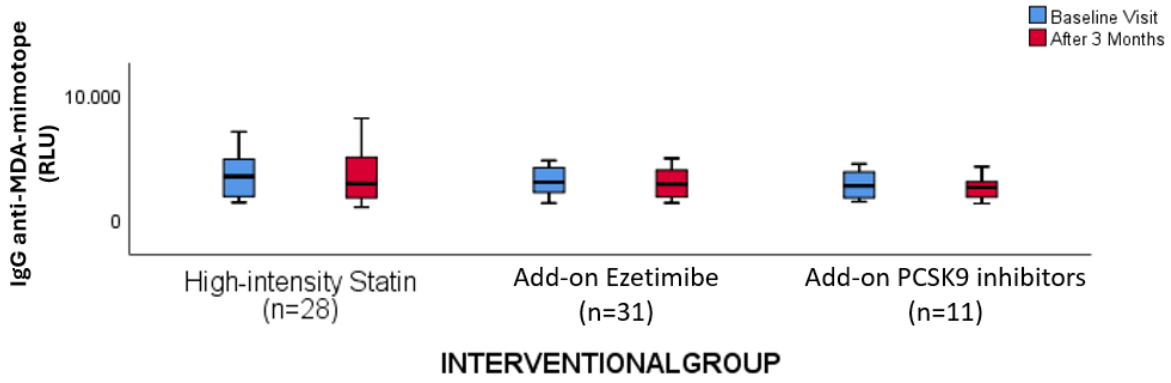

Figure 84. Effect of lipid-lowering treatment on the levels [median (IQR)] of IgG anti-malondialdehyde-mimotope (anti-MDA-mimotope).

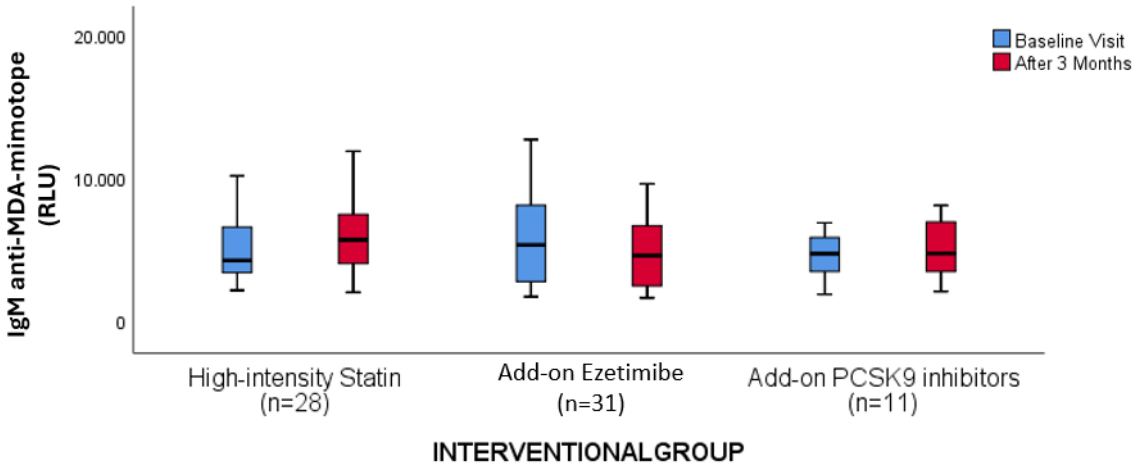

Figure 85. Effect of lipid-lowering treatment on the levels [median (IQR)] of IgM anti-malondialdehyde-mimotope (anti-MDA-mimotope).

Table 9. Effect of lipid-lowering drugs on apoB-immune complexes and malondialdehyde-modified mimotopes.

	Baseline Visit	After 3 Months	% Change within each group
IgG apoB-IC, RLU, median (IQR)			
High-intensity statin (n=28)	4326.5 (3821.5;5806.0)	3536.0 (2650.0;5112.0)	-18.3% ǂ
Add-on ezetimibe to statin (n=31)	4820.0 (3378.0;6455.0)	3976.0 (3099.0;4904.0)	-17.5% ǂ
Add-on PCSK9 inhibitor to statin and ezetimibe (n=11)	4895.0 (3598.0;5926.0)	3646.0 (2589.0;7838.0)	-25.5% ǂ
IgM apoB-IC, RLU, median (IQR)			
High-intensity statin (n=28)	2865.5 (2199.5; 3957.3)	3345.5 (2520.8; 4574.8)	+16.8%
Add-on ezetimibe to statin (n=31)	3297.0 (1806.0; 4364.0)	3236.0 (1866.0; 4806.0)	-1.9%
Add-on PCSK9 inhibitor to statin and ezetimibe (n=11)	3312.0 (2422.0; 4060.0)	3818.0 (2686.0; 5319.0)	+15.3%
IgG anti-MDA-mimotope, RLU, median (IQR)			
High-intensity statin (n=28)	3603.5 (1803.5; 4942.8)	3023.5 (1725.0; 5115.8)	-16.1%
Add-on ezetimibe to statin (n=31)	2996.0 (2043.0; 4201.0)	2831.0 (1793.0; 3992.0)	-5.5%
Add-on PCSK9 inhibitor to statin and ezetimibe (n=11)	2692.0 (1593.0; 3915.0)	2573.0 (1573.0; 3227.0)	-4.4%
IgM anti-MDA-mimotope, RLU, median (IQR)			
High-intensity statin (n=28)	4313.0 (3281.3; 7049.0)	5774.5 (4035.8; 7617.3)	+33.9%
Add-on ezetimibe to statin (n=31)	5349.0 (2729.0; 8177.0)	4606.0 (2425.0; 6806.0)	-13.9%
Add-on PCSK9 inhibitor to statin and ezetimibe (n=11)	4740.0 (3119.0; 6566.0)	4753.0 (2725.0; 7672.0)	+0.3%

ǂ $p < 0.05$ for the comparison within each group. Abbreviations: RLU; Relative Light Units, MDA-apoB; malondialdehyde-modified human apolipoprotein B-100, IgG apoB-IC; IgG apolipoproteinB-100-containing immune complexes, IgM apoB-IC; IgM apolipoproteinB-100-containing immune complexes, IgG MDA-mimotope; IgG autoantibodies to a malondialdehyde mimotope, IgM MDA-mimotope; IgM autoantibodies to a malondialdehyde mimotope, PCSK9; proprotein convertase subtilisin/kexin type 9

For the total sample, correlation analyses at baseline showed that there was not any association between IgG apoB-IC and apoB-100 (r = 0.077, p = 0.550) (Figure 86), Lp(a) (r = -0.091, p = 0.453) (Figure 87), or LDL-C (r = -0.049, p = 0.695) (Figure 88).

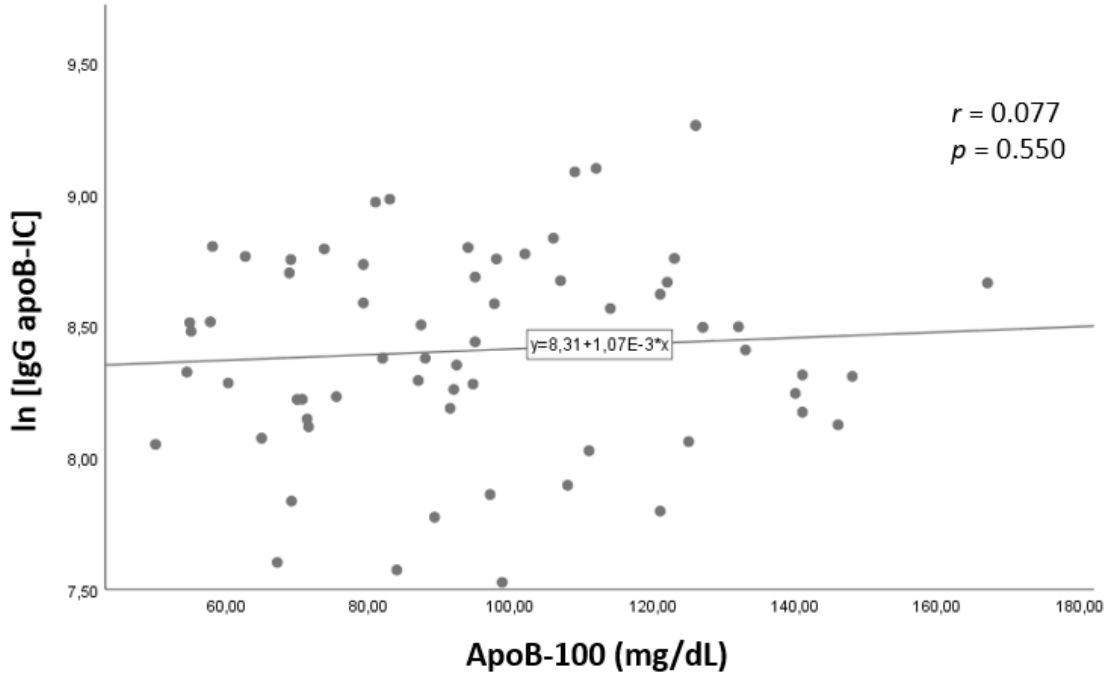

Figure 86. Correlation between IgG apoB-IC and apoB-100 levels at baseline for the whole sample.

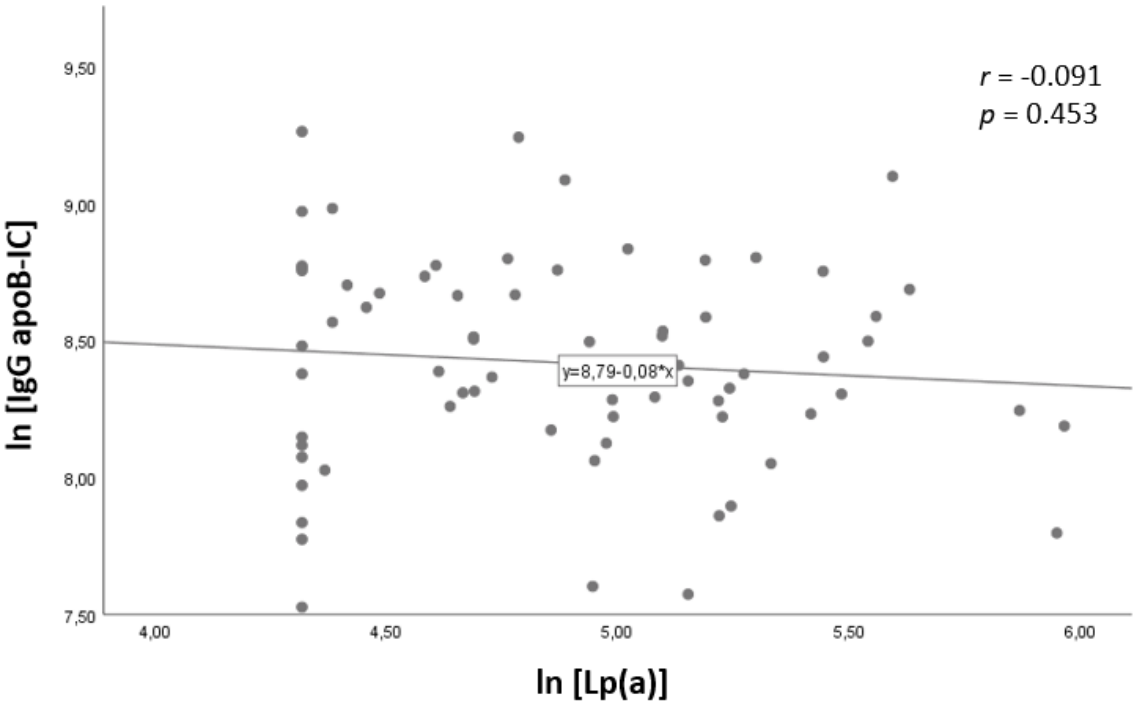

Figure 87. Correlation between IgG apoB-IC and lipoprotein(a) levels at baseline for the whole sample.

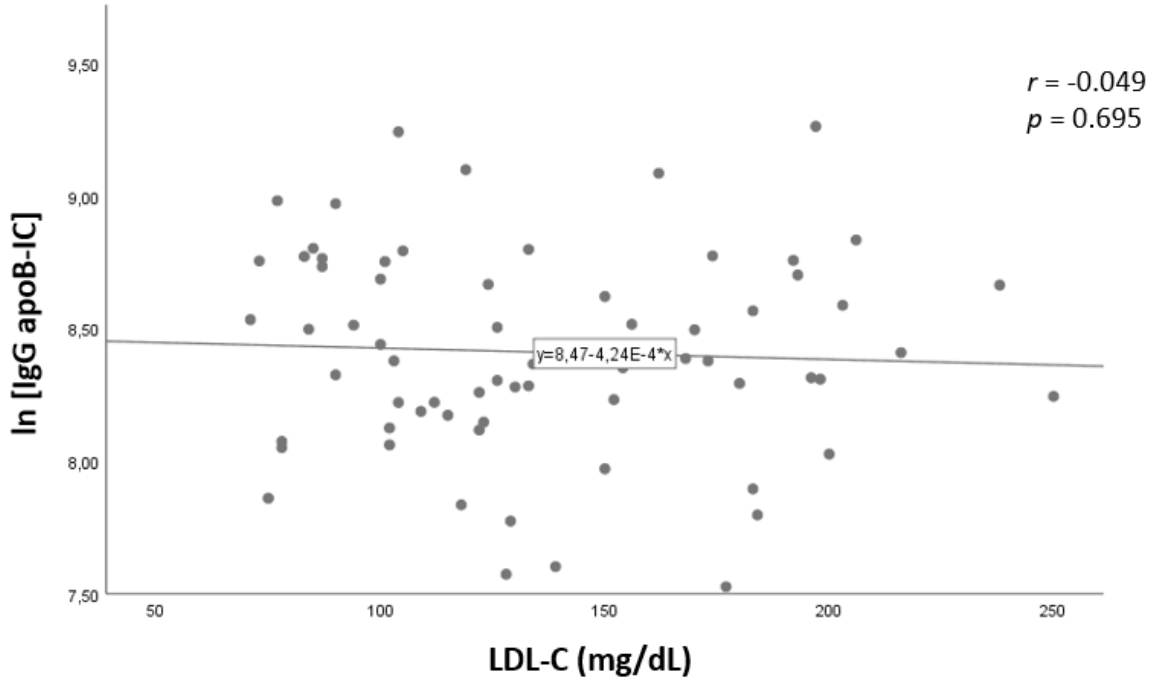

Figure 88. Correlation between IgG apoB-IC and low-density lipoprotein cholesterol (LDL-C) levels at baseline for the whole sample.

Similar patterns were observed at 3 months for the total sample, with IgG apoB-IC showing no significant correlations with either apoB-100 (r = -0.086, p = 0.488) (Figure 89), Lp(a) (r = -0.174, p = 0.150) (Figure 90), or LDL-C (r = -0.105, p = 0.392) (Figure 91).

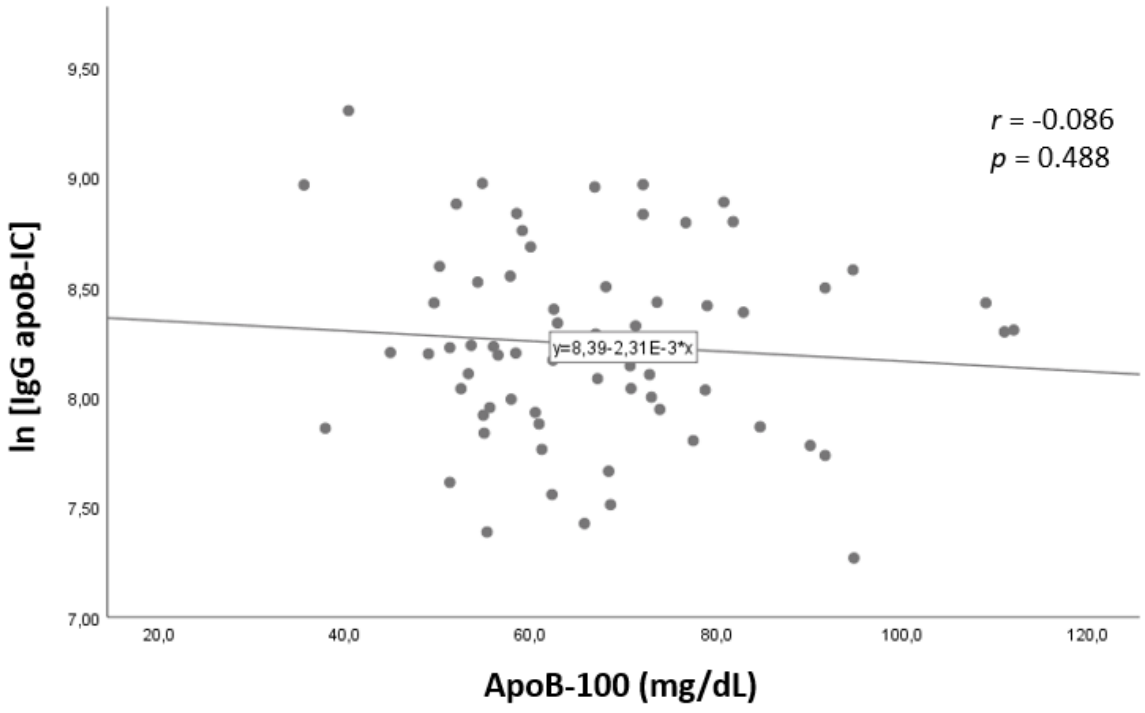

Figure 89. Correlation between IgG apoB-IC and apoB-100 levels at 3 months for the whole sample.

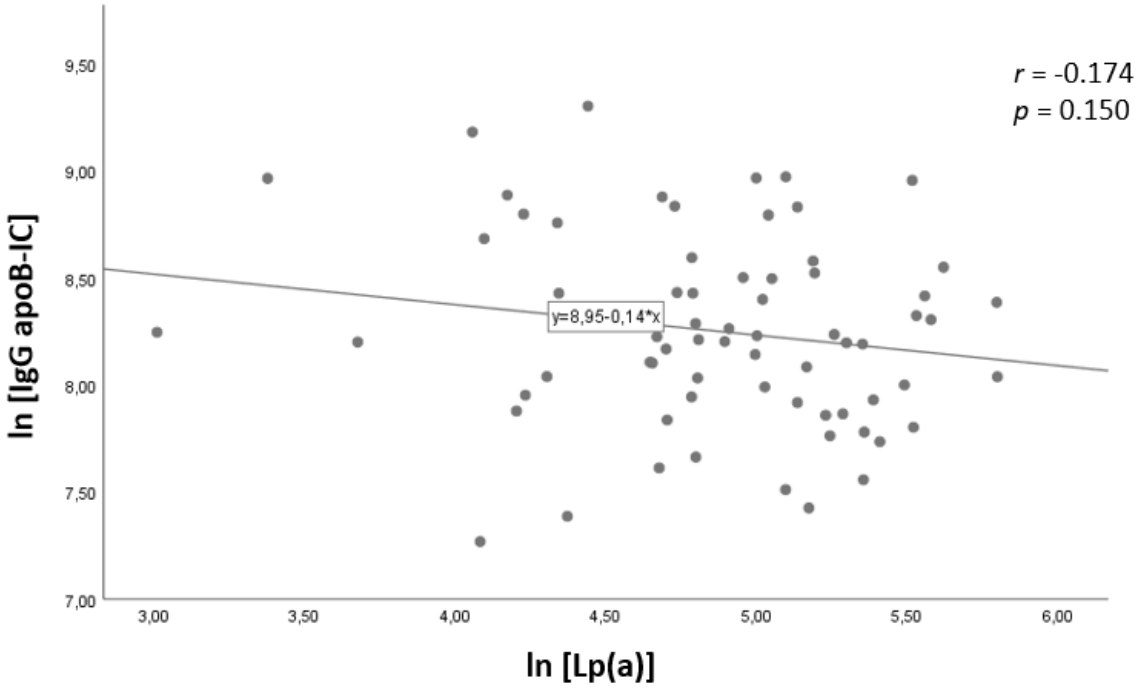

Figure 90. Correlation between IgG apoB-IC and lipoprotein(a) levels at 3 months for the whole sample.

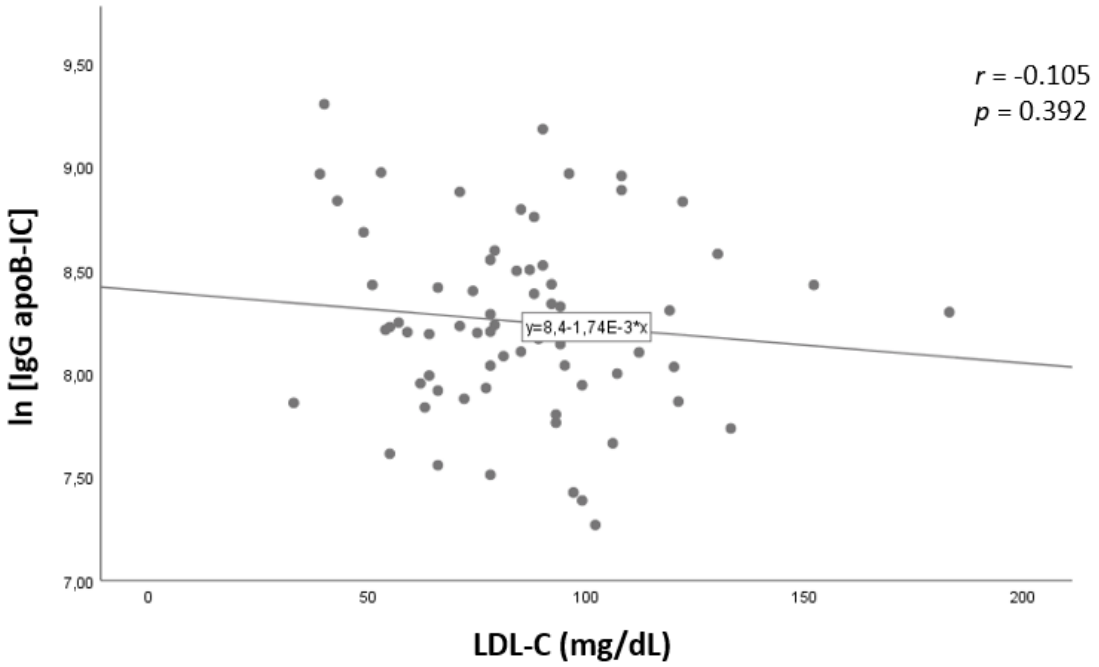

Figure 91. Correlation between IgG apoB-IC and low-density lipoprotein cholesterol (LDL-C) levels at 3 months for the whole sample.

Correlation analyses based on changes from baseline to follow-up for the whole sample revealed a significant negative association between changes in IgG apoB-IC and Lp(a) (r = -0.303, p = 0.011, Figure 92), but there was no significant associations between changes in IgG

apoB-IC and apoB-100 (r = -0.139, p = 0.286, Figure 93) or LDL-C (r = 0.071, p = 0.570) (Figure 94).

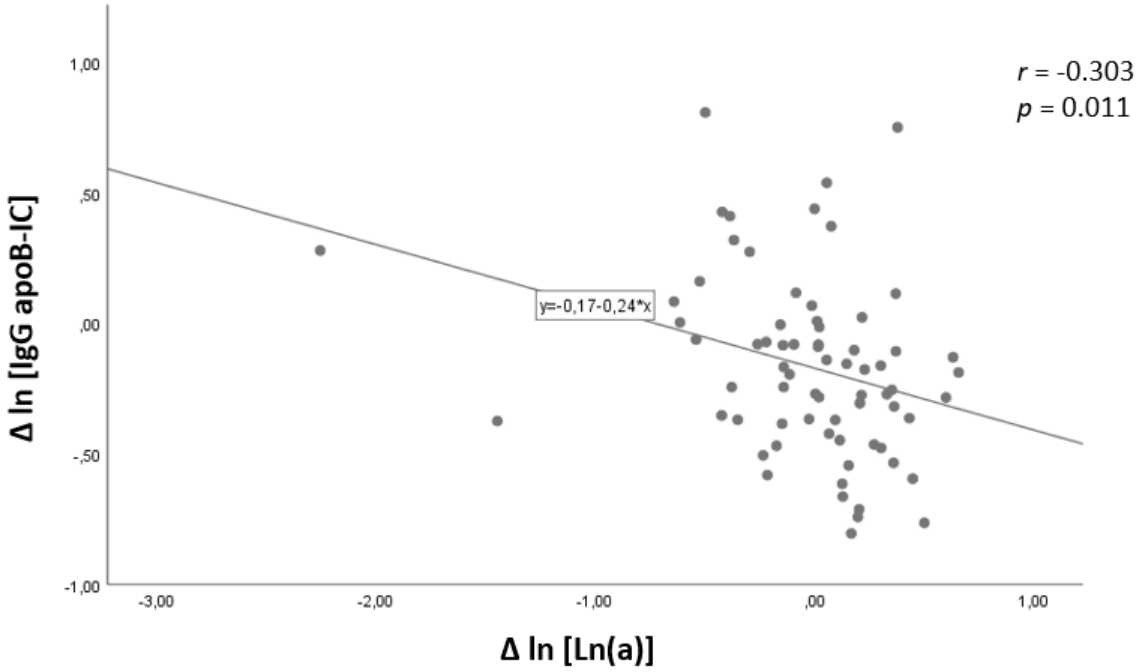

Figure 92. Correlation between changes in IgG apoB-IC and lipoprotein(a) levels for the whole sample.

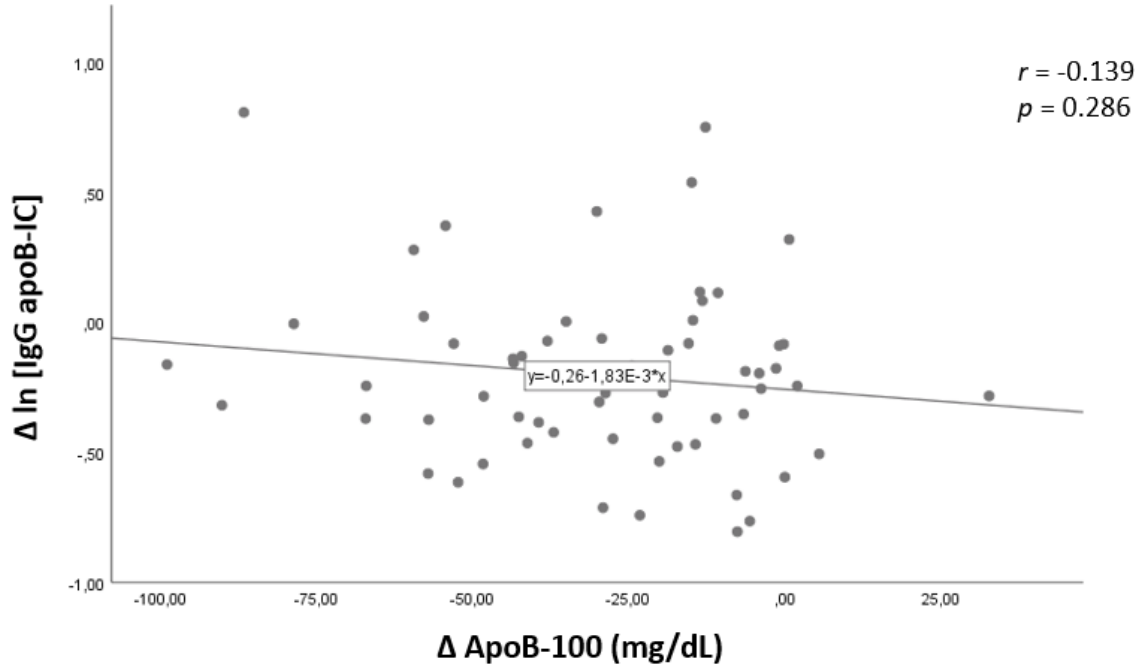

Figure 93. Correlation between changes in IgG apoB-IC and apoB-100 levels for the whole sample.

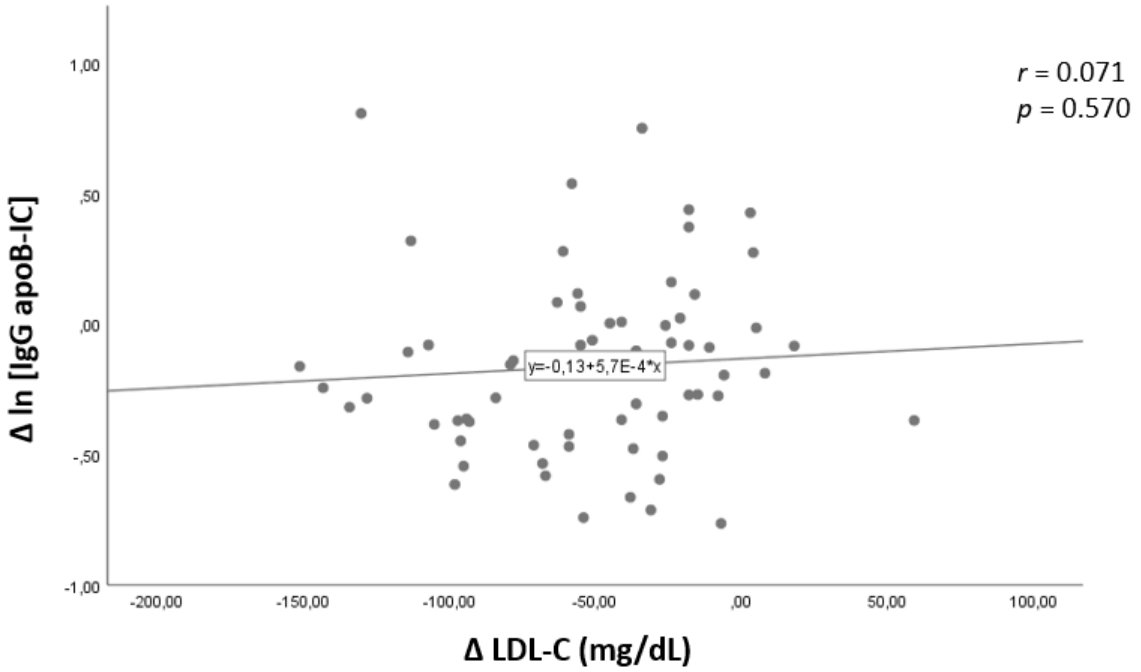

Figure 94. Correlation between changes in IgG apoB-IC and low-density lipoprotein cholesterol (LDL-C) levels for the whole sample.

For the high-intensity statin group, correlation analyses at baseline showed that there was not any association between IgG apoB-IC and apoB-100 (r = 0.208, p = 0.330) (Figure 95), Lp(a) (r = 0.024, p = 0.904) (Figure 96), or LDL-C (r = 0.179, p = 0.371) (Figure 97).

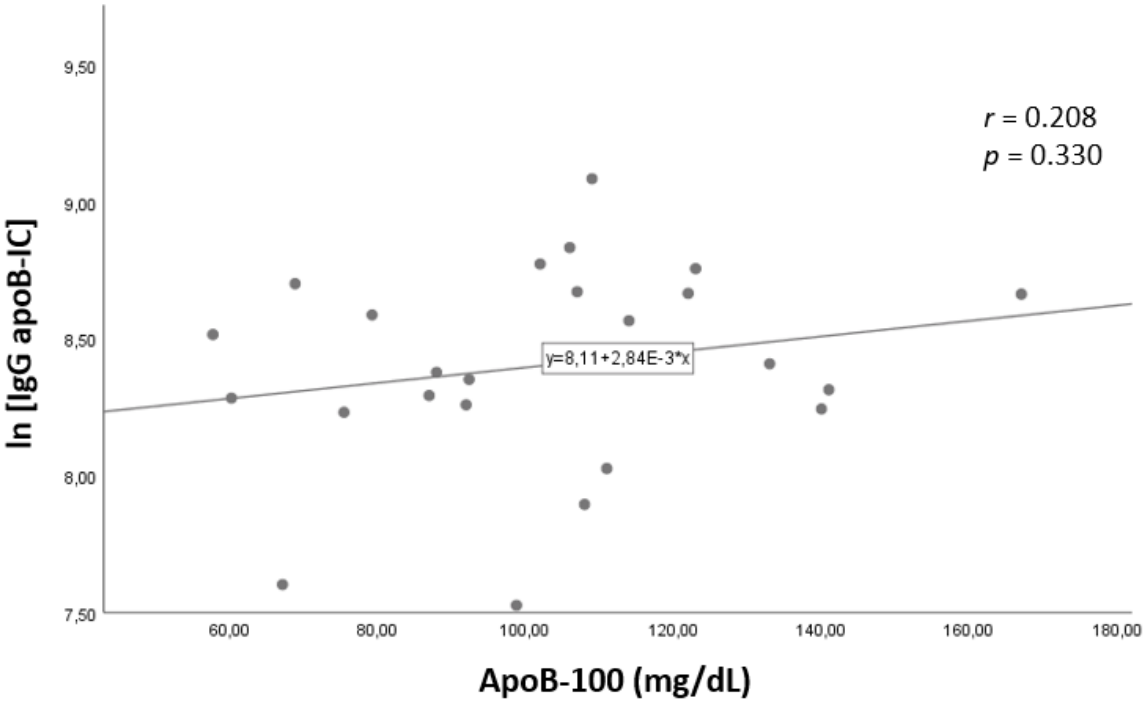

Figure 95. Correlation between IgG apoB-IC and apoB-100 levels at baseline for the high-intensity statin group.

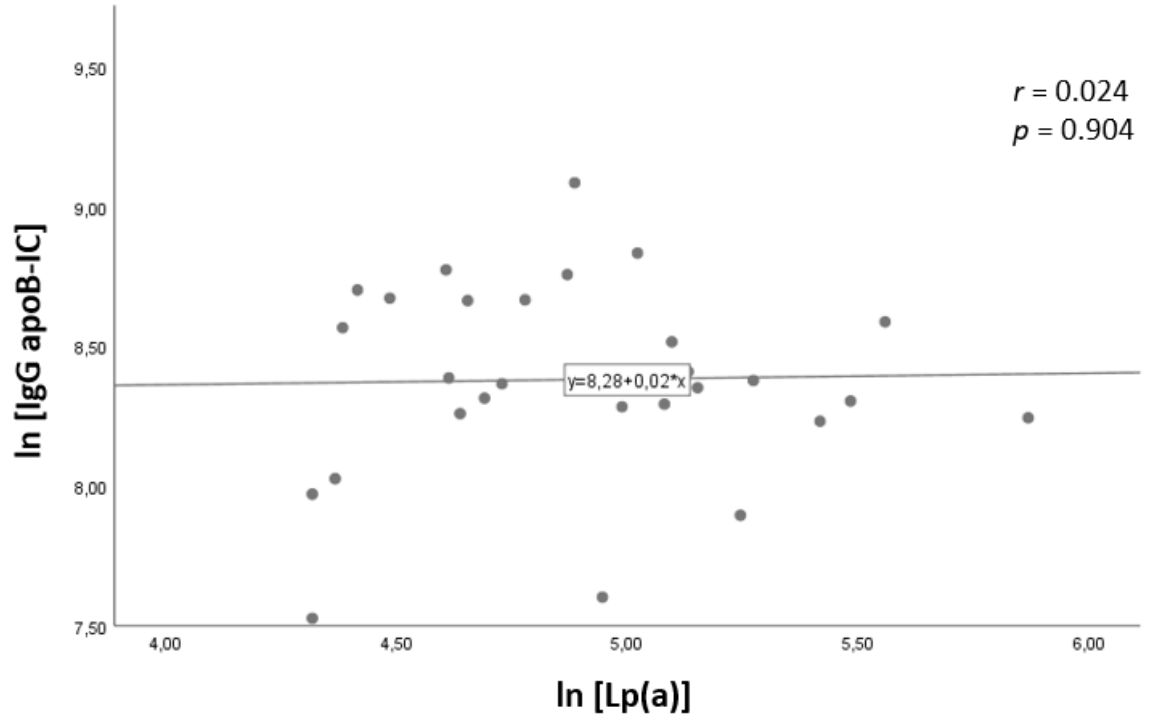

Figure 96. Correlation between IgG apoB-IC and lipoprotein(a) levels at baseline for the high-intensity statin group.

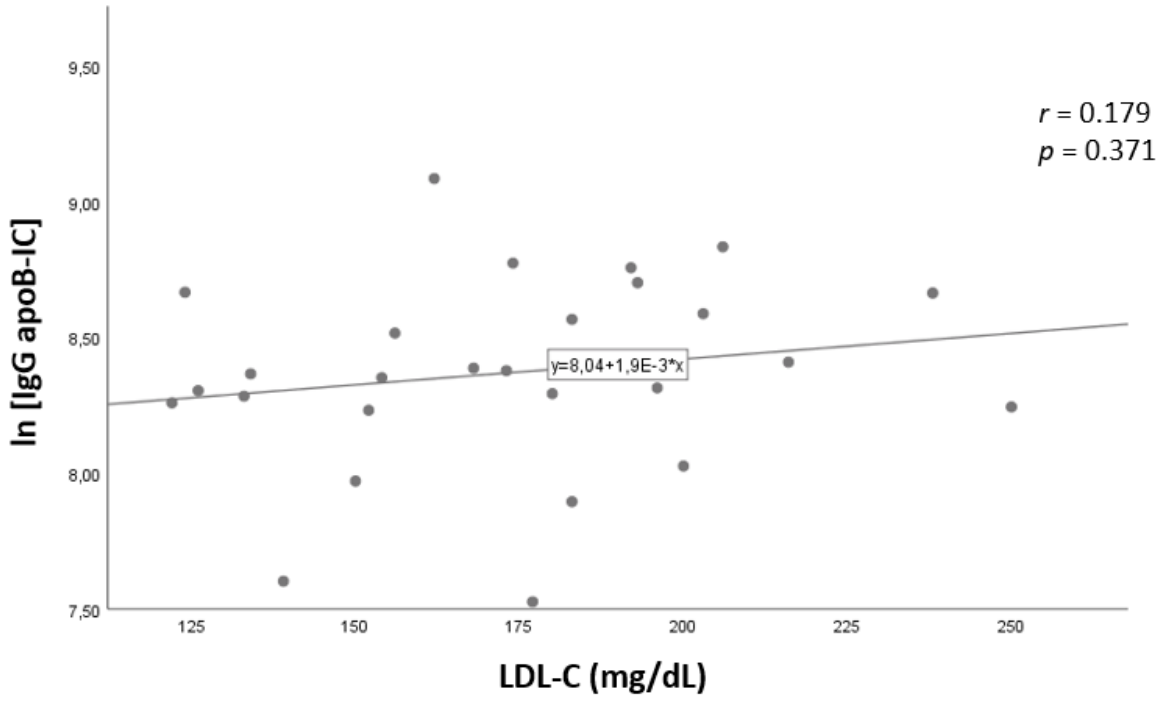

Figure 97. Correlation between IgG apoB-IC and low-density lipoprotein cholesterol (LDL-C) levels at baseline for the high-intensity statin group.

Similar patterns were observed at 3 months for the high-intensity statin group, with IgG apoB-IC showing no significant correlations with either apoB-100 (r = 0.167, p = 0.396)

(Figure 98), Lp(a) (r = 0.044, p = 0.824) (Figure 99), or LDL-C (r = 0.078, p = 0.701) (Figure 100).

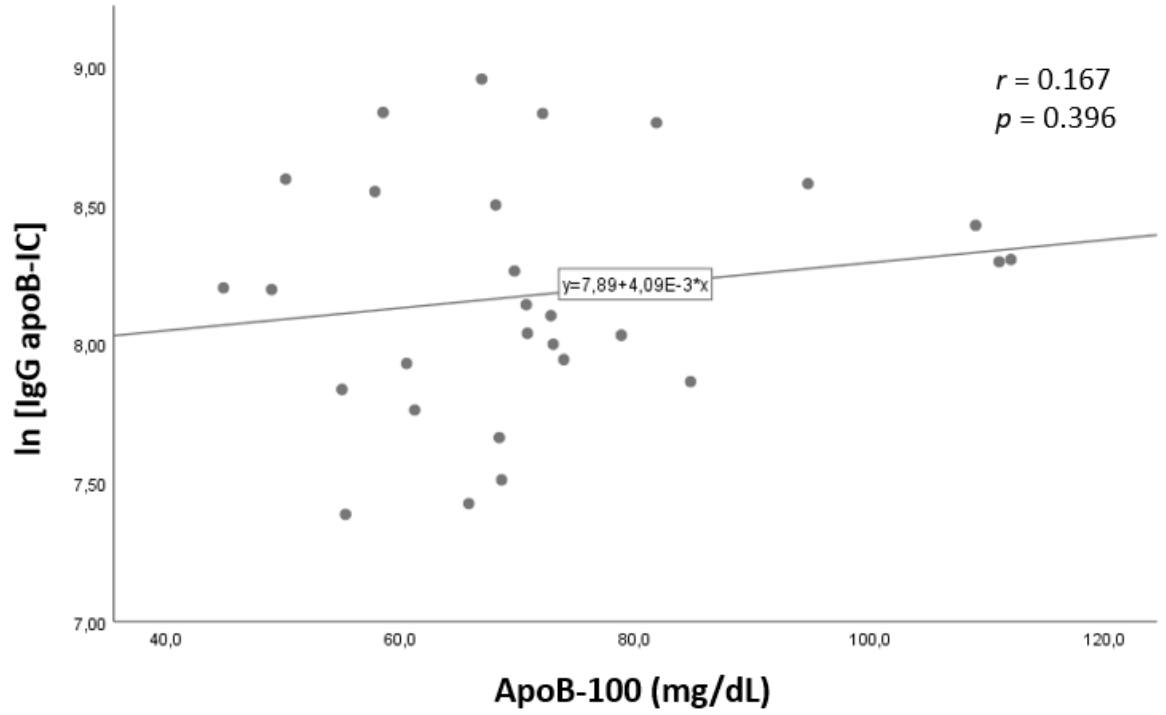

Figure 98. Correlation between IgG apoB-IC and apoB-100 levels at 3 months for the high-intensity statin group.

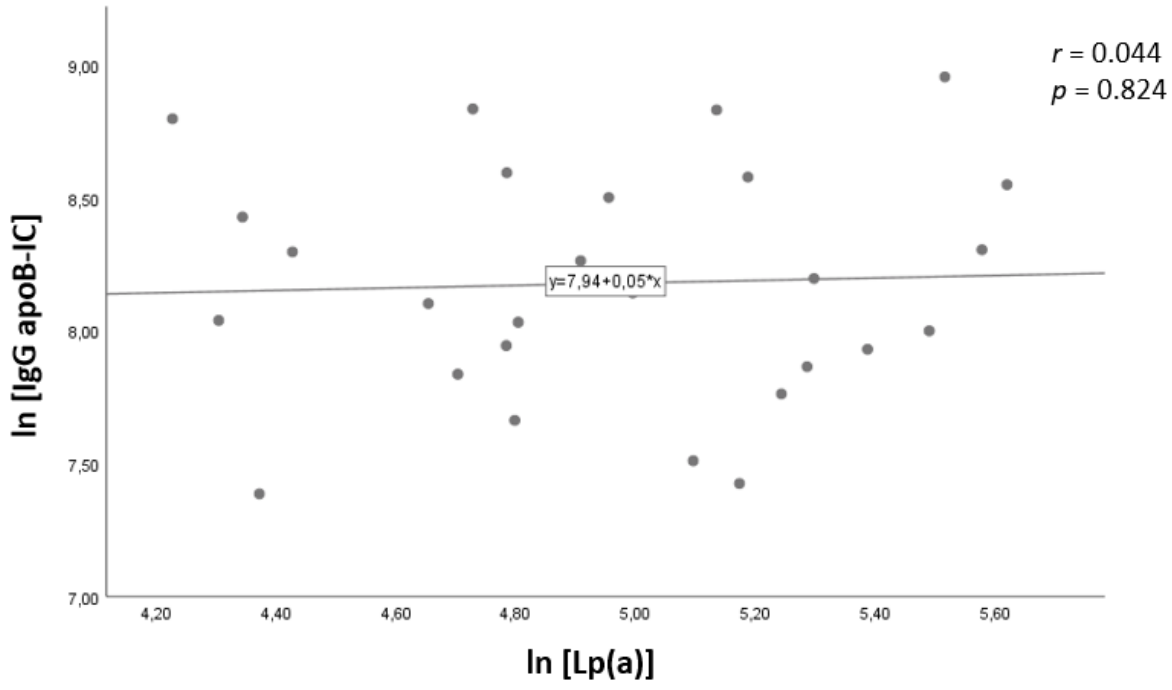

Figure 99. Correlation between IgG apoB-IC and lipoprotein(a) levels at 3 months for the high-intensity statin group.

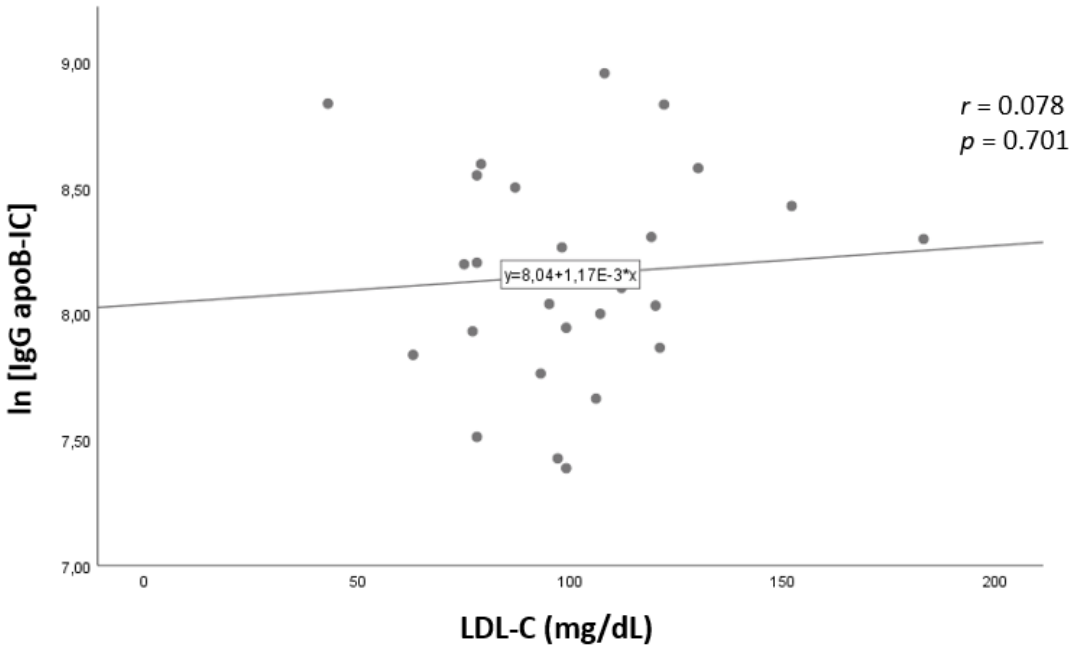

Figure 100. Correlation between IgG apoB-IC and low-density lipoprotein cholesterol (LDL-C) levels at 3 months for the high-intensity statin group.

Correlation analyses based on changes from baseline to follow-up for the high-intensity statin group revealed no significant associations between changes in IgG apoB-IC and apoB-100 (r = 0.219, p = 0.304) (Figure 101), Lp(a) (r = -0.196, p = 0.318) (Figure 102), or LDL-C (r = 0.113, p = 0.582) (Figure 103).

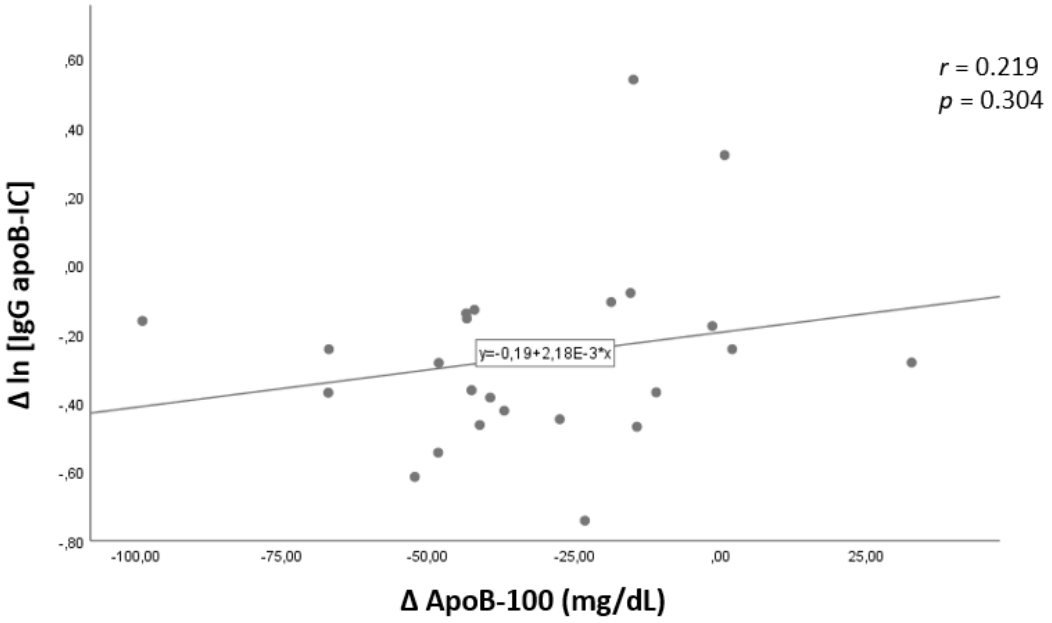

Figure 101. Correlation between changes in IgG apoB-IC and apoB-100 levels for the high-intensity statin group.

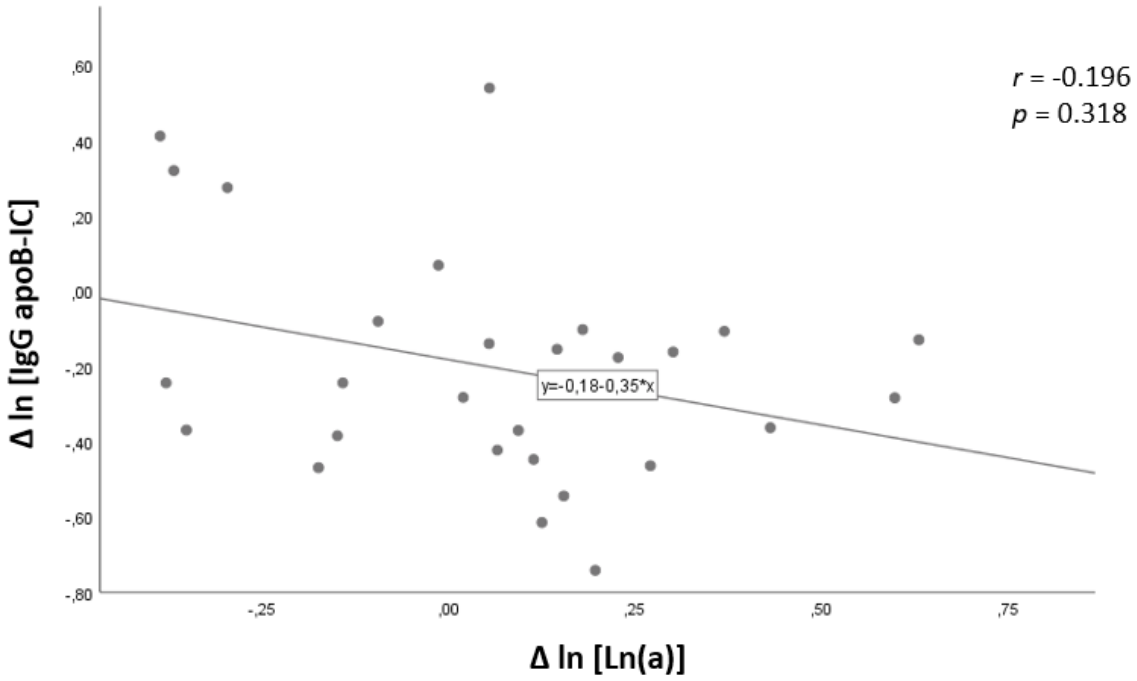

Figure 102. Correlation between changes in IgG apoB-IC and lipoprotein(a) levels for the high-intensity statin group.

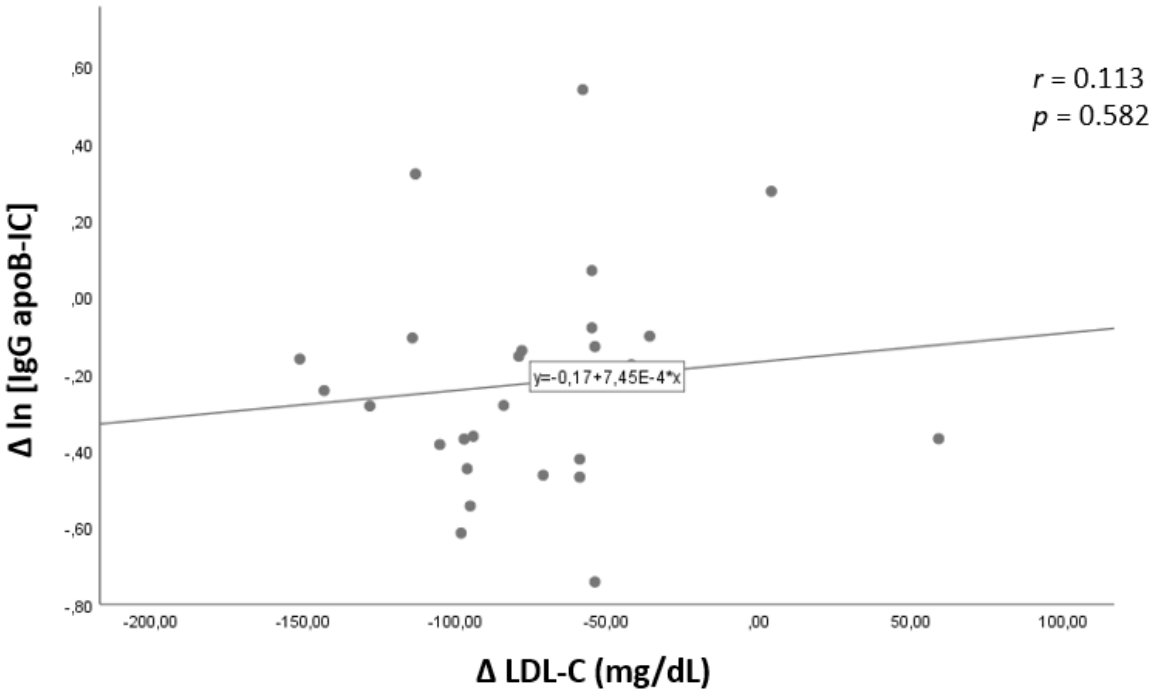

Figure 103. Correlation between changes in IgG apoB-IC and low-density lipoprotein cholesterol (LDL-C) levels for the high-intensity statin group.

For the add-on ezetimibe group, correlation analyses at baseline showed that there was not any association between IgG apoB-IC and apoB-100 (r = -0.096, p = 0.628) (Figure 104), Lp(a) (r = 0.005, p = 0.977) (Figure 105), or LDL-C (r = -0.227, p = 0.229) (Figure 106).

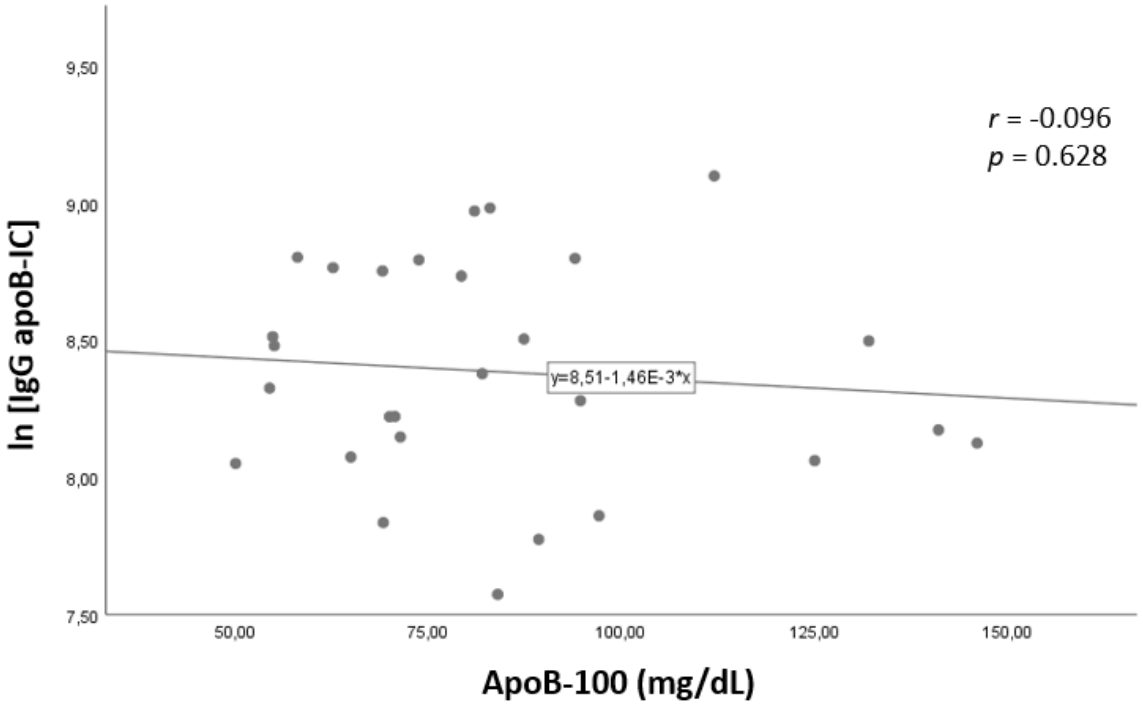

Figure 104. Correlation between IgG apoB-IC and apoB-100 levels at baseline for the add-on ezetimibe group.

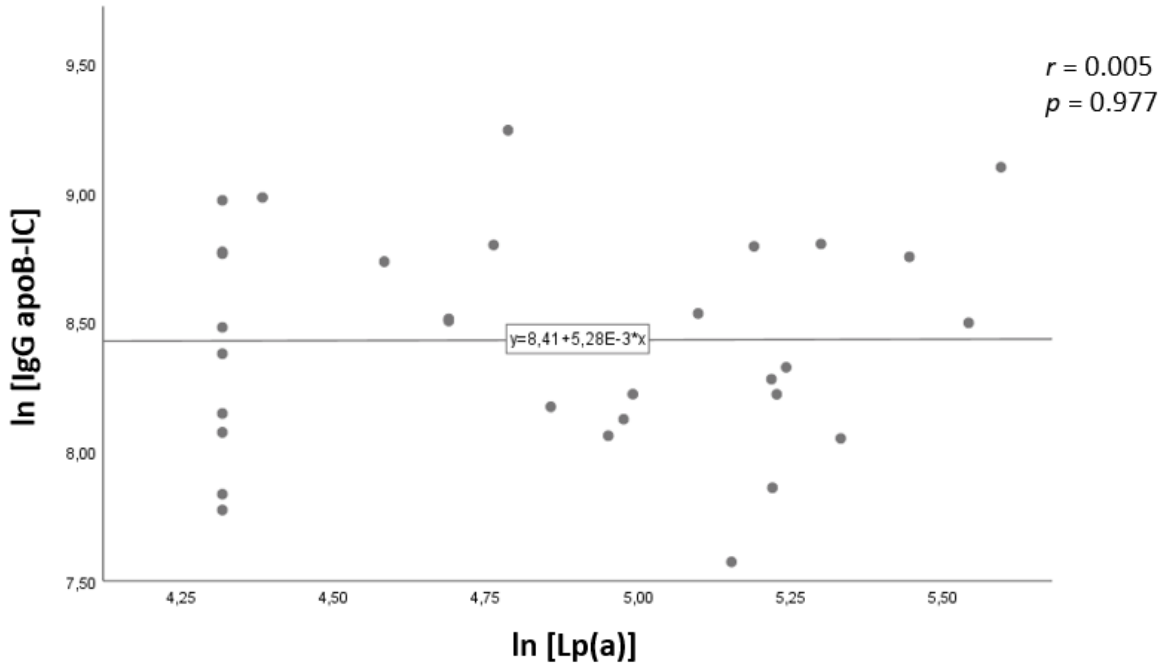

Figure 105. Correlation between IgG apoB-IC and lipoprotein(a) levels at baseline for the add-on ezetimibe group.

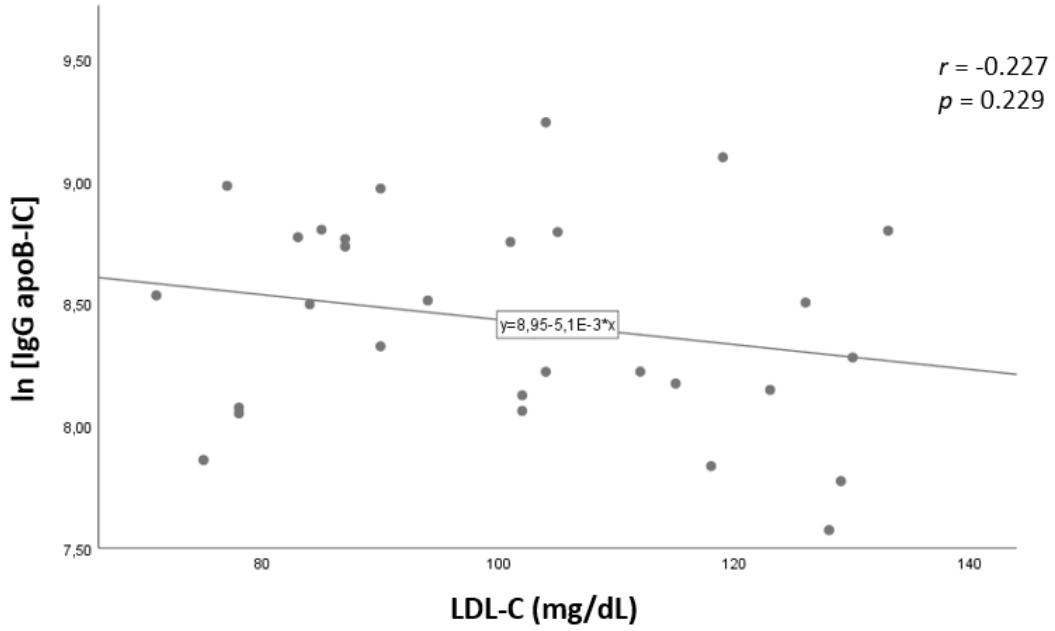

Figure 106. Correlation between IgG apoB-IC and low-density lipoprotein cholesterol (LDL-C) levels at baseline for the add-on ezetimibe group.

Similar patterns were observed at 3 months for the add-on ezetimibe group, with IgG apoB-IC showing no significant correlations with either apoB-100 (r = -0.009, p = 0.964) (Figure 107), Lp(a) (r = 0.047, p = 0.802) (Figure 108), or LDL-C (r = 0.131, p = 0.483) (Figure 109).

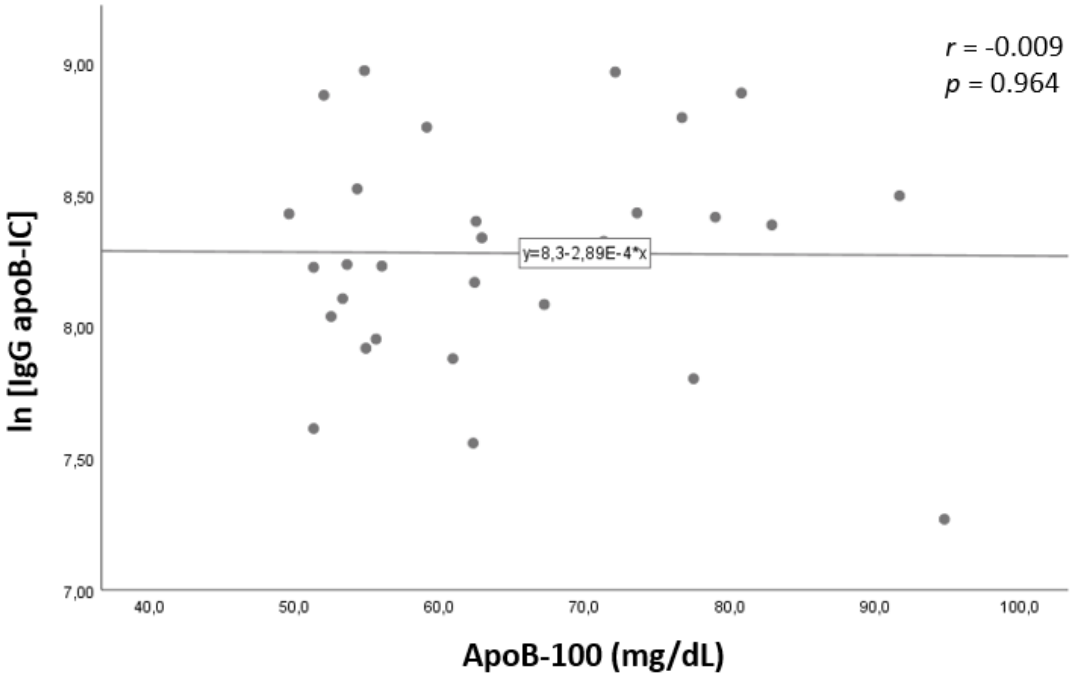

Figure 107. Correlation between IgG apoB-IC and apoB-100 levels at 3 months for the add-on ezetimibe group.

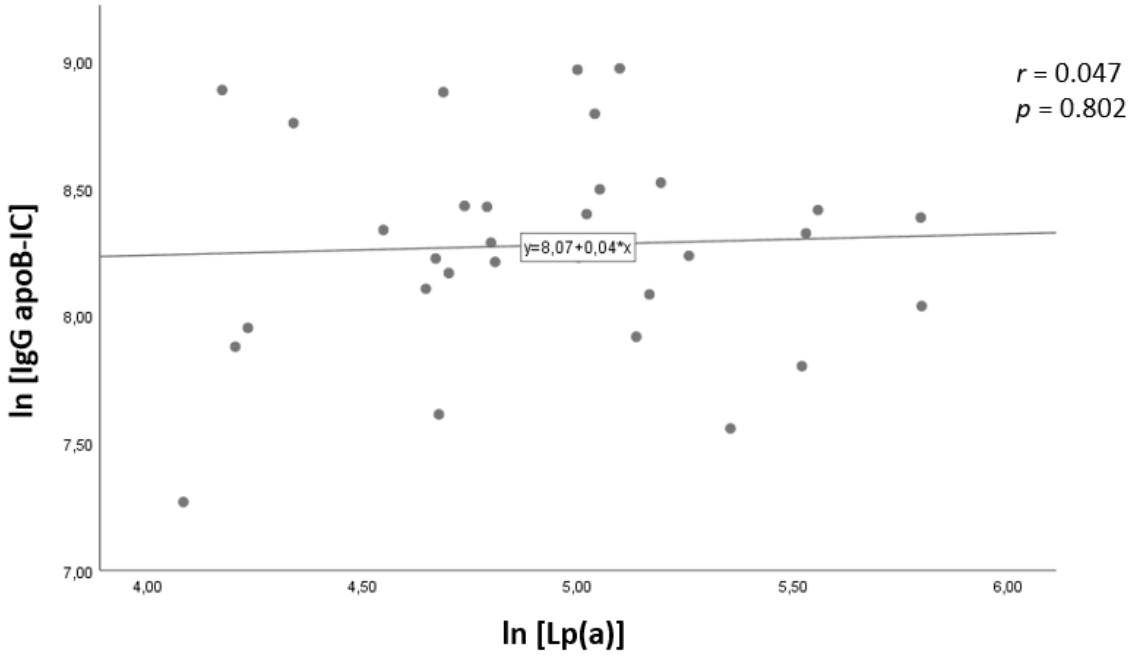

Figure 108. Correlation between IgG apoB-IC and lipoprotein(a) levels at 3 months for the add-on ezetimibe group.

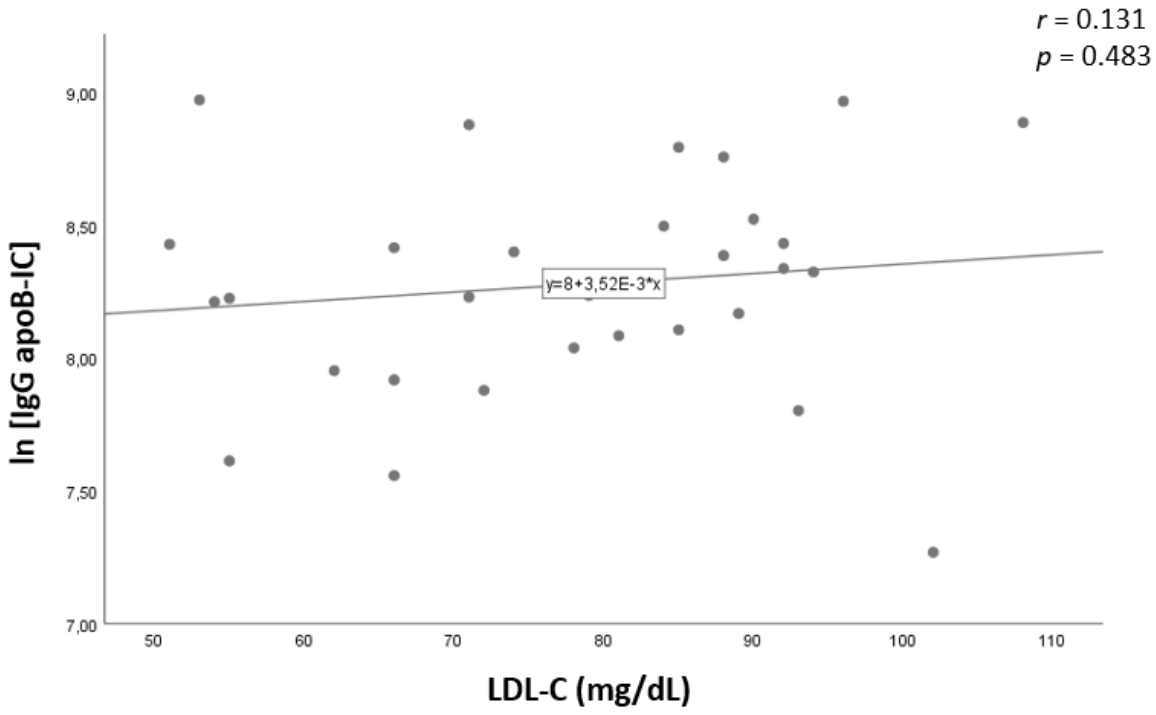

Figure 109. Correlation between IgG apoB-IC and low-density lipoprotein cholesterol (LDL-C) levels at 3 months for the add-on ezetimibe group.

Correlation analyses based on changes from baseline to follow-up for the add-on ezetimibe group revealed no significant associations between changes in IgG apoB-IC and apoB-100 (*r*

= -0.313, p = 0.112) (Figure 110), Lp(a) (r = -0.276, p = 0.133) (Figure 111), or LDL C (r = 0.192, p = 0.308) (Figure 112).

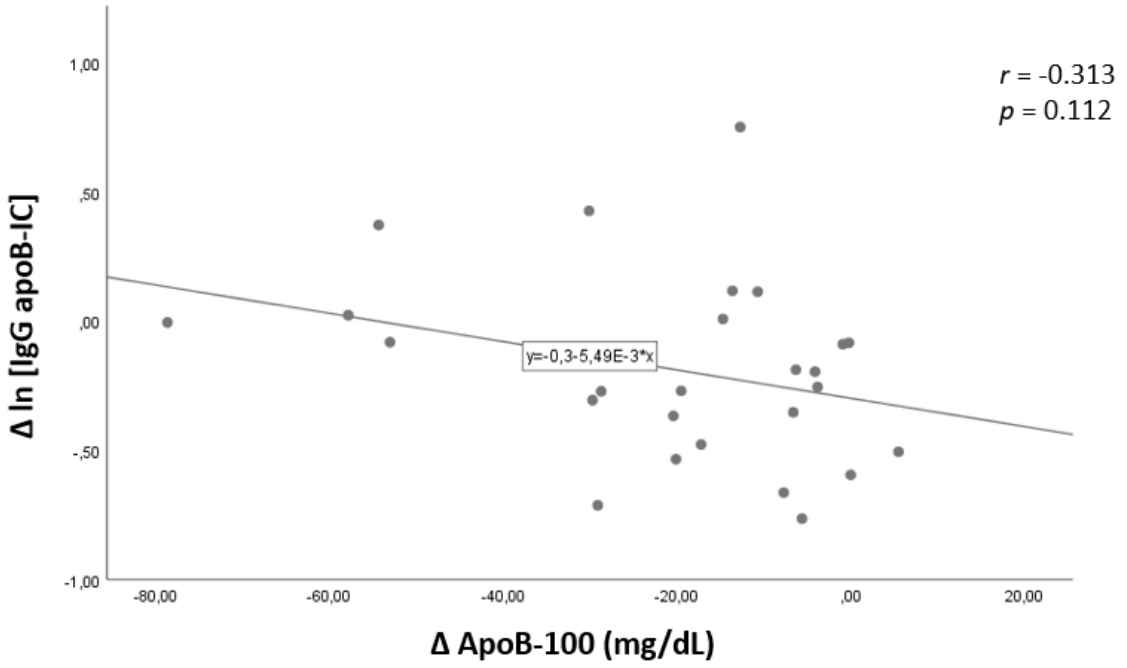

Figure 110. Correlation between changes in IgG apoB-IC and apoB-100 levels for the add-on ezetimibe group.

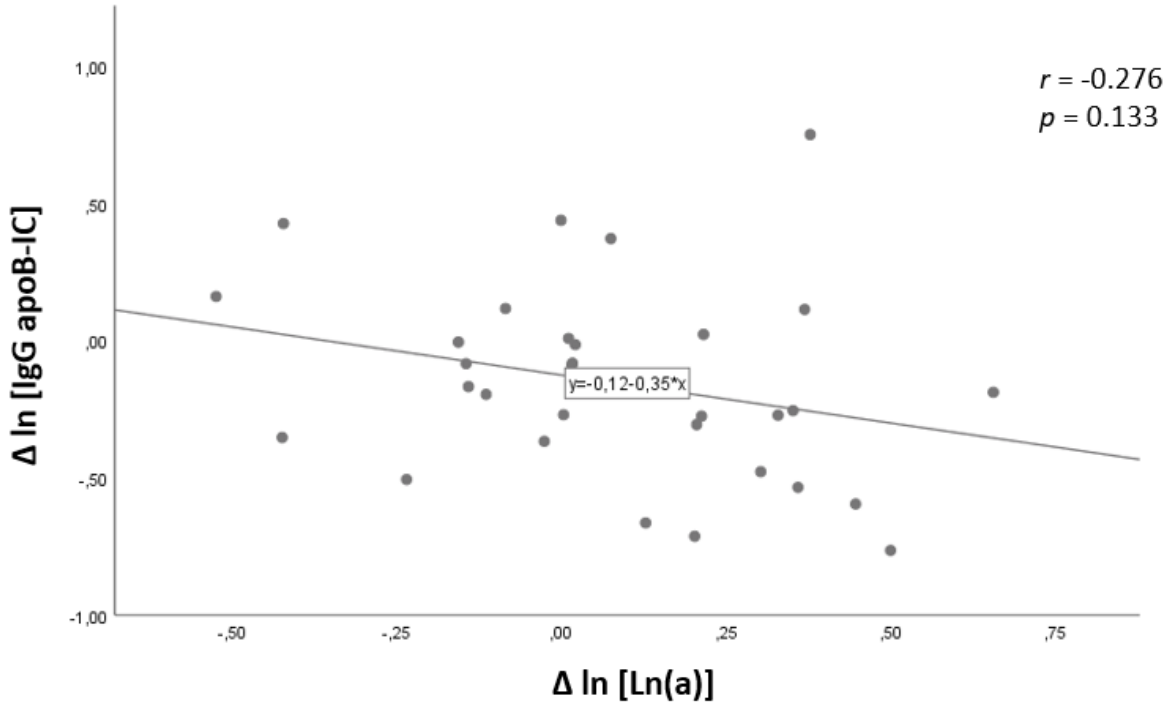

Figure 111. Correlation between changes in IgG apoB-IC and lipoprotein(a) levels for the add-on ezetimibe group.

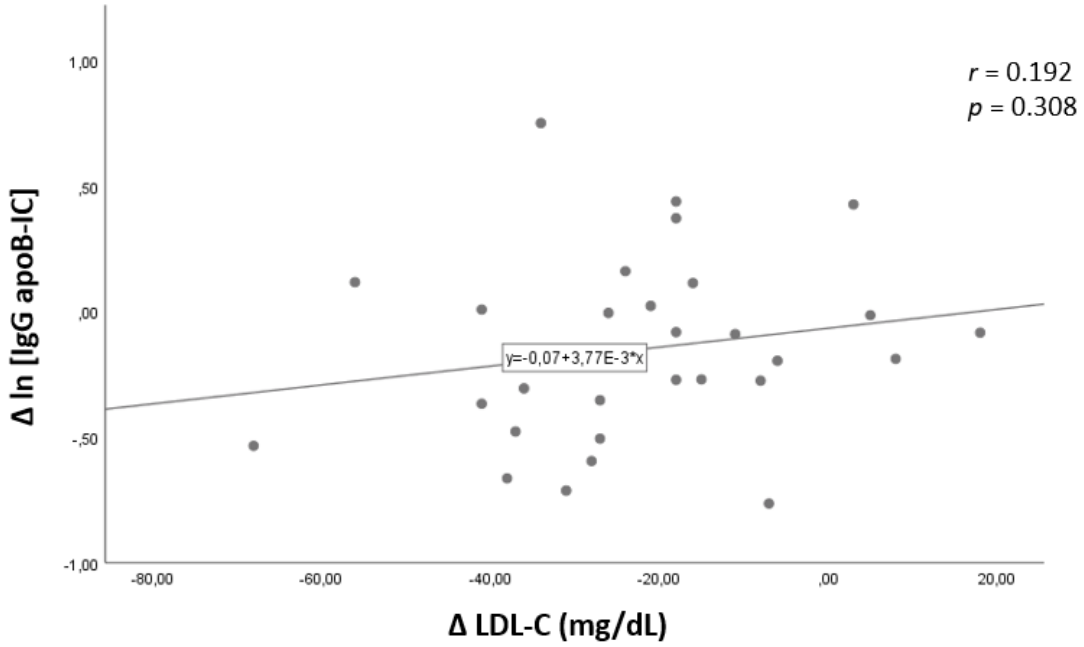

Figure 112. Correlation between changes in IgG apoB-IC and low-density lipoprotein cholesterol (LDL-C) levels for the add-on ezetimibe group.

For the add-on PCSK9 inhibitor group, correlation analyses at baseline showed that there was not any association between IgG apoB-IC and apoB-100 (r = 0.149, p = 0.661) (Figure 113), Lp(a) (r = -*0*.515, p = 0.105) (Figure 114), or LDL-C (r = -0.018, p = 0.961) (Figure 115).

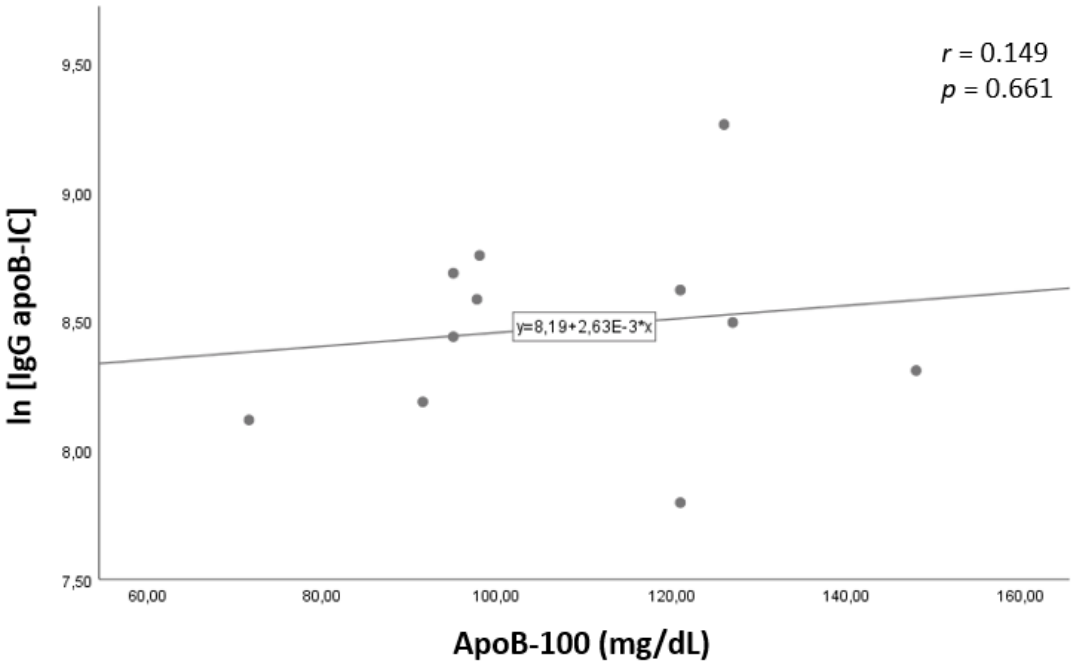

Figure 113. Correlation between IgG apoB-IC and apoB-100 levels at baseline for the add-on PCSK9 inhibitor group.

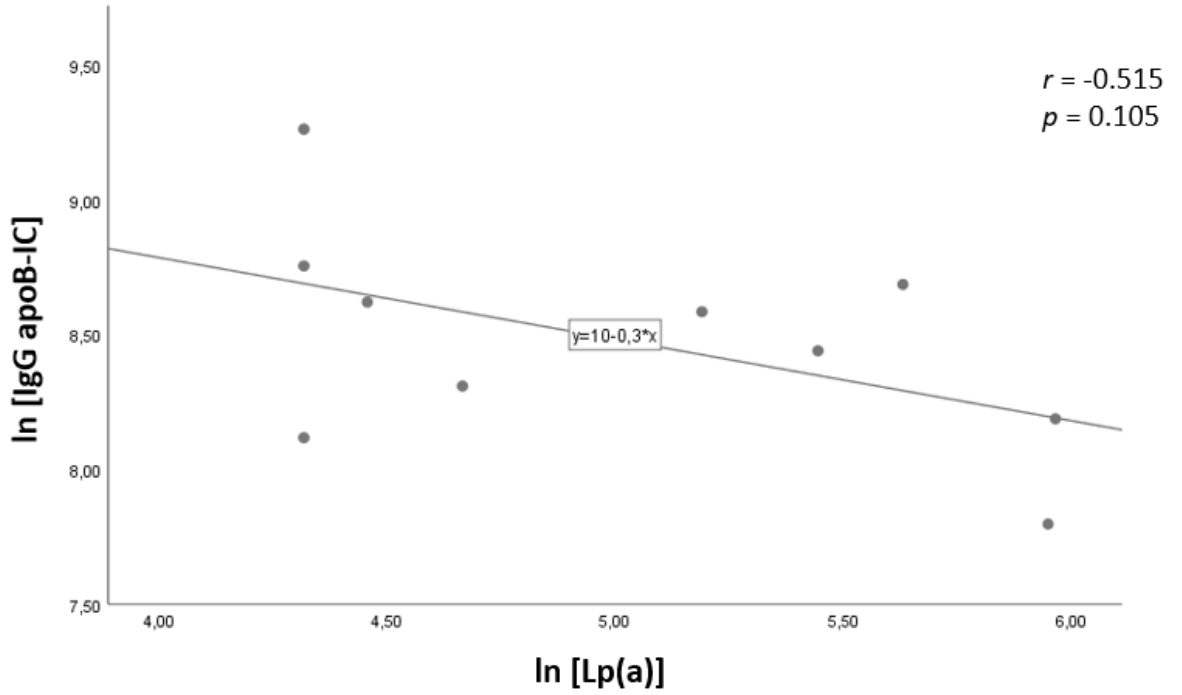

Figure 114. Correlation between IgG apoB-IC and lipoprotein(a) levels at baseline for the add-on PCSK9 inhibitor group.

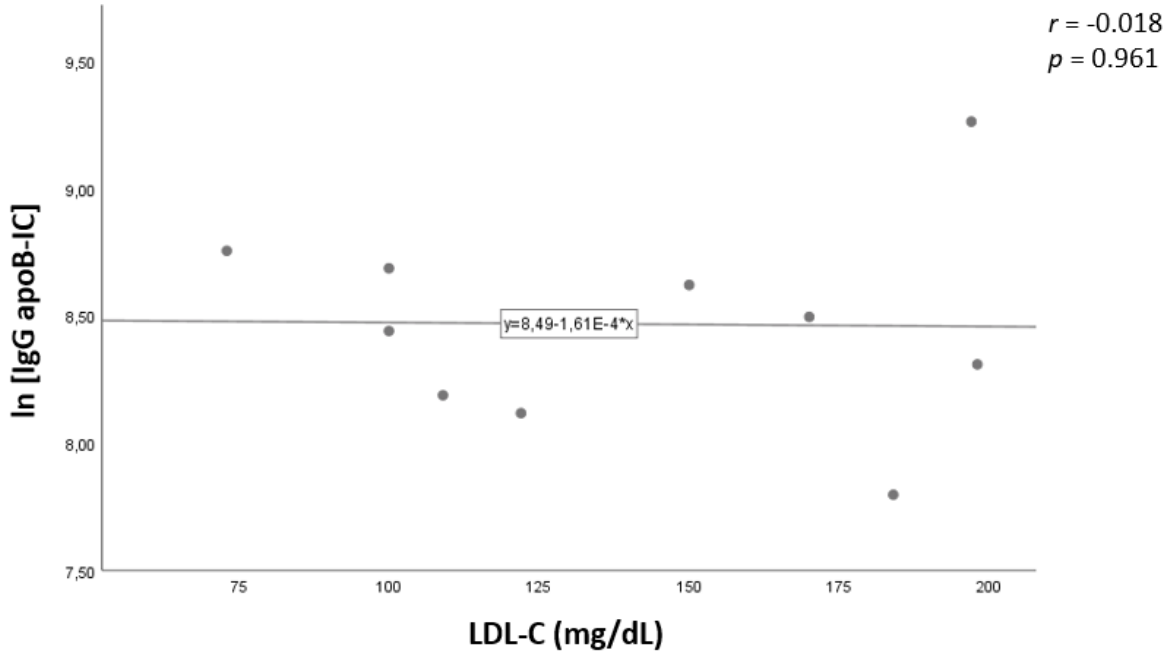

Figure 115. Correlation between IgG apoB-IC and low-density lipoprotein cholesterol (LDL-C) levels at baseline for the add-on PCSK9 inhibitor group.

At 3 months there was a significant correlation between IgG apoB-IC and apoB-100 (r = -0.637, p = 0.048) (Figure 116), whilst there was no correlation between IgG apoB-IC and Lp(a) (r = -0.545, p = 0.083) (Figure 117) or LDL-C (r = -0.310, p = 0.383) (Figure 118) for the add-on PCSK9 inhibitor group.

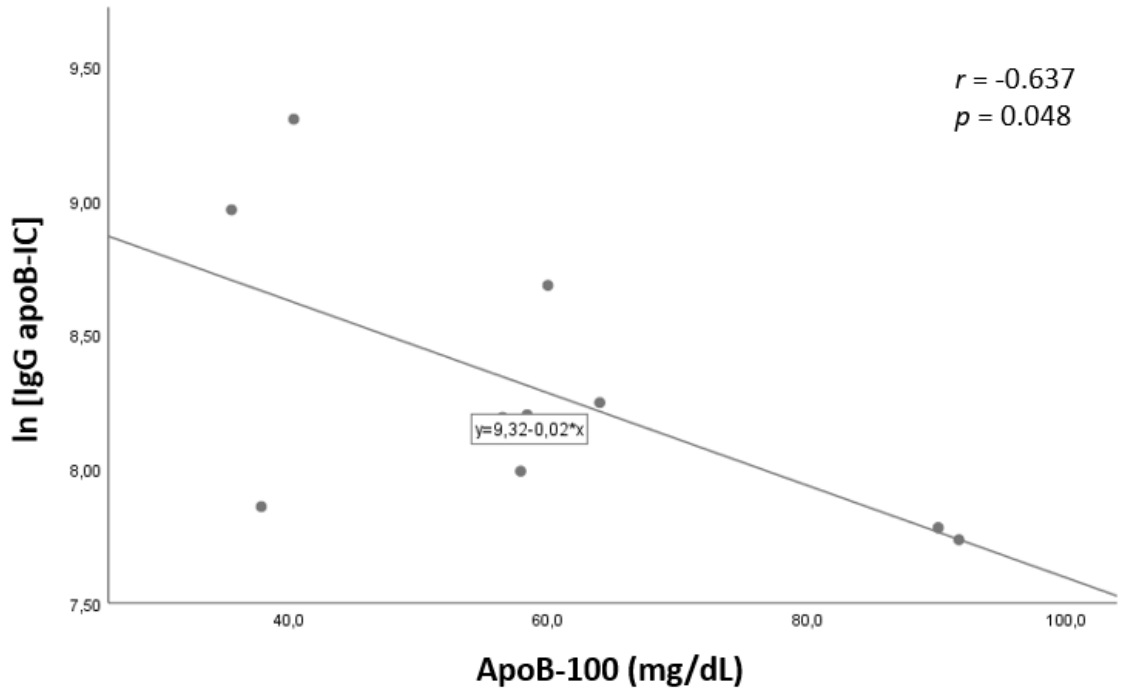

Figure 116. Correlation between IgG apoB-IC and apoB-100 levels at 3 months for the add-on PCSK9 inhibitor group.

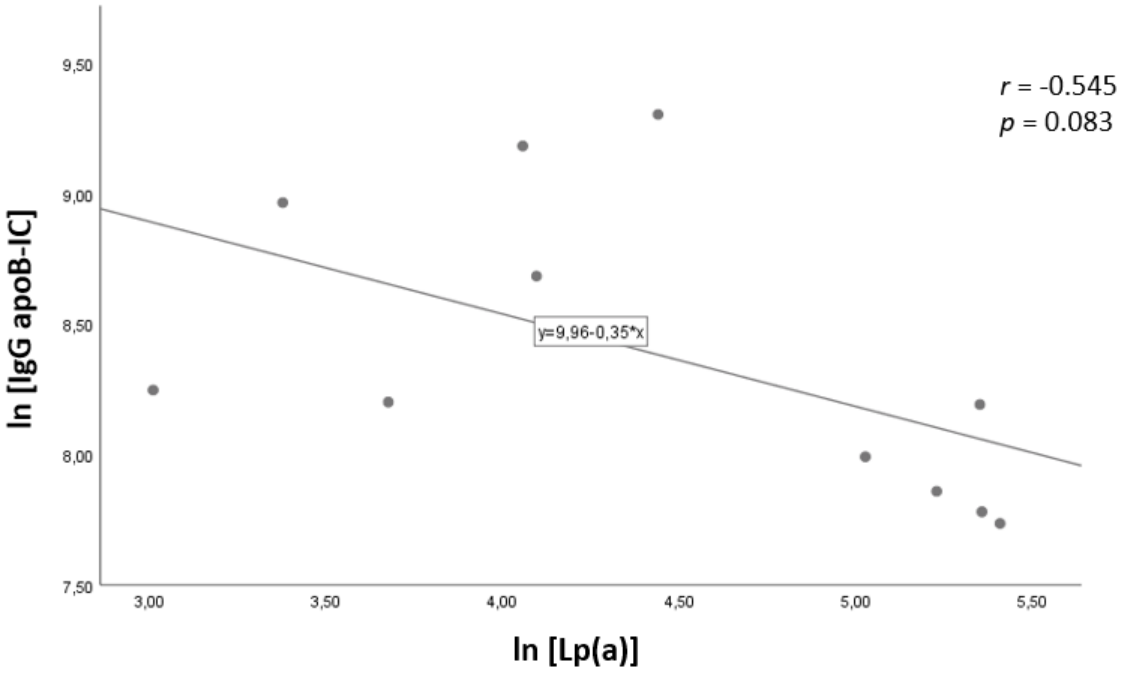

Figure 117. Correlation between IgG apoB-IC and lipoprotein(a) levels at 3 months for the add-on PCSK9 inhibitor group.

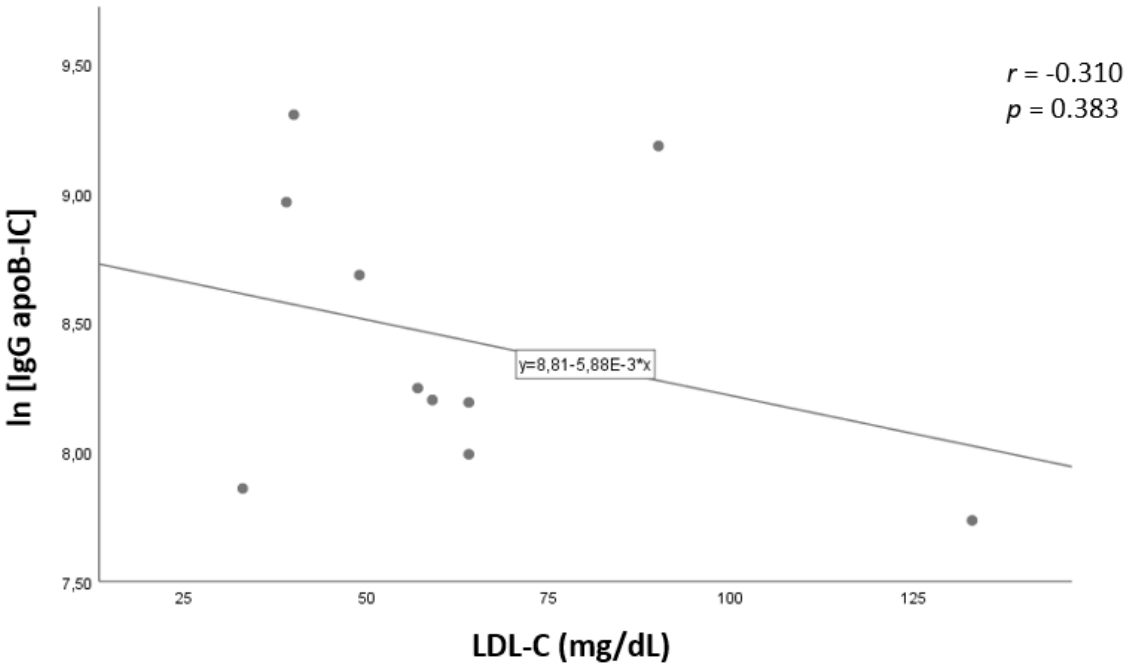

Figure 118. Correlation between IgG apoB-IC and low-density lipoprotein cholesterol (LDL-C) levels at 3 months for the add-on PCSK9 inhibitor group.

Correlation analyses based on changes from baseline to follow-up for the add-on PCSK9 inhibitor group revealed no significant associations between changes in IgG apoB-IC and apoB-100 (r = -0.376, p = 0.284) (Figure 119), Lp(a) (r = -0387, p = 0.239) (Figure 120), or LDL-C (r = -0.172, p = 0.635) (Figure 121).

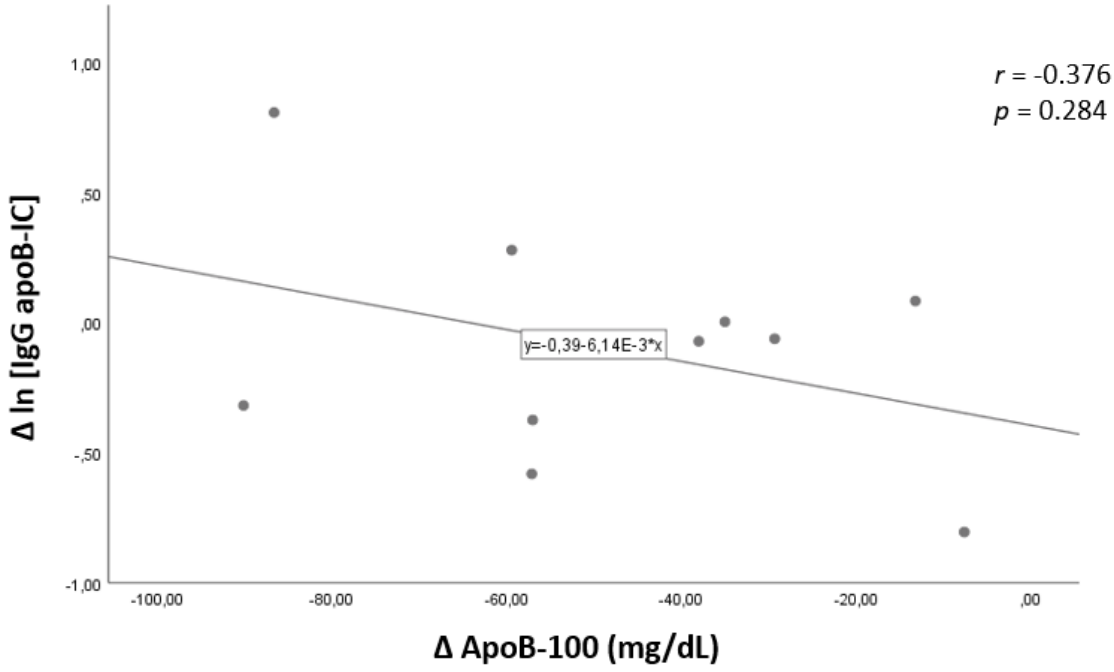

Figure 119. Correlation between changes in IgG apoB-IC and apoB-100 levels for the add-on PCSK9 inhibitor group.

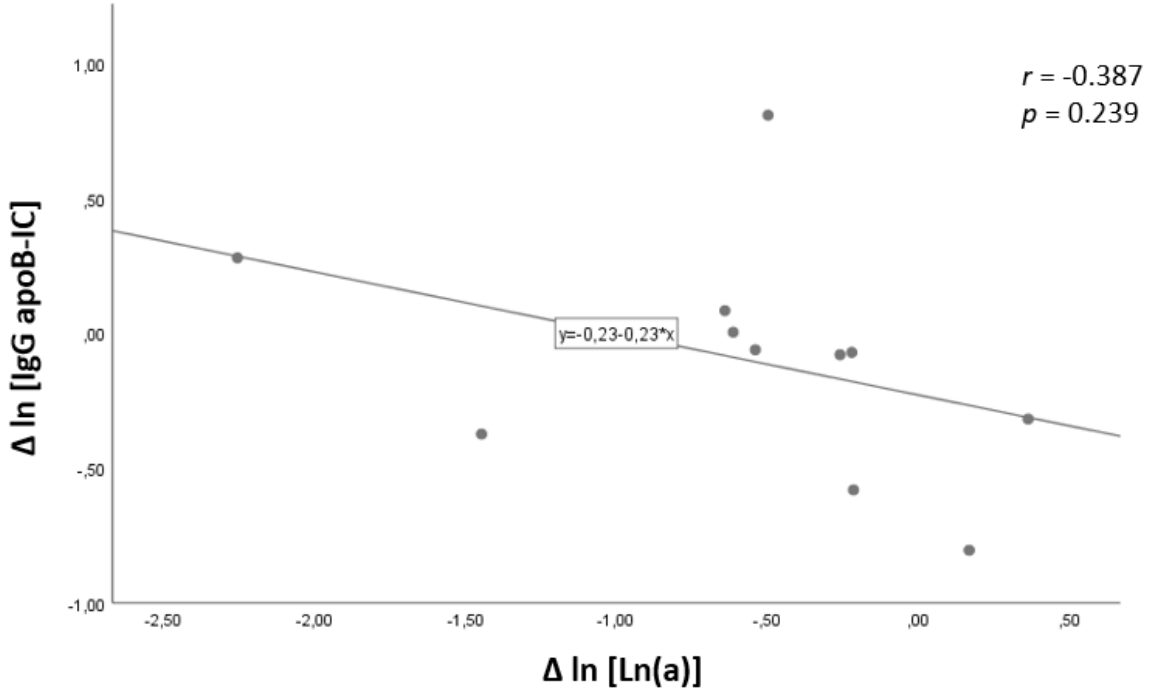

Figure 120. Correlation between changes in IgG apoB-IC and lipoprotein(a) levels for the add-on PCSK9 inhibitor group.

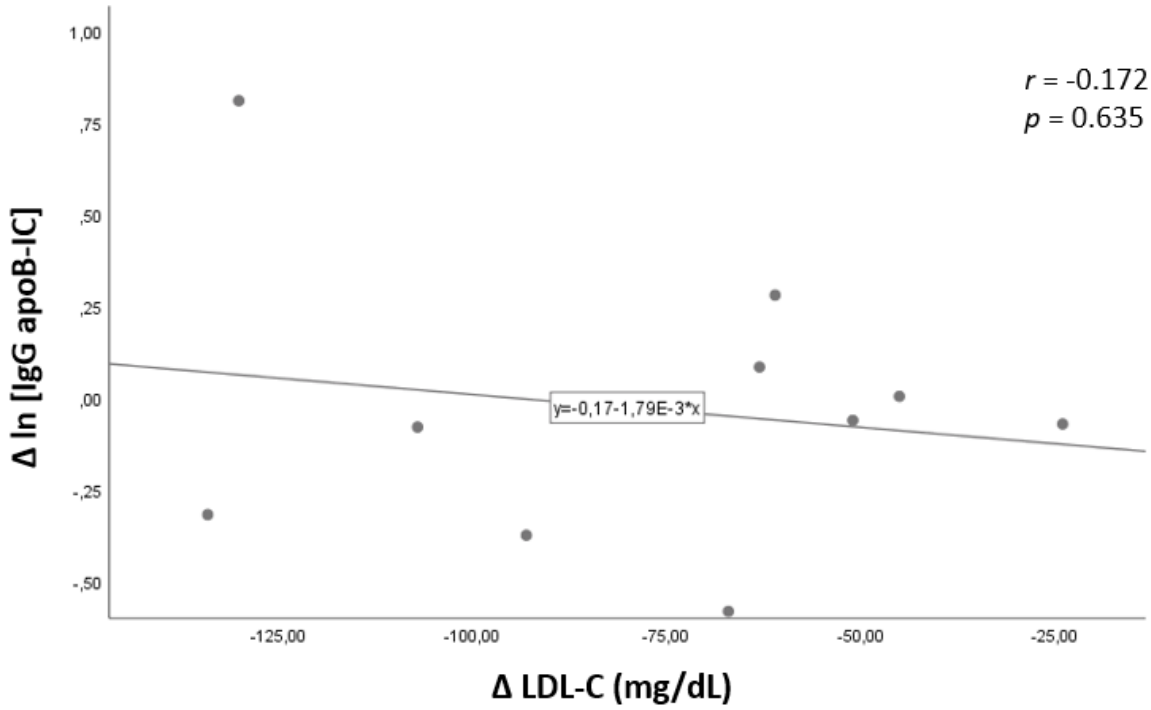

Figure 121. Correlation between changes in IgG apoB-IC and low-density lipoprotein cholesterol (LDL-C) levels for the add-on PCSK9 inhibitor group.

CHAPTER 6. DISCUSSION

1. Main findings

The present thesis demonstrated that add-on PCSK9 inhibitors achieved the highest LDL-C and apoB-100 reduction compared with high-intensity statin monotherapy and add-on ezetimibe in patients with elevated Lp(a) levels (≥75 nmol/L). Moreover, PCSK9 inhibitors were the only therapeutic class that significantly reduced Lp(a), while no differences were noted regarding the effect of lipid-lowering interventions on the participants' glycemic profile. Furhtermore, high-intensity statin therapy and add-on ezetimibe were associated with significant increases in OxPL-apoB and OxPL-apo(a) levels, despite non-significant changes in Lp(a) concentration. In contrast, add-on PCSK9 inhibition resulted in a significant reduction in Lp(a) levels without significant changes in OxPL-apoB or OxPL-apo(a). Moreover, high-intensity statins, add-on ezetimibe, and add-on PCSK9 inhibitors were each associated with significant reductions in IgG apoB-IC, while IgM apoB-IC and IgG and IgM anti-MDA-mimotope remained unchanged.

2. Effect on lipids

Elevated Lp(a) represents the most common inherited lipid disorder associated with ASCVD [66]. Approximately 90% of circulating Lp(a) levels are autosomal dominantly inherited and strongly determined by the LPA gene [17], which is fully expressed by early childhood [122]. Thereafter, Lp(a) levels remain largely stable and are not substantially influenced by diet, physical activity, or other environmental factors [33]. Evidence from experimental, observational, and genetic studies consistently demonstrates that increased Lp(a) is associated with CHD, ischemic stroke, peripheral artery disease, heart failure, CAVS, mitral valve stenosis, and possibly diabetic retinopathy [58, 63, 74, 300, 301]. Although Lp(a) thresholds have historically been proposed, a linear relationship between Lp(a) levels and ASCVD risk is now recognized [33].

At present, no medications are specifically approved for Lp(a) lowering [17, 21, 67]. Novel therapies targeting apo(a), including ASOs and siRNAs, have demonstrated reductions in Lp(a) exceeding 90%, although their cardiovascular benefit has not yet been established [153, 157]. However, a few available therapeutic agents have a modest effect on Lp(a) [17, 21, 67]. Although the effect of statin treatment on Lp(a) levels remains an area of controversy, it seems that statins slightly increase Lp(a) levels [124, 190, 302]. Most other lipid modifying agents, including ezetimibe do not significantly alter Lp(a) concentration [17, 21, 67], except

for PCSK9 inhibitors, which have been shown to reduce Lp(a) levels, by ~20-30% [35, 133, 134].

Our results concerning the effect of high intensity statins, ezetimibe and PCSK9 inhibitors on patients with elevated Lp(a) levels agree with past evidence derived from the general population. Similar to our study, statins reduce LDL-C by 30-63% [303], TGs by 14-33% [304], and increase HDL-C up to 10% [305] in patients with hypercholesterolemia, with atorvastatin 40-80 mg and rosuvastatin 20-40 mg being the most potent ones. Nevertheless, the effect of statin treatment on Lp(a) levels remains an area of controversy. Statin trials have shown mixed results regarding their impact on Lp(a) levels. A large meta-analysis of 6 trials including 5256 patients randomized to various statins or placebo indicated that most statins increase Lp(a) by 8-24%, although a significant heterogeneity in the response of Lp(a) levels to statin administration, as well as between different statins was reported [15]. On the other hand, another meta-analysis of 39 trials including 24,448 patients randomized to receive various statins or placebo indicated a non-significant trend towards an increase in Lp(a) by 0.1% in statin groups (vs placebo), with no significant differences among statins or different intensities of statins [122]. Similarly, our study indicated a nonsignificant increase in Lp(a) levels in the group of patients who received a high-intensity statin by 5.5%.

Ezetimibe, a cholesterol absorption inhibitor, has a modest LDL-C lowering efficacy; 17% when used alone [306] and 14-25% when used in combination with statins [307]. Its effect on Lp(a) remains controversial [103]. In a meta-analysis of 7 randomized controlled trials with ezetimibe monotherapy in 2237 patients with primary hypercholesterolemia, ezetimibe significantly reduced Lp(a) levels by 7.1%, whereas another meta-analysis including 11 double-blind randomized controlled trials with 1926 adults with hypercholesterolemia, showed that ezetimibe, either as monotherapy vs placebo or in combination with statin vs statin monotherapy, did not significantly alter Lp(a) concentration [103]. Our study showed that the addition of ezetimibe on high-intensity statin treatment increased nonsignificantly Lp(a) levels by 15.3%. This could be attributed at least in part to the small sample size of the study or to the different design of our study including only patients with elevated Lp(a) levels.

Regarding add-on PCSK9 inhibitors therapy, our results are in agreement with past evidence derived from FOURIER and the ODYSSEY OUTCOMES trials [308-311] showing that PCSK9 inhibitors are highly effective in lowering LDL-C levels by ~60% in patients on statin therapy, as well as Lp(a) levels by 20-30% [133, 134]. According to a post hoc analysis of the ODYSSEY OUTCOMES trial including statin-treated patients with LDL-C ~70 mg/dL, alirocumab was associated with a higher reduction in risk for MACEs in patients with

elevated Lp(a) levels (≥13.7 mg/dL) (adjusted treatment HR: 0.68, 95% confidence interval: 0.52-0.90) in comparison with those having lower Lp(a) levels (adjusted treatment HR: 1.11, 95% confidence interval: 0.83-1.49) [43]. Furthermore, it appears that Lp(a) reduction may have contributed to the clinical benefit [43]. In patients with LDL-C levels near 70 mg/dL, alirocumab produced similar LDL-C reductions regardless of baseline Lp(a), yet a significant reduction in MACE was observed only among those with elevated Lp(a). This dissociation implies that LDL-C lowering alone cannot fully explain the observed treatment effect. The authors note that because LDL-C and Lp(a) typically decrease in parallel with PCSK9 inhibition, separating their individual contributions is challenging; however, the magnitude of benefit in patients with elevated Lp(a) suggests either a direct effect of Lp(a) lowering or that elevated Lp(a) identifies a subgroup particularly responsive to therapy. Similarly, according to a post hoc analysis of the FOURIER trial, patients with higher baseline Lp(a) levels (>37 nmol/L) experienced greater absolute reductions in Lp(a) with evolocumab and tended to derive greater coronary benefit (HR: 0.77, 95% confidence interval: 0.67-0.88) in comparison with those having lower baseline Lp(a) levels (HR: 0.93, 95% confidence interval: 0.80-1.08) [108].

3. Effect on glycemic profile

Type 2 diabetes development is the caveat of statin therapy, mainly concerning high-intensity statins. A meta-analysis of clinical trials (13 randomized controlled endpoint trials of statins with 91140 participants) showed that statins were associated with an increased risk of newly developed diabetes, with the absolute risk increase by statins being 0.4% [7, 312]. It is worth noting, however, that the increase in type 2 diabetes development risk mainly concerns patients with predisposing factors, such as prediabetes and obesity and definitely this risk does not supersede the cardiovascular benefit of statins [7, 312]. In our study, high-intensity statin treatment had no significant effect on the participants' glycemic profile, but its small size and short follow-up, along with the lack of data on glycosylated hemoglobin test (HbA1c) levels consist major limitations.

Our results showing a neutral effect of the addition of ezetimibe on the glycemic profile of patients with elevated Lp(a) levels are similar to past evidence in the general population. In the IMPOVE-IT trial the addition of ezetimibe to simvastatin vs simvastatin alone did not increase the risk of new-onset diabetes (HR: 1.04, 95% confidence interval: 0.94-1.15) [313].

Furthermore, the results for PCSK9 inhibitors are also in agreement with the data from clinical trials so far. Published clinical studies to date have not demonstrated any correlation between PCSK9 inhibition, insulin resistance, plasma glucose levels, pancreas insufficiency, or increased rates of type 2 diabetes [135, 308, 314, 315]. However, genetic evidence suggests a more subtle relationship between PCSK9-mediated LDL-C lowering and glycemic risk. Mendelian randomization analyses have shown that LDL-C–lowering alleles in PCSK9 are associated with a modest increase in type 2 diabetes risk, similar to variants affecting HMGCR and LDL receptor pathways [316]. These findings imply that the diabetogenic signal may reflect a pathway-specific biological consequence of enhanced LDL receptor activity and hepatic cholesterol uptake rather than a direct pharmacologic toxicity of PCSK9 inhibition. Consistent with this hypothesis, additional genetic studies evaluating PCSK9 variants have reported small increases in fasting glucose or diabetes risk proxies despite favorable lipid effects [317].

It is worth noting that this is the first study concerning exclusively patients with elevated Lp(a) levels, especially considering the association between very low Lp(a) levels and incident type 2 diabetes, an association derived from large prospective epidemiological cohorts with robust duration of follow-up [104]. In addition, a non-significant trend towards a reduction in HOMA-IR index was noted with PCSK9 inhibitors. There was no correlation between the Lp(a) lowering and HOMA-IR lowering; therefore, the observed trend in HOMA-IR in the present study should be interpreted cautiously and may represent a chance finding or indirect metabolic effect unrelated to Lp(a) lowering. More evidence is needed to investigate the causality between Lp(a) and type 2 diabetes and whether Lp(a) reduction by treatment modalities can lead to incident type 2 diabetes [104].

4. Effect on OxPLs

High-intensity statin treatment resulted in a significant increase in both OxPL-apoB and OxPL-apo(a) levels by 2.9% and 100.8%, respectively. Data from the MIRACL trial, showed a reduction of OxPL-apoB levels by 29.7% after treatment with high-intensity atorvastatin in patients (n=1151) with recent acute coronary syndrome [198]. On the contrary, atorvastatin resulted in significant increases in OxPL-apoB by 22.9% in the VISION trial, in which 42 patients were randomized to pitavastatin 2 mg/day or atorvastatin 10 mg/day, with no effect on OxPL-apo(a) level 236]. In a study of 3896 patients treated with various statins an increase in OxPL-apoB by 23.8% was noted [229]. Of importance, OxPL-apoB levels increase

transiently with new statin therapy, peaking at 4 months and declining to below baseline thereafter [92]. Therefore, the 3-month follow-up of this study may coincide with the early peak in OxPL-apoB levels. It may be hypothesized that the reduction of LDL-C levels with high-intensity statin treatment ensues a mobilization of OxPLs from the atherosclerotic plaque into circulation, where they bind to apoB-100 particles. The increase in OxPL-apoB supports this possible mobilization. The substantial increase in OxPL-apo(a), despite unchanged Lp(a) concentration suggests that Lp(a) may become more enriched in OxPL following statin treatment. The mobilization of OxPLs from the vessel wall during high-intensity statin treatment can be attributed to several factors [92]. One key factor is the reduction in LDL-C, which changes lipid dynamics and increases OxPLs' affinity to other lipoprotein particles [92]. Additionally, statins have anti-inflammatory effects, which may help stabilize atherosclerotic plaques and reduce local inflammation [92]. This reduction in inflammation can lead to the mobilization of OxPLs as the inflammatory environment shifts [92]. Also, statin treatment improves endothelial function, potentially enhancing the endothelium's ability to process and clear OxPLs [92]. Although the mobilization of OxPLs from the vessel wall is a plausible hypothesis, it remains to be conclusively demonstrated. Statins have been associated with mixed effects on OxPL-apoB levels while limited data are available on their impact on OxPL-apo(a) levels.

The addition of ezetimibe to high-intensity statin led to a significant increase in OxPL-apoB and OxPL-apo(a) levels by 28.7% and 40.1%, respectively. As with high-intensity statin, the increase in OxPL-apo(a) is greater compared with the increase in Lp(a). The underlying mechanisms behind these changes may be like those hypothesized with high-intensity statin treatment. The combination of ezetimibe with simvastatin significantly increased OxPL-apoB in one study [229]. Furthermore, the addition of PCSK9 inhibitors resulted in a non-significant decrease in OxPL-apoB and OxPL-apo(a) levels. Similarly, the ANITSCHKOW study, in which 129 patients were randomized 1:1 to monthly evolocumab 420 mg or placebo for 16 weeks, resulted in no significant difference in the mean percent change in both OxPL-apoB and OxPL-apo(a) levels [134]. The EQUATOR study, in which 224 patients were randomized to RG7652 or placebo resulted in no significant difference in the mean percent change in OxPL-apoB [134]. However, a small but significant reduction in OxPL-apo(a) levels (-10.1 ± 31.3 nmol/L in the PCSK9 inhibitor group vs 30.6 ± 90.8 nmol/L in the placebo group; $p = 0.029$) was observed [318].

The ODYSSEY OUTCOMES trial provided further insights into the effect of PCSK9 inhibition on OxPL-apoB [319]. In that study, alirocumab reduced OxPL-apoB levels by a

median of 15% after 4 months of treatment compared to placebo [319]. Importantly, baseline OxPL-apoB strongly correlated with Lp(a) levels (r = 0.68, p < 0.001), suggesting that the majority of OxPL-apoB is carried on Lp(a) particles [319]. Elevated OxPL-apoB levels in the placebo group were predictive of MACEs, especially in people with low Lp(a) [319]. However, in the alirocumab group, neither baseline OxPL-apoB nor Lp(a) predicted MACEs, suggesting that PCSK9 inhibition mitigates the OxPL-apoB- and Lp(a)-mediated risk [319].
Notably, despite the substantial Lp(a) reduction with PCSK9 inhibitor, OxPL-apoB and OxPL-apo(a) levels did not decrease, which would be expected if Lp(a) was the major carrier of these species in plasma, raising questions about the underlying mechanisms. It should be noted that the assays used to measure OxPL-apoB and OxPL-apo(a) are designed to reflect the OxPL content per captured apoB or apo(a) particle, rather than total plasma OxPL levels. Therefore, the significant reduction in Lp(a) particle number may not necessarily lead to reduced OxPL-apo(a) values, particularly if the remaining particles maintain high OxPL content. Another possible explanation is that the composition of Lp(a) particles may be altered by PCSK9 inhibition, leading to a change in the types of phospholipids incorporated into these particles. Overall, our findings suggest that the initiation of a high-intensity statin or the addition of ezetimibe results in apoB-100 and Lp(a) particles that are more enriched in OxPLs, an effect that appears to be mitigated by the addition of a PCSK9 inhibitor. The reasons behind the differential effect of PCSK9 inhibitors on OxPLs compared to high-intensity statins and ezetimibe remain unclear. However, this may be attributed to the fact that PCSK9 inhibitor treatment was added on top of high-intensity statin and ezetimibe therapy, potentially leaving limited room for further increases in OxPLs. At both baseline and after 3 months of treatment, significant positive correlations were observed between Lp(a) and both OxPL-apo(a) and OxPL-apoB across the total study population, reinforcing that Lp(a) is the primary carrier of OxPLs in plasma. In contrast, LDL-C did not show significant correlations with OxPL-apoB suggesting that OxPL enrichment is largely independent of LDL-C concentrations. Correlation analyses for changes in these parameters showed a significant positive correlation between changes in OxPL-apo(a) and Lp(a), suggesting OxPL-apo(a) alteration may be partially explained by change in Lp(a) levels. Therefore, measuring OxPL enrichment per particle provides valuable information on the oxidative modification of individual lipoproteins, while assessing the total plasma OxPL burden would complement this by indicating the overall atherogenic and inflammatory potential. Both parameters are clinically relevant and may provide complementary insights into cardiovascular risk.

5. Effect on OxPL-PLG

Treatment with high-intensity statins had no effect on PLG levels but significantly decreased OxPL-PLG levels. The possible underlying mechanisms remain poorly understood. Prior studies have shown that PLG can carry OxPLs and that these OxPL modifications may interfere with PLG activation and fibrinolytic efficiency [84, 281]. Statin therapy has been reported to modestly enhance fibrinolytic activity, potentially through reductions in PAI-1 levels and improved endothelial function [10, 112, 280, 281]. The observed reduction in OxPL-PLG levels with high-intensity statin treatment may be relevant in this context, as prior studies have shown that OxPL adducts on PLG impair PLG function and fibrinolysis, and that their removal enhances fibrinolytic capacity [10, 112, 280, 281]. Add-on ezetimibe showed a similar pattern, with reduced OxPL-PLG but no significant change in total PLG. Treatment with PCSK9 inhibitors also showed a small, non-significant reduction in OxPL-PLG levels but not PLG, like high-intensity statin treatment and add-on ezetimibe. These findings suggest that lipid-lowering therapies may have a minor effect on OxPL modification of PLG, which could potentially enhance fibrinolysis and lower thrombotic risk, although this hypothesis requires direct functional studies. Overall, the clinical significance of changes in OxPL-PLG remains uncertain and warrants further investigation, especially given the central role of PLG in thrombosis and its emerging role as an OxPL carrier complementary to Lp(a).

6. Effect on IgG & IgM apoB-IC autoantibodies

In patients treated with high-intensity statins, we observed a significant reduction in IgG apoB-IC autoantibodies, while IgM apoB-IC autoantibody levels remained unchanged. These findings suggest that statins may exert selective immunomodulatory effects, particularly on the IgG response to apoB-IC. The reduction in IgG apoB-IC levels with high-intensity statin therapy may reflect statin-associated attenuation of chronic immune activation or reduced formation of circulating immune complexes. The absence of significant changes in IgM autoantibody levels aligns with prior evidence suggesting that these natural antibodies are predominantly involved in maintaining immune homeostasis and are less susceptible to modulation by short-term pharmacologic interventions [320].

Among individuals receiving ezetimibe added to high-intensity statin therapy, we also observed a significant reduction in IgG apoB-IC autoantibodies and no notable changes in IgM apoB-IC autoantibody levels. Although data specifically addressing ezetimibe effect on these autoantibodies are limited, it can be assumed that the significant drop in IgG apoB-IC

may reflect additive benefits of ezetimibe in lowering of oxidative neoepitopes. Furthermore, when PCSK9 inhibitors were added to statin and ezetimibe therapy we observed a significant reduction in IgG apoB-IC autoantibodies, but no significant change in IgM apoB-IC autoantibodies levels. Mechanistic studies indicate that PCSK9 inhibition dampens monocyte activation and inflammatory phenotype but does not necessarily reduce antibody responses or oxidation-specific biomarkers [321, 322].

Correlation analyses showed that individuals who experienced larger changes in Lp(a) tended to show smaller changes in IgG apoB-IC levels. This suggests that when Lp(a) –a major carrier of oxidation-specific epitopes– declines substantially, the antigenic stimulus for antibody production diminishes, stabilizing IgG responses. Conversely, in patients with smaller Lp(a) reductions, continued exposure to oxidation-specific epitopes may sustain immune complex formation, leading to more pronounced decreases in circulating IgG apoB-IC due to ongoing antibody-antigen complexing and clearance (Figure 45). Conversely, in individuals whose Lp(a) levels increased during follow-up, the enhanced availability of Lp(a)-associated oxidation-specific epitopes could potentially sustain or even augment immune stimulation. However, in our study the magnitude of Lp(a) increases was small, and therefore we did not observe a corresponding rise in IgG apoB-IC levels. Taken together, these observations suggest that both the direction and magnitude of Lp(a) change may influence circulating immune complex dynamics. These findings support the concept that apoB-100-containing lipoproteins [such as Lp(a)] serve as antigenic carriers of oxidation-specific epitopes that modulate adaptive immune responses [323].

With respect to MDA-mimotope autoantibodies, neither high-intensity statins, nor add-on ezetimibe, nor add-on PCSK9 inhibitors altered IgG or IgM responses to MDA-mimotope peptides. Data on these autoantibodies are limited, but they are thought to reflect recognition of specific MDA-related epitopes within oxLDL. Given that reductions in oxLDL with ezetimibe plus statins have been documented [324, 325], one might expect parallel effects on MDA-mimotope responses (Figure 122). However, the unchanged autoantibody titers in our study may indicate that either longer treatment duration is needed, or that these epitopes are less immunogenic and thus resistant to modulation by lipid lowering.

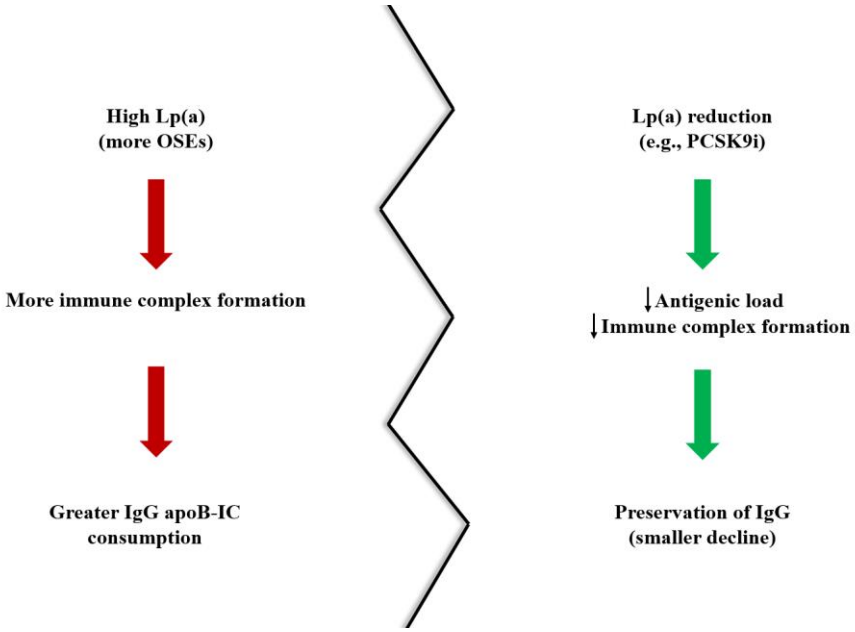

Figure 122. Potential explanation illustrating the relationship between lipoprotein(a) reduction and IgG apoB-IC decline. Abbreviations: IgG apoB-IC; IgG apolipoproteinB-100-containing immune complexes, Lp(a); lipoprotein(a), OSEs; oxidation-specific epitopes, PCSK9i; proprotein convertase subtilisin/kexin type 9 inhibitor.

8. Limitations of the present thesis

Major limitations of the present thesis include the small sample size and follow-up, along with the lack of data on dietary habits and physical activity of the patients. Another limitation is the lack of Lp(a) values before any therapeutic intervention in patients receiving ezetimibe or PCSK9 inhibitors. In addition, the inherent variability in Lp(a) and OxPL levels significantly limit the power and generalizability of this study as well as interpretation of non-significant findings. Therefore, these findings should be viewed as preliminary and hypothesis-generating and should be further validated with larger studies and longer follow-up periods. Furthermore, the lack of randomization and differences in baseline characteristics may have introduced confounding factors affecting treatment response despite statistical adjustment. Specifically, differences in baseline characteristics such as the higher prevalence of ASCVD and HeFH in the PCSK9 inhibitor group may have influenced OxPL dynamics independently of treatment effects. This non-randomized design limitation should be considered when interpreting study results. Patients who received add-on ezetimibe or add-on PCSK9 inhibitor were already on a stable lipid-lowering regimen prior to the addition of these agents. Consequently, the results presented reflect the additive effect of ezetimibe or PCSK9 inhibitors when incorporated into existing therapy, rather than their isolated efficacy. Finally, while we quantified OxPL enrichment per particle, we did not measure the total plasma OxPL

burden, which could have provided further insight into the overall atherogenic and inflammatory potential.

9. Implications and directions for future research

Lp(a) concentration, OxPL enrichment per particle, and downstream immune responses represent distinct but interconnected biological dimensions. Lipid-lowering therapies differentially affected these layers: add-on PCSK9 inhibition substantially reduced Lp(a) particle levels without proportionate reductions in OxPL enrichment, whereas high-intensity statins and add-on ezetimibe increased OxPL-apoB and OxPL-apo(a) despite minimal change in Lp(a). These findings suggest that Lp(a) particle number and particle composition are dissociable, and that short-term increases in OxPL enrichment may reflect redistribution or mobilization rather than increased oxidative burden.

Reductions in OxPL-PLG observed with high-intensity statins and add-on ezetimibe indicate that lipid-lowering therapy may influence thrombotic pathways, although the functional implications for fibrinolysis remain uncertain. In parallel, consistent reductions in IgG apoB-IC across treatment strategies, with preserved IgM responses, suggest selective modulation of adaptive immune responses to oxidation-specific epitopes rather than generalized immune suppression.

Future research should focus on longer-term, preferably randomized studies linking changes in Lp(a), OxPL enrichment, and immune biomarkers to clinical outcomes. Simultaneous assessment of OxPL enrichment per particle and total OxPL burden, incorporation of functional fibrinolytic assays, and extension of this biomarker framework to novel Lp(a)-lowering therapies will be essential to clarify the clinical relevance of these mechanistic findings.

CHAPTER 7. CONCLUSIONS

The present thesis examined the effect of hypolipidemic therapies on the lipidemic profile, the impact of high-intensity statins, add-on ezetimibe and add-on PCSK9 inhibitors on OxPL-apoB, OxPL-apo(a) and OxPL-PLG levels, and the effect of high-intensity statin treatment, add-on ezetimibe and add-on PCSK9 inhibitor therapy on IgG and IgM autoantibodies to apoB-IC as well as IgG and IgM anti-MDA-mimotope in patients with Lp(a) ≥75 nmol/L. In conclusion:

1. Add-on PCSK9 inhibitors were associated with the highest rates of LDL-C reduction compared with high-intensity statin treatment ± ezetimibe, and the only class to significantly lower Lp(a) in patients with elevated Lp(a) levels. Although these interventions did not affect participants' glycemic profile, longer follow up is needed.
2. Both high-intensity statins and add-on ezetimibe led to increases in OxPL-apoB and OxPL-apo(a) levels, while the addition of PCSK9 inhibitors did not significantly modify these parameters during a 3-month follow-up.
3. High-intensity statins, add-on ezetimibe and add-on PCSK9 inhibitors reduced IgG apoB-IC. No significant changes were observed in IgM apoB-IC or anti-MDA-mimotope levels.

ABSTRACT

Amalia Despoina Koutsogianni

Aim: The evaluation of the effect of lipid-lowering treatment on lipidemic, OxPLs levels and IgG/IgM apoB-IC and IgG/IgM anti-MDA-mimotope in individuals with elevated Lp(a) concentrations.

Material and methods: A prospective study including 70 patients with dyslipidemia and elevated Lp(a) levels (≥75 nmol/L) attending a Lipid Clinic in Greece. Subjects were allocated to the following therapies according to the national guidelines for cholesterol management: high-intensity statin monotherapy (n=28), ezetimibe added to high-intensity statin (n=31), and PCSK9 inhibitor added to high-intensity statin plus ezetimibe (n=11). Follow-up duration was 3 months.

Results: Mean age was 51 ± 15 years, 40% were male, 39% were diagnosed with HeFH, 16% with ASCVD, while 36%, 33% and 15% were at very high, high, and moderate cardiovascular risk, respectively. All interventions significantly reduced apoB-100, TC and LDL-C, but only add-on PCSK9 inhibitor group significantly reduced Lp(a) levels (add-on PCSK9 inhibitor: -43.1% vs add-on ezetimibe: +15.3% vs high-intensity statin: +5.5%, $p < 0.05$). Patients in the add-on PCSK9 inhibitor group achieved the highest rates of LDL-C reductions (add-on PCSK9 inhibitor: -55% vs add-on ezetimibe: -22% vs high-intensity statin: -43%). No significant effect on glycemic profile across treatment groups was noted. OxPL-apoB and OxPL-apo(a) significantly increased, while OxPL-PLG significantly decreased with both high-intensity statin monotherapy and add-on ezetimibe group. Add-on PCSK9 inhibitor group treatment was associated with no significant changes in OxPL-apoB, OxPL-apo(a) and OxPL-PLG levels. Significant reductions in IgG apoB-IC levels were observed following treatment with all regimens (by 18.3%, 17.5% and 25.5%, respectively, all $p < 0.05$). No significant changes in IgM apoB-IC, or IgG and IgM anti-MDA-mimotope levels were observed in any treatment group.

Conclusions: Add-on PCSK9 inhibitors were associated with the highest rates of LDL-C target achievement compared with high-intensity statin and add-on ezetimibe in high-risk patients with elevated Lp(a) and were the only class to significantly lower Lp(a) levels. In addition, both high-intensity statin and add-on ezetimibe significantly increased OxPL-apoB and OxPL-apo(a) levels, while significantly decreased OxPL-PLG levels. Add-on PCSK9 inhibitors had no significant effect on OxPLs levels.

Key words: Lipoprotein(a), Oxidized phospholipids, Plasminogen, Statins, Ezetimibe, PCSK9 inhibitors, autoantibodies, oxidative stress

ΠΕΡΙΛΗΨΗ

Σκοπός: Η αξιολόγηση της επίδρασης της υπολιπιδαιμικής αγωγής στο λιπιδαιμικό προφίλ, στα επίπεδα των οξειδωμένων φωσφολιπιδίων (OxPLs), καθώς και στα IgG/IgM σύμπλοκα ανοσοσφαιρίνης-οξειδωτικά τροποποιημένης από μηλονική διαλδεΰδη απολιποπρωτεΐνης B-100 (apoB-IC) και στα IgG/IgM αυτοαντισώματα έναντι μιμητικών επιτόπων παρουσία της μηλονικής διαλδεΰδης (anti-MDA-mimotope) σε άτομα με αυξημένα επίπεδα λιποπρωτεΐνης(a) [Lp(a)].

Υλικό και μέθοδοι: Προοπτική μελέτη παρακολούθησης που περιέλαβε 70 ασθενείς με δυσλιπιδαιμία και αυξημένα επίπεδα Lp(a) (≥75 nmol/L), οι οποίοι παρακολουθούνταν σε Ιατρείο Λιπιδίων στην Ελλάδα. Οι συμμετέχοντες κατανεμήθηκαν στις ακόλουθες θεραπευτικές παρεμβάσεις σύμφωνα με τις εθνικές κατευθυντήριες οδηγίες για τη διαχείριση της δυσπιπιδαιμίας: μονοθεραπεία με ισχυρή στατίνη (n=28), προσθήκη εζετιμίμπης σε ισχυρή στατίνη (n=31) και προσθήκη αναστολέα της προπρωτεϊνικής κονβερτάσης σουμπτιλισίνης/κεξίνης τύπου 9 (PCSK9) σε ισχυρή στατίνη και εζετιμίμπη (n=11). Η διάρκεια παρακολούθησης ήταν 3 μήνες.

Αποτελέσματα: Η μέση ηλικία ήταν 51 ± 15 έτη, το 40% ήταν άνδρες, το 39% είχε διαγνωσθεί με ετερόζυγο οικογενή υπερχοληστερολαιμία και το 16% με αθηροσκληρωτική καρδιαγγειακή νόσο, ενώ το 36%, 33% και 15% των ασθενών κατατάχθηκαν στις κατηγορίες του πολύ υψηλού, υψηλού και μέτριου καρδιαγγειακού κίνδυνου, αντίστοιχα. Όλες οι θεραπευτικές παρεμβάσεις οδήγησαν σε σημαντική μείωση της απολιποπρωτεΐνης B-100, της ολικής χοληστερόλης και της LDL-χοληστερόλης, ωστόσο μόνο η προσθήκη PCSK9 αναστολέα μείωσε σημαντικά τα επίπεδα της Lp(a) (προσθήκη PCSK9 αναστολέα: −43.1% έναντι προσθήκη εζετιμίμπης: +15.3% έναντι μονοθεραπεία με ισχυρή στατίνη: +5.5%, $p <$ 0,05). Δεν παρατηρήθηκε σημαντική επίδραση στο γλυκαιμικό προφίλ μεταξύ των θεραπευτικών ομάδων. Τα OxPL-apoB και OxPL-apo(a) αυξήθηκαν σημαντικά, ενώ τα OxPL-PLG μειώθηκαν σημαντικά τόσο με τη θεραπεία με μονοθεραπεία με ισχυρή στατίνη όσο και με την προσθήκη εζετιμίμπης. Η θεραπεία με προσθήκη PCSK9 αναστολέα δεν συσχετίστηκε με σημαντικές μεταβολές στα επίπεδα των OxPL-apoB, OxPL-apo(a) και OxPL-PLG. Παρατηρήθηκαν σημαντικές μειώσεις στα επίπεδα των IgG apoB-IC με όλες τις θεραπευτικές παρεμβάσεις (κατά 18,3%, 17,5% και 25,5% αντίστοιχα, όλα $p <$ 0,05). Δεν παρατηρήθηκαν σημαντικές μεταβολές στα IgM apoB-IC ούτε στα IgG και IgM anti-MDA-mimotope σε καμία θεραπευτική ομάδα.

Συμπεράσματα: Η θεραπεία με προσθήκη PCSK9 αναστολέα συσχετίστηκε με την υψηλότερη μείωση της LDL-C σε σύγκριση με την μονοθεραπεία με ισχυρή στατίνη και την προσθήκη εζετιμίμπης σε ασθενείς υψηλού κινδύνου με αυξημένα επίπεδα Lp(a) και αποτέλεσε τη μόνη θεραπευτική κατηγορία που μείωσε σημαντικά τα επίπεδα της Lp(a). Επιπλέον, τόσο η μονοθεραπεία με ισχυρή στατίνη όσο και η προσθήκη εζετιμίμπης αύξησαν σημαντικά τα επίπεδα των OxPL-apoB και OxPL-apo(a), ενώ μείωσαν σημαντικά τα επίπεδα των OxPL-PLG. Η θεραπεία με προσθήκη PCSK9 αναστολέα δεν είχε σημαντική επίδραση στα επίπεδα των OxPLs.

Λέξεις-κλειδιά: Λιποπρωτεΐνη(a), Οξειδωμένα φωσφολιπίδια, Πλασμινογόνο, Στατίνες, Εζετιμίμπη, Αναστολείς PCSK9, Αυτοαντισώματα, Οξειδωτικό στρες

PUBLICATIONS AND SCIENTIC ACTIVITY RELATED TO THE PRESENT THESIS

Peer-Reviewed Publications Derived from the Present Doctorate Thesis

A. Published Articles

1. Koutsogianni AD, Liberopoulos E, Tselepis AD. Lipoprotein(a): An update on its role in human health and disease. *J Atherosclerosis Prev Treat.* 2021;12(3):92–102. doi:10.53590/japt.02.1028.
2. Koutsogianni AD, Liberopoulos E, Tellis K, Tselepis AD. Oxidized phospholipids and lipoprotein(a): An update. *Eur J Clin Invest.* 2022;52(4):e13710. doi:10.1111/eci.13710.
3. Koutsogianni AD, Barkas F, Liberopoulos E. Metabolism and pathophysiology of lipoprotein(a). *Heart Vessels & Brain.* 2022;42:76–87.
4. Koutsogianni AD, Adamidis PS, Barkas F, Liberopoulos E, Su TC, Yamashita S, Liamis G, Rizzo M. Familial hypercholesterolemia and lipoprotein(a): A Gordian knot in cardiovascular prevention. *Metabolites.* 2022;12(11):1065. doi:10.3390/metabo12111065.
5. Koutsogianni AD, Liamis G, Liberopoulos E, Adamidis PS, Florentin M. Effects of lipid-modifying and other drugs on lipoprotein(a) levels—potent clinical implications. *Pharmaceuticals (Basel).* 2023;16(5):750. doi:10.3390/ph16050750.
6. Koutsogianni AD, Barkas F, Tellis C, Tselepis A, Liamis G, Liberopoulos E. Effect of lipid-lowering treatment on lipid and glycemic profile of patients with elevated lipoprotein(a) levels. *J Atherosclerosis Prev Treat.* 2023;14(3):1–11. doi:10.53590/japt.02.1046.
7. Koutsogianni AD, Barkas F, Tellis C, Tselepis A, Liamis G, Tsimikas S, Liberopoulos E. Effect of lipid-lowering medications on oxidized phospholipids in individuals with elevated lipoprotein(a). *Atheroscler Plus.* 2025;61:58–66. doi:10.1016/j.athplu.2025.09.003.

B. Articles Submitted-Under Review

1. Koutsogianni AD, Barkas F, Tellis C, Tselepis A, Liamis G, Tsimikas S, Liberopoulos E. Modulation of oxidation-related immune markers by lipid-lowering medications in individuals with elevated lipoprotein(a). Submitted to *Clinical Research in Cardiology*; under review.

2. Koutsogianni AD, Tatsis G, Telli C, Stamoulis K, Milionis C, Liberopoulos E, Tselepis AD, Tellis C. Impact of Lp(a) on HDL quality and functional properties. Submitted to *Applied Biosciences*; under review.

Published Abstracts

1. Koutsogianni AD, et al. Joint effect of familial hypercholesterolemia and hyperlipoproteinemia(a) on the risk of atherosclerotic cardiovascular disease. Atherosclerosis. 2022;355:e137. Abstract EP161 (#1103).
2. Koutsogianni AD, et al. Lipoprotein(a) and prevalent atherosclerotic cardiovascular disease in patients with type II diabetes. Atherosclerosis. 2022;355:e136. Abstract EP160 (#1092).
3. Koutsogianni AD, et al. The effect of statin monotherapy or add-on ezetimibe treatment on netosis in dyslipidemic patients. Atherosclerosis. 2023;379:117162. Abstract P045 (#1237).
4. Koutsogianni AD, et al. Hypolipidaemic treatment in patients with elevated lipoprotein(a). Atherosclerosis. 2023;379:117162. Abstract P153 (#172).
5. Koutsogianni AD, et al. Distribution and variation of lipoprotein(a) in patients attending a lipid clinic in Greece. Atherosclerosis. 2023;379:117162. Abstract P152 (#407).
6. Koutsogianni AD, et al. Impact of lipid-lowering medications on lipids, oxidized phospholipids and plasminogen levels in individuals with dyslipidemia and elevated lipoprotein(a). Atherosclerosis. 2024; Supplement. Abstract #071.

Accepted Abstracts (to be Presented)

1. Koutsogianni AD, et al. Impact of Lp(a) on HDL quality and functional properties. Accepted for presentation at the European Atherosclerosis Society (EAS) Congress 2026.
2. Koutsogianni AD, et al. Modulating oxidation-related immune markers: effects of lipid-lowering therapies in individuals with elevated lipoprotein(a). Accepted for presentation at the European Atherosclerosis Society (EAS) Congress 2026.

Conference Abstracts Related to the Present Doctorate Thesis

1. Koutsogianni AD, et al. Elevated lipoprotein(a) levels increase the risk of coronary heart disease in patients with familial hypercholesterolemia. Electronically posted announcement, 9th Panhellenic Congress of Working Groups, Hellenic Atherosclerosis Society, 2021.
2. Koutsogianni AD, et al. Correlation of lipoprotein(a) levels with the risk of cardiovascular disease in patients with type 2 diabetes. Electronically posted announcement, 9th Panhellenic Congress of Working Groups, Hellenic Atherosclerosis Society, 2021.

3. Koutsogianni AD, et al. Increased lipoprotein(a) multiplies coronary heart disease risk in patients with familial hypercholesterolemia. Abstract presentation, ESC Congress 2021 – The Digital Experience, 2021.

4. Koutsogianni AD, et al. Elevated lipoprotein(a) levels increase the cardiovascular risk in patients with type 2 diabetes. Poster presentation, 19th Panhellenic Congress of Diabetology, 2021.

5. Koutsogianni AD, et al. Lipoprotein(a) variability in patients with dyslipidemia. Electronically posted announcement with short presentation, 10th National Congress of the Hellenic Atherosclerosis Society, 2022.

6. Koutsogianni AD, et al. Study of the effect of hypolipidaemic treatment on the lipid profile of patients with elevated lipoprotein(a) levels. Electronically posted announcement with short presentation, 10th National Congress of the Hellenic Atherosclerosis Society, 2022.

7. Koutsogianni AD, et al. Effect of hypolipidaemic treatment on the glycemic profile of patients with elevated lipoprotein(a) levels. Electronically posted announcement, 10th National Congress of the Hellenic Atherosclerosis Society, 2022.

8. Koutsogianni AD, et al. Study of the effect of hypolipidemic treatment on the lipidemic and glycemic profile of patients with elevated lipoprotein(a) levels. Praised electronically posted announcement, 13th Panhellenic Congress of Cardio-Liver-Kidney Syndrome and Comorbidities, 2023.

9. Koutsogianni AD, et al. Qualitative characteristics of HDL in individuals with elevated lipoprotein(a). Electronically posted announcement, 11th National Congress of the Hellenic Atherosclerosis Society, 2024.

10. Koutsogianni AD, et al. Effect of lipid-lowering therapies on lipid, oxidized phospholipids and plasminogen levels in individuals with dyslipidemia and elevated lipoprotein(a) levels. Praised electronically posted announcement with short presentation, 11th National Congress of the Hellenic Atherosclerosis Society, 2024.

11. Koutsogianni AD, et al. Effect of hypolipidemic therapies on lipid, oxidized phospholipid and plasminogen levels in individuals with elevated lipoprotein(a) levels. Electronically posted announcement with short presentation, 11th National Congress of the Working Groups, Hellenic Atherosclerosis Society, 2025.

12. Koutsogianni AD, et al. Effect of hypolipidemic therapies on the levels of malondialdehyde-modified apolipoprotein B-100 and autoantibodies against its oxidized forms in individuals with elevated lipoprotein(a) levels. Electronically posted announcement

with short presentation, 11th National Congress of the Working Groups, Hellenic Atherosclerosis Society, 2025.

Invited Lectures and Scientific Presentations Related to the Present Doctorate Thesis

1. Oxidized phospholipids: their contribution in Lp(a) atherogenicity. Round Table: “From Basic Research to Clinical Practice”, 21st Medical Chemistry Conference, 2021.
2. The role of oxidized phospholipids of Lp(a). Special Topics in Basic and Clinical Research in Atherosclerosis and Metabolic Diseases, 10th National Congress of the Hellenic Atherosclerosis Society, 2022.
3. Effect of hypolipidemic treatment on lipids, oxidized phospholipids and plasminogen levels in patients with elevated lipoprotein(a) levels. 11th National Congress of the Hellenic Atherosclerosis Society, 2024.
4. Effect of hypolipidemic treatment on the lipidemic profile of individuals with elevated lipoprotein(a) levels. Congress of the Medical Society of Ioannina, 2024.
5. Effect of lipid-lowering treatment on oxidized phospholipids in patients with elevated lipoprotein(a) levels. Congress of the Medical Society of Ioannina, “Diagnostic Algorithms & Contemporary Therapeutic Approaches of Common and Rare Diseases in Internal Medicine”, 2025.

Awards and Distinctions Related to the Present Doctorate Thesis

1. Scholarship Award, Hellenic Atherosclerosis Society, supporting participation and presentation of research related to lipoprotein(a) and lipid-lowering therapies.
2. Praised electronically posted announcement with short presentation: Effect of lipid-lowering therapies on lipid, oxidized phospholipids and plasminogen levels in individuals with dyslipidemia and elevated lipoprotein(a) levels. 11th National Congress of the Hellenic Atherosclerosis Society, 2024.
3. Praised electronically posted announcement: Study of the effect of hypolipidemic treatment on the lipidemic and glycemic profile of patients with elevated lipoprotein(a) levels. 13th Panhellenic Congress of Cardio-Liver-Kidney Syndrome and Comorbidities, 2023.

REFERENCES

1. Berg K. A new serum type system in man–the LP system. *Acta Pathol Microbiol Scand*. 1963;59:369–382.
2. Kronenberg F. Human Genetics and the Causal Role of Lipoprotein(a) for Various Diseases. Cardiovasc Drugs Ther. 2016 Feb;30(1):87-100.
3. Trinder M, Uddin MM, Finneran P, Aragam KG, Natarajan P. Clinical Utility of Lipoprotein(a) and LPA Genetic Risk Score in Risk Prediction of Incident Atherosclerotic Cardiovascular Disease. JAMA Cardiol. 2021 Mar 1;6(3):287-295.
4. Boffa MB, Koschinsky ML. Lipoprotein(a) and cardiovascular disease. Biochem J. 2024 Oct 2;481(19):1277-1296.
5. Nurmohamed NS, Gaillard EL, Malkasian S, de Groot RJ, Ibrahim S, Bom MJ, Kaiser Y, Earls JP, Min JK, Kroon J, Planken RN, Danad I, van Rosendael AR, Choi AD, Stroes ESG, Knaapen P. Lipoprotein(a) and Long-Term Plaque Progression, Low-Density Plaque, and Pericoronary Inflammation. JAMA Cardiol. 2024 Sep 1;9(9):826-834.
6. Kronenberg F. Lipoprotein(a) and cardiovascular disease: Make use of the knowledge we have. Atherosclerosis. 2022 Dec;363:75-77.
7. Wang KL, Liu CJ, Chao TF, Huang CM, Wu CH, Chen SJ, Chen TJ, Lin SJ, Chiang CE. Statins, risk of diabetes, and implications on outcomes in the general population. J Am Coll Cardiol. 2012 Oct 2;60(14):1231-8.
8. Small AM, Pournamdari A, Melloni GEM, Scirica BM, Bhatt DL, Raz I, Braunwald E, Giugliano RP, Sabatine MS, Peloso GM, Marston NA, Natarajan P. Lipoprotein(a), C-Reactive Protein, and Cardiovascular Risk in Primary and Secondary Prevention Populations. JAMA Cardiol. 2024 Apr 1;9(4):385-391.
9. Thomas PE, Vedel-Krogh S, Kamstrup PR. High lipoprotein(a) is a risk factor for peripheral artery disease, abdominal aortic aneurysms, and major adverse limb events. Curr Opin Cardiol. 2024 Nov 1;39(6):511-519.
10. DeFilippis AP, Trainor PJ, Thanassoulis G, et al. Atherothrombotic factors and atherosclerotic cardiovascular events: the multi-ethnic study of atherosclerosis Eur Heart J. 2022 Mar 7;43(10):971-981. doi: 10.1093/eurheartj/ehab600.
11. Di MS, Lamina C, Coassin S, et al. Lipoprotein(a) and SARS-CoV-2 infections: susceptibility to infections, ischemic heart disease and thromboembolic events. J Intern Med. 2021. doi:10.1111/JOIM.13338.
12. Manzato M, Wright RS, Jaffe AS, Vasile VC. Lipoprotein (a): Underrecognized Risk with a Promising Future. Rev Cardiovasc Med. 2024; 25(11):393.

13. Yuen T, Mancini GBJ, Hegele RA, Pearson GJ. Consideration and Application of Lipoprotein(a) in the Risk Assessment of Atherosclerotic Cardiovascular Disease Risk in Adults. CJC Open. 2023 Nov 18;6(3):597-606.
14. Tsimikas S. Lipoprotein(a) in the Year 2024: A Look Back and a Look Ahead. Arterioscler Thromb Vasc Biol. 2024 Jul;44(7):1485-1490.
15. Tsimikas S, Karwatowska-Prokopczuk E, Gouni-Berthold I, et al. Lipoprotein(a) reduction in persons with cardiovascular disease. N Engl J Med. 2020;382(3):244-255. doi:10.1056/NEJMOA1905239.
16. Cegla J, Neely *R*, France M, et al. HEART UK consensus statement on Lipoprotein(a): A call to action. Atherosclerosis. 2019;291:62-70. doi:10.1016/J.ATHER OSCLE ROSIS.2019.10.011.
17. Jawi M, Frohlich J, Chan S. Lipoprotein(a) the Insurgent: A New Insight into the Structure, Function, Metabolism, Pathogenicity, and Medications Affecting Lipoprotein(a) Molecule. J Lipids. 2020;2020:1-26. doi:10.1155/2020/3491764.
18. Tsimikas S. A Test in Context: Lipoprotein(a): Diagnosis, Prognosis, Controversies, and Emerging Therapies. J Am Coll Cardiol. 2017 Feb 14;69(6):692-711.
19. Ellis KL, Boffa MB, Sahebkar A, Koschinsky ML, Watts GF. The renaissance of lipoprotein(a): Brave new world for preventive cardiology? Prog Lipid Res. 2017 Oct;68:57-82.
20. Coassin S, Kronenberg F. Lipoprotein(a) beyond the kringle IV repeat polymorphism: The complexity of genetic variation in the LPA gene. Atherosclerosis. 2022 May;349:17-35.
21. Koutsogianni AD, Liberopoulos E, Tellis K, Tselepis AD. Oxidized phospholipids and lipoprotein(a): An update. Eur J Clin Invest. 2022 Apr;52(4):e13710.
22. Coassin S, Erhart G, Weissensteiner H, Guimarães de Araújo ME, Lamina C, Schönherr S, et al. A novel but frequent variant in LPA KIV-2 is associated with a pronounced Lp(a) and cardiovascular risk reduction. Eur Heart J. 2017 Jun;38(23):1823-31.
23. Di Maio S, Grüneis *R*, Streiter G, Lamina C, Maglione M, Schoenherr S, et al. Investigation of a nonsense mutation located in the complex KIV-2 copy number variation region of apolipoprotein(a) in 10,910 individuals. Genome Med. 2020 Aug;12(1):74.
24. Schachtl-Riess JF, Kheirkhah A, Grüneis *R*, Di Maio S, Schoenherr S, Streiter G, et al. Frequent LPA KIV-2 Variants Lower Lipoprotein(a) Concentrations and protect against coronary artery disease. J Am Coll Cardiol. 2021 Aug;78(5):437-49.

25. Koschinsky ML, Boffa MB. Genetics to the Rescue: Sophisticated Approaches Provide Critical Insights Into the Determination of Lp(a) Levels. J Am Coll Cardiol. 2021 Aug;78(5):450-52.
26. Schmidt K, Noureen A, Kronenberg F, Utermann G. Structure, function, and genetics of lipoprotein (a). J Lipid Res. 2016 Aug;57(8):1339-59.
27. Ernst A, Helmhold M, Brunner C, Pethö-Schramm A, Armstrong VW, Müller HJ. Identification of two functionally distinct lysine-binding sites in kringle 37 and in kringles 32-36 of human apolipoprotein(a). J Biol Chem. 1995 Mar;270(11):6227-34.
28. Gabel BR, May LF, Marcovina SM, Koschinsky ML. Lipoprotein(a) assembly. Quantitative assessment of the role of apo(a) kringle IV types 2-10 in particle formation. Arterioscler Thromb Vasc Biol. 1996 Dec;16(12):1559-67.
29. Marcovina SM, Clouet-Foraison N, Koschinsky ML, et al. Development of an LC-MS/ MS Proposed Candidate Reference Method for the Standardization of Analytical Methods to Measure Lipoprotein(a). Clin Chem. 2021;67(3):490-499. doi:10.1093/CLINC HEM/HVAA324.
30. Reyes-Soffer G, Ginsberg HN, Berglund L, Duell PB, Heffron SP, Kamstrup PR, Lloyd-Jones DM, Marcovina SM, Yeang C, Koschinsky ML; American Heart Association Council on Arteriosclerosis, Thrombosis and Vascular Biology; Council on Cardiovascular Radiology and Intervention; and Council on Peripheral Vascular Disease. Lipoprotein(a): A Genetically Determined, Causal, and Prevalent Risk Factor for Atherosclerotic Cardiovascular Disease: A Scientific Statement From the American Heart Association. Arterioscler Thromb Vasc Biol. 2022 Jan;42(1):e48-e60.
31. Gencer B, Kronenberg F, Stroes E, Mach F. Lipoprotein(a): the revenant. Eur Heart J. 2017;38(20):1553-1560. doi:10.1093/ EURHE ARTJ/EHX033.
32. Mehta A, Jain V, Saeed A, Saseen JJ, Gulati M, Ballantyne CM, Virani SS. Lipoprotein(a) and ethnicities. Atherosclerosis. 2022 May;349:42-52.
33. Kronenberg F, Mora S, Stroes ESG, Ference BA, Arsenault BJ, Berglund L, Dweck MR, Koschinsky M, Lambert G, Mach F, McNeal CJ, Moriarty PM, Natarajan P, Nordestgaard BG, Parhofer KG, Virani SS, von Eckardstein A, Watts GF, Stock JK, Ray KK, Tokgözoğlu LS, Catapano AL. Lipoprotein(a) in atherosclerotic cardiovascular disease and aortic stenosis: a European Atherosclerosis Society consensus statement. Eur Heart J. 2022 Oct 14;43(39):3925-3946.

34. 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 May;290(18):11649-62.
35. Raal FJ, Giugliano RP, Sabatine MS, Koren MJ, Blom D, Seidah NJ, 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 Jun;57(6):1086-96.
36. Sharma M, Redpath GM, Williams MJ, McCormick SP. Recycling of Apolipoprotein(a) After PlgRKT-Mediated Endocytosis of Lipoprotein(a). Circ Res. 2017 Mar 31;120(7):1091-1102.
37. Yang XP, Amar MJ, Vaisman B, Bocharov AV, Vishnyakova TG, Freeman LA, Kurlander RJ, Patterson AP, Becker LC, Remaley AT. Scavenger receptor-BI is a receptor for lipoprotein(a). J Lipid Res. 2013 Sep;54(9):2450-7.
38. Boffa MB, Koschinsky ML. Lipoprotein (a): Truly a direct prothrombotic factor in cardiovascular disease? J Lipid Res. 2016 May;57(5):745-57.
39. Mach F, Koskinas KC, Roeters van Lennep JE, Tokgözoğlu L, Badimon L, Baigent C, Benn M, Binder CJ, Catapano AL, De Backer GG, Delgado V, Fabin N, Ference BA, Graham IM, Landmesser U, Laufs U, Mihaylova B, Nordestgaard BG, Richter DJ, Sabatine MS; ESC/EAS Scientific Document Group. 2025 Focused Update of the 2019 ESC/EAS Guidelines for the management of dyslipidaemias. Eur Heart J. 2025 Nov 7;46(42):4359-4378.
40. Simony SB, Mortensen MB, Langsted A, Afzal S, Kamstrup PR, Nordestgaard BG. Sex differences of lipoprotein(a) levels and associated risk of morbidity and mortality by age: The Copenhagen General Population Study. Atherosclerosis. 2022 Aug;355:76-82.
41. Langsted A, Nordestgaard BG, Kamstrup PR. Low lipoprotein(a) levels and risk of disease in a large, contemporary, general population study. Eur Heart J. 2021 Mar;42(12):1147-56.
42. Gudbjartsson DF, Thorgeirsson G, Sulem P, Helgadottir A, Gylfason A, Saemundsdottir J, Bjornsson E, Norddahl GL, Jonasdottir A, Jonasdottir A, Eggertsson HP, Gretarsdottir S, Thorleifsson G, Indridason OS, Palsson R, Jonasson F, Jonsdottir I, Eyjolfsson GI, Sigurdardottir O, Olafsson I, Danielsen R, Matthiasson SE, Kristmundsdottir S, Halldorsson BV, Hreidarsson AB, Valdimarsson EM, Gudnason T, Benediktsson R, Steinthorsdottir V, Thorsteinsdottir U, Holm H, Stefansson K. Lipoprotein(a) Concentration and Risks of Cardiovascular Disease and Diabetes. J Am Coll Cardiol. 2019 Dec 17;74(24):2982-2994.

43. Schwartz GG, Gabriel Steg P, Bhatt DL, Bittner VA, Diaz R, Goodman SG, Jukema JW, Kim YU, Li QH, Manvelian G, Pordy R, Sourdille T, White HD, Szarek M; ODYSSEY OUTCOMES Committees and Investigators. Clinical Efficacy and Safety of Alirocumab After Acute Coronary Syndrome According to Achieved Level of Low-Density Lipoprotein Cholesterol: A Propensity Score-Matched Analysis of the ODYSSEY OUTCOMES Trial. Circulation. 2021 Mar 16;143(11):1109-1122.
44. Lippi G, Guidi G. Lipoprotein(a): from ancestral benefit to modern pathogen? QJM. 2000 Feb;93(2):75-84.
45. Bianconi V, Mannarino MR, Figorilli F, Pirro M, Sahebkar A. The complex interplay of lipoprotein(a) and cancer in the era of emerging therapeutics. Semin Cancer Biol. 2025 Nov;116:96-107.
46. Reyes-Soffer G, Westerterp M. Beyond Lipoprotein(a) plasma measurements: Lipoprotein(a) and inflammation. Pharmacol Res [Internet]. 2021 Jul [accessed 2021 Aug 19];169:105689. Available from: https://pubmed.ncbi. nlm.nih.gov/34033878.
47. Durlach V, Bonnefont-Rousselot D, Boccara F, Varret M, Di-Filippo Charcosset M, Cariou B, Valero R, Charriere S, Farnier M, Morange PE, Meilhac O, Lambert G, Moulin P, Gillery P, Beliard-Lasserre S, Bruckert E, Carrié A, Ferrières J, Collet X, Chapman MJ, Anglés-Cano E. Lipoprotein(a): Pathophysiology, measurement, indication and treatment in cardiovascular disease. A consensus statement from the Nouvelle Société Francophone d'Athérosclérose (NSFA). Arch Cardiovasc Dis. 2021 Dec;114(12):828-847.
48. Li Q, Xu J, Xiong Z, Ying Q. Between Scylla and Charybdis - Enigmatic role of lipoprotein(a) in atherosclerotic cardiovascular disease and type 2 diabetes mellitus. Diabetes Res Clin Pract. 2025 Nov;229:112940.
49. Siekmeier *R*, Scharnagl H, Kostner GM, Grammar T, Stojakovic T, März W. Lipoprotein (a) - structure, epidemiology, function and diagnostics of a cardiovascular risk marker. The Open Clinical Chemistry Journal. 2008 Nov;1:79-91.
50. Tomoi Y, Takahara M, Soga Y, Hirano T, Tsunoda K, Katsuki T, Ando K. Impact of High Lipoprotein(a) Levels on Delayed Wound Healing in Patients With Chronic Limb-Threatening Ischemia After Peripheral Endovascular Therapy. Circ J. 2025 Apr 25;89(5):550-556.
51. 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 Mar 21;16(1):38.

52. Dzobo KE, Kraaijenhof JM, Stroes ESG, Nurmohamed NS, Kroon J. Lipoprotein(a): An underestimated inflammatory mastermind. Atherosclerosis. 2022 May;349:101-109.
53. Felici N, Liu D, Maret J, Restrepo M, Borovskiy Y, Hajj J, Chung W, Laudanski K. Long-Term Abnormalities of Lipid Profile After a Single Episode of Sepsis. Front Cardiovasc Med. 2021 Nov 15;8:674248.
54. Filippas-Ntekouan S, Liberopoulos E, Elisaf M. Lipid testing in infectious diseases: possible role in diagnosis and prognosis. Infection. 2017 Oct;45(5):575-88.
55. Rader DJ, Bajaj A. Lipoprotein(a) and Oxidized Phospholipids: Partners in Crime or Individual Perpetrators in Cardiovascular Disease? J Am Coll Cardiol. 2023 May 9;81(18):1793-1796.
56. Tsioulos G, Kounatidis D, Vallianou NG, Poulaki A, Kotsi E, Christodoulatos GS, Tsilingiris D, Karampela I, Skourtis A, Dalamaga M. Lipoprotein(a) and Atherosclerotic Cardiovascular Disease: Where Do We Stand? Int J Mol Sci. 2024 Mar 21;25(6):3537.
57. Doherty S, Hernandez S, Rikhi R, Mirzai S, De Los Reyes C, McIntosh S, Block RC, Shapiro MD. Lipoprotein(a) as a Causal Risk Factor for Cardiovascular Disease. Curr Cardiovasc Risk Rep. 2025;19(1):8.
58. Kaltoft M, Sigvardsen PE, Afzal S, Langsted A, Fuchs A, Kühl JT, Køber L, Kamstrup PR, Kofoed KF, Nordestgaard BG. Elevated lipoprotein(a) in mitral and aortic valve calcification and disease: The Copenhagen General Population Study. Atherosclerosis. 2022 May;349:166-174.
59. Xu R, Wang Z, Dong J, Yu M, Zhou Y. Lipoprotein(a) and panvascular disease. Lipids Health Dis. 2025 May 24;24(1):186.
60. Gencer B, Mach F. Lipoprotein(a): the perpetual supporting actor. Eur Heart J. 2018 Jul;39(27):2597-99.
61. Bhatia HS, Dalal S, Ross E. Peripheral arterial disease associated with elevated lipoprotein(a): a review of the evidence and treatment approaches. Curr Opin Lipidol. 2025 Oct 1;36(5):238-250.
62. Arnold M, Schweizer J, Nakas CT, Schütz V, Westphal LP, Inauen C, et al. Lipoprotein(a) is associated with large artery atherosclerosis stroke aetiology and stroke recurrence among patients below the age of 60 years: Results from the BIOSIGNAL study. Eur Heart J. 2021 Jun;42(22):2186-96.

63. Langsted A, Kamstrup PR, Nordestgaard BG. High lipoprotein(a) and high risk of mortality. Eur Heart J. 2019 Sep 1;40(33):2760-2770.
64. Stürzebecher PE, Schorr JJ, Klebs SHG, Laufs U. Trends and consequences of lipoprotein(a) testing: Cross-sectional and longitudinal health insurance claims database analyses. Atherosclerosis. 2023 Feb;367:24-33.
65. Zierfuss B, Höbaus C, Feldscher A, Hannes A, Mrak D, Koppensteiner R, Stangl H, Schernthaner GH. Lipoprotein (a) and long-term outcome in patients with peripheral artery disease undergoing revascularization. Atherosclerosis. 2022 Dec;363:94-101.
66. Nordestgaard BG, Langsted A. Lipoprotein (a) as a cause of cardiovascular disease: Insights from epidemiology, genetics, and biology. J Lipid Res. 2016 Nov;57(11):1953-75.
67. Koutsogianni AD, Liamis G, Liberopoulos E, Adamidis PS, Florentin M. Effects of Lipid-Modifying and Other Drugs on Lipoprotein(a) Levels-Potent Clinical Implications. Pharmaceuticals (Basel). 2023 May 16;16(5):750.
68. Trinder M, Uddin M, Finneran *P*, Aragam KG, Natarajan *P*. Clinical utility of lipoprotein(a) and LPA genetic risk score in risk prediction of incident atherosclerotic cardiovascular disease. JAMA Cardiol. 2020 Oct;6(3):1-9.
69. Orsó E, Ahrens N, Kilali´c D, Schmitz G. Familial hypercholesterolemia and lipoprotein(a) hyperlipidemia as independent and combined cardiovascular risk factors. Atheroscler Suppl. 2009 Dec;10(5):74-8.
70. Schmitz G, Orsó E. Lipoprotein(a) hyperlipidemia as cardiovascular risk factor: pathophysiological aspects. Clin Res Cardiol Suppl. 2015;10(Suppl 1):21-5.
71. Anastasiou G, Sakka E, Blathra E, Kalivi A, Elisaf M, Liamis G, et al. Lipoprotein(a): A concealed precursor of increased cardiovascular risk? A real-world regional lipid clinic experience. Arch Med Res. 2021; 52(4):397-404.
72. Anagnostis *P*, Siolos *P*, Krikidis D, Goulis DG, Stevenson JC. Should we consider lipoprotein (a) in cardiovascular disease risk assessment in patients with familial hypercholesterolaemia? Curr Pharm Des. 2018; 4(31):3665-3671.
73. McCormick SPA, Schneider WJ. Lipoprotein(a) catabolism: A case of multiple receptors. Pathology. 2019 Feb;51(2):155-164.
74. Malaguarnera M, Vacante M, Russo C, et al. Lipoprotein(a) in cardiovascular diseases. Biomed Res Int. 2013;2013:1-9. doi:10.1155/2013/650989.

75. Makris A, Barkas F, Sfikakis PP, Liberopoulos E, Filippatos TD, Ray KK, Agouridis AP. Lipoprotein(a), Interleukin-6 inhibitors, and atherosclerotic cardiovascular disease: Is there an association? Atheroscler Plus. 2023;5 4:1-6.
76. Yuliasih Y, Rachma B, Sutanto H. Lipoprotein(a) at the crossroads of inflammation and atherosclerosis in rheumatoid arthritis: A narrative review. J Clin Lipidol. 2025 Dec 4:S1933-2874(25)00525-2.
77. Fanshawe AE, Ibrahim M. The current status of lipoprotein (a) in pregnancy: A literature review. J Cardiol. 2013 Feb;61(2):99-106.
78. Chennamsetty I, Claudel T, Kostner KM, Baghdasaryan A, Kratky D, Levak-Frank S, et al. Farnesoid X receptor represses hepatic human APOA gene expression. J Clin Invest. [Internet]. 2011 Sep [accessed 2021 Aug 27];121(9):3724-34. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3163948/.
79. Kattoor AJ, Kanuri SH, Mehta JL. Role of Ox-LDL and LOX-1 in Atherogenesis. Curr Med Chem. 2019;26(9):1693-1700.
80. Rosenson RS, Goonewardena SN. The residual risk Odyssey: From LDL to Lp(a). J Am Coll Cardiol. 2021 Aug;78(5):434-6.
81. Borén B, Chapman MJ, Krauss RM, Packard CJ, Bentzon JF, Binder CJ, et al. Low-density lipoproteins cause atherosclerotic cardiovascular disease: Pathophysiological, genetic, and therapeutic insights: A consensus statement from the European Atherosclerosis Society Consensus Panel. Eur Heart J. 2020 Jun;41(24):2313-30.
82. Tasdighi E, Adhikari R, Almaadawy O, Leucker TM, Blaha MJ. LP(a): Structure, Genetics, Associated Cardiovascular Risk, and Emerging Therapeutics. Annu Rev Pharmacol Toxicol. 2024 Jan 23;64:135-157.
83. Bhatia HS, Becker RC, Leibundgut G, Patel M, Lacaze P, Tonkin A, Narula J, Tsimikas S. Lipoprotein(a), platelet function and cardiovascular disease. Nat Rev Cardiol. 2024 May;21(5):299-311.
84. Berliner JA, Leitinger N, Tsimikas S. The role of oxidized phospholipids in atherosclerosis. J Lipid Res. 2009 Apr;50(Suppl):S207-12.
85. Tsimikas S, Witztum JL. The role of oxidized phospholipids in mediating lipoprotein(a) atherogenicity. Curr Opin Lipidol. 2008 Aug;19(4):369-77.
86. Capoulade *R*, Mahmut A, Tastet L, Arsenault M, Bédard E, Dumesnil JG, et al. Impact of plasma Lp-PLA2 activity on the progression of aortic stenosis the PROGRESSA study. JACC Cardiovasc Imaging. 2015 Jan;8(1):26-33.

87. Bochkov VN, Oskolkova OV, Birukov KG, Levonen A-L, Binder CJ, Stöckl J. Generation and Biological Activities of Oxidized Phospholipids. Antioxid Redox Signal. 2010;12(8):1009-1059. doi:10.1089/ARS.2009.2597.
88. Capoulade *R*, Yeang C, Chan KL, Pibarot *P*, Tsimikas S. Association of Mild to Moderate Aortic Valve Stenosis Progression With Higher Lipoprotein(a) and Oxidized Phospholipid Levels: Secondary Analysis of a Randomized Clinical Trial. JAMA Cardiol. 2018;3(12):1212-1217. doi:10.1001/JAMAC ARDIO.2018.3798.
89. Kamstrup PR, Hung MY, Witztum JL, Tsimikas S, Nordestgaard BG. Oxidized Phospholipids and Risk of Calcific Aortic Valve Disease: The Copenhagen General Population Study. Arterioscler Thromb Vasc Biol. 2017;37(8):1570-1578. doi:10.1161/ATVBAHA.116.308761.
90. Rao F, Schork AJ, Maihofer AX, Nievergelt CM, Marcovina S, Miller ER, et al. Heritability of biomarkers of oxidized lipoproteins: Twin pair study. Arterioscler Thromb Vasc Biol. 2015 Jul;35(7):1704-11.
91. Tselepis AD. Oxidized phospholipids and lipoprotein-associated phospholipase A2 as important determinants of Lp(a) functionality and pathophysiological role. J Biomed Res. 2018;32(1):13-22. doi:10.7555/JBR.31.20160009.
92. Tsimikas S, Witztum JL. Oxidized phospholipids in cardiovascular disease. Nat Rev Cardiol. 2024 Mar;21(3):170-191.
93. Pantazi D, Tellis C, Tselepis AD. Oxidized phospholipids and lipoprotein-associated phospholipase A2 (Lp-PLA2) in atherosclerotic cardiovascular disease: An update. Biofactors. 2022 Nov;48(6):1257-1270.
94. Qin T, Ma TY, Huang K, Lu SJ, Zhong JH, Li JJ. Lipoprotein (a)-Related Inflammatory Imbalance: A Novel Horizon for the Development of Atherosclerosis. Curr Atheroscler Rep. 2024 Aug;26(8):383-394.
95. Wiesner *P*, Tafelmeier M, Chittka D, Choi SH, Zhang L, Byun YS, et al. MCP-1 binds to oxidized LDL and is carried by lipoprotein(a) in human plasma. J Lipid Res. 2013 Jul;54(7):1877-83.
96. Lampsas S, Xenou M, Oikonomou E, Pantelidis P, Lysandrou A, Sarantos S, Goliopoulou A, Kalogeras K, Tsigkou V, Kalpis A, Paschou SA, Theofilis P, Vavuranakis M, Tousoulis D, Siasos G. Lipoprotein(a) in Atherosclerotic Diseases: From Pathophysiology to Diagnosis and Treatment. Molecules. 2023 Jan 18;28(3):969.

97. Sotiriou SN, Orlova VV, Al-Fakhri N, Ihanus E, Economopoulou M, Isermann B, et al. Lipoprotein(a) in atherosclerotic plaques recruits inflammatory cells through interaction with Mac-1 integrin. FASEB J. 2006 Jan;20:559-561.
98. Yung YC, Stoddard NC, Chun J. LPA receptor signaling: pharmacology, physiology, and pathophysiology. J Lipid Res. 2014 Jul;55(7):1192-214.
99. De Oliveira Sá MPB, Cavalcanti LRP, Perazzo ÁM, Gomes RAF, Clavel MA, Pibarot *P*, et al. Calcific aortic valve stenosis and atherosclerotic calcification. Current Atherosclerosis Reports. 2020 Jan;22(2):2.
100. Tsimikas S. Elevated lipoprotein(a) and the risk of stroke in children, young adults, and the elderly. Eur Heart J. 2021 Jun 7;42(22):2197-2200.
101. Kamstrup PR, Tybjærg-Hansen A, Nordestgaard BG. Extreme lipoprotein(a) levels and improved cardiovascular risk prediction. J Am Coll Cardiol. 2013 Mar 19;61(11):1146-56.
102. Waldmann E, Parhofer KG. Lipoprotein apheresis to treat elevated lipoprotein (a). J Lipid Res. 2016 Oct;57(10):1751-7.
103. Sahebkar A, Simental-Mendia LE, Watts GF, Serban MC, Banach M, Lipid and blood pressure meta-analysis collaboration (LBPMC) group. Comparison of the effects of fibrates versus statins on plasma lipoprotein(a) concentrations: A systematic review and meta-analysis of head-to-head randomized controlled trials. BMC Med. 2017 Feb;15(1):22.
104. Tsimikas S, Fazio S, Ferdinand KC, Ginsberg HN, Koschinsky ML, Marcovina SM, Moriarty PM, Rader DJ, Remaley AT, Reyes-Soffer G, Santos RD, Thanassoulis G, Witztum JL, Danthi S, Olive M, Liu L. NHLBI Working Group Recommendations to Reduce Lipoprotein(a)-Mediated Risk of Cardiovascular Disease and Aortic Stenosis. J Am Coll Cardiol. 2018 Jan 16;71(2):177-192.
105. Ladenson PW, Kristensen JD, Ridgway EC, Olsson AG, Carlsson B, Klein I, et al. Use of the thyroid hormone analogue eprotirome in statin-treated dyslipidemia. N Engl J Med. 2010 Mar;362(10):906-16.
106. Guyton JR. Niacin in cardiovascular prevention: mechanisms, efficacy, and safety. Curr Opin Lipidol. 2007 Aug;18(4):415-20.
107. Rader DJ, Kastelein JJ. Lomitapide and mipomersen: Two first-in-class drugs for reducing low-density lipoprotein cholesterol in patients with homozygous familial hypercholesterolemia. Circulation. 2014 Mar;129(9):1022-32.

108. O'Donoghue ML, Fazio S, Giugliano RP, Stroes ESG, Kanevsky E, Gouni-Berthold I, et al. Lipoprotein(a), PCSK9 Inhibition, and cardiovascular Risk. Circulation. 2019 Mar;139(12):1483-92.
109. Bittner VA, Szarek M, Aylward PE, Bhatt DL, Diaz *R*, Edelberg JM, et al. Effect of alirocumab on lipoprotein(a) and cardiovascular risk after acute coronary syndrome. J Am Coll Cardiol. 2020 Jan;75(2):133-44.
110. Ray KK, Wright RS, Kallend D, Koenig W, Leiter LA, Raal FJ, et al. Two phase 3 trials of inclisiran in patients with elevated ldl cholesterol. N Engl J Med. 2020 Apr;382(16):1507-19.
111. Puri *R*, Nissen SE, Arsenault BJ, John JSt, Riesmeyer JS, Ruotolo G, et al. Effect of C-reactive protein on lipoprotein(a)- associated cardiovascular risk in optimally treated patients with high-risk vascular disease: A prespecified secondary analysis of the ACCELERATE trial. JAMA Cardiol. 2020 Oct;5(10):1136-43.
112. Sahebkar A, Simental-Mendia LE, Stefanutti C, Pirro M. Supplementation with coenzyme Q10 reduces plasma lipoprotein(a) concentrations but not other lipid indices: A systematic review and metaanalysis. Pharmacol Res. 2016 Mar;105:198-209.
113. Liu L, Zhao SP, Cheng YC, Li YL: Xuezhikang decreases serum lipoprotein(a) and C-reactive protein concentrations in patients with coronary heart disease. Clin Chem. 2003 Aug;49(8):1347-52.
114. Momtazi-Borojeni AA, Katsiki N, Pirro M, Banach M, Rasadi KA, Sahebkar A. Dietary natural products as emerging lipoprotein(a)-lowering agents. J Cell Physiol. 2019 Aug;234(8):12581-94.
115. Florentin M, Elisaf MS, Rizos CV, Bilianou E, Pitsavos C, Liberopoulos EN: L-Carnitine/Simvastatin reduces lipoprotein (a) levels compared with simvastatin monotherapy: A randomized double-blind placebo-controlled study. Lipids. 2017 Jan;52(1):1-9.
116. Awad K, Mikhailidis DP, Katsiki N, Muntner *P*, Banach M. Lipid and blood pressure meta-analysis collaboration (LBPMC) group. Effect of ezetimibe monotherapy on plasma lipoprotein(a) concentrations in patients with primary hypercholesterolemia: A systematic review and meta-analysis of randomized controlled trials. Drugs. 2018 Mar;78(4):453-62.
117. Jenner JL, Jacques PF, Seman LJ, Schaefer EJ. Ascorbic acid supplementation does not lower plasma lipoprotein(a) concentrations. Atherosclerosis. 2000 Aug;151(2):541-4.

118. Nozue T, Michishita I, Mizuguchi I. Effects of ezetimibe on remnant-like particle cholesterol, lipoprotein (a), and oxidized low-density lipoprotein in patients with dyslipidemia. J Atheroscler Thromb. 2010 Feb;17(1):37-44.
119. Roeseler E, Julius U, Heigl F, Spitthoever R, Heutling D, Breitenberger P, Leebmann J, Lehmacher W, Kamstrup PR, Nordestgaard BG, Maerz W, Noureen A, Schmidt K, Kronenberg F, Heibges A, Klingel R; Pro(a)LiFe-Study Group. Lipoprotein Apheresis for Lipoprotein(a)-Associated Cardiovascular Disease: Prospective 5 Years of Follow-Up and Apolipoprotein(a) Characterization. Arterioscler Thromb Vasc Biol. 2016 Sep;36(9):2019-27.
120. Kayıkcıoglu M, Kısmalı E, Can L, Payzin S. Long-term follow-up in patients with homozygous familial hypercholesterolemia; 13-year experience of a university hospital lipid clinic. Turk Kardiyol Dern Ars. 2014 Oct;42(7):599-611.
121. Kolovou G, Kolovou V, Bilianou H, Goumas G, Foussas S, Grapsa E, Garoufi A, Karavolias G, Mavrogieni S, Melidonis A, Milionis H, Rallidis L, Richter D, Skoumas I, Tousoulis D, Vlachopoulos C, Liberopoulos E. Lipoprotein apheresis: a Hellenic consensus on its clinical use. Hellenic J Cardiol. 2021 Nov-Dec;62(6):460-462.
122. de Boer LM, Oorthuys AOJ, Wiegman A, Langendam MW, Kroon J, Spijker R, Zwinderman AH, Hutten BA. Statin therapy and lipoprotein(a) levels: a systematic review and meta-analysis. Eur J Prev Cardiol. 2022 May 5;29(5):779-792.
123. Willeit P, Ridker PM, Nestel PJ, Simes J, Tonkin AM, Pedersen TR, Schwartz GG, Olsson AG, Colhoun HM, Kronenberg F, Drechsler C, Wanner C, Mora S, Lesogor A, Tsimikas S. Baseline and on-statin treatment lipoprotein(a) levels for prediction of cardiovascular events: individual patient-data meta-analysis of statin outcome trials. Lancet. 2018 Oct 13;392(10155):1311-1320.
124. Khera AV, Everett BM, Caulfield MP, Hantash FM, Wohlgemuth J, Ridker PM, Mora S. Lipoprotein(a) concentrations, rosuvastatin therapy, and residual vascular risk: an analysis from the JUPITER Trial (Justification for the Use of Statins in Prevention: an Intervention Trial Evaluating Rosuvastatin). Circulation. 2014 Feb 11;129(6):635-42.
125. Villard EF, Thedrez A, Blankenstein J, Croyal M, Tran TT, Poirier B, Le Bail JC, Illiano S, Nobécourt E, Krempf M, Blom DJ, Marais AD, Janiak P, Muslin AJ, Guillot E, Lambert G. PCSK9 Modulates the Secretion But Not the Cellular Uptake of Lipoprotein(a) Ex Vivo: An Effect Blunted by Alirocumab. JACC Basic Transl Sci. 2016 Oct;1(6):419-427.

126. Awan Z, Seidah NG, MacFadyen JG, Benjannet S, Chasman DI, Ridker PM, Genest J. Rosuvastatin, proprotein convertase subtilisin/kexin type 9 concentrations, and LDL cholesterol response: the JUPITER trial. Clin Chem. 2012 Jan;58(1):183-9.
127. Scanu AM, Bamba R. Niacin and lipoprotein(a): facts, uncertainties, and clinical considerations. Am J Cardiol. 2008 Apr 17;101(8A):44B-47B.
128. Parish S, Hopewell JC, Hill MR, Marcovina S, Valdes-Marquez E, Haynes R, Offer A, Pedersen TR, Baigent C, Collins R, Landray M, Armitage J; HPS2-THRIVE Collaborative Group. Impact of Apolipoprotein(a) Isoform Size on Lipoprotein(a) Lowering in the HPS2-THRIVE Study. Circ Genom Precis Med. 2018 Feb;11(2):e001696.
129. Chemello K, Chan DC, Lambert G, Watts GF. Recent advances in demystifying the metabolism of lipoprotein(a). Atherosclerosis. 2022; 349:82-91.
130. Anderson TJ, Boden WE, Desvigne-Nickens P, Fleg JL, Kashyap ML, McBride R, Probstfield JL; AIM-HIGH Investigators. Safety profile of extended-release niacin in the AIM-HIGH trial. N Engl J Med. 2014 Jul 17;371(3):288-90.
131. Landray MJ, Haynes R, Armitage J. Niacin for reduction of cardiovascular risk. N Engl J Med. 2014 Nov 13;371(20):1943-4.
132. Baragetti A, Da Dalt L, Norata GD. New insights into the therapeutic options to lower lipoprotein(a). Eur J Clin Invest. 2024 Sep;54(9):e14254.
133. Gaudet D, Kereiakes DJ, McKenney JM, Roth EM, Hanotin C, Gipe D, Du Y, Ferrand AC, Ginsberg HN, Stein EA. Effect of alirocumab, a monoclonal proprotein convertase subtilisin/kexin 9 antibody, on lipoprotein(a) concentrations (a pooled analysis of 150 mg every two weeks dosing from phase 2 trials). Am J Cardiol. 2014 Sep 1;114(5):711-5.
134. Stiekema LCA, Stroes ESG, Verweij SL, Kassahun H, Chen L, Wasserman SM, Sabatine MS, Mani V, Fayad ZA. Persistent arterial wall inflammation in patients with elevated lipoprotein(a) despite strong low-density lipoprotein cholesterol reduction by proprotein convertase subtilisin/kexin type 9 antibody treatment. Eur Heart J. 2019 Sep 1;40(33):2775-2781.
135. Sabatine MS, Leiter LA, Wiviott SD, Giugliano RP, Deedwania P, De Ferrari GM, Murphy SA, Kuder JF, Gouni-Berthold I, Lewis BS, Handelsman Y, Pineda AL, Honarpour N, Keech AC, Sever PS, Pedersen TR. Cardiovascular safety and efficacy of the PCSK9 inhibitor evolocumab in patients with and without diabetes and the effect of evolocumab on glycaemia and risk of new-onset diabetes: a prespecified analysis of the

FOURIER randomised controlled trial. Lancet Diabetes Endocrinol. 2017 Dec;5(12):941-950.

136. Schwartz GG, Steg PG, Szarek M, Bhatt DL, Bittner VA, Diaz R, Edelberg JM, Goodman SG, Hanotin C, Harrington RA, Jukema JW, Lecorps G, Mahaffey KW, Moryusef A, Pordy R, Quintero K, Roe MT, Sasiela WJ, Tamby JF, Tricoci P, White HD, Zeiher AM; ODYSSEY OUTCOMES Committees and Investigators. Alirocumab and Cardiovascular Outcomes after Acute Coronary Syndrome. N Engl J Med. 2018 Nov 29;379(22):2097-2107.
137. Szarek M, Bittner VA, Aylward P, Baccara-Dinet M, Bhatt DL, Diaz R, Fras Z, Goodman SG, Halvorsen S, Harrington RA, Jukema JW, Moriarty PM, Pordy R, Ray KK, Sinnaeve P, Tsimikas S, Vogel R, White HD, Zahger D, Zeiher AM, Steg PG, Schwartz GG; ODYSSEY OUTCOMES Investigators. Lipoprotein(a) lowering by alirocumab reduces the total burden of cardiovascular events independent of low-density lipoprotein cholesterol lowering: ODYSSEY OUTCOMES trial. Eur Heart J. 2020 Nov 21;41(44):4245-4255.
138. Xie S, Galimberti F, Olmastroni E, Carugo S, Catapano AL, Casula M; META-LIPID Group. Effect of lipid-lowering therapies on lipoprotein(a) levels: a comprehensive meta-analysis of randomized controlled trials. Atherosclerosis. 2025 Sep;408:120420.
139. Liberopoulos E. Lipoprotein(a) reduction with proprotein convertase subtilisin/kexin type 9 inhibitors: An unsolved mystery. Eur J Prev Cardiol. 2021 Jul 23;28(8):813-815.
140. Norata GD, Ballantyne CM, Catapano AL. New therapeutic principles in dyslipidaemia: focus on LDL and Lp(a) lowering drugs. Eur Heart J. 2013 Jun;34(24):1783-9.
141. HPS3/TIMI55–REVEAL Collaborative Group; Bowman L, Hopewell JC, Chen F, Wallendszus K, Stevens W, Collins R, Wiviott SD, Cannon CP, Braunwald E, Sammons E, Landray MJ. Effects of Anacetrapib in Patients with Atherosclerotic Vascular Disease. N Engl J Med. 2017 Sep 28;377(13):1217-1227.
142. Nicholls SJ, Ditmarsch M, Kastelein JJ, Rigby SP, Kling D, Curcio DL, Alp NJ, Davidson MH. Lipid lowering effects of the CETP inhibitor obicetrapib in combination with high-intensity statins: a randomized phase 2 trial. Nat Med. 2022 Aug;28(8):1672-1678.
143. Nicholls SJ, Nelson AJ, Ditmarsch M, Kastelein JJP, Ballantyne CM, Ray KK, Navar AM, Nissen SE, Harada-Shiba M, Curcio DL, Neild A, Kling D, Hsieh A, Butters J, Ference BA, Laufs U, Banach M, Mehran R, Catapano AL, Huo Y, Szarek M, Balinskaite V, Davidson MH; BROADWAY Investigators. Safety and Efficacy of

Obicetrapib in Patients at High Cardiovascular Risk. N Engl J Med. 2025 Jul 3;393(1):51-61.

144. Sarraju A, Brennan D, Hayden K, Stronczek A, Goldberg AC, Michos ED, McGuire DK, Mason D, Tercek G, Nicholls SJ, Kling D, Neild AL, Kastelein J, Davidson M, Ditmarsch M, Nissen SE. Fixed-dose combination of obicetrapib and ezetimibe for LDL cholesterol reduction (TANDEM): a phase 3, randomised, double-blind, placebo-controlled trial. Lancet. 2025 May 17;405(10491):1757-1768.
145. Eraikhuemen N, Lazaridis D, Dutton MT. Emerging Pharmacotherapy to Reduce Elevated Lipoprotein(a) Plasma Levels. Am J Cardiovasc Drugs. 2021 May;21(3):255-265.
146. Wu H, Lima WF, Zhang H, Fan A, Sun H, Crooke ST. Determination of the role of the human RNase H1 in the pharmacology of DNA-like antisense drugs. J Biol Chem. 2004 Apr 23;279(17):17181-9.
147. Crooke ST. Molecular Mechanisms of Antisense Oligonucleotides. Nucleic Acid Ther. 2017; 27(2):70-77.
148. Viney NJ, van Capelleveen JC, Geary RS, Xia S, Tami JA, Yu RZ, et al. Antisense oligonucleotides targeting apolipoprotein(a) in people with raised lipoprotein(a): two randomised, double-blind, placebo-controlled, dose-ranging trials. Lancet [Internet]. 2016 Nov [accessed 2021 Feb 17];388(10057):2239-53. Available from: https://pubmed.ncbi.nlm.nih.gov/27665230/.
149. Graham MJ, Viney N, Crooke RM, Tsimikas S. Antisense inhibition of apolipoprotein (a) to lower plasma lipoprotein (a) levels in humans. J Lipid Res. 2016 Mar;57(3):340-51. doi: 10.1194/jlr.R052258. Epub 2015 Nov 4. Erratum in: J Lipid Res. 2016 Dec;57(12):2235.
150. Tsimikas S, Viney NJ, Hughes SG, Singleton W, Graham MJ, Baker BF, Burkey JL, Yang Q, Marcovina SM, Geary RS, Crooke RM, Witztum JL. Antisense therapy targeting apolipoprotein(a): a randomised, double-blind, placebo-controlled phase 1 study. Lancet. 2015 Oct 10;386(10002):1472-83.
151. Yu RZ, Graham MJ, Post N, Riney S, Zanardi T, Hall S, Burkey J, Shemesh CS, Prakash TP, Seth PP, Swayze EE, Geary RS, Wang Y, Henry S. Disposition and Pharmacology of a GalNAc3-conjugated ASO Targeting Human Lipoprotein (a) in Mice. Mol Ther Nucleic Acids. 2016 May 3;5(5):e317.
152. O'Donoghue ML, Rosenson RS, Gencer B, López JAG, Lepor NE, Baum SJ, Stout E, Gaudet D, Knusel B, Kuder JF, Ran X, Murphy SA, Wang H, Wu Y, Kassahun H,

Sabatine MS; OCEAN(a)-DOSE Trial Investigators. Small Interfering RNA to Reduce Lipoprotein(a) in Cardiovascular Disease. N Engl J Med. 2022 Nov 17;387(20):1855-1864.

153. Koren MJ, Moriarty PM, Baum SJ, Neutel J, Hernandez-Illas M, Weintraub HS, Florio M, Kassahun H, Melquist S, Varrieur T, Haldar SM, Sohn W, Wang H, Elliott-Davey M, Rock BM, Pei T, Homann O, Hellawell J, Watts GF. Preclinical development and phase 1 trial of a novel siRNA targeting lipoprotein(a). Nat Med. 2022 Jan;28(1):96-103.
154. Rider DA, Eisermann M, Löffler K, Aleku M, Swerdlow DI, Dames S, Hauptmann J, Morrison E, Lindholm MW, Schubert S, Campion G. Pre-clinical assessment of SLN360, a novel siRNA targeting LPA, developed to address elevated lipoprotein (a) in cardiovascular disease. Atherosclerosis. 2022 May;349:240-247.
155. Nissen SE, Wolski K, Watts GF, Koren MJ, Fok H, Nicholls SJ, Rider DA, Cho L, Romano S, Melgaard C, Rambaran C. Single Ascending and Multiple-Dose Trial of Zerlasiran, a Short Interfering RNA Targeting Lipoprotein(a): A Randomized Clinical Trial. JAMA. 2024 May 14;331(18):1534-1543.
156. Nissen SE, Wang Q, Nicholls SJ, Navar AM, Ray KK, Schwartz GG, Szarek M, Stroes ESG, Troquay R, Dorresteijn JAN, Fok H, Rider DA, Romano S, Wolski K, Rambaran C. Zerlasiran-A Small-Interfering RNA Targeting Lipoprotein(a): A Phase 2 Randomized Clinical Trial. JAMA. 2024 Dec 17;332(23):1992-2002.
157. Nissen SE, Linnebjerg H, Shen X, Wolski K, Ma X, Lim S, Michael LF, Ruotolo G, Gribble G, Navar AM, Nicholls SJ. Lepodisiran, an Extended-Duration Short Interfering RNA Targeting Lipoprotein(a): A Randomized Dose-Ascending Clinical Trial. JAMA. 2023 Dec 5;330(21):2075-2083.
158. Nicholls SJ, Nissen SE, Fleming C, Urva S, Suico J, Berg PH, Linnebjerg H, Ruotolo G, Turner PK, Michael LF. Muvalaplin, an Oral Small Molecule Inhibitor of Lipoprotein(a) Formation: A Randomized Clinical Trial. JAMA. 2023 Sep 19;330(11):1042-1053.
159. Nicholls SJ, Ni W, Rhodes GM, Nissen SE, Navar AM, Michael LF, Haupt A, Krege JH. Oral Muvalaplin for Lowering of Lipoprotein(a): A Randomized Clinical Trial. JAMA. 2025 Jan 21;333(3):222-231.
160. Boffelli D, Zajchowski DA, Yang Z, Lawn RM. Estrogen modulation of apolipoprotein(a) expression. Identification of a regulatory element. J Biol Chem. 1999 May 28;274(22):15569-74
161. Anagnostis P, Galanis P, Chatzistergiou V, Stevenson JC, Godsland IF, Lambrinoudaki I, Theodorou M, Goulis DG. The effect of hormone replacement therapy and tibolone on

lipoprotein (a) concentrations in postmenopausal women: A systematic review and meta-analysis. Maturitas. 2017; 99:27-36.

162. Fogacci F, Borghi C, Davinelli S, Scapagnini G, Cicero AFG. Impact of anti-oestrogen therapy on lipoprotein(a) in postmenopausal women: a systematic review and meta-analysis of double-blind placebo-controlled clinical studies. Endocrine. 2023; 80(2):292-302.
163. Shlipak MG, Simon JA, Vittinghoff E, Lin F, Barrett-Connor E, Knopp RH, Levy RI, Hulley SB. Estrogen and progestin, lipoprotein(a), and the risk of recurrent coronary heart disease events after menopause. JAMA. 2000 Apr 12;283(14):1845-52.
164. Marcovina SM, Lippi G, Bagatell CJ, Bremner WJ. Testosterone-induced suppression of lipoprotein(a) in normal men; relation to basal lipoprotein(a) level. Atherosclerosis. 1996 Apr 26;122(1):89-95.
165. Giannoulis MG, Jackson N, Shojaee-Moradie F, Sonksen PH, Martin FC, Umpleby AM. Effects of growth hormone and/or testosterone on very low density lipoprotein apolipoprotein B100 kinetics and plasma lipids in healthy elderly men: a randomised controlled trial. Growth Horm IGF Res. 2006 Oct-Dec;16(5-6):308-17.
166. Hartgens F, Rietjens G, Keizer HA, Kuipers H, Wolffenbuttel BH. Effects of androgenic-anabolic steroids on apolipoproteins and lipoprotein (a). Br J Sports Med. 2004 Jun;38(3):253-9.
167. Liberopoulos E, Karabina SA, Tselepis A, Bairaktari E, Nicolaides C, Pavlidis N, Elisaf M. Are the effects of tamoxifen on the serum lipid profile modified by apolipoprotein E phenotypes? Oncology. 2002;62(2):115-20.
168. Kotwal A, Cortes T, Genere N, Hamidi O, Jasim S, Newman CB, Prokop LJ, Murad MH, Alahdab F. Treatment of Thyroid Dysfunction and Serum Lipids: A Systematic Review and Meta-analysis. J Clin Endocrinol Metab. 2020 Dec 1;105(12):dgaa672.
169. Angelin B, Kristensen JD, Eriksson M, Carlsson B, Klein I, Olsson AG, Chester Ridgway E, Ladenson PW. Reductions in serum levels of LDL cholesterol, apolipoprotein B, triglycerides and lipoprotein(a) in hypercholesterolaemic patients treated with the liver-selective thyroid hormone receptor agonist eprotirome. J Intern Med. 2015 Mar;277(3):331-342.
170. Zhao X, Wang D, Qin L. Lipid profile and prognosis in patients with coronary heart disease: a meta-analysis of prospective cohort studies. BMC Cardiovasc Disord. 2021 Feb 3;21(1):69.

171. Harrison SA, Bedossa P, Guy CD, Schattenberg JM, Loomba R, Taub R, Labriola D, Moussa SE, Neff GW, Rinella ME, Anstee QM, Abdelmalek MF, Younossi Z, Baum SJ, Francque S, Charlton MR, Newsome PN, Lanthier N, Schiefke I, Mangia A, Pericàs JM, Patil R, Sanyal AJ, Noureddin M, Bansal MB, Alkhouri N, Castera L, Rudraraju M, Ratziu V; MAESTRO-NASH Investigators. A Phase 3, Randomized, Controlled Trial of Resmetirom in NASH with Liver Fibrosis. N Engl J Med. 2024 Feb 8;390(6):497-509.
172. Glynn N, Halsall DJ, Boran G, Cook P, McDermott JH, Smith D, Tormey W, Thompson CJ, O'Gorman D, McKenna MJ, Agha A. Growth hormone replacement may influence the biological action of thyroid hormone on liver and bone tissue. Growth Horm IGF Res. 2021 Apr-Jun;57-58:101393.
173. Chasman DI, Paré G, Mora S, Hopewell JC, Peloso G, Clarke R, Cupples LA, Hamsten A, Kathiresan S, Mälarstig A, Ordovas JM, Ripatti S, Parker AN, Miletich JP, Ridker PM. Forty-three loci associated with plasma lipoprotein size, concentration, and cholesterol content in genome-wide analysis. PLoS Genet. 2009 Nov;5(11):e1000730.
174. Lacaze P, Bakshi A, Riaz M, Polekhina G, Owen A, Bhatia HS, Natarajan P, Wolfe R, Beilin L, Nicholls SJ, Watts GF, McNeil JJ, Tonkin AM, Tsimikas S. Aspirin for Primary Prevention of Cardiovascular Events in Relation to Lipoprotein(a) Genotypes. J Am Coll Cardiol. 2022 Oct 4;80(14):1287-1298.
175. Schultz O, Oberhauser F, Saech J, Rubbert-Roth A, Hahn M, Krone W, Laudes M. Effects of inhibition of interleukin-6 signalling on insulin sensitivity and lipoprotein (a) levels in human subjects with rheumatoid diseases. PLoS One. 2010 Dec 13;5(12):e14328.
176. Müller N, Schulte DM, Türk K, Freitag-Wolf S, Hampe J, Zeuner R, Schröder JO, Gouni-Berthold I, Berthold HK, Krone W, Rose-John S, Schreiber S, Laudes M. IL-6 blockade by monoclonal antibodies inhibits apolipoprotein (a) expression and lipoprotein (a) synthesis in humans. J Lipid Res. 2015 May;56(5):1034-42.
177. Enkhmaa B, Anuurad E, Zhang W, Li CS, Kaplan R, Lazar J, Merenstein D, Karim R, Aouizerat B, Cohen M, Butler K, Pahwa S, Ofotokun I, Adimora AA, Golub E, Berglund L. Effect of antiretroviral therapy on allele-associated Lp(a) level in women with HIV in the Women's Interagency HIV Study. J Lipid Res. 2018 Oct;59(10):1967-1976.
178. Santos HO, Kones R, Rumana U, Earnest CP, Izidoro LFM, Macedo RCO. Lipoprotein(a): Current Evidence for a Physiologic Role and the Effects of Nutraceutical Strategies. Clin Ther. 2019 Sep;41(9):1780-1797.

179. Enkhmaa B, Petersen KS, Kris-Etherton PM, Berglund L. Diet and Lp(a): Does Dietary Change Modify Residual Cardiovascular Risk Conferred by Lp(a)? Nutrients. 2020 Jul 7;12(7):2024.
180. Clevidence BA, Judd JT, Schaefer EJ, Jenner JL, Lichtenstein AH, Muesing RA, Wittes J, Sunkin ME. Plasma lipoprotein (a) levels in men and women consuming diets enriched in saturated, cis-, or trans-monounsaturated fatty acids. Arterioscler Thromb Vasc Biol. 1997 Sep;17(9):1657-61.
181. Silaste ML, Rantala M, Alfthan G, Aro A, Witztum JL, Kesäniemi YA, Hörkkö S. Changes in dietary fat intake alter plasma levels of oxidized low-density lipoprotein and lipoprotein(a). Arterioscler Thromb Vasc Biol. 2004 Mar;24(3):498-503.
182. Ebbeling CB, Knapp A, Johnson A, Wong JMW, Greco KF, Ma C, Mora S, Ludwig DS. Effects of a low-carbohydrate diet on insulin-resistant dyslipoproteinemia-a randomized controlled feeding trial. Am J Clin Nutr. 2022 Jan 11;115(1):154-162.
183. Austin A, Warty V, Janosky J, Arslanian S. The relationship of physical fitness to lipid and lipoprotein(a) levels in adolescents with IDDM. Diabetes Care. 1993 Feb;16(2):421-5.
184. Theodorou AA, Panayiotou G, Volaklis KA, Douda HT, Paschalis V, Nikolaidis MG, Smilios I, Toubekis A, Kyprianou D, Papadopoulos I, Tokmakidis SP. Aerobic, resistance and combined training and detraining on body composition, muscle strength, lipid profile and inflammation in coronary artery disease patients. Res Sports Med. 2016 Jul-Sep;24(3):171-84.
185. Ho J, Adam S, Liu Y, et al. Effect of bariatric surgery on plasma levels of oxidised phospholipids, biomarkers of oxidized LDL and lipoprotein(a). J Clin Lipidol. 2021;15(2):320-331.doi:10.1016/J.JACL.2020.12.002.
186. Lin J, Ji Y, Wang G, Ma X, Yao Z, Han X, Chen J, Chen J, Huang W, Xu G, Peng D, Yan P, Qiao P, He Y, Tang Y, Wang M, Zhang M, Yu J, Hao Y, Ma C. Efficacy and safety of ongericimab in Chinese patients with heterozygous familial hypercholesterolemia: A randomized, double-blind, placebo-controlled phase 3 trial. Atherosclerosis. 2025 Apr;403:119120.
187. Wang B, Shen Y, Zhai L, Xia X, Gu HM, Wang M, Zhao Y, Chang X, Alabi A, Xing S, Deng S, Liu B, Wang G, Qin S, Zhang DW. Atherosclerosis-associated hepatic secretion of VLDL but not PCSK9 is dependent on cargo receptor protein Surf4. J Lipid Res. 2021;62:100091.

188. Hao Q, Aertgeerts B, Guyatt G, Bekkering GE, Vandvik PO, Khan SU, Rodondi N, Jackson R, Reny JL, Al Ansary L, Van Driel M, Assendelft WJJ, Agoritsas T, Spencer F, Siemieniuk RAC, Lytvyn L, Heen AF, Zhao Q, Riaz IB, Ramaekers D, Okwen PM, Zhu Y, Dawson A, Ovidiu MC, Vanbrabant W, Li S, Delvaux N. PCSK9 inhibitors and ezetimibe for the reduction of cardiovascular events: a clinical practice guideline with risk-stratified recommendations. BMJ. 2022 May 4;377:e069066.
189. Cuchel M, Meagher EA, du Toit Theron H, Blom DJ, Marais AD, Hegele RA, Averna MR, Sirtori CR, Shah PK, Gaudet D, Stefanutti C, Vigna GB, Du Plessis AM, Propert KJ, Sasiela WJ, Bloedon LT, Rader DJ; Phase 3 HoFH Lomitapide Study investigators. Efficacy and safety of a microsomal triglyceride transfer protein inhibitor in patients with homozygous familial hypercholesterolaemia: a single-arm, open-label, phase 3 study. Lancet. 2013 Jan 5;381(9860):40-6.
190. Arsenault BJ, Dubé MP, Brodeur MR, de Oliveira Moraes AB, Lavoie V, Kernaleguen AE, Guauque-Olarte S, Mathieu P, Pibarot P, Messika-Zeitoun D, Bossé Y, Rhainds D, Rhéaume E, Tardif JC. Evaluation of links between high-density lipoprotein genetics, functionality, and aortic valve stenosis risk in humans. Arterioscler Thromb Vasc Biol. 2014 Feb;34(2):457-62.
191. Freigang S. The regulation of inflammation by oxidized phospholipids. Eur J Immunol. 2016;46(8):1818-1825. doi:10.1002/EJI.20154 5676.
192. Friedli O, Freigang S. Cyclopentenone-containing oxidized phospholipids and their isoprostanes as pro-resolving mediators of inflammation. Biochim Biophys Acta Mol Cell Biol Lipids. 2017;1862(4):382-392. doi:10.1016/J.BBALIP.2016.07.006.
193. Koschinsky ML, Boffa MB. Oxidized phospholipid modification of lipoprotein(a): Epidemiology, biochemistry and pathophysiology. Atherosclerosis. 2022 May;349:92-100.
194. Byun YS, Lee JH, Arsenault BJ, Yang X, Bao W, DeMicco D, Laskey R, Witztum JL, Tsimikas S; TNT Trial Investigators. Relationship of oxidized phospholipids on apolipoprotein B-100 to cardiovascular outcomes in patients treated with intensive versus moderate atorvastatin therapy: the TNT trial. J Am Coll Cardiol. 2015; 65:1286-1295.
195. Yano Y, Shimokawa K, Okada Y, Noma A. Immunolocalization of Lipoprotein(a) in Wounded Tissues. J Histochem Cytochem. 2016;45(4):559-568. doi:10.1177/00221 55497 04500408.

196. Que X, Hung MY, Yeang C, et al. Oxidized phospholipids are proinflammatory and proatherogenic in hypercholesterolaemic mice. Nature. 2018;558(7709):301-306. doi:10.1038/S41586-018-0198-8.
197. Gilliland TC, Liu Y, Mohebi R, Miksenas H, Haidermota S, Wong M, Hu X, Cristino JR, Browne A, Plutzky J, Tsimikas S, Januzzi JL Jr, Natarajan P. Lipoprotein(a), Oxidized Phospholipids, and Coronary Artery Disease Severity and Outcomes. J Am Coll Cardiol. 2023 May 9;81(18):1780-1792.
198. Tsimikas S, Lau H, Han K, et al. Percutaneous coronary intervention results in acute increases in oxidized phospholipids and lipoprotein(a): short-term and long-term immunologic responses to oxidized low-density lipoprotein. Circulation. 2004;109(25):3164-3170. doi:10.1161/01.CIR.00001 30844.01174.55.
199. Sun X, Seidman JS, Zhao P, Troutman TD, Spann NJ, Que X, Zhou F, Liao Z, Pasillas M, Yang X, Magida JA, Kisseleva T, Brenner DA, Downes M, Evans RM, Saltiel AR, Tsimikas S, Glass CK, Witztum JL. Neutralization of Oxidized Phospholipids Ameliorates Non-alcoholic Steatohepatitis. Cell Metab. 2020 Jan 7;31(1):189-206.e8.
200. Itabe H, Obama T. The Oxidized Lipoproteins In Vivo: Its Diversity and Behavior in the Human Circulation. Int J Mol Sci. 2023 Mar 17;24(6):5747.
201. de Mello Barros Pimentel MV, Bertolami A, Fernandes LP, Barroso LP, Castro IA. Could a lipid oxidative biomarker be applied to improve risk stratification in the prevention of cardiovascular disease? Biomed Pharmacother. 2023 Apr;160:114345.
202. Wonisch W, Tatzber F, Lindschinger M, Falk A, Resch U, Mörkl S, Zarkovic N, Cvirn G. Overview of Clinical Relevance of Antibodies Against Oxidized Low-Density Lipoprotein (oLAb) Within Three Decades by ELISA Technology. Antioxidants (Basel). 2024 Dec 19;13(12):1560.
203. Wu T, Willett WC, Rifai N, Shai I, Manson JE, Rimm EB. Is plasma oxidized low-density lipoprotein, measured with the widely used antibody 4E6, an independent predictor of coronary heart disease among U.S. men and women? J Am Coll Cardiol. 2006 Sep 5;48(5):973-9.
204. Tsironis LD, Katsouras C, Lourida E, et al. Reduced PAF-acetylhydrolase activity associated with Lp(a) in patients with coronary artery disease. Atherosclerosis. 2004;177(1):193-201. doi:10.1016/J.ATHER OSCLE ROSIS.2004.07.030.
205. White H, Held C, Stewart *R*, et al. Darapladib for preventing ischemic events in stable coronary heart disease. N Engl J Med. 2014;370(18):1702-1711. doi:10.1056/NEJMO A1315878.

206. O'Donoghue ML, Braunwald E, White HD, Lukas MA, Tarka E, Steg PG, Hochman JS, Bode C, Maggioni AP, Im K, Shannon JB, Davies RY, Murphy SA, Crugnale SE, Wiviott SD, Bonaca MP, Watson DF, Weaver WD, Serruys PW, Cannon CP; SOLID-TIMI 52 Investigators; Steen DL. Effect of darapladib on major coronary events after an acute coronary syndrome: the SOLID-TIMI 52 randomized clinical trial. JAMA. 2014 Sep 10;312(10):1006-15.

207. Byun YS, Yang X, Bao W, DeMicco D, Laskey R, Witztum JL, Tsimikas S; SPARCL Trial Investigators. Oxidized Phospholipids on Apolipoprotein B-100 and Recurrent Ischemic Events Following Stroke or Transient Ischemic Attack. J Am Coll Cardiol. 2017; 69(2):147-158.

208. Tsimikas S, Witztum JL, Miller ER, et al. High-dose atorvastatin reduces total plasma levels of oxidized phospholipids and immune complexes present on apolipoprotein B-100 in patients with acute coronary syndromes in the MIRACL trial. Circulation. 2004;110(11):1406-1412. doi:10.1161/01.CIR.00001 41728.23033.B.

209. Tsimikas S, Brilakis ES, Miller ER, et al. Oxidized phospholipids, Lp(a) lipoprotein, and coronary artery disease. N Engl J Med. 2005;353(1):46-57. doi:10.1056/NEJMO A043175.

210. Tsimikas S, Clopton *P*, Brilakis ES, et al. Relationship of Oxidized Phospholipids on Apolipoprotein B-100 Particles to Race, Apolipoprotein(a) Isoform Size and Cardiovascular Risk Factors: Results from the Dallas Heart Study. Circulation. 2009;119(13):1711-1719.doi:10.1161/CIRCU LATIO NAHA.108.836940.

211. Tsimikas S, Mallat Z, Talmud *P*, et al. Oxidation-specific biomarkers lipoprotein(a), and risk of fatal and nonfatal coronary events. J Am Coll Cardiol. 2010;56(12):946-955. doi:10.1016/J. JACC.2010.04.048.

212. Despres AA, Perrot N, Poulin A, Tastet L, Shen M, Chen HY, Bourgeois R, Trottier M, Tessier M, Guimond J, Nadeau M, Engert JC, Thériault S, Bossé Y, Witztum JL, Couture P, Mathieu P, Dweck MR, Tsimikas S, Thanassoulis G, Pibarot P, Clavel MA, Arsenault BJ. Lipoprotein(a), Oxidized Phospholipids, and Aortic Valve Microcalcification Assessed by 18F-Sodium Fluoride Positron Emission Tomography and Computed Tomography. CJC Open. 2019 Apr 12;1(3):131-140.

213. Zheng KH, Tsimikas S, Pawade T, Kroon J, Jenkins WSA, Doris MK, White AC, Timmers NKLM, Hjortnaes J, Rogers MA, Aikawa E, Arsenault BJ, Witztum JL, Newby DE, Koschinsky ML, Fayad ZA, Stroes ESG, Boekholdt SM, Dweck MR. Lipoprotein(a)

and Oxidized Phospholipids Promote Valve Calcification in Patients With Aortic Stenosis. J Am Coll Cardiol. 2019 May 7;73(17):2150-2162.

214. Capoulade *R*, Torzewski M, Mayr M, et al. ApoCIII-Lp(a) complexes in conjunction with Lp(a)-OxPL predict rapid progression of aortic stenosis. Heart. 2020;106(10):738-745. doi:10.1136/HEART JNL-2019- 315840.
215. Girard A, Gaillard E, Puri R, Capoulade R, Chan KL, Paulin A, Manikpurage HD, Dumesnil J, Tam JW, Teo KK, Couture C, Wareham NJ, Clavel MA, Stroes ESG, Mathieu P, Thériault S, Tsimikas S, Pibarot P, Boekholdt SM, Arsenault BJ. Impact of C-reactive protein levels on lipoprotein(a)-associated aortic stenosis incidence and progression. Eur Heart J Open. 2023 Mar 30;3(2):oead032.
216. Lee SR, Prasad A, Choi YS, Xing C, Clopton P, Witztum JL, Tsimikas S. LPA Gene, Ethnicity, and Cardiovascular Events. Circulation. 2017 Jan 17;135(3):251-263.
217. Hoekstra M, Chen HY, Rong J, Dufresne L, Yao J, Guo X, Tsai MY, Tsimikas S, Post WS, Vasan RS, Rotter JI, Larson MG, Thanassoulis G, Engert JC. Genome-Wide Association Study Highlights APOH as a Novel Locus for Lipoprotein(a) Levels-Brief Report. Arterioscler Thromb Vasc Biol. 2021 Jan;41(1):458-464.
218. Volgman AS, Koschinsky ML, Mehta A, Rosenson RS. Genetics and Pathophysiological Mechanisms of Lipoprotein(a)-Associated Cardiovascular Risk. J Am Heart Assoc. 2024 Jun 18;13(12):e033654.
219. Reyes-Soffer G, Yeang C, Michos ED, Boatwright W, Ballantyne CM. High lipoprotein(a): Actionable strategies for risk assessment and mitigation. Am J Prev Cardiol. 2024 Apr 3;18:100651.
220. Enkhmaa B, Berglund L. Non-genetic influences on lipoprotein(a) concentrations. Atherosclerosis. 2022 May;349:53-62.
221. Atallah M, Harb T, Nasrallah N, Jones SR, Gerstenblith G, Tsimikas S, Leucker TM. Oxidized phospholipid dynamics in the early post-infarction period: Effects of PCSK9 inhibition with evolocumab. Atherosclerosis. 2025 Oct;409:120469.
222. Clarke R, Hammami I, Sherliker P, Valdes-Marquez E, Watkins H, Hill M, Yang X, Tsimikas S, Hopewell JC; PROCARDIS Consortium. Oxidized phospholipids on apolipoprotein B-100 versus plasminogen and risk of coronary heart disease in the PROCARDIS study. Atherosclerosis. 2022 Aug;354:15-22.
223. Afzal Z, Hira S, Cao H. Emerging Lp(a)-Lowering therapies: Is muvalaplin a potential breakthrough? Int J Cardiol Cardiovasc Risk Prev. 2024; 21:200262.

224. Wu MD, Moccetti F, Brown E, Davidson BP, Atkinson T, Belcik JT, Giraud G, Duell PB, Fazio S, Tavori H, Tsimikas S, Lindner JR. Lipoprotein Apheresis Acutely Reverses Coronary Microvascular Dysfunction in Patients With Severe Hypercholesterolemia. JACC Cardiovasc Imaging. 2019 Aug;12(8 Pt 1):1430-1440.
225. Kiechl S, Willeit J, Mayr M, et al. Oxidized phospholipids, lipoprotein(a), lipoprotein-associated phospholipase A2 activity, and 10-year cardiovascular outcomes: prospective results from the Bruneck study. Arterioscler Thromb Vasc Biol.2007;27(8):1788-1795. doi:10.1161/ATVBA HA.107.145805.
226. Tsimikas S, Duff GW, Berger PB, Rogus J, Huttner K, Clopton P, Brilakis E, Kornman KS, Witztum JL. Pro-inflammatory interleukin-1 genotypes potentiate the risk of coronary artery disease and cardiovascular events mediated by oxidized phospholipids and lipoprotein(a). J Am Coll Cardiol. 2014 May 6;63(17):1724-34.
227. Saleheen D, Haycock PC, Zhao W, Rasheed A, Taleb A, Imran A, Abbas S, Majeed F, Akhtar S, Qamar N, Zaman KS, Yaqoob Z, Saghir T, Rizvi SNH, Memon A, Mallick NH, Ishaq M, Rasheed SZ, Memon FU, Mahmood K, Ahmed N, Frossard P, Tsimikas S, Witztum JL, Marcovina S, Sandhu M, Rader DJ, Danesh J. Apolipoprotein(a) isoform size, lipoprotein(a) concentration, and coronary artery disease: a mendelian randomisation analysis. Lancet Diabetes Endocrinol. 2017 Jul;5(7):524-533.
228. Arai K, Orsoni A, Mallat Z, et al. Acute impact of apheresis on oxidized phospholipids in patients with familial hypercholesterolemia. J Lipid Res. 2012;53(8):1670-1678. doi:10.1194/JLR.P027235.
229. Yeang C, Hung MY, Byun YS, Clopton *P*, Yang X, Witztum JL, et al. Effect of therapeutic interventions on oxidized phospholipids on apolipoprotein B100 and lipoprotein(a). J Clin Lipidol. 2016 May-Jun;10(3):594-603.
230. Khan TZ, Hsu LY, Arai AE, Rhodes S, Pottle A, Wage R, Banya W, Gatehouse PD, Giri S, Collins P, Pennell DJ, Barbir M. Apheresis as novel treatment for refractory angina with raised lipoprotein(a): a randomized controlled cross-over trial. Eur Heart J. 2017 May 21;38(20):1561-1569.
231. Konishi K, Miyake T, Furukawa S, Senba H, Kanzaki S, Nakaguchi H, Yukimoto A, Nakamura Y, Watanabe T, Koizumi Y, Yoshida O, Tokumoto Y, Hirooka M, Kumagi T, Abe M, Matsuura B, Hiasa Y. Advanced fibrosis of non-alcoholic steatohepatitis affects the significance of lipoprotein(a) as a cardiovascular risk factor. Atherosclerosis. 2020 Apr;299:32-37.

232. Faghihnia N, Tsimikas S, Miller ER, Witztum JL, Krauss RM. Changes in lipoprotein(a), oxidized phospholipids, and LDL subclasses with a low-fat high-carbohydrate diet. J Lipid Res. 2010 Nov;51(11):3324-30.
233. Choi SH, Chae A, Miller E, Messig M, Ntanios F, DeMaria AN, Nissen SE, Witztum JL, Tsimikas S. Relationship between biomarkers of oxidized low-density lipoprotein, statin therapy, quantitative coronary angiography, and atheroma: volume observations from the REVERSAL (Reversal of Atherosclerosis with Aggressive Lipid Lowering) study. J Am Coll Cardiol. 2008; 52(1):24-32.
234. Ky B, Burke A, Tsimikas S, Wolfe ML, Tadesse MG, Szapary PO, Witztum JL, FitzGerald GA, Rader DJ. The influence of pravastatin and atorvastatin on markers of oxidative stress in hypercholesterolemic humans. J Am Coll Cardiol. 2008 Apr 29;51(17):1653-62.
235. Rodenburg J, Vissers MN, Wiegman A, Miller ER, Ridker PM, Witztum JL, Kastelein JJ, Tsimikas S. Oxidized low-density lipoprotein in children with familial hypercholesterolemia and unaffected siblings: effect of pravastatin. J Am Coll Cardiol. 2006 May 2;47(9):1803-10.
236. Yoshida H, Shoda T, Yanai H, Ikewaki K, Kurata H, Ito K, Furutani N, Tada N, Witztum JL, Tsimikas S. Effects of pitavastatin and atorvastatin on lipoprotein oxidation biomarkers in patients with dyslipidemia. Atherosclerosis. 2013 Jan;226(1):161-4.
237. Watts GF, Chan DC, Pang J, Ma L, Ying Q, Aggarwal S, Marcovina SM, Barrett PHR. PCSK9 Inhibition with alirocumab increases the catabolism of lipoprotein(a) particles in statin-treated patients with elevated lipoprotein(a). Metabolism. 2020 Jun;107:154221.
238. Bergmark C, Dewan A, Orsoni A, Merki E, Miller ER, Shin MJ, et al. A novel function of lipoprotein [a] as a preferential carrier of oxidized phospholipids in human plasma. J Lipid Res. 2008 Oct;49(10):2230-9.
239. Berg K, Dahlen G, Christophersen B, Cook T, Kjekshus J, Pedersen T. Lp(a) lipoprotein level predicts survival and major coronary events in the Scandinavian Simvastatin Survival Study. Clin Genet. 1997 Nov;52(5):254-61.
240. Zhu L, Fang Y, Gao B, Jin X, Zheng J, He Y, Huang J. Effect of an increase in Lp(a) following statin therapy on cardiovascular prognosis in secondary prevention population of coronary artery disease. BMC Cardiovasc Disord. 2022 Nov 8;22(1):474. doi: 10.1186/s12872-022-02932-y. Erratum in: BMC Cardiovasc Disord. 2022 Dec 30;22(1):577.

241. O'Donoghue ML, Rosenson RS, López JAG, Lepor NE, Baum SJ, Stout E, Gaudet D, Knusel B, Kuder JF, Murphy SA, Wang H, Wu Y, Shah T, Wang J, Wilmanski T, Sohn W, Kassahun H, Sabatine MS; OCEAN(a)-DOSE Trial Investigators. The Off-Treatment Effects of Olpasiran on Lipoprotein(a) Lowering: OCEAN(a)-DOSE Extension Period Results. J Am Coll Cardiol. 2024 Aug 27;84(9):790-797.
242. Rosenson RS, López JAG, Gaudet D, Baum SJ, Stout E, Lepor NE, Park JG, Murphy SA, Knusel B, Wang J, Wilmanski T, Wang H, Wu Y, Kassahun H, Sabatine MS, O'Donoghue ML; OCEAN(a)-DOSE Trial Investigators. Olpasiran, Oxidized Phospholipids, and Systemic Inflammatory Biomarkers: Results From the OCEAN(a)-DOSE Trial. JAMA Cardiol. 2025 May 1;10(5):482-486.
243. Nissen SE, Ni W, Shen X, Wang Q, Navar AM, Nicholls SJ, Wolski K, Michael L, Haupt A, Krege JH; ALPACA Trial Investigators. Lepodisiran - A Long-Duration Small Interfering RNA Targeting Lipoprotein(a). N Engl J Med. 2025 May 1;392(17):1673-1683.
244. Bhatia HS, Yeang C, Baruch A, Yang X, Stroes ESG, Tsimikas S. PCSK9 Inhibition and Oxidized Phospholipids. J Am Coll Cardiol. 2021;78(12):1288-1289. doi:10.1016/J.JACC.2021.07.031.
245. Boffa MB, Marar TT, Yeang C, Viney NJ, Xia S, Witztum JL, Koschinsky ML, Tsimikas S. Potent reduction of plasma lipoprotein (a) with an antisense oligonucleotide in human subjects does not affect ex vivo fibrinolysis. J Lipid Res. 2019 Dec;60(12):2082-2089.
246. Dai H, Zhu Y, Chen Z, Yan R, Liu J, He Z, Zhang L, Zhang F, Yan S. Impact of alirocumab/evolocumab on lipoprotein (a) concentrations in patients with familial hypercholesterolaemia: a systematic review and meta-analysis of randomized controlled trials. Endokrynol Pol. 2023;74(3):234-242.
247. Fogelstrand P, Borén J. Retention of atherogenic lipoproteins in the artery wall and its role in atherogenesis. Nutr Metab Cardiovasc Dis. 2012 Jan;22(1):1-7.
248. Rodriguez M, Zheng Z. Connecting impaired fibrinolysis and dyslipidemia. Res Pract Thromb Haemost. 2024 Apr 5;8(3):102394.
249. Rubenstein DA, Yin W. Platelet-Activation Mechanisms and Vascular Remodeling. Compr Physiol. 2018 Jun 18;8(3):1117-1156.
250. Zhang Z, Rodriguez M, Zheng Z. Clot or Not? Reviewing the Reciprocal Regulation Between Lipids and Blood Clotting. Arterioscler Thromb Vasc Biol. 2024 Mar;44(3):533-544.

251. Langsted A, Kamstrup PR, Nordestgaard BG. High Lipoprotein(a) and Low Risk of Major Bleeding in Brain and Airways in the General Population: a Mendelian Randomization Study. Clin Chem. 2017 Nov;63(11):1714-1723.
252. Romagnuolo R, DeMarco K, Scipione CA, Boffa MB, Koschinsky ML. Apolipoprotein(a) inhibits the conversion of Glu-plasminogen to Lys-plasminogen on the surface of vascular endothelial and smooth muscle cells. Thromb Res. 2018 Sep;169:1-7.
253. Moriarty PM, Gorby LK, Stroes ES, Kastelein JP, Davidson M, Tsimikas S. Lipoprotein(a) and Its Potential Association with Thrombosis and Inflammation in COVID-19: a Testable Hypothesis. Curr Atheroscler Rep. 2020 Jul 25;22(9):48.
254. Mackman N, Antoniak S, Wolberg AS, Kasthuri R, Key NS. Coagulation Abnormalities and Thrombosis in Patients Infected With SARS-CoV-2 and Other Pandemic Viruses. Arterioscler Thromb Vasc Biol. 2020 Sep;40(9):2033-2044.
255. Lippi G, Szergyuk I, de Oliveira MHS, Benoit SW, Benoit JL, Favaloro EJ, Henry BM. The role of lipoprotein(a) in coronavirus disease 2019 (COVID-19) with relation to development of severe acute kidney injury. J Thromb Thrombolysis. 2022 Apr;53(3):581-585.
256. Nurmohamed NS, Collard D, Reeskamp LF, Kaiser Y, Kroon J, Tromp TR; Amsterdam UMC Covid-19 Biobank; van den Born BH, Coppens M, Vlaar APJ, Beudel M, van de Beek D, van Es N, Moriarty PM, Tsimikas S, Stroes ESG. Lipoprotein(a), venous thromboembolism and COVID-19: A pilot study. Atherosclerosis. 2022 Jan;341:43-49.
257. Grundy SM, Stone NJ, Bailey AL, Beam C, Birtcher KK, Blumenthal RS, Braun LT, de Ferranti S, Faiella-Tommasino J, Forman DE, Goldberg R, Heidenreich PA, Hlatky MA, Jones DW, Lloyd-Jones D, Lopez-Pajares N, Ndumele CE, Orringer CE, Peralta CA, Saseen JJ, Smith SC Jr, Sperling L, Virani SS, Yeboah J. 2018 AHA/ACC/AACVPR/AAPA/ABC/ACPM/ADA/AGS/APhA/ASPC/NLA/PCNA. Guideline on the Management of Blood Cholesterol: A Report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines. Circulation. 2019 Jun 18;139(25):e1082-e1143.
258. Schol-Gelok S, de Maat MPM, Biedermann JS, van Gelder T, Leebeek FWG, Lijfering WM, van der Meer FJM, Rijken DC, Versmissen J, Kruip MJHA. Rosuvastatin use increases plasma fibrinolytic potential: a randomised clinical trial. Br J Haematol. 2020;190:916–22.

259. Undas A, Celinska-Lowenhoff M, Lowenhoff T, Szczeklik A. Statins, fenofibrate, and quinapril increase clot permeability and enhance fibrinolysis in patients with coronary artery disease. J Thromb Haemost. 2006;4:1029–36.

260. Marston NA, Gurmu Y, Melloni GEM, Bonaca M, Gencer B, Sever PS, Pedersen TR, Keech AC, Roselli C, Lubitz SA, Ellinor PT, O'Donoghue ML, Giugliano RP, Ruff CT, Sabatine MS. The effect of PCSK9 (Proprotein Convertase Subtilisin/Kexin Type 9) inhibition on the risk of venous thromboembolism. Circulation. 2020;141: 1600–7.

261. Avellone G, Di Garbo V, Cordova *R*, Piliego T, Raneli G, De Simone *R*, Bompiani GD. Improvement of fibrinolysis and plasma lipoprotein levels induced by gemfibrozil in hypertriglyceridemia. Blood Coagul Fibrinolysis. 1995;6:543–8.

262. Brown SL, Sobel BE, Fujii S. Attenuation of the synthesis of plasminogen activator inhibitor type 1 by niacin. A potential link between lipid lowering and fibrinolysis. Circulation. 1995;92: 767–72.

263. Canner PL, Berge KG, Wenger NK, Stamler J, Friedman L, Prineas RJ, Friedewald W. Fifteen year mortality in Coronary Drug Project patients: long-term benefit with niacin. J Am Coll Cardiol. 1986;8:1245–55.

264. Lagace TA. PCSK9 and LDLR degradation: regulatory mechanisms in circulation and in cells. Curr Opin Lipidol. 2014;25:387–93.

265. Andersen *P*, Smith *P*, Seljeflot I, Brataker S, Arnesen H. Effects of gemfibrozil on lipids and haemostasis after myocardial infarction. Thromb Haemost. 1990; 63:174-177.

266. Becher T, Schulze TJ, Schmitt M, Trinkmann F, El-Battrawy I, Akin I, Kälsch T, Borggrefe M, Stach K. Ezetimibe inhibits platelet activation and uPAR expression on endothelial cells. Int J Cardiol. 2017 Jan 15;227:858-862.

267. Pinkosky SL, Newton RS, Day EA, Ford RJ, Lhotak S, Austin RC, Birch CM, Smith BK, Filippov S, Groot PHE, Steinberg GR, Lalwani ND. Liver-specific ATP-citrate lyase inhibition by bempedoic acid decreases LDL-C and attenuates atherosclerosis. Nat Commun. 2016 Nov 28;7:13457.

268. Cappellari M, Bovi *P*, Moretto G, Zini A, Nencini *P*, Sessa M, Furlan M, Pezzini A, Orlandi G, Paciaroni M, Tassinari T, Procaccianti G, Di Lazzaro V, Bettoni L, Gandolfo C, Silvestrelli G, Rasura M, Martini G, Melis M, Calloni MV, et al. The THRombolysis and STatins (THRaST) study. Neurology. 2013;80:655–61.

269. Shapiro MD, Tavori H, Fazio S. PCSK9: from basic science discoveries to clinical trials. Circ Res. 2018;122:1420–38.

270. Marston NA, Oyama K, Jarolim P, Tang M, Sever PS, Keech AC, Lira Pineda A, Wang H, Giugliano RP, Sabatine MS, Morrow DA. Combining High-Sensitivity Troponin With the American Heart Association/American College of Cardiology Cholesterol Guidelines to Guide Evolocumab Therapy. Circulation. 2021 Jul 20;144(3):249-251.
271. Undas A, Ciesla-Dul M, Drazkiewicz T, Sadowski J. Altered fibrin clot properties are associated with residual vein obstruction: effectsof lipoprotein(a) and apolipoprotein(a) isoform. Thromb Res. 2012;130:e184–7.
272. Undas A, Zawilska K, Ciesla-Dul M, Lehmann-Kopydłowska A, Skubiszak A, Ciepłuch K, Tracz W. Altered fibrin clot structure/ function in patients with idiopathic venous thromboembolism and in their relatives. Blood. 2009;114:4272–8.
273. Avellone G, Di Garbo V, Panno AV, Cordova *R*, Lepore *R*, Strano A. Changes induced by gemfibrozil on lipidic, coagulative and fibrinolytic pattern in patients with type IV hyperlipoproteinemia. Int Angiol. 1988;7:270–7.
274. Skuza AA, Polak M, Undas A. Elevated lipoprotein(a) as a new risk factor of cerebral venous sinus thrombosis: association with fibrin clot properties. J Thromb Thrombolysis. 2019;47:8–15.
275. Mani *P*, Rohatgi A. Niacin therapy, HDL cholesterol, and cardiovascular disease: is the HDL hypothesis defunct? Curr Atheroscler Rep. 2015;17:43.
276. Investigators GA. The effects of tissue plasminogen activator, streptokinase, or both on coronary-artery patency, ventricular function, and survival after acute myocardial infarction. N Engl J Med. 1993;329:1615–22.
277. Lin TC, Lin YK, Chen CI, Chan L, Chi NF, Yuan RY, Sheu JJ, Wei CR, Tsai JP, Yeh TH. Serum lipid level is not associated with symptomatic intracerebral hemorrhage after intravenous thrombolysis for acute ischemic stroke. PeerJ. 2018 Nov 23;6:e6021.
278. Hegele RA, Freeman MR, Langer A, Connelly PW, Armstrong PW. Acute reduction of lipoprotein(a) by tissue-type plasminogen activator. Circulation. 1992;85:2034–8.
279. Libby P, Buring JE, Badimon L, Hansson GK, Deanfield J, Bittencourt MS, Tokgözoğlu L, Lewis EF. Atherosclerosis. Nat Rev Dis Primers. 2019 Aug 16;5(1):56.
280. DeFilippis AP, Chernyavskiy I, Amraotkar AR, Trainor PJ, Kothari S, Ismail I, Hargis CW, Korley FK, Leibundgut G, Tsimikas S, Rai SN, Bhatnagar A. Circulating levels of plasminogen and oxidized phospholipids bound to plasminogen distinguish between atherothrombotic and non-atherothrombotic myocardial infarction. J Thromb Thrombolysis. 2016 Jul;42(1):61-76.

281. Leibundgut G., Arai K., Orsoni A., Yin H., Scipione C., Miller E.R., et al. Oxidized phospholipids are present on plasminogen, affect fibrinolysis and increase following acute myocardial infarction. J Am Coll Cardiol. 2012 Apr 17;59(16):1426.
282. Tsimikas S., Hall J.L. Lipoprotein(a) as a potential causal genetic risk factor of cardiovascular disease: a rationale for increased efforts to understand its pathophysiology and develop targeted therapies. J Am Coll Cardiol. 2012 Aug 21;60(8):716–721.
283. Ajoolabady A, Pratico D, Lin L, Mantzoros CS, Bahijri S, Tuomilehto J, Ren J. Inflammation in atherosclerosis: pathophysiology and mechanisms. Cell Death Dis. 2024; 15(11):817.
284. Nègre-Salvayre A, Salvayre R. Reactive Carbonyl Species and Protein Lipoxidation in Atherogenesis. Antioxidants (Basel). 2024 Feb 14;13(2):232.
285. Fredrikson GN, Hedblad B, Berglund G, Alm R, Ares M, Cercek B, Chyu KY, Shah PK, Nilsson J. Identification of immune responses against aldehyde-modified peptide sequences in apoB associated with cardiovascular disease. Arterioscler Thromb Vasc Biol. 2003 May 1;23(5):872-8.
286. Luquain-Costaz C, Delton I. Oxysterols in Vascular Cells and Role in Atherosclerosis. Adv Exp Med Biol. 2024;1440:213-229.
287. Marchini T, Malchow S, Caceres L, El Rabih AAH, Hansen S, Mwinyella T, Spiga L, Piepenburg S, Horstmann H, Olawale T, Li X, Mitre LS, Gissler MC, Bugger H, Zirlik A, Heidt T, Hilgendorf I, Stachon P, von Zur Muehlen C, Bode C, Wolf D. Circulating Autoantibodies Recognizing Immunodominant Epitopes From Human Apolipoprotein B Associate With Cardiometabolic Risk Factors, but Not With Atherosclerotic Disease. Front Cardiovasc Med. 2022 Apr 11;9:826729.
288. Asciutto G, Wigren M, Fredrikson GN, Mattisson IY, Grönberg C, Alm R, Björkbacka H, Dias NV, Edsfeldt A, Gonçalves I, Nilsson J. Apolipoprotein B-100 Antibody Interaction With Atherosclerotic Plaque Inflammation and Repair Processes. Stroke. 2016 Apr;47(4):1140-3.
289. Amir S, Hartvigsen K, Gonen A, Leibundgut G, Que X, Jensen-Jarolim E, Wagner O, Tsimikas S, Witztum JL, Binder CJ. Peptide mimotopes of malondialdehyde epitopes for clinical applications in cardiovascular disease. J Lipid Res. 2012; 53(7):1316-1326.
290. Fagerberg B, Barregard L, Sallsten G, Forsgard N, Ostling G, Persson M, Borné Y, Engström G, Hedblad B. Cadmium exposure and atherosclerotic carotid plaques--results from the Malmö diet and Cancer study. Environ Res. 2015 Jan;136:67-74.

291. Katsiki N, Filippatos T, Vlachopoulos C, Panagiotakos D, Milionis H, Tselepis A, Garoufi A, Rallidis L, Richter D, Nomikos T, Kolovou G, Kypreos K, Chrysohoou C, Tziomalos K, Skoumas I, Koutagiar I, Attilakos A, Papagianni M, Boutari C, Kotsis V, Pitsavos C, Elisaf M, Tsioufis K, Liberopoulos E. Executive summary of the Hellenic Atherosclerosis Society guidelines for the diagnosis and treatment of dyslipidemias - 2023. Atheroscler Plus. 2024 Feb 17;55:74-92.
292. European Society of Cardiology, 2022: Halvorsen S, Mehilli J, Cassese S, Hall TS, Abdelhamid M, Barbato E, De Hert S, de Laval I, Geisler T, Hinterbuchner L, Ibanez B, Lenarczyk R, Mansmann UR, McGreavy P, Mueller C, Muneretto C, Niessner A, Potpara TS, Ristić A, Sade LE, Schirmer H, Schüpke S, Sillesen H, Skulstad H, Torracca L, Tutarel O, Van Der Meer P, Wojakowski W, Zacharowski K; ESC Scientific Document Group. 2022 ESC Guidelines on cardiovascular assessment and management of patients undergoing non-cardiac surgery. Eur Heart J. 2022; 43(39):3826-3924.
293. Williams B, Mancia G, Spiering W, Rosei EA, Azizi M, Burnier M, et al. 2018 ESC/ESH Guidelines for the management of arterial hypertensionThe Task Force for the management of arterial hypertension of the European Society of Cardiology (ESC) and the European Society of Hypertension (ESH). Eur Heart J. 2018 Sep;39(33):3021-104.
294. McGowan MP, Hosseini Dehkordi SH, Moriarty PM, Duell PB. Diagnosis and Treatment of Heterozygous Familial Hypercholesterolemia. J Am Heart Assoc. 2019 Dec 17;8(24):e013225.
295. Levey AS, Stevens LA, Schmid CH, Zhang YL, Castro AF 3rd, Feldman HI, Kusek JW, Eggers P, Van Lente F, Greene T, Coresh J; CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration). A new equation to estimate glomerular filtration rate. Ann Intern Med. 2009 May 5;150(9):604-12.
296. Son DH, Lee HS, Lee YJ, Lee JH, Han JH. Comparison of triglyceride-glucose index and HOMA-IR for predicting prevalence and incidence of metabolic syndrome. Nutr Metab Cardiovasc Dis. 2022 Mar;32(3):596-604.
297. Willeit P, Yeang C, Moriarty PM, Tschiderer L, Varvel SA, McConnell JP, Tsimikas S. Low-Density Lipoprotein Cholesterol Corrected for Lipoprotein(a) Cholesterol, Risk Thresholds, and Cardiovascular Events. J Am Heart Assoc. 2020 Dec;9(23):e016318.
298. Marcovina SM, Navabi N, Allen S, Gonen A, Witztum JL, Tsimikas S. Development and validation of an isoform-independent monoclonal antibody-based ELISA for measurement of lipoprotein(a). J Lipid Res. 2022 Aug;63(8):100239.

299. Marcovina S, Smith S, Kornel J, Yang X, Tsimikas S. Analytical validation of monoclonal antibody-based ELISA methods for OxPL-apoB and OxPL-apo(a). J Lipid Res. 2026 Jan 7;67(2):100976.
300. Kamstrup PR, Tybjærg-Hansen A, Nordestgaard BG. Genetic evidence that lipoprotein(a) associates with atherosclerotic stenosis rather than venous thrombosis. Arterioscler Thromb Vasc Biol. 2012 Jul;32(7):1732-41.
301. Langsted A, Nordestgaard BG, Kamstrup PR. Elevated Lipoprotein(a) and Risk of Ischemic Stroke. J Am Coll Cardiol. 2019 Jul 9;74(1):54-66.
302. Fraley AE, Schwartz GG, Olsson AG, Kinlay S, Szarek M, Rifai N, Libby P, Ganz P, Witztum JL, Tsimikas S; MIRACL Study Investigators. Relationship of oxidized phospholipids and biomarkers of oxidized low-density lipoprotein with cardiovascular risk factors, inflammatory biomarkers, and effect of statin therapy in patients with acute coronary syndromes: Results from the MIRACL (Myocardial Ischemia Reduction With Aggressive Cholesterol Lowering) trial. J Am Coll Cardiol. 2009 Jun 9;53(23):2186-96.
303. Stroes E. Statins and LDL-cholesterol lowering: an overview. Curr Med Res Opin. 2005;21 Suppl 6:S9-16.
304. Jones PH, Davidson MH, Stein EA, Bays HE, McKenney JM, Miller E, Cain VA, Blasetto JW; STELLAR Study Group. Comparison of the efficacy and safety of rosuvastatin versus atorvastatin, simvastatin, and pravastatin across doses (STELLAR* Trial). Am J Cardiol. 2003 Jul 15;92(2):152-60.
305. Barter PJ, Brandrup-Wognsen G, Palmer MK, Nicholls SJ. Effect of statins on HDL-C: a complex process unrelated to changes in LDL-C: analysis of the VOYAGER Database. J Lipid Res. 2010 Jun;51(6):1546-53.
306. Knopp RH, Dujovne CA, Le Beaut A, Lipka LJ, Suresh R, Veltri EP; Ezetimbe Study Group. Evaluation of the efficacy, safety, and tolerability of ezetimibe in primary hypercholesterolaemia: a pooled analysis from two controlled phase III clinical studies. Int J Clin Pract. 2003 Jun;57(5):363-8.
307. Davidson MH, McGarry T, Bettis R, Melani L, Lipka LJ, LeBeaut AP, Suresh R, Sun S, Veltri EP. Ezetimibe coadministered with simvastatin in patients with primary hypercholesterolemia. J Am Coll Cardiol. 2002 Dec 18;40(12):2125-34.
308. Robinson JG, Colhoun HM, Bays HE, Jones PH, Du Y, Hanotin C, Donahue S. Efficacy and safety of alirocumab as add-on therapy in high-cardiovascular-risk patients with hypercholesterolemia not adequately controlled with atorvastatin (20 or 40 mg) or

rosuvastatin (10 or 20 mg): design and rationale of the ODYSSEY OPTIONS Studies. Clin Cardiol. 2014 Oct;37(10):597-604.

309. Giugliano RP, Desai NR, Kohli P, Rogers WJ, Somaratne R, Huang F, Liu T, Mohanavelu S, Hoffman EB, McDonald ST, Abrahamsen TE, Wasserman SM, Scott R, Sabatine MS; LAPLACE-TIMI 57 Investigators. Efficacy, safety, and tolerability of a monoclonal antibody to proprotein convertase subtilisin/kexin type 9 in combination with a statin in patients with hypercholesterolaemia (LAPLACE-TIMI 57): a randomised, placebo-controlled, dose-ranging, phase 2 study. Lancet. 2012 Dec 8;380(9858):2007-17.
310. Blom DJ, Hala T, Bolognese M, Lillestol MJ, Toth PD, Burgess L, Ceska R, Roth E, Koren MJ, Ballantyne CM, Monsalvo ML, Tsirtsonis K, Kim JB, Scott R, Wasserman SM, Stein EA; DESCARTES Investigators. A 52-week placebo-controlled trial of evolocumab in hyperlipidemia. N Engl J Med. 2014 May 8;370(19):1809-19.
311. Farnier M, Gaudet D, Valcheva V, Minini P, Miller K, Cariou B. Efficacy of alirocumab in high cardiovascular risk populations with or without heterozygous familial hypercholesterolemia: Pooled analysis of eight ODYSSEY Phase 3 clinical program trials. Int J Cardiol. 2016; 223:750-757.
312. Ridker PM, Pradhan A, MacFadyen JG, Libby P, Glynn RJ. Cardiovascular benefits and diabetes risks of statin therapy in primary prevention: an analysis from the JUPITER trial. Lancet. 2012 Aug 11;380(9841):565-71.
313. Cannon CP, Blazing MA, Giugliano RP, McCagg A, White JA, Theroux P, Darius H, Lewis BS, Ophuis TO, Jukema JW, De Ferrari GM, Ruzyllo W, De Lucca P, Im K, Bohula EA, Reist C, Wiviott SD, Tershakovec AM, Musliner TA, Braunwald E, Califf RM; IMPROVE-IT Investigators. Ezetimibe Added to Statin Therapy after Acute Coronary Syndromes. N Engl J Med. 2015; 372(25):2387-97.
314. Colhoun HM, Ginsberg HN, Robinson JG, Leiter LA, Müller-Wieland D, Henry RR, Cariou B, Baccara-Dinet MT, Pordy R, Merlet L, Eckel RH. No effect of PCSK9 inhibitor alirocumab on the incidence of diabetes in a pooled analysis from 10 ODYSSEY Phase 3 studies. Eur Heart J. 2016 Oct 14;37(39):2981-2989.
315. Sabatine MS, Underberg JA, Koren M, Baum SJ. Focus on PCSK9 Inhibitors: From Genetics to Clinical Practice. Postgrad Med. 2016 Oct;128 Suppl 1:31-9.
316. Ference BA, Robinson JG, Brook RD, Catapano AL, Chapman MJ, Neff DR, Voros S, Giugliano RP, Davey Smith G, Fazio S, Sabatine MS. Variation in PCSK9 and HMGCR and Risk of Cardiovascular Disease and Diabetes. N Engl J Med. 2016 Dec 1;375(22):2144-2153.

317. Schmidt AF, Pearce LS, Wilkins JT, Overington JP, Hingorani AD, Casas JP. PCSK9 monoclonal antibodies for the primary and secondary prevention of cardiovascular disease. Cochrane Database Syst Rev. 2017 Apr 28;4(4):CD011748.
318. Baruch A, Luca D, Kahn RS, Cowan KJ, Leabman M, Budha NR, Chiu CPC, Wu Y, Kirchhofer D, Peterson A, Davis JC Jr, Tingley WG. A phase 1 study to evaluate the safety and LDL cholesterol-lowering effects of RG7652, a fully human monoclonal antibody against proprotein convertase subtilisin/kexin type 9. Clin Cardiol. 2017 Jul;40(7):503-511.
319. Tsimikas S, Szarek M, Cobbaert CM, Romijn F, Jukema JW, Bhatt DL, Bittner VA, Diaz R, Fazio S, Garon G, Yuan C, Gong XM, Goodman SG, White HD, Witztum JL, Steg PG, Schwartz GG; ODYSSEY OUTCOMES Investigators. Oxidized Phospholipids, Lipoprotein(a), and Cardiovascular Outcomes After Acute Coronary Syndrome. Circulation. 2025 Dec 16;152(24):1666-1678.
320. Dunér P, To F, Berg K, Alm R, Björkbacka H, Engelbertsen D, Fredrikson GN, Nilsson J, Bengtsson E. Immune responses against aldehyde-modified laminin accelerate atherosclerosis in Apoe-/- mice. Atherosclerosis. 2010 Oct;212(2):457-65.
321. Bernelot Moens SJ, Neele AE, Kroon J, van der Valk FM, Van den Bossche J, Hoeksema MA, Hoogeveen RM, Schnitzler JG, Baccara-Dinet MT, Manvelian G, de Winther MPJ, Stroes ESG. PCSK9 monoclonal antibodies reverse the pro-inflammatory profile of monocytes in familial hypercholesterolaemia. Eur Heart J. 2017 May 21;38(20):1584-1593.
322. Wu Y, Shi J, Su Q, Yang Z, Qin L. Correlation Between Circulating PCSK9 Levels and Gestational Diabetes Mellitus in a Chinese Population. Front Endocrinol (Lausanne). 2022 Apr 13;13:826757.
323. Gerdes N, Klingenberg R. Mini-Review: Immunogenic epitopes in apolipoprotein B-100 for atheroprotective immunization. Front Cardiovasc Med. 2024 Aug 15;11:1448664.
324. Azar RR, Badaoui G, Sarkis A, Azar M, Aydanian H, Harb S, Achkouty G, Kassab R. Effect of ezetimibe/atorvastatin combination on oxidized low density lipoprotein cholesterol in patients with coronary artery disease or coronary artery disease equivalent. Am J Cardiol. 2010 Jul 15;106(2):193-7.
325. Jamialahmadi T, Baratzadeh F, Reiner Ž, Simental-Mendía LE, Xu S, Susekov AV, Santos RD, Sahebkar A. The Effects of Statin Dose, Lipophilicity, and Combination of Statins plus Ezetimibe on Circulating Oxidized Low-Density Lipoprotein Levels: A

Systematic Review and Meta-Analysis of Randomized Controlled Trials. Mediators Inflamm. 2021 Sep 4;2021:9661752.