frontiers | Research Topics

Implications of lipids and modified lipoproteins in atherogenesis: From mechanisms towards novel diagnostic and therapeutic targets

Edited by
Alexander Akhmedov, Chu-Huang Chen and Tatsuya Sawamura

Published in
Frontiers in Cardiovascular Medicine

ISSN 1664-8714
ISBN 978-2-8325-2973-7
DOI 10.3389/978-2-8325-2973-7

About Frontiers

Frontiers is more than just an open access publisher of scholarly articles: it is a pioneering approach to the world of academia, radically improving the way scholarly research is managed. The grand vision of Frontiers is a world where all people have an equal opportunity to seek, share and generate knowledge. Frontiers provides immediate and permanent online open access to all its publications, but this alone is not enough to realize our grand goals.

Frontiers journal series

The Frontiers journal series is a multi-tier and interdisciplinary set of open-access, online journals, promising a paradigm shift from the current review, selection and dissemination processes in academic publishing. All Frontiers journals are driven by researchers for researchers; therefore, they constitute a service to the scholarly community. At the same time, the *Frontiers journal series* operates on a revolutionary invention, the tiered publishing system, initially addressing specific communities of scholars, and gradually climbing up to broader public understanding, thus serving the interests of the lay society, too.

Dedication to quality

Each Frontiers article is a landmark of the highest quality, thanks to genuinely collaborative interactions between authors and review editors, who include some of the world's best academicians. Research must be certified by peers before entering a stream of knowledge that may eventually reach the public - and shape society; therefore, Frontiers only applies the most rigorous and unbiased reviews. Frontiers revolutionizes research publishing by freely delivering the most outstanding research, evaluated with no bias from both the academic and social point of view. By applying the most advanced information technologies, Frontiers is catapulting scholarly publishing into a new generation.

What are Frontiers Research Topics?

Frontiers Research Topics are very popular trademarks of the *Frontiers journals series*: they are collections of at least ten articles, all centered on a particular subject. With their unique mix of varied contributions from Original Research to Review Articles, Frontiers Research Topics unify the most influential researchers, the latest key findings and historical advances in a hot research area.

Find out more on how to host your own Frontiers Research Topic or contribute to one as an author by contacting the Frontiers editorial office: frontiersin.org/about/contact

Implications of lipids and modified lipoproteins in atherogenesis: From mechanisms towards novel diagnostic and therapeutic targets

Topic editors
Alexander Akhmedov — University of Zurich, Switzerland
Chu-Huang Chen — Texas Heart Institute, United States
Tatsuya Sawamura — Shinshu University, Japan

Topic coordinator
Simon Kraler — University of Zurich, Switzerland

Citation
Akhmedov, A., Chen, C.-H., Sawamura, T., eds. (2023). *Implications of lipids and modified lipoproteins in atherogenesis: From mechanisms towards novel diagnostic and therapeutic targets*. Lausanne: Frontiers Media SA.
doi: 10.3389/978-2-8325-2973-7

Table of contents

frontiers | Frontiers in Cardiovascular Medicine

TYPE Editorial
PUBLISHED 24 July 2023
DOI 10.3389/fcvm.2023.1245716

OPEN ACCESS

EDITED AND REVIEWED BY
Elena Osto,
Medical University of Graz, Austria

*CORRESPONDENCE
Chu-Huang Chen
✉ cchen@texasheart.org
Alexander Akhmedov
alexander.akhmedov@uzh.ch

RECEIVED 23 June 2023
ACCEPTED 14 July 2023
PUBLISHED 24 July 2023

CITATION
Kraler S, Sawamura T, Harn GY-S, Chen C-H and Akhmedov A (2023) Editorial: Implications of lipids and modified lipoproteins in atherogenesis: from mechanisms towards novel diagnostic and therapeutic targets.
Front. Cardiovasc. Med. 10:1245716.
doi: 10.3389/fcvm.2023.1245716

Editorial: Implications of lipids and modified lipoproteins in atherogenesis: from mechanisms towards novel diagnostic and therapeutic targets

Simon Kraler[1], Tatsuya Sawamura[2], Grace Yen-Shin Harn[3], Chu-Huang Chen[3,4]* and Alexander Akhmedov[1]*

[1]Center for Molecular Cardiology, University of Zurich, Schlieren, Switzerland, [2]Department of Molecular Pathophysiology, Shinshu University School of Medicine, Shinshu University, Matsumoto, Japan, [3]HEART, Health Resource Technology, LLC, Houston, TX, United States, [4]Vascular and Medical Research, The Texas Heart Institute, Houston, TX, United States

KEYWORDS

low-density lipoprotein, inflammation, biomarkers, risk stratification, residual cardiovascular risks, high-density lipoproteins, triglycerides

Editorial on the Research Topic
Implications of lipids and modified lipoproteins in atherogenesis: from mechanisms towards novel diagnostic and therapeutic targets

1. Introduction

Atherosclerosis is the leading cause of morbidity and mortality worldwide. Despite the unprecedented gains over the past decades, patients with established atherosclerotic cardiovascular disease (ASCVD) remain at high risk of recurrent ischemic events despite optimal management (1–3). Deepening insights into the underlying mechanisms provide unique opportunities to refine previous concepts of atherosclerosis pathobiology with the ultimate goal to improve its prevention and treatment and eventually patient outcomes (4). The atherogenicity of apolipoprotein-B containing lipoproteins is well established, but recent studies have shed new light on the importance of lipid quality in the development of atherosclerosis and its clinical complications (5, 6).

With this Research Topic, we have assembled an issue of compelling articles, comprising original research, case reports, editorials, and state-of-the-art reviews, with the aim to give the readers of *Frontiers in Cardiovascular Medicine* a comprehensive overview of the role of modified lipoproteins in the different phases of atherogenesis. Indeed, this Research Topic not only deepens our understanding of lipid-driven mechanisms underpinning ASCVD but also provides insights into novel concepts to address the high burden of ASCVD.

2. Low-density lipoproteins, high-density lipoproteins, and triglycerides

With the accumulation of evidence on sex-specific differences in atherosclerosis pathobiology, clinical tools to guide sex-specific patient care are gaining increasing

attention in recent years (7). The narrative review article by Wang and He highlights specific differences in lipoprotein metabolism and associated risk factors in women and men. For example, women tend to have higher high-density lipoprotein cholesterol (HDL-C) levels, which are observationally linked to lower ASCVD risk; however, at the same time, women tend to have higher triglyceride levels, which are associated with higher cardiovascular risk. Furthermore, sex hormones and reproductive factors may affect lipoprotein metabolism and, thus, the risk of major adverse cardiovascular events (MACE). Gaining a better understanding of sex-specific differences may open novel avenues for clinical interventions that could improve the prevention and treatment of ASCVD.

Taking this concept a step further, Dietrich et al. have emphasized the importance of sex hormones and sex-specific effects of HDL and its interaction with endothelial cells. In addition to their immunomodulatory, anti-inflammatory, and anti-oxidative properties, HDL particles are thought to provide vasculoprotective effects by promoting vasorelaxation and regulating vascular lipid metabolism. An improved understanding of how sex-specific factors affect these interactions may be useful in developing personalized approaches for preventing and treating ASCVD.

In the review article by Tirandi et al. the potential role of physical activity in regulating the expression of proprotein convertase subtilisin/kexin type 9 (PCSK9), a key regulator of LDL-C levels, is discussed. In a related study, Wang et al. investigated potential associations between PCSK9 levels and platelet reactivity in individuals not receiving statin or antiplatelet therapy. The authors reported a significant positive correlation between PCSK9 and platelet reactivity, suggesting that inhibiting PCSK9 may attenuate platelet reactivity and thus MACE risk in patients at high ASCVD risk. This discovery encourages further research on the pleiotropic effects of PCSK9 inhibition in caring for patients with established ASCVD.

The retrospective study by Wang and He assessed the correlation between cardiometabolic risk factors and obesity in 103 patients with familial hypercholesterolemia. Their findings demonstrate the potential of using novel biomarkers for risk stratification and personalized management of moderate to high-risk patients, such as those with familial hypercholesterolemia. Similarly, the study by Zeng et al. emphasizes the importance of considering non-HDL-C as a risk factor in men not receiving statin therapy. The authors describe a U-shaped relationship between non-HDL-C levels and all-cause and cardiovascular mortality, suggesting that both low and high levels of non-HDL-C are associated with increased mortality risk, a finding that was similarly reported for LDL-C (8).

Xu et al.'s study focused on comparing established formulas used for calculating LDL-C levels in fasting and postprandial states in the Chinese population. Notably, the Friedewald formula provided the highest accuracy for determining fasting LDL-C levels, whereas the Sampson formula performed better for measuring postprandial LDL-C levels. These findings may stimulate further research in this direction to accurately assess cardiovascular risk and eventually refine interventions in the Chinese population.

In an important review on macrophage-mediated pinocytotic engulfment of lipoproteins, Miyazaki highlights a receptor-independent endocytic pathway for foam cell formation. This process appears to occur when lipoproteins accumulate around inflammatory cells and involves plasma membrane ruffling, small GTPases, and cytoskeletal rearrangement. Although native LDL may not be the main driver of foam cell formation, further experimental studies are necessary to identify the master regulator of lipoprotein engulfment by macrophages to improve our understanding of its role in ASCVD pathogenesis.

3. Quality of lipoproteins

One area of research that has gained increasing attention in recent years is the role of modified lipoproteins in the life cycle of atherosclerotic plaque. Modified lipids include oxidized low-density lipoprotein (oxLDL), small-dense LDL, triglyceride-rich LDL, electronegative LDL, and very low-density lipoprotein (VLDL) (5, 9–11).

Lee et al.'s review focuses on VLDL, a potential driver of cardiometabolic diseases. The most electronegative VLDL subclass exerts cytotoxic effects on the endothelium and has been linked to chronic coronary syndromes and atrial remodeling in patients with metabolic syndrome. The review article highlights the significance of postprandial VLDL modification and the need for further investigation into the role of VLDL subclasses in the pathobiology of cardiometabolic diseases.

In a Bayesian network analysis focused on biomarkers of coronary atherosclerosis, Voros et al. have highlighted the importance of triglyceride-rich LDL particles in ASCVD development. In the 665 patients included in the analysis, LDL-triglycerides were directly linked to carotid atherosclerosis in over 95% of the models. Interestingly, genetic variants in the LIPC gene (encoding hepatic lipase) were associated with LDL-triglyceride levels and the presence of atherosclerotic plaque. These findings suggest that triglyceride-rich LDL particles may play a crucial role in atherosclerosis development, thus providing a potential target for future studies.

In a Chinese population, Liu et al. explored potential associations between remnant cholesterol (RC) and new-onset carotid plaques. The authors concluded that elevated RC levels are strongly associated with the presence of new-onset carotid plaques relative to other lipid parameters, particularly in those with low LDL-C levels. This study highlights the importance of considering RC in predicting residual cardiovascular risk, such as in those with low levels of LDL-C. A meta-analysis by Tian et al. further evaluated the prognostic value of RC in patients with chronic coronary syndromes. They reported an increasing risk of MACE with higher RC levels, although no significant association with all-cause mortality was identified.

The review article by Shen et al. provides insights into the impact of dyslipidemia on coronary collateral formation during diabetic states. The authors note that both altered serum lipid profiles and glycoxidative modification of lipoproteins contribute to endothelial dysfunction and blunt endothelial progenitor cell responses and interfere with the growth and maturation of collateral vessels in diabetic patients with chronic coronary syndromes.

The above review article and the aforementioned work demonstrate the significance of lipids in atherosclerosis and its acute and chronic clinical sequelae (Miyazaki, Voros et al. Shen et al.). Further research in this area may lead to innovative strategies for preventing and treating ASCVD and ultimately improving patient outcomes.

Hong et al. conducted a systematic review and meta-analysis to evaluate the relationship between oxLDL and cardiovascular disease in patients with chronic inflammatory conditions. The analysis of three observational studies comprising a total of 1,060 participants showed that circulating oxLDL levels are increased in participants with ASCVD during chronic inflammatory conditions. Indeed, their study suggests that oxLDL may be a useful biomarker for risk stratification of patients with established cardiovascular disease driven by chronic inflammation.

Since the introduction of the traditional risk factor concept in the Framingham Study (12), cholesterol-rich lipoproteins have been extensively examined, with instrumental variable approaches now allowing for causal inference using observational data. In the innovative study by Jin et al., the causal role of cholesterol efflux capacity in chronic coronary syndromes, acute myocardial infarction, and ischemic stroke was examined using Mendelian randomization. Considering the potential limitations of their approach, these findings suggest that increased cholesterol efflux capacity reduces the risk of chronic coronary syndromes and myocardial infarction but has a weaker causal effect on ischemic stroke, likely because of its more heterogeneous pathobiology.

Collectively, the studies by Wang et al. Zeng et al. Xu et al. Liu et al. Tian et al. and Jin et al. highlight the importance of considering multiple factors in assessing cardiovascular risk with potential future therapeutic implications. In addition to traditional risk factors, non-HDL-C and RC concentrations may provide valuable insights into a patient's overall cardiovascular risk.

These studies also highlight the interplay of lipoproteins, physical activity, sex-specific determinants, and emerging biomarkers in cardiovascular health and disease (Wang and He). Although the traditional risk factor concept has long prevailed, it might have resulted in an oversimplified understanding of ASCVD. An improved understanding of ASCVD pathobiology offers unique opportunities to further improve patient care and to mitigate residual cardiovascular risk.

In their review article, Durrington et al. highlight a critical role of HDL-contained serum paraoxonase-1 (PON1) in protecting against harmful LDL oxidation, a mechanism that drives early phases of ASCVD. Accordingly, reduced serum PON1 activity is associated with dyslipidemic, diabetic, and inflammatory states. Low PON1 levels are further linked to adverse cardiovascular events, particularly in patients with diabetes and established ASCVD, providing a conceptual framework to study functional determinants of HDL to reduce residual cardiovascular risk.

The articles by Wang and He, Dietrich et al., Tirandi et al., Lee et al., Hong et al., and Durrington et al. underscore the significance of biomarkers in cardiovascular disease prevention and personalized patient care. The identification of novel biomarkers can aid in personalized risk assessment and provide a basis for targeted interventions in high-risk patients. Moreover, these studies suggest that novel biomarkers, including oxLDL, PON1, and electronegative VLDL, may serve as complementary tools for the identification of patients at particularly high ASCVD risk who may benefit from intensive secondary prevention measures.

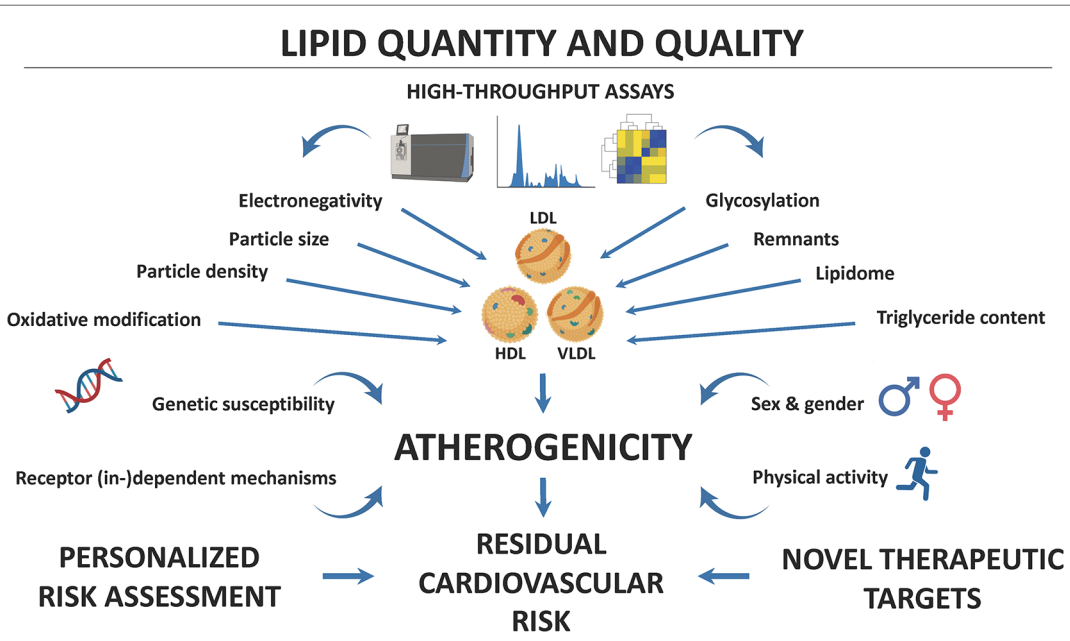


FIGURE 1
Lipids and their modifications contribute importantly to the pathogenesis of atherosclerotic cardiovascular disease (ASCVD). High-throughput technologies, including lipidomics, now allow the in-depth characterization of lipoprotein structure and function. Both physical and chemical characteristics modulate the atherogenic effects of blood lipids, but genetic susceptibility, sex and gender, physical activity, and receptor (in-) dependent mechanisms can determine their (cardio-)vascular effects. A better understanding of how these biomarkers contribute to ASCVD pathogenesis may allow for personalized risk assessment and the identification of novel therapeutic targets, aimed at reducing residual cardiovascular risk and thus improving outcomes of patients at particularly high cardiovascular risk.

However, it is essential to recognize that these biomarkers should not be used in isolation but should be considered alongside other clinical tools, including risk scores. Moreover, the clinical utility of novel biomarkers for risk assessment must undergo rigorous validation in different populations and settings, taking into account potential limitations inherent in the design of the studies noted above.

Together, the studies presented in this Research Topic highlight the importance of biomarkers in the prevention and management of ASCVD and its complications (**Figure 1**). The identification and rigorous validation of novel biomarkers could assist in personalized risk assessment to guide targeted interventions in high-risk populations. To that end, high-throughput assays are needed for qualitatively assessing changes in the structure and function of lipoproteins to work toward the clinical implementation of multidimensional lipid profiling. The herein proposed research pursuit is crucial for developing effective prevention and management strategies for patients at risk for or with established ASCVD with the ultimate goal of reducing the burden of residual cardiovascular risk.

Author contributions

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

Acknowledgments

The authors thank Rebecca Bartow, of The Texas Heart Institute in Houston, TX, for editorial assistance.

Conflict of interest

GH and CH-H were employed by HEART, Health Resource Technology, LLC.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The handling editor EO declared a past collaboration with the author AA.

Publisher's note

References

1. Kraler S, Wenzl FA, Luscher TF. Repurposing colchicine to combat residual cardiovascular risk: the LoDoCo2 trial. *Eur J Clin Invest*. (2020) 50:e13424. doi: 10.1111/eci.13424

2. Sabatine MS, Giugliano RP, Keech AC, Honarpour N, Wiviott SD, Murphy SA, et al. Evolocumab and clinical outcomes in patients with cardiovascular disease. *N Engl J Med*. (2017) 376:1713–22. doi: 10.1056/NEJMoa1615664

3. Schwartz GG, Steg PG, Szarek M, Bhatt DL, Bittner VA, Diaz R, et al. Alirocumab and cardiovascular outcomes after acute coronary syndrome. *N Engl J Med*. (2018) 379:2097–107. doi: 10.1056/NEJMoa1801174

4. Kraler S, Libby P, Evans PC, Akhmedov A, Schmiady MO, Reinehr M, et al. Resilience of the internal mammary artery to atherogenesis: shifting from risk to resistance to address unmet needs. *Arterioscler Thromb Vasc Biol*. (2021) 41:2237–51. doi: 10.1161/ATVBAHA.121.316256

5. Kraler S, Wenzl FA, Vykoukal J, Fahrmann JF, Shen M-Y, Chen D-Y, et al. Low-density lipoprotein electronegativity and risk of death after acute coronary syndromes: a case-cohort analysis. *Atherosclerosis*. (2023) 376:43–52. doi: 10.1016/j.atherosclerosis.2023.05.014

6. Ruuth M, Nguyen SD, Vihervaara T, Hilvo M, Laajala TD, Kondadi PK, et al. Susceptibility of low-density lipoprotein particles to aggregate depends on particle lipidome, is modifiable, and associates with future cardiovascular deaths. *Eur Heart J*. (2018) 39:2562–73. doi: 10.1093/eurheartj/ehy319

7. Wenzl FA, Kraler S, Ambler G, Weston C, Herzog SA, Raber L, et al. Sex-specific evaluation and redevelopment of the GRACE score in non-ST-segment elevation acute coronary syndromes in populations from the UK and Switzerland: a multinational analysis with external cohort validation. *Lancet*. (2022) 400:744–56. doi: 10.1016/S0140-6736(22)01483-0

8. Johannesen CDL, Langsted A, Mortensen MB, Nordestgaard BG. Association between low density lipoprotein and all cause and cause specific mortality in Denmark: prospective cohort study. *Br Med J*. (2020) 371:m4266. doi: 10.1136/bmj.m4266

9. Chan HC, Ke LY, Chu CS, Lee AS, Shen MY, Cruz MA, et al. Highly electronegative LDL from patients with ST-elevation myocardial infarction triggers platelet activation and aggregation. *Blood*. (2013) 122:3632–41. doi: 10.1182/blood-2013-05-504639

10. Kraler S, Wenzl FA, Georgiopoulos G, Obeid S, Liberale L, von Eckardstein A, et al. Soluble lectin-like oxidized low-density lipoprotein receptor-1 predicts premature death in acute coronary syndromes. *Eur Heart J*. (2022) 43:1849–60. doi: 10.1093/eurheartj/ehac143

11. Shen MY, Chen FY, Hsu JF, Fu RH, Chang CM, Chang CT, et al. Plasma L5 levels are elevated in ischemic stroke patients and enhance platelet aggregation. *Blood*. (2016) 127:1336–45. doi: 10.1182/blood-2015-05-646117

12. Kannel WB, Dawber TR, Kagan A, Revotskie N, Stokes J 3rd. Factors of risk in the development of coronary heart disease–six year follow-up experience. The framingham study. *Ann Intern Med*. (1961) 55:33–50. doi: 10.7326/0003-4819-55-1-33

frontiers | Frontiers in Cardiovascular Medicine

ORIGINAL RESEARCH
published: 04 July 2022
doi: 10.3389/fcvm.2022.891148

Differential Effects of Genetically Determined Cholesterol Efflux Capacity on Coronary Artery Disease and Ischemic Stroke

Aoming Jin[1,2†], *Mengxing Wang*[1,2†], *Weiqi Chen*[1,2], *Hongyi Yan*[1,2], *Xianglong Xiang*[1,2] and *Yuesong Pan*[1,2*]

[1] Department of Neurology, Beijing Tiantan Hospital, Capital Medical University, Beijing, China, [2] China National Clinical Research Center for Neurological Diseases, Beijing Tiantan Hospital, Capital Medical University, Beijing, China

OPEN ACCESS

Edited by:
Chu-Huang Chen,
Texas Heart Institute, United States

Reviewed by:
C. M. Schooling,
City University of New York,
United States
Adria Arboix,
Sacred Heart University
Hospital, Spain

***Correspondence:**
Yuesong Pan
yuesongpan@ncrcnd.org.cn

[†]These authors have contributed equally to this work and share first authorship

Specialty section:
This article was submitted to Lipids in Cardiovascular Disease, a section of the journal Frontiers in Cardiovascular Medicine

Received: 07 March 2022
Accepted: 14 June 2022
Published: 04 July 2022

Citation:
Jin A, Wang M, Chen W, Yan H, Xiang X and Pan Y (2022) Differential Effects of Genetically Determined Cholesterol Efflux Capacity on Coronary Artery Disease and Ischemic Stroke.
Front. Cardiovasc. Med. 9:891148.
doi: 10.3389/fcvm.2022.891148

Background: Observational studies indicated that cholesterol efflux capacity (CEC) of high-density lipoprotein (HDL) is inversely associated with cardiovascular events, independently of the HDL cholesterol concentration. The aim of the study is to examine the casual relevance of CEC for coronary artery disease (CAD) and myocardial infarction (MI), and compare it with that for ischemic stroke and its subtypes using a Mendelian randomization approach.

Methods: We performed a 2-sample Mendelian randomization to estimate the casual relationship of CEC with the risk of CAD, MI, and ischemic stroke. A CEC-related genetic variant (rs141622900) and other five genetic variants were used as the instrumental variables. Association of genetic variants with CAD were estimated in a GWAS involving 60,801 CAD cases and 123,504 controls. They were then compared with the associations of these variants with ischemic stroke and its subtypes (large vessel, small vessel, and cardioembolic) involving 40,585 ischemic stroke cases and 406,111 controls.

Results: Using the SNP of rs141622900 as the instrument, a 1-SD increase in CEC was associated with 45% lower risk for CAD (odds ratio [OR] 0.55, 95% confidence interval [CI] 0.44–0.69, $p < 0.001$) and 33% lower risk for MI (odds ratio [OR] 0.67, 95% CI 0.52–0.87, $p = 0.002$). By contrast, the causal effect of CEC was much weaker for ischemic stroke (odds ratio [OR] 0.79, 95% CI 0.64–0.97, $p = 0.02$; p for heterogeneity $= 0.03$) and, in particular, for cardioembolic stroke (p for heterogeneity $= 0.006$) when compared with that for CAD. Results using five genetic variants as the instrument also indicated consistently weaker effects on ischemic stroke than on CAD.

Conclusion: Genetic predicted higher CEC may be associated with decreased risk of CAD. However, the casual association of CEC with ischemic stroke and specific subtypes would need to be validated in further Mendelian randomization studies.

Keywords: cholesterol efflux capacity, Mendelian randomization, coronary artery disease, stroke, genetics

INTRODUCTION

Epidemiologic studies have shown an inverse relationship between high-density lipoprotein (HDL) cholesterol levels and cardiovascular disease (1); however, recent clinical trials (2, 3) and Mendelian randomization (MR) studies (4, 5) failed to established a clear causal association between HDL cholesterol and cardiovascular disease. This led to the hypothesis that the atheroprotective role of HDL lies in its function rather than in its concentrations (6).

The most important measure of HDL function is cholesterol efflux capacity (CEC), the ability of HDL to reverse cholesterol transport from peripheral cells (7). Previous cohort and case-control studies showed that CEC was inversely associated with atherosclerosis and the incidence of cardiovascular events in the general population, independently of the HDL cholesterol concentration (8–11). However, observational epidemiological studies may suffer from confounding and selection bias that represent obstacles to valid causal inference (12, 13). The causal association between CEC and cardiovascular diseases is still controversial. Furthermore, ischemic stroke had a heterogeneous mechanism and may have different cause and risk factors from coronary artery disease (CAD) (14). Previous MR studies have showed a weaker effect on ischemic stroke than on CAD for some lipid metabolic factors, such as low-density lipoprotein cholesterol and proprotein convertase subtilisin/kexin type 9 (PCSK9) variants (15, 16). Therefore, the relative effects of CEC on CAD and ischemic stroke needs further investigation.

MR study, using genetic variants as instrumental variables, is a method that can control potential confounders and reverse causation that may bias observational studies, and make stronger causal inferences between an exposure and risk of diseases (12). In the present study, we aimed to use MR analysis to examine the causal relevance of CEC for CAD and myocardial infarction (MI), and compares it with that for ischemic stroke and its subtypes.

MATERIALS AND METHODS

Study Design

A two-sample MR analysis using CEC-related genetic variants as instrumental variable was designed to evaluate the causal effect between CEC and risk of CAD and ischemic stroke (**Supplementary Figure 1**). Summary-level data on the exposure (CEC) were derived from a recent published genome-wide association study (GWAS) of up to 5,293 European individuals (17) and data on the outcome (CAD and ischemic stroke) were obtained from GWASs of up to 446,696 European individuals (18, 19). **Table 1** and **Supplementary Table 1** shows the characteristics of these GWASs. Approval of ethics committee and written informed consent were obtained before data collection in the original GWASs.

Genetic Instrumental Variables

We used 6 single nucleotide polymorphisms (SNPs) associated with CEC identified through GWAS by Low-Kam et al. (17) as the instrumental variables. Low-Kam et al. (17) tested the genetic association between 4 CEC measures and genotypes at >9 million common autosomal DNA sequence variants in 5,293 French Canadians. They identified 10 genome-wide significant signals ($P < 6.25 \times 10^{-9}$) representing 7 loci. Among the 7 loci, 2 loci (near the *PPP1CB/PLB1* and *RBFOX3/ENPP7* genes) only reached genome-wide significance in the model further adjusted for HDL-C and triglyceride levels which may lead to false positive associations in the GWAS context (i.e., collider bias). Other 5 loci (*CETP*, *LIPC*, *LPL*, *APOA1/C3/A4/A5*, and *APOE/C1/C2/C4*) harbored genes with important roles in lipid biology and reached genome-wide significance in the model adjusted for sex, age squared, coronary artery disease status, experimental batches, statin treatment, and the first 10 principal components. Except for the *APOE/C1/C2/C4* variant, association of other 4 loci disappeared when correcting for HDL-C and triglyceride levels. Only the SNP of rs141622900 in *APOE/C1/C2/C4* locus reached genome-wide significance in both two models and was used as the instrument. In sensitivity analysis, we used the most significant SNP in each of the 5 loci (rs77069344, rs2070895, rs247616, rs964184, and rs445925) as the instrument. These 5 SNPs were in different genomic regions and not in linkage disequilibrium ($r^2 < 0.1$). The 1 SNP (rs141622900) instrument explained 0.9% and the 5 SNPs instrument explained 5.3% of the variance in CEC (F statistic = 59.2 and 45.9, respectively, indicating sufficient strength of the instruments). **Table 2** shows the characteristics and associations of these included SNPs with CEC.

Outcomes

Summary statistics for the association of each CEC-related SNP with the CAD and MI were extracted from the Coronary ARtery DIsease Genome-wide Replication And Meta-Analysis Plus Coronary Artery Disease Genetics (CARDIoGRAMplusC4D) 1000 Genomes-based GWAS (17). The CARDIoGRAMplusC4D 1000 Genomes-based GWAS interrogated 9.4 million variants in up to 60,801 CAD cases and 123,504 controls from 48 studies of predominantly European ancestry. Summary statistics for the association of the included SNPs with ischemic stroke and the 3 main subtypes of ischemic stroke (large artery stroke [LAS], small vessel stroke [SVS], cardioembolic stroke [CES]) were extracted from the GWAS of Multiancestry Genome-wide Association Study of Stroke (MEGASTROKE) consortium (19). The MEGASTROKE consortium tested ∼8 million SNPs and indels with minor-allele frequency ≥0.01 in up to 67,162 stroke cases and 454,450 controls from 29 studies, predominantly European ancestry (40,585 cases; 406,111 controls). This GWAS involved 34,217 cases with LAS, 5,386 cases with SVS and 7,193 cases with CES of European ancestry. The associations of the 6 individual SNPs for CEC with CAD and MI, and ischemic stroke and its subtypes are presented in **Tables 3**, **4**, respectively.

Statistical Analysis

Per-allele effects of the selected SNPs on CEC and disease outcomes were extracted from the GWASs and used to estimate the causal effect of CEC on outcomes using two-sample MR analyses. Using the SNP of rs141622900 as the instrument, Wald ratio method were used to obtain effect estimate by dividing the SNP-outcome estimate by the SNP-CEC estimate. Standard error were estimated using the Delta method by dividing

TABLE 1 | Characteristics of the GWAS studies used in this study.

Phenotype	Consortium	*N*	Ethnicity	Genotype data	PMID
Exposure					
Cholesterol efflux capacity (CEC)		Up to 5,293 individuals	European	GWAS array	30369316
Outcomes					
Coronary artery disease (CAD)	CARDIoGRAMplusC4D	Up to 184,305 individuals	European	GWAS array	26343387
Myocardial infarction (MI)	CARDIoGRAMplusC4D	Up to 171,876 individuals	European	GWAS array	26343387
Ischemic stroke (IS)	MEGASTROKE	Up to 446,696 individuals	European	GWAS array	29531354
Large artery stroke (LAS)	MEGASTROKE	Up to 440,328 individuals	European	GWAS array	29531354
Small vessel stroke (SVS)	MEGASTROKE	Up to 411,497 individuals	European	GWAS array	29531354
Cardioembolic stroke (CES)	MEGASTROKE	Up to 413,304 individuals	European	GWAS array	29531354

TABLE 2 | Characteristics of the included SNP loci associated with cholesterol efflux capacity.

SNP	Locus	Chromosome (Position) (hg19)	EA/OA	EAF	CEC	Beta	SE	*p*
rs77069344	*LPL*	8 (19 821 782)	G/T	0.099	J774 basal	0.2008	0.0327	7.96×10^{-10}
rs2070895	*LIPC*	15 (58 723 939)	A/G	0.230	J774 basal	0.1424	0.0232	8.49×10^{-10}
rs247616	*CETP*	16 (56 989 590)	T/C	0.314	J774 basal	0.1466	0.0211	4.08×10^{-12}
rs964184	*APOA1/C3/A4/A5*	11 (116 648 917)	C/G	0.857	J774 ABCA1 dependent	0.2019	0.0281	6.78×10^{-13}
rs445925	*APOE/C1/C2/C4*	19 (45 415 640)	A/G	0.114	J774 ABCA1 dependent	0.2155	0.0303	1.20×10^{-12}
rs141622900	*APOE/C1/C2/C4*	19 (45 426 792)	A/G	0.058	BHK stimulated	0.2833	0.0417	1.03×10^{-11}

SNP, single nucleotide polymorphism; EA, effect allele; OA, other allele; EAF, effect allele frequency; CEC, cholesterol efflux capacity; SE, standard error. The unit of beta coefficients is SD increase of CEC per allele.

the SNP-outcome standard error by the SNP-CEC estimate (20). When using the 5 SNPs as the instruments, we used a conventional inverse-variance weighted (IVW) MR analysis with multiplicative random effects assuming all genetic variants are valid instruments. In IVW method, the SNP-outcome estimate is regressed on the SNP-CEC estimate, weighted by the inverse-variance of SNP-outcome estimate and with the y-axis intercept is fixed to zero (21). We further conducted methodologic sensitivity analyses using MR-Egger, simple median, weighted median methods using the 5 SNPs as the instruments, which are more robust to the inclusion of pleiotropic or invalid instruments. MR-Egger method can assess and control for directional pleiotropic bias and provide an pleiotropy-corrected effect estimate in which genetic variants are permitted to be invalid instrumental variables (22). The median methods can provide a consistent effect estimate using the median of the empirical distribution function of individual SNP ratio estimates in which up to 50% of the genetic variants are permitted to be invalid instruments (23). Presences of heterogeneity between causal effects of individual variants and comparisons between the causal effects of CEC on CAD vs. ischemic stroke were tested using the Cochran Q statistic and I^2 index in the IVW analysis (23). Evidence of pleiotropic effects were assessed using intercepts of the MR-Egger regression (22). Moreover, multivariable two-sample MR were performed to adjust for major causes of survival (smoking, body mass index, and blood pressure) using the 5 SNPs as the instruments (24). Multivariable MR analysis was used to assess whether the associations between genetic predisposition to CEC and ischemic stroke may be affected by selection bias (25). The above analysis were conducted in the UK Biobank GWAS and FinnGen GWAS as sensitivity analyses.

TABLE 3 | Genetic association of cholesterol efflux capacity related genetic variants with coronary artery disease and myocardial infarction in the CARDIoGRAMplusC4D consortium.

SNPs	EA/OA	Coronary artery disease			Myocardial infarction		
		Beta	SE	*p*	Beta	SE	*p*
rs77069344	G/T	−0.0514	0.0158	0.001	−0.0651	0.0176	0.000
rs2070895	A/G	0.0372	0.0108	0.001	0.0414	0.0121	0.001
rs247616	T/C	−0.0312	0.0103	0.002	−0.0280	0.0114	0.014
rs964184	C/G	−0.0500	0.0124	0.000	−0.0488	0.0139	0.000
rs445925	A/G	−0.0858	0.0187	0.000	−0.0664	0.0214	0.002
rs141622900	A/G	−0.1421	0.0278	0.000	−0.0963	0.0315	0.002

*EA, effect allele; OA, other allele; SE, standard error; SNP, single nucleotide polymorphism. The unit of beta coefficients is log-odds per allele. The odds ratio = exp(Beta), upper bound of odds ratio = exp(Beta+1.96*SE), and lower bound of odds ratio = exp(Beta-1.96*SE).*

The percentage of variance explained in CEC was estimated by 2× (effect on CEC)^2 × minor allele frequency × (1- minor allele frequency) (16). A power analysis was performed using a web-based application (https://sb452.shinyapps.io/power/). Effect estimates of CEC-outcome (CAD, MI, ischemic stroke and its subtypes) are presented as odds ratios (ORs) with their 95% confidence intervals (CIs) of outcome per 1-SD genetically higher CEC. To account for multiple testing, a Bonferroni-corrected significance level of $p < 0.0083$ (i.e., 0.05/6 for 6 outcomes)

TABLE 4 | Genetic association of cholesterol efflux capacity related genetic variants with ischemic stroke and its subtypes in the MEGASTROKE consortium.

SNPs	EA/OA	Ischemic stroke			LAS			SVS			CES		
		Beta	SE	p	Beta	SE	p	Beta	SE	P	Beta	SE	p
rs77069344	G/T	0.0153	0.0160	0.339	0.0450	0.0396	0.256	0.0062	0.0371	0.868	0.0207	0.0316	0.514
rs2070895	A/G	−0.0033	0.0121	0.783	−0.0587	0.0304	0.054	0.0367	0.0278	0.187	0.0136	0.0235	0.563
rs247616	T/C	0.0082	0.0110	0.455	−0.0168	0.0276	0.542	−0.0119	0.0258	0.645	0.0108	0.0212	0.609
rs964184	C/G	0.0181	0.0152	0.233	0.0060	0.0373	0.872	−0.0071	0.0349	0.838	0.0122	0.0297	0.681
rs445925	A/G	−0.0298	0.0184	0.106	−0.0723	0.0461	0.117	−0.0365	0.0413	0.378	−0.0362	0.0350	0.301
rs141622900	A/G	−0.0579	0.0254	0.023	−0.0963	0.0679	0.156	−0.0793	0.0598	0.185	0.0178	0.0509	0.727

*EA, effect allele; OA, other allele; LAS, large artery stroke; SVS, small vessel stroke; CES, cardioembolic stroke; SE, standard error; SNP, single nucleotide polymorphism. The unit of beta coefficients is log-odds per allele. The odds ratio = exp(Beta), upper bound of odds ratio = exp(Beta+1.96*SE), and lower bound of odds ratio = exp(Beta-1.96*SE).*

was predefined as statistically significant evidence for a causal association. All analyses were conducted with R 3.5.1 (R Development Core Team).

RESULTS

Genetically determined 1-SD increase in CEC was casually associated with a substantial decrease in risk of CAD (OR = 0.55, 95% CI: 0.44–0.69, $p < 0.001$) and MI (OR = 0.67, 95% CI: 0.52–0.87, $p = 0.002$); but, by contrast, was not causally associated with ischemic stroke (OR = 0.79, 95% CI: 0.64–0.97, $p = 0.02$) or any separate subtype of ischemic stroke (LAS: OR = 0.67, 95% CI: 0.39–1.17, $p = 0.16$; SVS: OR = 1.08, 95% CI: 0.71–1.63, $p = 0.73$; CES: OR = 0.72, 95% CI: 0.44–1.17, $p = 0.18$) at the Bonferroni-adjusted level of significance ($p < 0.0083$) using the SNP of rs141622900 as the instrument (**Figure 1**). The effect of CEC on ischemic stroke was weaker than that on CAD (p for heterogeneity = 0.03, $I^2 = 80\%$), and in particular on CES (p for heterogeneity = 0.006, $I^2 = 87\%$), whereas the effects of CEC on LAS and SVS were compatible with the magnitude of the effect observed for CAD (p for heterogeneity = 0.53 and 0.34, respectively). The effects of CEC on ischemic stroke and its subtypes were compatible with the magnitude of the effect for MI (p for heterogeneity = 0.34, 1.00, 0.80, and 0.06, respectively). These analyses had a >99, >99, 70, and 82% power to detect a 30% decrease in risk of ischemic stroke, LAS, SVS, and CES (equivalent to the upper limit of the CI for CAD), respectively; this can exclude a causal effect of CEC on ischemic stroke and CES of the same magnitude as on CAD, and indicate comparable effects of CEC on LAS, SVS, and CAD. Whereas, the power to detect a 13% decrease in risk of ischemic stroke, LAS, SVS, and CES (equivalent to the upper limit of the CI for MI) was 72, 65, 16, and 20%, respectively; this indicated comparatively little power for comparable effects of CEC on ischemic stroke, particular stroke subtypes and MI.

Similar disparate associations of CEC were observed with the risk of CAD (OR = 0.85, 95% CI: 0.79–0.90, $p < 0.001$) and MI (OR = 0.86, 95% CI: 0.80–0.92, $p < 0.001$), compared to ischemic stroke (OR = 1.02, 95% CI: 0.95–1.09, $p = 0.58$) and its subtypes (LAS: OR = 0.90, 95% CI: 0.76–1.07, $p = 0.25$; SVS: OR = 1.00, 95% CI: 0.85–1.17, $p = 0.95$; CES: OR = 1.04, 95% CI: 0.91–1.19, $p = 0.60$), using the IVW methods with the 5-SNPs instrument (**Figure 1**). The effect of CEC on ischemic stroke was weaker than that on CAD (p for heterogeneity <0.001, $I^2 = 93\%$) and MI (p for heterogeneity <0.001, $I^2 = 91\%$), and in particular on CES (p for heterogeneity = 0.008, $I^2 = 86\%$; p for heterogeneity = 0.01, $I^2 = 83\%$), whereas the effects of CEC on LAS and SVS were compatible with the magnitude of the effect observed for CAD (p for heterogeneity = 0.49 and 0.07, respectively) and MI (p for heterogeneity = 0.58 and 0.10, respectively). These analyses had a >99, 99, 42, and 53% power to detect a 10% decrease in risk of ischemic stroke, LAS, SVS, and CES (equivalent to the upper limit of the CI for CAD and MI), respectively; this can exclude a causal effect of CEC on ischemic stroke of the same magnitude as on CAD and MI, and indicate comparable effects of CEC on LAS, CAD, and MI.

Significant association for CAD and MI and insignificant association for ischemic stroke and its subtypes were also observed in sensitivity analyses using the MR-Egger, simple median and weighted median methods using the 5-SNPs instrument (**Table 5**). Evidence of heterogeneity was observed for the outcome of CAD and MI ($Q = 42.5$, $p < 0.001$; $Q = 35.4$, $p < 0.001$) but not for the outcome of ischemic stroke or its subtypes ($Q = 5.3$, $p = 0.26$; $Q = 6.6$, $p = 0.16$; $Q = 2.8$, $p = 0.59$; $Q = 2.0$, $p = 0.74$) in the IVW analysis. MR-Egger regression showed no evidence of directional pleiotropy for the association of CEC with all disease outcomes (all p value for intercept >0.05) (**Table 5**). In sensitivity analyses using FinnGen GWAS data, significant associations for ischemic heart disease and MI and insignificant association for ischemic stroke and CES were observed using the SNP of rs141622900 as the instrument. However, no significant associations for the outcomes was observed in the IVW analysis using the 5-SNPs instrument (**Supplementary Table 2**). In the multivariable MR adjusting for major causes of survival using the 5-SNPs instrument, the associations of CEC with ischemic stroke and its subtypes remained insignificant, which were similar to the estimates by the IVW method (**Supplementary Table 3**). Similar results of insignificant associations for ischemic stroke were observed in the UK Biobank data (**Supplementary Table 4**).

DISCUSSION

The present study is the first large-scale assessment and comparison of causal relevance of CEC and the risk of vascular

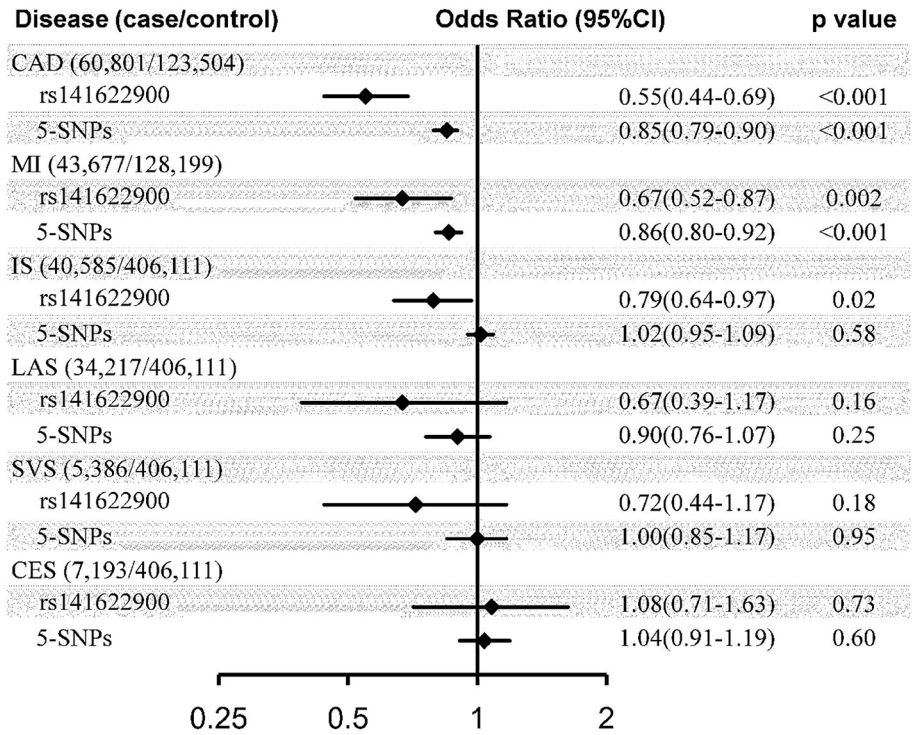


FIGURE 1 | Causal effect estimates of genetically predicted cholesterol efflux capacity on coronary artery disease and stroke. Estimates represented odds ratio (95% CI) per SD genetically higher cholesterol efflux capacity derived from Wald ratio method using rs141622900 as the instrument and inverse-variance weighted method using 5-SNPs (rs77069344, rs2070895, rs247616, rs964184, and rs445925) as the instrument. CI, confidence interval; SNP, single nucleotide polymorphism; CAD, coronary artery disease; MI, myocardial infarction; IS, ischemic stroke; LAS, large artery stroke; SVS, small vessel stroke; CES, cardioembolic stroke.

disease using Mendelian randomization approach. The results showed that genetic predicted higher CEC may be associated with decreased risk of CAD. However, the casual association of CEC with ischemic stroke and specific subtypes would need to be validated in further Mendelian randomization studies.

Our findings of inverse relationship between CEC and CAD and MI are consistent with several meta-analysis summarizing previous observational studies (10, 26, 27). Although results from the majority of studies were in line with the hypothesis that higher CEC is associated with lower risk of CAD (9, 11, 28–31), a study by Li showed that increased HDL-mediated CEC was paradoxically associated with increased risk for incident myocardial infarction or stroke, which based on the study population undergoing coronary angiography (32). Moreover, the German Diabetes Dialysis Study (4D Study) failed to observe an significant association of CEC with the composite outcome (cardiac death, nonfatal MI, and stroke) in patients with end-stage renal disease (33). The CEC was quantified using human THP-1-derived macrophage foam cells loaded with cholesterol, which was different from cAMP (cyclic adenosine monophosphate)-stimulated murine J774 macrophages employed by other studies in the general population (8, 11). The reasons for the apparent discrepancies among previous studies in the relationship between CEC and CAD are unclear but could be ascribed to difference in sample size, study population, study design, and methods for CEC measurements across studies. Considering the heterogeneity between observational studies and potential confounders that warrant caution, MR studies using genetic variants as instrumental variables could provide more robust evidence for the causal relationship of CEC and the health outcome of interest. The present study showed genetic predicted higher CEC was associated with lower CAD risk, which supports the direct causal association between CEC and CAD.

Our study does not support a causal role of CEC in ischemic stroke. Few studies have investigated the association between CEC and ischemic stroke and its subtypes expect two cohort studies with inconsistent results (9, 29). Results from the MESA (Multi-Ethnic Study of Atherosclerosis) cohort showed no relationship of cholesterol mass efflux capacity with stroke or with non-hemorrhagic stroke. However, a small subgroup ($n = 37$) of the Dallas Heart study reported an inverse association between CEC and stroke. Using genetic variants related to CEC as the instrument, the association between CEC and ischemic stroke was examined directly in our study. However, we found no evidence of significant causal relationships between CEC and ischemic stroke and its subtypes. In the present Mendelian randomization study, the estimates of CEC with ischemic stroke might be biased by sample selection on surviving exposure of interest and on surviving competing risk of the outcome (24). However, the results of multivariable MR analyses conducted by adjusting for potential causes of survival were consistent

TABLE 5 | MR statistical sensitivity analyses using 5 SNPs as the instrumental variables*.

Outcome	MR-Egger				Simple median		Weighted median	
	OR (95% CI)	*P*	Intercept (95% CI)	*p* value for intercept	OR (95% CI)	*P*	OR (95% CI)	*p*
CAD (60,801/123,504)	0.35 (0.14–0.89)	0.03	0.156 (−0.007, 0.319)	0.06	0.78 (0.71–0.86)	<0.001	0.79 (0.71–0.87)	<0.001
MI (43,677/128,199)	0.34 (0.13–0.89)	0.03	0.161 (−0.004, 0.326)	0.056	0.79 (0.70–0.88)	<0.001	0.79 (0.71–0.88)	<0.001
IS (40,585/406,111)	0.97 (0.57–1.65)	0.92	0.008 (−0.083, 0.100)	0.86	1.06 (0.96–1.17)	0.26	1.06 (0.97–1.16)	0.21
LAS (34,217/406,111)	1.60 (0.43–6.00)	0.49	−0.101 (−0.330, 0.128)	0.39	0.89 (0.69–1.16)	0.39	0.92 (0.72–1.17)	0.49
SVS (5,386/406,111)	0.66 (0.26–1.64)	0.37	0.074 (−0.086, 0.233)	0.37	0.97 (0.78–1.19)	0.74	0.96 (0.78–1.19)	0.72
CES (7,193/406,111)	0.79 (0.36–1.71)	0.55	0.048 (−0.086, 0.182)	0.48	1.08 (0.90–1.28)	0.41	1.08 (0.91–1.28)	0.41

*MR, mendelian randomization; OR, odds ratio; CI, confidence interval; SNP, single nucleotide polymorphism; CAD, coronary artery disease; MI, myocardial infarction; IS, ischemic stroke; LAS, large artery stroke; SVS, small vessel stroke; CES, cardioembolic stroke. *Five SNPs in the instrument included rs77069344, rs2070895, rs247616, rs964184, and rs445925.*

with the main results. As with any selection bias correction by multivariable adjustment, it may not be feasible to recover the valid estimate. We repeated the analyses in the UK Biobank data and FinnGen data, respectively. Both results were similar to the main results in the present study. Larger scale Mendelian randomization studies are still needed to clarify the genetic effects of CEC on ischemic stroke and assess any heterogeneity between ischemic stroke subtypes, which may shed light on the relationship of CEC on ischemic stroke further. Furthermore, in these future studies, the effects of genetically determined CEC on ischemic stroke could be compared between patients with vascular cognitive impairment vs. patients with non-cognitive impairment.

The HDL-mediated CEC is the ability to remove excess cholesterol from lipid-laden macrophages representing the first crucial step within the process of reverse cholesterol transport (7). Reverse cholesterol transport plays an important role in atheroprotective mechanism by facilitating the removal of cholesterol in the arterial wall and the subsequent decrease in the proinflammatory response (34, 35). Our study found that the effect of CEC was weaker on ischemic stroke than CAD, which were consistently observed in such comparisons in the effects of other blood lipid on vascular disease in recent Mendelian studies. A Mendelian study suggested that the effects of LDL cholesterol on ischemic stroke was weaker than that on coronary heart disease (16). Moreover, another Mendelian study showed PCSK9 genetic variants had smaller associations with risk of ischemic stroke than with risk of coronary heart disease (15). The potential explanation for the difference between the effects of CEC on CAD and ischemic stroke is the biological differences in these disease process. Ischemic stroke involves phenotypic heterogeneity, with different biological pathways for LAS, SVS, and CES, compared to the more homogenous CAD phenotype (36). Moreover, a review reported that hematological disorders were the most frequent etiology of cerebral infarcts of unusual cause (37). Except for the usual cerebrovascular risk factors such as hypertension, diabetes mellitus, and dyslipidemia, other newly factors could be considered of the causal relevance for ischemic stroke. Furthermore, differences in the distribution of risk factors as well as patient characteristics between CARDIoGRAMPlusC4D and METASTROKE consortium may partly explain the different effects for CAD and IS observed in the study. Additionally, insufficient statistical power due to small sample size, especially for stroke subtypes, may be considered as another reason. Anacetrapib, an CETP (cholesteryl ester transfer protein) inhibitor that was developed for increasing HDL cholesterol levels and promoting CEC to a greater degree, was shown a significant reduction in CAD in the REVEAL trial (38). Although it met its primary endpoint, the small improvement against the main goal and the safety of CETP inhibitor had become a point of contention consistently. Finally, Anacetrapib was not filed for approval with the US Food and Drug Administration. Our study provided supporting evidence for the causal relationship between CEC and risk of CAD, indicating potential intervention targets to the increase of CEC for improving cardiovascular outcomes.

The present Mendelian randomization analyses relies on three underlying assumptions. First, we identified 6 CEC-related SNPs ($P < 6.25 \times 10^{-9}$) served as instruments in the MR analysis that satisfied the first assumption. Second, we did not find that the 5-SNPs in the study were associated with other key lipids including low-density lipoprotein, triglycerides, total cholesterol, and apolipoprotein B based on GWAS datasets. And the results of multivariable MR analyses and sensitivity analyses using the UK Biobank data and FinnGen data were consistent with the main results. Thus, these results increase confidence in the validity of the second assumption that there is no confounding (measured or unmeasured) of the genetic variants with the outcome. Third, all the genetic variants were not directly associated with the outcomes (all $P > 5 \times 10^{-8}$), which suggested that the third assumption was not violated.

Our study has several limitations. First, the study was conducted based on datasets of predominantly European ancestry and generalization of the results to other populations of non-European ancestry was limited. However, the uniformity of the included subjects may minimize the risk of bias by population admixture. Second, the sample sizes of ischemic stroke subtypes were still relatively small, specifically for SVS and CES. Insignificant association between CEC and ischemic stroke subtypes could be attributed to insufficient statistical power. However, most estimates were consistent using different MR approaches, which suggests that the observed associations are not by chance. Third, although the GWAS that we used to identify all CEC-related SNPs represents the first and largest

effort to identify genome-wide significant loci associated with CEC, sample size remains modest ($N = 5{,}293$ participants), and therefore there is a limitation of power to find weak effect variants. In the main analysis, only one SNP (rs141622900) was used as the instrument. The SNP is strongly associated with many key lipids relevant to cardiovascular disease, such as low-density lipoprotein and apolipoprotein B. The pleiotropic effects of the CEC-related SNPs on other key lipids was unable to be assessed by two-sample multivariable MR in the present study because of the lack of data. However, we have conducted MR-Egger regression using 5 CEC-related SNPs as the instrumental variables to assess evidence of pleiotropic effects in the study. Though the results showed no evidence of directional pleiotropy, further studies are needed to validate the associations of CEC with disease outcomes. Forth, we performed multivariable MR and sensitivity analyses to correct selection bias. However, recovering the valid estimates of CEC for ischemic stroke has not been fully addressed. Finally, Mendelian randomization has been considered as an alternative approach for causal inferences with a lot of advantages compared to randomized controlled trials. However, it cannot replace randomized controlled trials in establishing a claim of causality (39). Future clinical trials are still needed with sufficient statistical power to validate the causal relationship of CEC.

The study examined causal relationships between CEC and risk of vascular disease using MR analysis, and suggests that genetic predicted higher CEC may be associated with decreased risk of CAD. However, the casual association of CEC with ischemic stroke and specific subtypes would need to be validated in further Mendelian randomization studies.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding authors.

ETHICS STATEMENT

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

AJ and MW performed the study, analyzed the data, wrote the paper, and reviewed drafts of the paper. WC and XX wrote the paper and reviewed drafts of the paper. HY analyzed the data, prepared figures and tables, and reviewed drafts of the paper. YP conceived and designed the study, performed the study, analyzed the data, wrote the paper, and reviewed drafts of the paper. All authors contributed to the article and approved the submitted version.

FUNDING

This work was supported by grants from the National Natural Science Foundation of China (81971091 and 81901177), Beijing Hospitals Authority Youth Programme (QML20190501 and QML20200501), Young Elite Scientist Sponsorship Program from China Association for Science and Technology (2019QNRC001), Beijing Tiantan Hospital, Capital Medical University (2018-YQN-1 and 2020MP01) and Outstanding Young Talents Project of Capital Medical University (A2105).

ACKNOWLEDGMENTS

Data on cholesterol efflux capacity were derived from the GWAS performed by Low-Kam et al. Data on coronary artery disease/myocardial infarction have been contributed by CARDIoGRAMplusC4D investigators and obtained from www.CARDIOGRAMPLUSC4D.ORG. Data on stroke have been contributed by MEGASTROKE investigators and obtained from http://megastroke.org. The MEGASTROKE project received funding from sources specified at http://megastroke.org/acknowledgements.html.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcvm.2022.891148/full#supplementary-material

REFERENCES

1. Huxley RR, Barzi F, Lam TH, Czernichow S, Fang X, Welborn T, et al. Isolated low levels of high-density lipoprotein cholesterol are associated with an increased risk of coronary heart disease: an individual participant data meta-analysis of 23 studies in the Asia-Pacific region. *Circulation.* (2011) 124:2056–64. doi: 10.1161/CIRCULATIONAHA.111.028373
2. Keene D, Price C, Shun-Shin MJ, Francis DP. Effect on cardiovascular risk of high density lipoprotein targeted drug treatments niacin, fibrates, and CETP inhibitors: meta-analysis of randomised controlled trials including 117,411 patients. *BMJ.* (2014) 349:g4379. doi: 10.1136/bmj.g4379
3. Boekholdt SM, Arsenault BJ, Hovingh GK, Mora S, Pedersen TR, Larosa JC, et al. Levels and changes of HDL cholesterol and apolipoprotein A-I in relation to risk of cardiovascular events among statin-treated patients: a meta-analysis. *Circulation.* (2013) 128:1504–12. doi: 10.1161/CIRCULATIONAHA.113.002670
4. Voight BF, Peloso GM, Orho-Melander M, Frikke-Schmidt R, Barbalic M, Jensen MK, et al. Plasma HDL cholesterol and risk of myocardial infarction: a mendelian randomisation study. *Lancet.* (2012) 380:572–80. doi: 10.1016/S0140-6736(12)60312-2
5. Holmes MV, Asselbergs FW, Palmer TM, Drenos F, Lanktree MB, Nelson CP, et al. Mendelian randomization of blood lipids for coronary heart disease. *Eur Heart J.* (2015) 36:539–50. doi: 10.1093/eurheartj/eht571
6. Anastasius M, Kockx M, Jessup W, Sullivan D, Rye KA, Kritharides L. Cholesterol efflux capacity: an introduction for clinicians. *Am Heart J.* (2016) 180:54–63. doi: 10.1016/j.ahj.2016.07.005

7. Rosenson RS, Brewer HB Jr, Davidson WS, Fayad ZA, Fuster V, Goldstein J, et al. Cholesterol efflux and atheroprotection: advancing the concept of reverse cholesterol transport. *Circulation.* (2012) 125:1905–19. doi: 10.1161/CIRCULATIONAHA.111.066589
8. Khera AV, Cuchel M, de la Llera-Moya M, Rodrigues A, Burke MF, Jafri K, et al. Cholesterol efflux capacity, high-density lipoprotein function, and atherosclerosis. *N Engl J Med.* (2011) 364:127–35. doi: 10.1056/NEJMoa1001689
9. Rohatgi A, Khera A, Berry JD, Givens EG, Ayers CR, Wedin KE, et al. HDL cholesterol efflux capacity and incident cardiovascular events. *N Engl J Med.* (2014) 371:2383–93. doi: 10.1056/NEJMoa1409065
10. Ye H, Xu G, Ren L, Peng J. Cholesterol efflux capacity in coronary artery disease: a meta-analysis. *Coron Artery Dis.* (2020) 31:642–9. doi: 10.1097/MCA.0000000000000886
11. Saleheen D, Scott R, Javad S, Zhao W, Rodrigues A, Picataggi A, et al. Association of HDL cholesterol efflux capacity with incident coronary heart disease events: a prospective case-control study. *Lancet Diabetes Endocrinol.* (2015) 3:507–13. doi: 10.1016/S2213-8587(15)00126-6
12. Lawlor DA, Harbord RM, Sterne JA, Timpson N, Davey Smith G. Mendelian randomization: using genes as instruments for making causal inferences in epidemiology. *Stat Med.* (2008) 27:1133–63. doi: 10.1002/sim.3034
13. Bareinboim E, Pearl J. Causal inference and the data-fusion problem. *Proc Natl Acad Sci U S A.* (2016) 113:7345–52. doi: 10.1073/pnas.1510507113
14. Boehme AK, Esenwa C, Elkind MS. Stroke risk factors, genetics, and prevention. *Circ Res.* (2017) 120:472–95. doi: 10.1161/CIRCRESAHA.116.308398
15. Hopewell JC, Malik R, Valdés-Márquez E, Worrall BB, Collins R; METASTROKE Collaboration of the ISGC. Differential effects of PCSK9 variants on risk of coronary disease and ischaemic stroke. *Eur Heart J.* (2018) 39:354–9. doi: 10.1093/eurheartj/ehx373
16. Valdes-Marquez E, Parish S, Clarke R, Stari T, Worrall BB; METASTROKE Consortium of the ISGC, et al. Relative effects of LDL-C on ischemic stroke and coronary disease: a Mendelian randomization study. *Neurology.* (2019) 92:e1176–87. doi: 10.1212/WNL.0000000000007091
17. Low-Kam C, Rhainds D, Lo KS, Barhdadi A, Boulé M, Alem S, et al. Variants at the APOE /C1/C2/C4 locus modulate cholesterol efflux capacity independently of high-density lipoprotein cholesterol. *J Am Heart Assoc.* (2018) 7:e009545. doi: 10.1161/JAHA.118.009545
18. Nikpay M, Goel A, Won HH, Hall LM, Willenborg C, Kanoni S, et al. A comprehensive 1,000 Genomes-based genome-wide association meta-analysis of coronary artery disease. *Nat Genet.* (2015) 47:1121–30. doi: 10.1038/ng.3396
19. Malik R, Chauhan G, Traylor M, Sargurupremraj M, Okada Y, Mishra A, et al. Multiancestry genome-wide association study of 520,000 subjects identifies 32 loci associated with stroke and stroke subtypes. *Nat Genet.* (2018) 50:524–37. doi: 10.1038/s41588-018-0058-3
20. Thomas DC, Lawlor DA, Thompson JR. Re: estimation of bias in nongenetic observational studies using "Mendelian triangulation" by Bautista et al. *Ann Epidemiol.* (2007) 17:511–3. doi: 10.1016/j.annepidem.2006.12.005
21. Burgess S, Butterworth A, Thompson SG. Mendelian randomization analysis with multiple genetic variants using summarized data. *Genet Epidemiol.* (2013) 37:658–65. doi: 10.1002/gepi.21758
22. Bowden J, Davey Smith G, Burgess S. Mendelian randomization with invalid instruments: effect estimation and bias detection through Egger regression. *Int J Epidemiol.* (2015) 44:512–25. doi: 10.1093/ije/dyv080
23. Bowden J, Davey Smith G, Haycock PC, Burgess S. Consistent estimation in mendelian randomization with some invalid instruments using a weighted median estimator. *Genet Epidemiol.* (2016) 40:304–14. doi: 10.1002/gepi.21965
24. Schooling CM, Lopez PM, Yang Z, Zhao JV, Au Yeung SL, Huang JV. Use of multivariable mendelian randomization to address biases due to competing risk before recruitment. *Front Genet.* (2020) 11:610852. doi: 10.3389/fgene.2020.610852
25. Smit RAJ, Trompet S, Dekkers OM, Jukema JW, le Cessie S. Survival bias in mendelian randomization studies: a threat to causal inference. *Epidemiology.* (2019) 30:813–6. doi: 10.1097/EDE.0000000000001072
26. Qiu C, Zhao X, Zhou Q, Zhang Z. High-density lipoprotein cholesterol efflux capacity is inversely associated with cardiovascular risk: a systematic review and meta-analysis. *Lipids Health Dis.* (2017) 16:212. doi: 10.1186/s12944-017-0604-5
27. Soria-Florido MT, Schroder H, Grau M, Fito M, Lassale C. High density lipoprotein functionality and cardiovascular events and mortality: a systematic review and meta-analysis. *Atherosclerosis.* (2020) 302:36–42. doi: 10.1016/j.atherosclerosis.2020.04.015
28. Soria-Florido MT, Castaner O, Lassale C, Estruch R, Salas-Salvado J, Martinez-Gonzalez MA, et al. Dysfunctional high-density lipoproteins are associated with a greater incidence of acute coronary syndrome in a population at high cardiovascular risk: a nested case-control study. *Circulation.* (2020) 141:444–53. doi: 10.1161/CIRCULATIONAHA.119.041658
29. Shea S, Stein JH, Jorgensen NW, McClelland RL, Tascau L, Shrager S, et al. Cholesterol mass efflux capacity, incident cardiovascular disease, and progression of carotid plaque. *Arterioscler Thromb Vasc Biol.* (2019) 39:89–96. doi: 10.1161/ATVBAHA.118.311366
30. Ebtehaj S, Gruppen EG, Bakker SJL, Dullaart RPF, Tietge UJF. HDL (High-Density Lipoprotein) cholesterol efflux capacity is associated with incident cardiovascular disease in the general population. *Arterioscler Thromb Vasc Biol.* (2019) 39:1874–83. doi: 10.1161/ATVBAHA.119.312645
31. Zhang J, Xu J, Wang J, Wu C, Xu Y, Wang Y, et al. Prognostic usefulness of serum cholesterol efflux capacity in patients with coronary artery disease. *Am J Cardiol.* (2016) 117:508–14. doi: 10.1016/j.amjcard.2015.11.033
32. Li XM, Tang WH, Mosior MK, Huang Y, Wu Y, Matter W, et al. Paradoxical association of enhanced cholesterol efflux with increased incident cardiovascular risks. *Arterioscler Thromb Vasc Biol.* (2013) 33:1696–705. doi: 10.1161/ATVBAHA.113.301373
33. Kopecky C, Ebtehaj S, Genser B, Drechsler C, Krane V, Antlanger M, et al. HDL cholesterol efflux does not predict cardiovascular risk in hemodialysis patients. *J Am Soc Nephrol.* (2017) 28:769–75. doi: 10.1681/ASN.2016030262
34. Vallejo-Vaz AJ, Ray KK. Cholesterol efflux capacity as a novel biomarker for incident cardiovascular events: has high-density lipoprotein been resuscitated? *Circ Res.* (2015) 116:1646–8. doi: 10.1161/CIRCRESAHA.115.305938
35. Feig JE, Hewing B, Smith JD, Hazen SL, Fisher EA. High-density lipoprotein and atherosclerosis regression: evidence from preclinical and clinical studies. *Circ Res.* (2014) 114:205–13. doi: 10.1161/CIRCRESAHA.114.300760
36. Mendelson SJ, Prabhakaran S. Diagnosis and management of transient ischemic attack and acute ischemic stroke: a review. *JAMA.* (2021) 325:1088–98. doi: 10.1001/jama.2020.26867
37. Arboix A, Jiménez C, Massons J, Parra O, Besses C. Hematological disorders: a commonly unrecognized cause of acute stroke. *Expert Rev Hematol.* (2016) 9:891–901. doi: 10.1080/17474086.2016.1208555
38. Bowman L, Hopewell JC, Chen F, Wallendszus K, Stevens W, Collins R, et al. Effects of anacetrapib in patients with atherosclerotic vascular disease. *N Engl J Med.* (2017) 377:1217–27. doi: 10.1056/NEJMoa1706444
39. Plotnikov D, Guggenheim JA. Mendelian randomisation and the goal of inferring causation from observational studies in the vision sciences. *Ophthalmic Physiol Opt.* (2019) 39:11–25. doi: 10.1111/opo.12596

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers | Frontiers in Cardiovascular Medicine

ORIGINAL RESEARCH
published: 07 July 2022
doi: 10.3389/fcvm.2022.903481

U-Shaped Relationship of Non-HDL Cholesterol With All-Cause and Cardiovascular Mortality in Men Without Statin Therapy

Rui-Xiang Zeng[1,2], *Jun-Peng Xu*[1,2], *Yong-Jie Kong*[1,2], *Jia-Wei Tan*[1,2], *Li-Heng Guo*[1,2] *and Min-Zhou Zhang*[1,2*]

[1] *The Second Clinical College of Guangzhou University of Chinese Medicine, Guangzhou, China,* [2] *Department of Critical Care Medicine, Guangdong Provincial Hospital of Chinese Medicine, Guangzhou, China*

OPEN ACCESS

Edited by:
Alexander Akhmedov, University of Zurich, Switzerland

Reviewed by:
Yang Peng, The University of Queensland, Australia
Dongfeng Zhang, Qingdao University, China

***Correspondence:**
Min-Zhou Zhang
minzhouzhang@gzucm.edu.cn

Specialty section:
This article was submitted to Lipids in Cardiovascular Disease, a section of the journal Frontiers in Cardiovascular Medicine

Received: *24 March 2022*
Accepted: *06 June 2022*
Published: *07 July 2022*

Citation:
Zeng R-X, Xu J-P, Kong Y-J, Tan J-W, Guo L-H and Zhang M-Z (2022) U-Shaped Relationship of Non-HDL Cholesterol With All-Cause and Cardiovascular Mortality in Men Without Statin Therapy. Front. Cardiovasc. Med. 9:903481. doi: 10.3389/fcvm.2022.903481

Background: Non-HDL-C is well established causal risk factor for the progression of atherosclerotic cardiovascular disease. However, there remains a controversial pattern of how non-HDL-C relates to all-cause and cardiovascular mortality, and the concentration of non-HDL-C where the risk of mortality is lowest is not defined.

Methods: A population-based cohort study using data from the National Health and Nutrition Examination Survey (NHANES) from 1999 to 2014. Male participants without statin therapy were divided into the six groups according to non-HDL-C levels (<100, 100–129, 130–159, 160–189, 190–219, ≥220 mg/dl). Multivariable Cox proportional hazards models were conducted with a hazard ratio (HR) and corresponding 95% confidence interval (CI). To further explore the relationship between non-HDL-C and mortality, Kaplan–Meier survival curves, restricted cubic spline curves, and subgroup analysis were performed.

Results: Among 12,574 individuals (average age 44.29 ± 16.37 years), 1,174(9.34%) deaths during a median follow-up 98.38 months. Both low and high non-HDL-C levels were significantly associated with increased risk of all-cause and cardiovascular mortality, indicating a U-shaped association. Threshold values were detected at 144 mg/dl for all-cause mortality and 142 mg/dl for cardiovascular mortality. Below the threshold, per 30 mg/dl increase in non-HDL-C reduced a 28 and 40% increased risk of all-cause ($p < 0.0001$) and cardiovascular mortality ($p = 0.0037$), respectively. Inversely, above the threshold, per 30 mg/dl increase in non-HDL-C accelerated risk of both all-cause mortality (HR 1.11, 95% CI 1.03–1.20, $p = 0.0057$) and cardiovascular mortality (HR 1.30, 95% CI 1.09–1.54, $p = 0.0028$).

Conclusions: Non-HDL-C was U-shaped related to all-cause and cardiovascular mortality among men without statin therapy.

Keywords: non-HDL cholesterol, all-cause mortality, cardiovascular mortality, U-shaped relationship, men

INTRODUCTION

Non-high-density lipoprotein cholesterol (non-HDL-C), which represents the total cholesterol content of apolipoprotein B-containing lipoproteins, includes very low-density lipoproteins (VLDL) and their metabolic remnants, intermediate-density lipoproteins (IDL), lipoprotein(a), and low-density lipoproteins (LDL) (1, 2). Non-HDL-C is well established causal risk factor for the development of atherosclerotic cardiovascular disease (1, 3, 4). Two decades ago, non-HDL-C was highlighted as an important secondary lipid therapeutic goal in the United States National Cholesterol Education Program's Adult Treatment Panel (5). Furthermore, the National Lipid Association and International Atherosclerosis Society recently recommended that non-HDL-C should be an equal target to LDL-cholesterol (LDL-C) in patients with atherosclerotic cardiovascular disease (6). Preponderantly, non-HDL-C trajectories remain fairly flat, and non-HDL-C between age 25 and 40 years is sufficient to confidently categorise individuals as high or low non-HDL-C for the next 25 to 30 years (3). Moreover, non-HDL-C can be accurately calculated in a non-fasting specimen, without incurring additional expense (7).

A systematic review and meta-analysis by our team previously demonstrated that the increased levels of non-HDL-C were significantly associated with an increased risk of mortality in patients with coronary heart disease (CHD) (8), which was similar to previous research findings in population without cardiovascular diseases (9) or patients with diabetes (10). Interestingly, a U-shaped relationship was also recently identified in different populations (11, 12). Studies detected the U-shaped association of non-HDL-C with all-cause and cardiovascular mortality among patients with hypertension or chronic kidney disease (CKD) stages 3–5 (11, 12), non-HDL-C was U-shaped associated with mortality among male hypertension in subgroup analysis, but not in female (12). Differently, one study focused on the men population, from the Israeli National Death Registry demonstrated a positive association that non-HDL-C as a useful predictor of cardiovascular disease mortality in 22 years follow-up (13). Hence, for more clearly understand how non-HDL-C relates to all-cause and cardiovascular mortality in men, the aim of the present study used data from the large population representative surveys to determine the relationship of non-HDL-C with all-cause and cardiovascular mortality, and the concentration of non-HDL-C associated with the lowest risk of mortality in men without statin therapy.

MATERIALS AND METHODS

Study Population

We performed a population-based cohort study using data from the National Health and Nutrition Examination Survey (NHANES), and data were combined across 8 continuous NHANES cycles: 1999–2000, 2001–2002, 2003–2004, 2005–2006, 2007–2008, 2009–2010, 2011–2012, and 2013–2014. NHANES is a series of national surveys to evaluate the health status of the United States population with a complex, stratified, multistage, probability sampling method. The Centres for Disease Control and Prevention ratified the study protocols, and all the participants provided written informed consent. Detailed about the NHANES has been published elsewhere (14, 15).

The total number of participants in primary survey was 82,091. After excluding participants for age <18 (n = 34,735), female (n = 24,534) or without follow-up data (n = 1,085), and those in baseline with cancer or missing data (n = 3,534), acute myocardial infarction (AMI) or missing data (n = 952), heart failure (HF) or miss data (n = 331) and excluding because covariates were unavailable (n = 2,455), finally excluding for baseline with statin therapy (n = 1,891). Hence, 12,574 individuals were included in our final analysis (**Figure 1**).

Covariates

Fasting samples obtained from peripheral venous blood were stored at −20°C and shipped weekly for laboratory analyses. Non-HDL-C levels were calculated from total cholesterol (TC) minus HDL cholesterol (HDL-C). The measurement of TC used with an enzymatic assay method, and HDL-C was used with a heparin-manganese precipitation method or a direct immunoassay technique. Further detailed information about the collection of blood samples and lipid concentration measurement is available in another study (16). In addition, creatinine, haemoglobin, and glycated haemoglobin A1c (HbA1c) measurements were based on standardised procedures. Demographic variables such as age, gender, body weight, height, race/ethnicity (Mexican American, other Hispanic, non-Hispanic White, non-Hispanic Black, other race), education (Lower than high school, high school, more than high school), were acquired according to the household interview. Information on smoking status (current smoker, former smoker, and never smoked), and history of disease had been assessed at baseline by standard examinations, and questionnaires were administered by trained health technicians, interviewers, and physicians. The mean blood pressure was calculated as the average of three valid measurements. Nutritional status (e.g., energy intake, protein intake, carbohydrate intake, total fat intake) was acquired according to the dietary interview. A "multiple pass" 24-h dietary interview format was used to collect detailed information about all foods and beverages, which were used to estimate the total intake of energy, nutrients, and non-nutrient food components. More information is available at www.cdc.gov/nchs/nhanes.

Outcomes

The primary determination of mortality for eligible participants is based upon matching survey records to the records from the National Death Index (NDI), and other sources including the Social Security Administration, the Centres for Medicare and Medicaid Services, data collection, and for the follow-up surveys of the National Centre for Health Statistics, ascertainment of death certificates are also incorporated. Participants were eligible for mortality follow-up based on matching identifying information during their NHANES interviews, such as the last 4 digits of social security number, full name, date of birth, state of birth, state of residence, marital status, race, and sex (17). All-cause mortality and cardiovascular mortality were the endpoints of the present study. The mortality status of individuals was

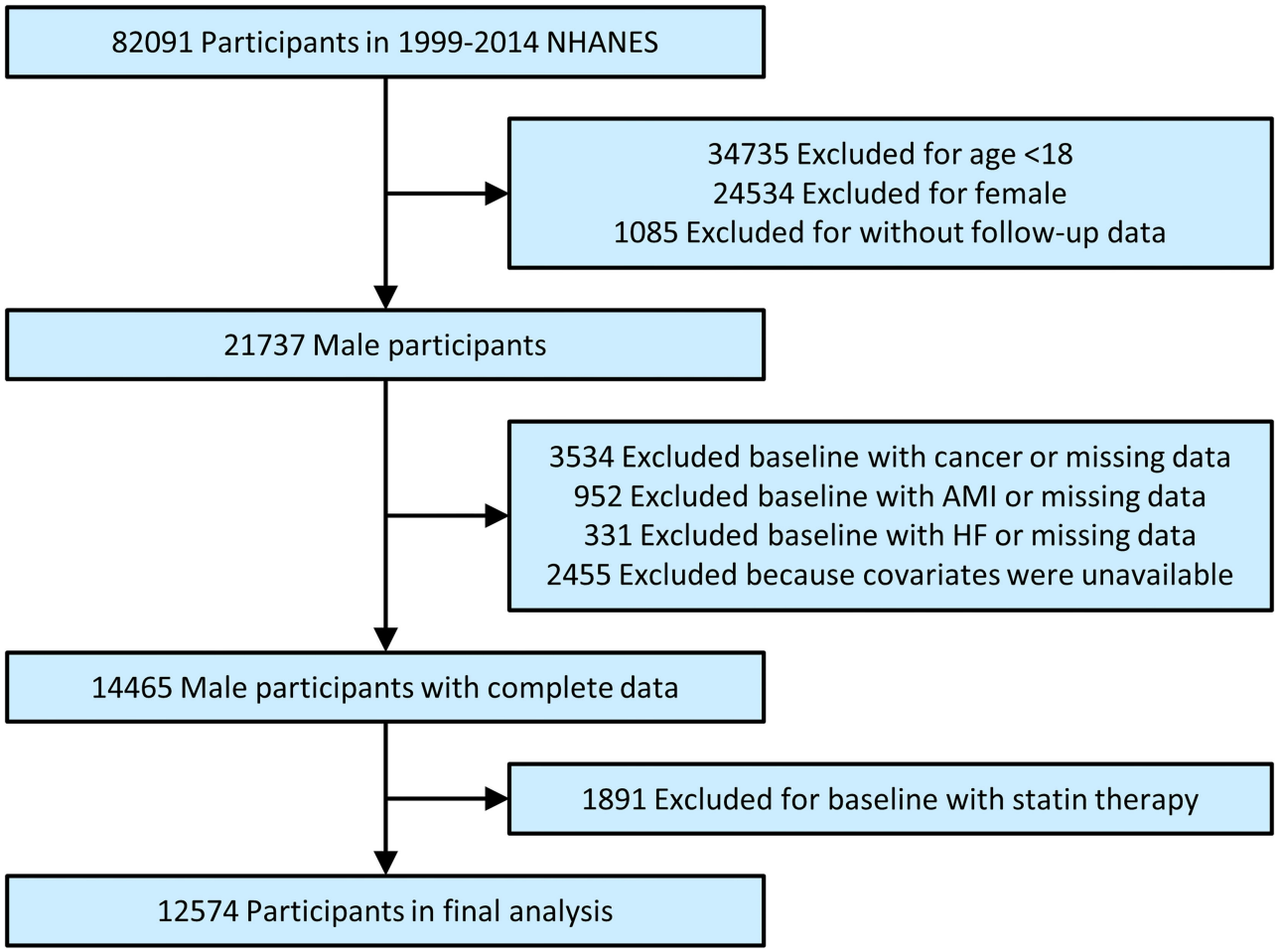


FIGURE 1 | Study flowchart. NHANES, National health and nutrition examination survey; AMI, acute myocardial infarction; HF, heart failure.

obtained from data from the NDI through December 31, 2015. This study classified causes of mortality referred to the codes of the international statistical classification of diseases, 10th revision (ICD-10). Cardiovascular mortality was defined by the ICD-10 codes for as I00-I09, I11, I13, and I20-I51. When treating cardiovascular mortality as an outcome, the deaths due to other causes were censored.

Statistical Analysis

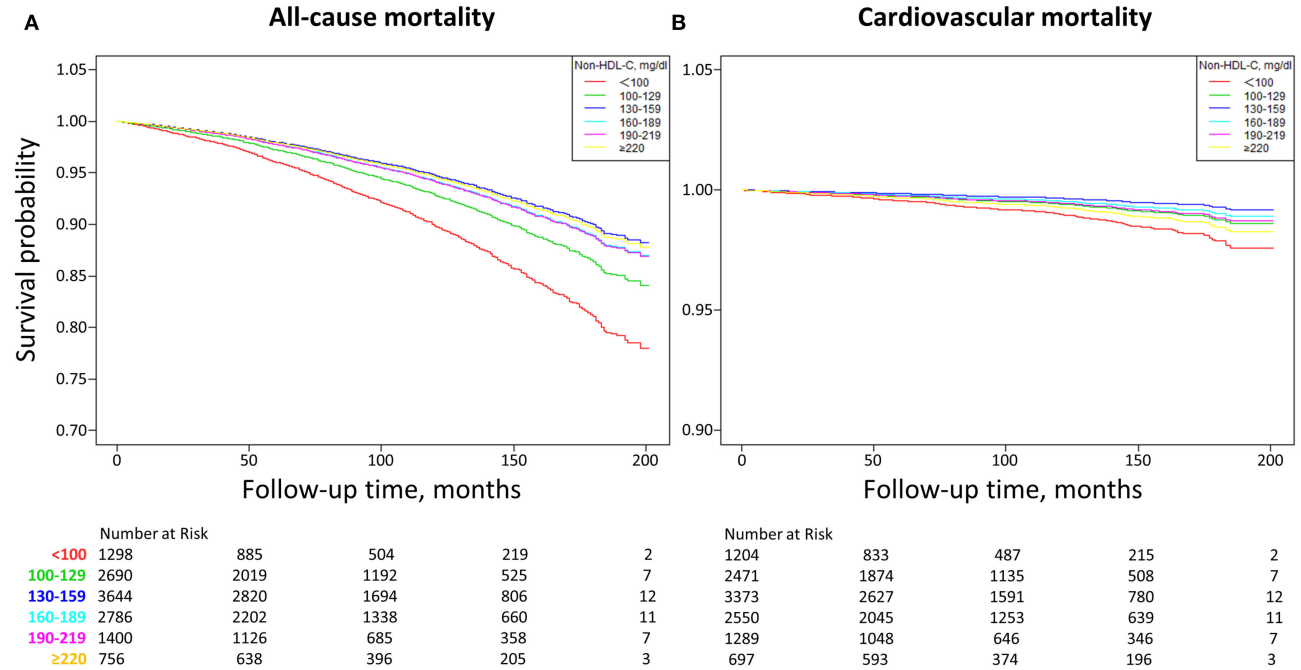
The data were presented as mean values with standard deviation (SD), the median with interquartile ranges, or frequencies with percentages, as appropriate. Comparisons of the differences between groups were made with one-way ANOVA, chi-square tests, or Kruskal–Wallis H-tests by the classification of non-HDL-C levels (<100, 100–129, 130–159, 160–189, 190–219, ≥220 mg/dl). Survival analysis according to non-HDL-C stratification was performed using standardised Kaplan–Meier curves. The proportional hazard assumption was examined and met by plotting the log minus log survival curves and survival times. The multivariable Cox proportional hazards models were used for exploring the association of non-HDL-C with all-cause and cardiovascular mortality. In model 1, there was no adjustment. In model 2, we adjusted for age and race. In model 3, we adjusted for age, race, education, body mass index (BMI), systolic blood pressure, diastolic blood pressure, smoking, diabetes, hypertension, CHD, stroke, creatinine, haemoglobin, HbA1c, triglycerides, energy intake, protein intake, carbohydrate intake, and total fat intake. Restricted cubic spline models were used for nonlinear relationships with knots at 5, 35, 65, and 95 percentiles of non-HDL-C. If the relationships were non-linear, the difference of relationships at the threshold was detected by two piecewise linear regression models. The point with the highest likelihood among all the possible values was chosen to define the threshold value. The differences in the results when applying one-line or two piecewise linear regression models were compared by a logarithmic likelihood ratio test. Furthermore, the subgroup analysis includes age (<65 or ≥65 years), race (White, Black, or other race), education (lower than high school, high school, more than high school), BMI (<25 or ≥25 kg/m2), smoking (yes or no), diabetes (yes or no), hypertension (yes or

TABLE 1 | Baseline characteristics according to non-HDL-C levels.

		Non-HDL-C, mg/dl						*P*-value
	Total	**<100**	**100–129**	**130–159**	**160–189**	**190–219**	**≥220**	
N	12,574	1,298	2,690	3,644	2,786	1,400	756	
Age, years	44.29 ± 16.37	36.87 ± 16.98	41.77 ± 17.42	45.24 ± 16.47	46.79 ± 15.43	46.93 ± 14.10	47.26 ± 12.92	<0.001
Race								<0.001
White	5,562 (44.23%)	534 (41.14%)	1,138 (42.30%)	1,671 (45.86%)	1,230 (44.15%)	626 (44.71%)	363 (48.02%)	
Black	2,539 (20.19%)	437 (33.67%)	663 (24.65%)	685 (18.80%)	461 (16.55%)	185 (13.21%)	108 (14.29%)	
Other	4,473 (35.57%)	327 (25.19%)	889 (33.05%)	1,288 (35.35%)	1,095 (39.30%)	589 (42.07%)	285 (37.70%)	
Education								<0.001
Lower than high school	3,565 (28.35%)	322 (24.81%)	738 (27.43%)	977 (26.81%)	863 (30.98%)	417 (29.79%)	248 (32.80%)	
High school	3,027 (24.07%)	330 (25.42%)	645 (23.98%)	863 (23.68%)	644 (23.12%)	361 (25.79%)	184 (24.34%)	
More than high school	5,982 (47.57%)	646 (49.77%)	1,307 (48.59%)	1,804 (49.51%)	1,279 (45.91%)	622 (44.43%)	324 (42.86%)	
Body mass index, kg/m^2	28.09 ± 5.69	25.30 ± 5.21	26.92 ± 5.94	28.47 ± 5.84	29.04 ± 5.37	29.29 ± 4.96	29.44 ± 4.91	<0.001
SBP, mmHg	124.19 ± 16.20	120.82 ± 15.59	122.57 ± 15.83	124.33 ± 16.35	125.22 ± 16.35	126.02 ± 15.49	127.92 ± 16.89	<0.001
DBP, mmHg	72.83 ± 11.86	68.65 ± 11.77	70.76 ± 11.60	72.77 ± 11.89	74.34 ± 11.25	75.41 ± 11.65	77.21 ± 11.86	<0.001
Smoking	6,673 (53.07%)	660 (50.85%)	1,378 (51.23%)	1,888 (51.81%)	1,456 (52.26%)	810 (57.86%)	481 (63.62%)	<0.001
Diabetes	765 (6.08%)	68 (5.24%)	171 (6.36%)	208 (5.71%)	171 (6.14%)	85 (6.07%)	62 (8.20%)	0.116
Hypertension	2,921 (23.23%)	217 (16.72%)	568 (21.12%)	867 (23.79%)	695 (24.95%)	359 (25.64%)	215 (28.44%)	<0.001
Coronary heart disease	99 (0.79%)	8 (0.62%)	26 (0.97%)	22 (0.60%)	27 (0.97%)	9 (0.64%)	7 (0.93%)	0.428
Stroke	178 (1.42%)	9 (0.69%)	33 (1.23%)	57 (1.56%)	41 (1.47%)	23 (1.64%)	15 (1.98%)	0.133
Creatinine, mg/dl	86.46 ± 34.07	87.25 ± 33.31	86.99 ± 31.75	85.80 ± 26.28	86.57 ± 30.04	86.60 ± 55.42	85.77 ± 38.44	0.689
Haemoglobin, g/l	15.23 ± 1.18	14.82 ± 1.30	15.07 ± 1.24	15.24 ± 1.16	15.35 ± 1.11	15.46 ± 1.05	15.54 ± 1.14	<0.001
HbA1c, %	5.58 ± 0.99	5.33 ± 0.72	5.46 ± 0.84	5.55 ± 0.89	5.63 ± 0.94	5.75 ± 1.18	6.08 ± 1.67	<0.001
Triglyceride, mg/dl	124.04 (82.04–198.02)	66.01 (49.00–92.06)	92.06 (66.98–131.04)	122.00 (86.03–176.05)	156.02 (108.00–231.07)	196.07 (134.05–293.09)	265.58 (178.09–456.62)	<0.001
TC, mg/dl	199.05 ± 42.07	139.13 ± 20.04	167.38 ± 16.61	192.14 ± 15.25	218.15 ± 14.29	245.97 ± 13.75	290.67 ± 42.03	<0.001
HDL-C, mg/dl	47.84 ± 14.00	55.01 ± 17.04	51.57 ± 15.05	47.56 ± 13.19	44.94 ± 11.96	43.74 ± 11.35	41.96 ± 10.90	<0.001
LDL-C, mg/dl	121.54 ± 35.37	69.56 ± 12.96	95.66 ± 12.49	118.73 ± 14.48	142.30 ± 14.99	165.13 ± 16.73	202.66 ± 38.87	<0.001
Non-HDL-C, mg/dl	151.21 ± 43.54	84.13 ± 12.14	115.81 ± 8.54	144.57 ± 8.65	173.22 ± 8.48	202.23 ± 8.50	248.70 ± 41.75	<0.001
Energy intake, kcal	2,562.21 ± 1,148.91	2,698.28 ± 1,208.16	2,599.52 ± 1,201.38	2,554.34 ± 1,129.65	2,497.82 ± 1,080.05	2,537.78 ± 1,172.34	2,516.30 ± 1,127.45	<0.001
Protein intake, gm	98.00 ± 49.24	100.23 ± 53.75	99.25 ± 51.72	97.09 ± 47.13	96.43 ± 47.57	99.31 ± 48.32	97.44 ± 49.75	0.088
Carbohydrate intake, gm	307.40 ± 147.76	326.10 ± 155.89	310.65 ± 152.37	308.76 ± 148.86	299.71 ± 137.13	301.92 ± 151.54	295.73 ± 138.69	<0.001
Total fat intake, gm	94.34 ± 53.27	97.27 ± 55.11	95.99 ± 55.48	93.91 ± 52.47	92.72 ± 50.79	93.45 ± 53.11	93.17 ± 54.83	0.073
All-cause mortality	1,174 (9.34%)	115 (8.86%)	258 (9.59%)	312 (8.56%)	277 (9.94%)	134 (9.57%)	78 (10.32%)	0.381
Cardiovascular mortality	184 (1.46%)	21 (1.62%)	39 (1.45%)	41 (1.13%)	41 (1.47%)	23 (1.64%)	19 (2.51%)	0.101

Non-HDL-C, non-high-density lipoprotein cholesterol; N, number; SBP, systolic blood pressure; DBP, diastolic blood pressure; HbA1c, glycated haemoglobin A1c; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol.

Values are expressed as the mean ± SD, the median with interquartile range or n (%).



Number at Risk						Number at Risk				
<100	1298	885	504	219	2	1204	833	487	215	2
100-129	2690	2019	1192	525	7	2471	1874	1135	508	7
130-159	3644	2820	1694	806	12	3373	2627	1591	780	12
160-189	2786	2202	1338	660	11	2550	2045	1253	639	11
190-219	1400	1126	685	358	7	1289	1048	646	346	7
≥220	756	638	396	205	3	697	593	374	196	3

FIGURE 2 | Kaplan-Meier survival curves of non-HDL-C with all-cause **(A)** and cardiovascular **(B)** mortality. Non-HDL-C, non-high-density lipoprotein cholesterol. **(A)** Other groups vs. non-HDL-C <100 mg/dl (all $P < 0.005$). **(B)** non-HDL-C = 130–159 or 160–189 mg/dl vs. non-HDL-C <100 mg/dl (all $P < 0.005$), non-HDL-C = 100–129 mg/dl vs. non-HDL-C <100 mg/dl ($P = 0.0443$), non-HDL-C = 190–219 mg/dl vs. non-HDL-C <100 mg/dl ($P = 0.0546$), non-HDL-C ≥220 mg/dl vs. non-HDL-C <100 mg/dl ($P = 0.3687$).

no). All statistical analyses were performed using R version 3.3.2 (R Foundation for Statistical Computing, Vienna, Austria), and $p < 0.05$ was considered statistically significant.

RESULTS

Baseline Characteristics

Finally, 12,574 individuals (average age 44.29 ± 16.37 years) were included in this analysis. **Table 1** demonstrates baseline characteristics according to non-HDL-C levels. There were significant subgroup differences in age, race, education, BMI, systolic blood pressure, diastolic blood pressure, smoking, hypertension, haemoglobin, HbA1C, triglycerides, TC, HDL-C, LDL-C, energy intake, and carbohydrate intake (all p <0.001), except for diabetes, CHD, stroke, creatinine, protein intake, total fat intake, all-cause, and cardiovascular mortality. We found 1,174 (9.34%) all-cause deaths and 184 (1.46%) cardiovascular deaths during a median follow-up of 98.38 ± 53.78 months.

Relationships of Non-HDL-C With All-Cause and Cardiovascular Mortality

As demonstrated in **Figure 2**, Kaplan–Meier survival curves were diverged according to non-HDL-C stratification. The highest risk for all-cause mortality was observed when non-HDL-C <100 mg/dl, compared to other groups (all $p < 0.005$). Besides, more risk for cardiovascular mortality was only observed when non-HDL-C <100 mg/dl, compared to when non-HDL-C = 130-159 or 160-189 mg/dl (all $P < 0.005$). The optimal non-HDL-C concentration range was between 130 and 159 mg/dl for a lower risk of all-cause and cardiovascular death.

Table 2 summarised the multivariable Cox regression results. When non-HDL-C was treated as a continuous variable, per 30 mg/dl increment in non-HDL-C corresponded to the hazard ratio (HR) (95% confidence interval, CI) as 0.94 (95% CI 0.90–0.99, $p = 0.0193$) for all-cause mortality and 1.03 (95% CI 0.91–1.16, $p = 0.6826$) for cardiovascular mortality in model 3. When non-HDL-C was treated as a categorical variable, non-HDL-C = 130-159 mg/dl as a reference, the fully adjusted HRs for all-cause mortality were 1.98 (95% CI 1.59–2.48, $p < 0.0001$), 1.38 (95% CI 1.17–1.62, $p = 0.0002$), 1.11 (95% CI 0.95–1.31, $p = 0.2007$), 1.12 (95% CI 0.91–1.38, $p = 0.2807$) and 1.04 (95% CI 0.79–1.36, $p = 0.8018$) for non-HDL-C levels <100, 100–129, 160–189, 190–219, and ≥220 mg/dl, respectively. Meanwhile, for cardiovascular mortality, the fully adjusted HRs were 2.99 (95% CI 1.73–5.16, $p < 0.0001$), 1.72 (95% CI 1.10–2.69, $p = 0.0169$), 1.36 (95% CI 0.88–2.11, $p = 0.1636$), 1.61 (95% CI 0.94–2.73, $p = 0.0811$), and 2.16 (95% CI 1.17–3.98, $p = 0.0134$) for non-HDL-C levels <100, 100–129, 160–189, 190–219, and ≥ 220 mg/dl, respectively.

Non-Linear Relationships of Non-HDL-C With All-Cause and Cardiovascular Mortality

As shown in **Figure 3**, the multivariable adjusted restrictive cubic curves confirmed that the relationships of non-HDL-C

TABLE 2 | Multivariable cox regression analysis of non-HDL-C with all-cause and cardiovascular mortality.

	Model 1 HR (95% CI), *P*	Model 2 HR (95% CI), *P*	Model 3 HR (95% CI), *P*
All-cause mortality			
Non-HDL-C (per 30 mg/dl increment)	1.01 (0.97, 1.05) 0.6087	0.95 (0.91, 0.99) 0.0207	0.94 (0.90, 0.99) 0.0193
Non-HDL-C group, mg/dl			
<100	1.20 (0.97, 1.49) 0.0926	2.18 (1.76, 2.71) <0.0001	1.98 (1.59, 2.48) <0.0001
100–129	1.18 (1.00, 1.39) 0.0465	1.45 (1.22, 1.71) <0.0001	1.38 (1.17, 1.63) 0.0002
130–159	Reference	Reference	Reference
160–189	1.13 (0.96, 1.32) 0.1518	1.12 (0.95, 1.31) 0.1758	1.11 (0.95, 1.31) 0.2007
190–219	1.06 (0.87, 1.30) 0.5705	1.16 (0.95, 1.42) 0.1493	1.12 (0.91, 1.38) 0.2807
≥220	1.09 (0.85, 1.40) 0.4902	1.24 (0.97, 1.60) 0.0857	1.04 (0.79, 1.36) 0.8018
P for trend	0.3574	<0.0001	<0.0001
Cardiovascular mortality			
Non-HDL-C (per 30 mg/dl increment)	1.08 (0.98, 1.19) 0.1076	1.05 (0.94, 1.18) 0.3504	1.03 (0.91, 1.16) 0.6826
Non-HDL-C group, mg/dl			
<100	1.64 (0.97, 2.77) 0.0667	3.15 (1.85, 5.36) <0.0001	2.99 (1.73, 5.16) <0.0001
100–129	1.36 (0.88, 2.11) 0.1699	1.66 (1.07, 2.58) 0.0241	1.72 (1.10, 2.69) 0.0169
130–159	Reference	Reference	Reference
160–189	1.27 (0.83, 1.96) 0.2726	1.32 (0.86, 2.04) 0.2039	1.36 (0.88, 2.11) 0.1636
190–219	1.38 (0.83, 2.31) 0.2128	1.63 (0.97, 2.72) 0.0633	1.61 (0.94, 2.73) 0.0811
≥220	2.03 (1.18, 3.50) 0.0107	2.52 (1.45, 4.35) 0.0010	2.16 (1.17, 3.98) 0.0134
P for trend	0.5151	0.8498	0.5035

Non-HDL-C, non-high-density lipoprotein cholesterol; HR, hazard ratio; CI, confidence interval.
Model 1: no adjustment; Model 2: adjusted for age and race; Model 3: adjusted for age, race, education, body mass index, systolic blood pressure, diastolic blood pressure, smoking, diabetes, hypertension, coronary heart disease, stroke, creatinine, haemoglobin, glycated haemoglobin A1c, triglycerides, energy intake, protein intake, carbohydrate intake, and total fat intake.

TABLE 3 | The results of two piecewise linear regression model of non-HDL-C with all-cause and cardiovascular mortality.

	All-cause mortality HR (95% CI) *P*	Cardiovascular mortality HR (95% CI) *P*
Cut-off value	144	142
<Cut-off value (as continuous variables, per 30 mg/dl increment)	0.72 (0.62, 0.82) <0.0001	0.60 (0.42, 0.85) 0.0037
≥Cut-off value (as continuous variables, per 30 mg/dl increment)	1.11 (1.03, 1.20) 0.0057	1.30 (1.09, 1.54) 0.0028

Non-HDL-C, non-high-density lipoprotein cholesterol; HR, hazard ratio; CI, confidence interval.
The two piecewise linear regression models were adjusted for adjusted for age, race, education, body mass index, systolic blood pressure, diastolic blood pressure, smoking, diabetes, hypertension, coronary heart disease, stroke, creatinine, haemoglobin, glycated haemoglobin A1c, triglycerides, energy intake, protein intake, carbohydrate intake, and total fat intake.

with all-cause and cardiovascular mortality were U-shaped (All p for likelihood ratio test < 0.0001). The threshold values of non-HDL-C related to the lowest risk in multivariable adjusted analyses were 144 mg/dl for all-cause mortality and 142 mg/dl for cardiovascular mortality. As shown in **Table 3**, below the threshold, per 30 mg/dl increase in non-HDL-C reduced a 28% increased risk of all-cause mortality ($p < 0.0001$) and a 40% increased risk of cardiovascular mortality ($p = 0.0037$). Inversely, above the threshold, per 30 mg/dl increase in non-HDL-C accelerated risk of both all-cause mortality (HR 1.11, 95% CI 1.03–1.20, $p = 0.0057$) and cardiovascular mortality (HR 1.30, 95% CI 1.09–1.54, $p = 0.0028$).

Subgroups Analysis of the Risk of All-Cause and Cardiovascular Mortality

The stratified analyses are demonstrated in **Figure 4** (Detail data as shown in **Supplementary Table S1**). The non-linear relationships for all-cause mortality with statistical significance were found among participants who were aged <65 years old, and race (White). Besides, the non-linear relationships for cardiovascular mortality with statistical significance were found among participants who were race (White), BMI $\geq$ 25 kg/m^{2,} and without diabetes. When non-HDL-C $\geq$ 142 mg/dl, per 30 mg/dl increase in non-HDL-C increased risk of cardiovascular mortality were 1.41-fold for aged <65 years old ($p = 0.0040$), 1.38-fold for race (White) ($p = 0.0182$) and 1.68-fold for race

(Black) ($p = 0.0017$), 1.57-fold for high school education ($p = 0.0002$), 1.67-fold for smoking ($p = 0.0002$), 1.31-fold for participants without diabetes ($p = 0.0054$) and 1.41-fold for participants with hypertension ($p = 0.0038$).

DISCUSSION

The novel finding of the study is that both low and high non-HDL-C levels were significantly associated with increased risk of all-cause and cardiovascular mortality among men without statin therapy in U-shaped relationships. Furthermore, we confirmed that the non-HDL-C level was related to the lowest risk of all-cause and cardiovascular mortality at threshold values of 144 and 142 mg/dl, respectively. These new results are probable to have implications for the interpretation of levels of non-HDL-C in clinical practise.

Global age-standardised mean non-HDL-C remained almost unchanged from 1980 to 2018, and high non-HDL-C was responsible for an estimated 3.9 million worldwide deaths from ischemic heart disease and ischemic stroke in 2017, accounting for a third of deaths from these causes (18). Undoubtedly, it is particularly urgent to clearly understand the relationship of non-HDL-C stratification with death, and locate the best threshold values of non-HDL-C. One of our major findings is that once non-HDL-C levels are greater than threshold values, is closely contributed to higher mortality. This finding is similar to previous several studies in different populations (8, 9, 11, 13, 19–21). The potential explanation for this finding is that extremely high non-HDL-C levels play a role in accelerated atherosclerosis, leading to an increased risk of death (22). In United States population study, non-HDL-C may be best suited for the prediction of future coronary artery calcium (CAC) progression, especially since non-HDL-C levels $\geq$ 190 mg/dl are consistently associated with significant CAC progression in the overall population (β 16.4, 95%CI −5.63 to 27.2, $p = 0.003$) (23). One genetic study finding is that levels of non-HDL-C are associated with the extent of coronary atherosclerosis. Besides, the mutations of some genes like LDLR, apolipoprotein B, and proprotein convertase subtilisin/kexin type 9 (PCSK9), can result in hypercholesterolemia, and guidelines suggested that non-HDL-C $\geq$ 220 mg/dl could possibly imply hereditary genetic hypercholesterolemia (11, 24). Patients with hypercholesterolemia have increased non-HDL-C and are more prone to suffer from atherosclerotic cardiovascular and cardiovascular death (11, 23).

Moreover, the present study contributed evidence that lower non-HDL-C is also closely related to higher mortality in men without statin therapy, and indicated a U-shaped association. Although the disparity in the study population, in accordance with our results, several previous studies have observed the U-shaped association between non-HDL-C and mortality (11, 12). One study by Cheng and colleagues analysing data from NHANES demonstrated that relatively higher or lower non-HDL-C concentrations were linked to increased mortality, and the lowest risk was found at threshold values of 158 and 190 mg/dl for all-cause and cardiovascular mortality, respectively. The difference in threshold estimates might be attributed to different study populations, of which all patients in the study by Cheng et al. were hypertension, and had relatively higher non-HDL-C levels (25). Likewise, the U-shaped relationships between non-HDL-C and the risk of all-cause and cardiovascular mortality have been shown in patients with CKD and the optimal non-HDL-C concentration range was between 116.2 and 143.9 mg/dl (11). Similarly, a study of a general population cohort also found a U-shaped association between levels of LDL-C and the risk of all-cause mortality (26). In patients with CHD, a paradoxical association existed between baseline non-HDL-C and long-term all-cause mortality, but disappeared after taking into account the effects of malnutrition, indicating that the worse long-term prognosis in the low non-HDL-C group (<2.2 mmol/L) was mainly mediated by the underlying effect of malnutrition (27). Apart from that, an inverse association between cholesterol and mortality has been demonstrated in the elderly (28, 29). Another population-based register study including 118,160 subjects without statin therapy found that high lipoprotein levels were associated with lower mortality indicating that high lipoprotein levels do not seem to be definitely harmful in the general population (29). Similarly, participants with low serum TC seem to have a lower survival rate than participants with an elevated cholesterol level, irrespective of concomitant diseases or health status (28). Unexpectedly, the finding of a U-shaped association in our study is inconsistent with a positive association in another study (13). The differences between the two studies in the population (United States or Israel), sample size (13,562 or 4,832), and follow-up time (98.38 ± 53.78 months or 22.1 ± 3.2 years) may result in different conclusions.

However, the underlying mechanism of U-shaped association is not clear. First, one possible reason is that the participants with the lowest cholesterol levels had a poorer health status (28), or debilitation and illness have been hypothesised to cause a decrease in levels of cholesterol (26, 30, 31). Second, higher HDL-C equals to low non-HDL-C levels according to the calculation formula, extremely high HDL-C increases mortality in the general population by analysing the data from NHANES (12). The genetic variation of particular genes and variation of the size or function of HDL particles may be the underlying mechanism (12). Finally, the U-shaped association between lipoprotein levels and mortality may be similar to the obesity paradox, which is largely explained by methodological issues, including reverse causation. No matter how, more studies are needed to clarify the exact mechanism of the U-shaped association.

The advantage of the present study lies in its relatively large sample size and long-term follow-up. Regarding clinical importance, our novel findings are conducive to understanding the risk stratification of non-HDL-C and remind us that when initiating lipid-lowering therapy in clinical practise, attention should be paid to assessing the absolute risk of atherosclerotic cardiovascular disease (26, 32, 33), rather than starting treatment based solely on a moderate increase in levels of a specific lipid marker. Anyway, there are still some limitations to this study. First, during long-term follow-up, only a single measurement of serum non-HDLC concentration at

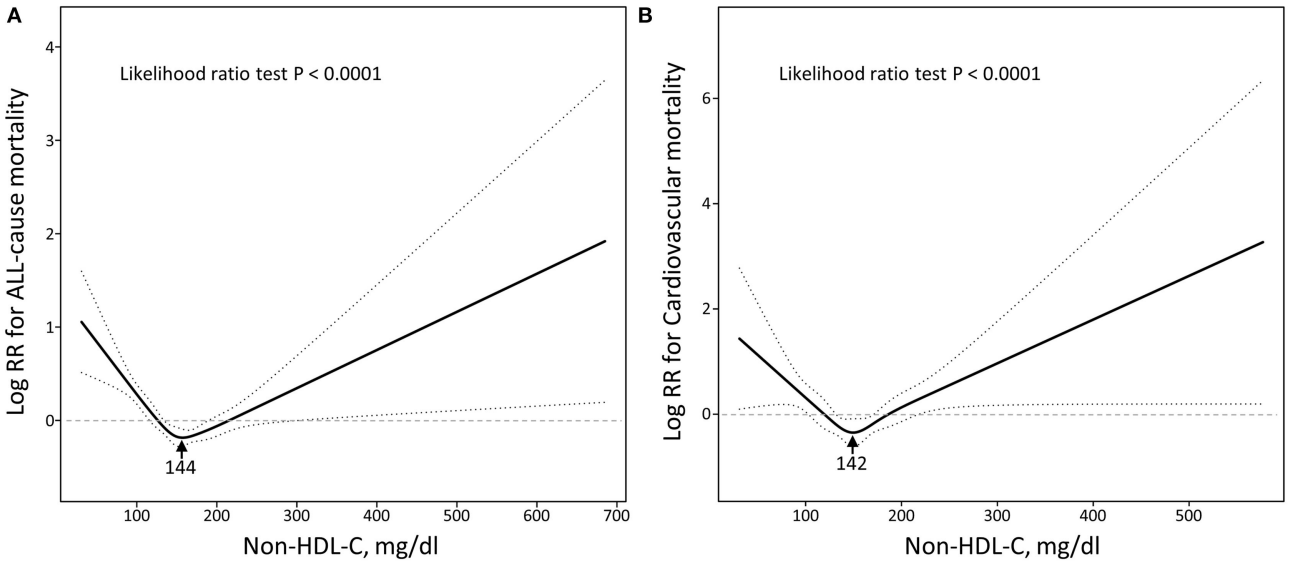


FIGURE 3 | Restricted cubic spine models of non-HDL-C with all-cause **(A)** and cardiovascular **(B)** mortality. Non-HDL-C, non-high-density lipoprotein cholesterol. Restricted cubic spine models were adjusted for adjusted for age, race, education, body mass index, systolic blood pressure, diastolic blood pressure, smoking, diabetes, hypertension, coronary heart disease, stroke, creatinine, haemoglobin, glycated haemoglobin A1c, triglycerides, energy intake, protein intake, carbohydrate intake, and total fat intake.

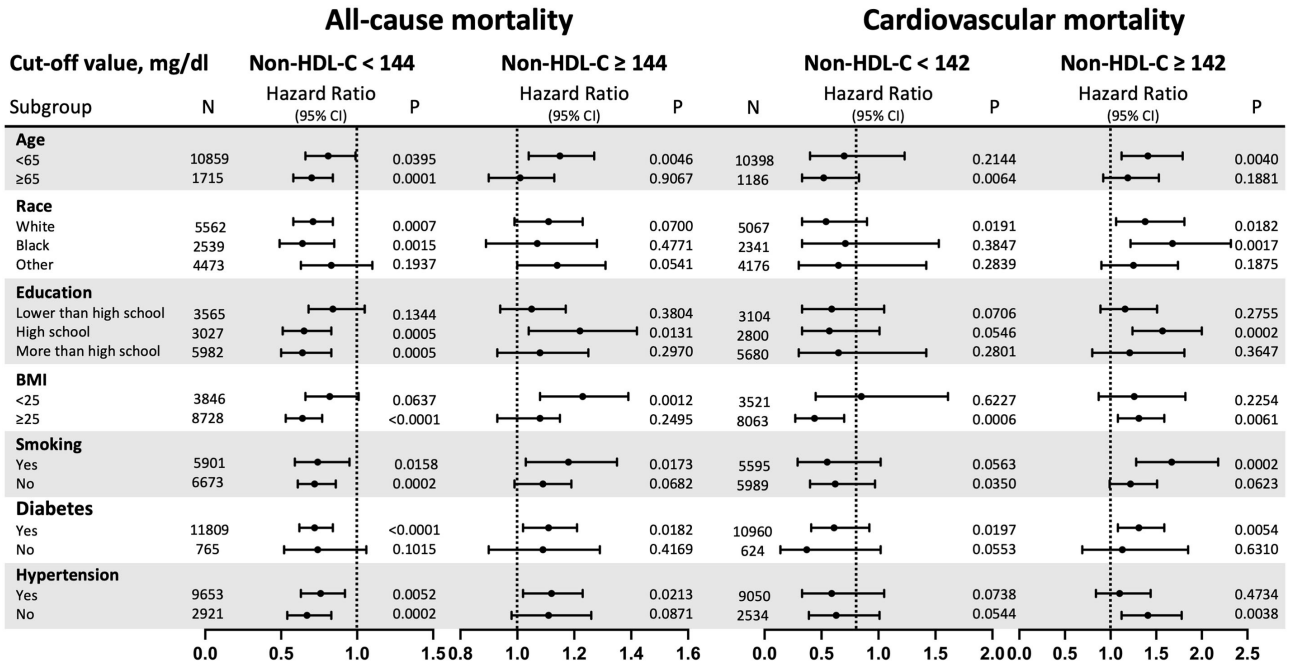


FIGURE 4 | Subgroup analysis. Non-HDL-C, non-high-density lipoprotein cholesterol; HR, hazard ratio; CI, confidence interval; BMI, body mass index. Results are expressed as multivariable-adjusted HR in continuous analyses (Non-HDL-C per 30 mg/dl increment). When analysing a subgroup variable, age, race, education, BMI, systolic blood pressure, diastolic blood pressure, smoking, diabetes, hypertension, coronary heart disease, stroke, creatinine, haemoglobin, glycated haemoglobin A1c, triglycerides, energy intake, protein intake, carbohydrate intake, and total fat intake were all adjusted except the variable itself.

baseline is available, leading to potential bias and failure to evaluate the affection of non-HDL-C trajectories on mortality. Second, although we adjusted many relevant confounding variables that were considered to influence mortality, residual confounders and hidden comorbidities might have been not eliminated. Finally, our study was performed in a nationally

representative sample of men in the United States, so our results may not be easily extrapolated to the population in other regions.

CONCLUSION

From a population-based cohort study base on the national representative database, our study demonstrated that non-HDL-C was U-shaped and related to all-cause and cardiovascular mortality among men without statin therapy. The more clear risk stratification of non-HDL-C and comprehensive strategic management to deal with dyslipidemia deserves further investigation for confirmation.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: https://www.cdc.gov/nchs/nhanes/index.htm.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the US Centres for Disease Control and Prevention ratified the study protocols. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

R-XZ, J-PX, Y-JK, L-HG, and M-ZZ: conceived and designed the study. R-XZ, J-PX, and J-WT: collected and analysed the data. R-XZ: drafted the paper. M-ZZ: revised the manuscript. All authors have reviewed the final manuscript. All authors contributed to the article and approved the submitted version.

FUNDING

This work was partially supported by the National Natural Scientific Foundation (No. 82004135 to R-XZ), the Research Fund for Zhaoyang Talents of Guangdong Provincial Hospital of Chinese Medicine (No. ZY2022KY03 to R-XZ), and the Specific Research Fund for TCM Science and Technology of Guangzhou Provincial Hospital of Chinese Medicine (YN10101915 to M-ZZ).

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcvm.2022.903481/full#supplementary-material

REFERENCES

1. Brunner FJ, Waldeyer C, Ojeda F, Salomaa V, Kee F, Sans S, et al. Application of non-HDL cholesterol for population-based cardiovascular risk stratification: results from the Multinational Cardiovascular Risk Consortium. *Lancet.* (2019) 394:2173–83. doi: 10.1016/S0140-6736(19)32519-X
2. Emerging Risk Factors C, Di Angelantonio E, Sarwar N, Perry P, Kaptoge S, Ray KK, et al. Major lipids, apolipoproteins, and risk of vascular disease. *JAMA.* (2009) 302:1993–2000. doi: 10.1001/jama.2009.1619
3. Pencina KM, Thanassoulis G, Wilkins JT, Vasan RS, Navar AM, Peterson ED, et al. Trajectories of Non-HDL Cholesterol Across Midlife: implications for cardiovascular prevention. *J Am Coll Cardiol.* (2019) 74:70–9. doi: 10.1016/j.jacc.2019.04.047
4. Yusuf S, Joseph P, Rangarajan S, Islam S, Mente A, Hystad P, et al. Modifiable risk factors, cardiovascular disease, and mortality in 155 722 individuals from 21 high-income, middle-income, and low-income countries (PURE): a prospective cohort study. *Lancet.* (2020) 395:795–808. doi: 10.1016/S0140-6736(19)32008-2
5. National Cholesterol Education Program Expert Panel on Detection E, Treatment of High Blood Cholesterol in A. Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) final report. *Circulation.* (2002) 106:3143–421. doi: 10.1161/circ.106.25.3143
6. Expert Dyslipidemia P, Grundy SM. An International Atherosclerosis Society Position Paper: global recommendations for the management of dyslipidemia. *J Clin Lipidol.* (2013) 7:561–5. doi: 10.1016/j.jacl.2013.10.001
7. Zeng RX, Li XL, Zhang MZ, Guo YL, Zhu CG, Guo LH, et al. Non-HDL cholesterol is a better target for predicting periprocedural myocardial injury following percutaneous coronary intervention in type 2 diabetes. *Atherosclerosis.* (2014) 237:536–43. doi: 10.1016/j.atherosclerosis.2014.10.030
8. Liao P, Zeng R, Zhao X, Guo L, Zhang M. Prognostic value of non-high-density lipoprotein cholesterol for mortality in patients with coronary heart disease: a systematic review and meta-analysis. *Int J Cardiol.* (2017) 227:950–55. doi: 10.1016/j.ijcard.2016.10.106
9. Cui Y, Blumenthal RS, Flaws JA, Whiteman MK, Langenberg P, Bachorik PS, et al. Non-high-density lipoprotein cholesterol level as a predictor of cardiovascular disease mortality. *Arch Intern Med.* (2001) 161:1413–9. doi: 10.1001/archinte.161.11.1413
10. Li C, Ford ES, Tsai J, Zhao G, Balluz LS. Gidding SS. Serum non-high-density lipoprotein cholesterol concentration and risk of death from cardiovascular diseases among US adults with diagnosed diabetes: the Third National Health and Nutrition Examination Survey linked mortality study Cardiovasc Diabetol. (2011) 10:46. doi: 10.1186/1475-2840-10-46
11. Chiu H, Wu PY, Huang JC, Tu HP, Lin MY, Chen SC, et al. There is a U shaped association between non high density lipoprotein cholesterol with overall and cardiovascular mortality in chronic kidney disease stage 3-5. *Sci Rep.* (2020) 10:12749. doi: 10.1038/s41598-020-69794-2
12. Cheng Q, Liu XC, Chen CL, Huang YQ, Feng YQ, Chen JY. The U-Shaped Association of Non-High-Density Lipoprotein Cholesterol Levels With All-Cause and Cardiovascular Mortality Among Patients With Hypertension. *Front Cardiovasc Med.* (2021) 8:707701. doi: 10.3389/fcvm.2021.707701
13. Harari G, Green MS, Magid A, Zelber-Sagi S. Usefulness of Non-High-Density Lipoprotein Cholesterol as a Predictor of Cardiovascular Disease Mortality in Men in 22-Year Follow-Up. *Am J Cardiol.* (2017) 119:1193–98. doi: 10.1016/j.amjcard.2017.01.008
14. Ford ES, Giles WH, Dietz WH. Prevalence of the metabolic syndrome among US adults: findings from the third National Health and Nutrition Examination Survey. *JAMA.* (2002) 287:356–9. doi: 10.1001/jama.287.3.356
15. Palmer MK, Toth PP. Trends in lipids, obesity, metabolic syndrome, and diabetes mellitus in the United States: An NHANES Analysis (2003-2004 to 2013-2014). *Obesity (Silver Spring).* (2019) 27:309–14. doi: 10.1002/oby.22370
16. Yan YQ, Chen J, Huang YQ. A Non-linear association of high-density lipoprotein cholesterol with all-cause and cause-specific mortality in diabetic patients. *Diabetes Metab Syndr Obes.* (2021) 14:2851–62. doi: 10.2147/DMSO.S313006

17. National Center for Health Statistics. Office of Analysis and Epidemiology. *The Linkage of National Center for Health Statistics Survey Data to the National Death Index – 2015 Linked Mortality File (LMF): Methodology Overview and Analytic Considerations.* (2019). Hyattsville, Maryland. Available online at: https://www.cdc.gov/nchs/data-linkage/mortality-methods.htm (accessed May 11, 2022).
18. Collaboration NCDRF. Repositioning of the global epicentre of non-optimal cholesterol. *Nature.* (2020) 582:73–7. doi: 10.1038/s41586-020-2338-1
19. Echida Y, Ogawa T, Otsuka K, Ando Y, Nitta K. Serum non-high-density lipoprotein cholesterol (non-HDL-C) levels and cardiovascular mortality in chronic hemodialysis patients. *Clin Exp Nephrol.* (2012) 16:767–72. doi: 10.1007/s10157-012-0615-5
20. Huang YQ, Liu XC, Lo K, Liu L, Yu YL, Chen CL, et al. The U Shaped Relationship Between High-Density Lipoprotein Cholesterol and All-Cause or Cause-Specific Mortality in Adult Population. *Clin Interv Aging.* (2020) 15:1883–96. doi: 10.2147/CIA.S271528
21. Lee EY, Yang Y, Kim HS, Cho JH, Yoon KH, Chung WS, et al. Effect of visit-to-visit LDL-, HDL-, and non-HDL-cholesterol variability on mortality and cardiovascular outcomes after percutaneous coronary intervention. *Atherosclerosis.* (2018) 279:1–9. doi: 10.1016/j.atherosclerosis.2018.10.012
22. Stone NJ, Robinson JG, Lichtenstein AH, Bairey Merz CN, Blum CB, Eckel RH, et al. 2013 ACC/AHA guideline on the treatment of blood cholesterol to reduce atherosclerotic cardiovascular risk in adults: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines. *Circulation.* (2014) 129:S1–45. doi: 10.1161/01.cir.0000437738.63853.7a
23. Zalawadiya SK, Veeranna V, Panaich S, Kottam A, Afonso L. Non-high-density lipoprotein cholesterol and coronary artery calcium progression in a multiethnic US population. *Am J Cardiol.* (2014) 113:471–4. doi: 10.1016/j.amjcard.2013.10.027
24. Defesche JC, Gidding SS, Harada-Shiba M, Hegele RA, Santos RD, Wierzbicki AS. Familial hypercholesterolaemia. *Nat Rev Dis Primers.* (2017) 3:17093. doi: 10.1038/nrdp.2017.93
25. Contreras F, Lares M, Castro J, Velasco M, Rojas J, Guerra X, et al. Determination of non-HDL cholesterol in diabetic and hypertensive patients. *Am J Ther.* (2010) 17:337–40. doi: 10.1097/MJT.0b013e3181c1233c
26. Johannesen CDL, Langsted A, Mortensen MB, Nordestgaard BG. Association between low density lipoprotein and all cause and cause specific mortality in Denmark: prospective cohort study. *BMJ.* (2020) 371:m4266. doi: 10.1136/bmj.m4266
27. Wang B, Guo Z, Li H, Zhou Z, Lu H, Ying M, et al. Non-HDL cholesterol paradox and effect of underlying malnutrition in patients with coronary artery disease: a 41,182 cohort study. *Clin Nutr.* (2022) 41:723–30. doi: 10.1016/j.clnu.2022.01.027
28. Tuikkala P, Hartikainen S, Korhonen MJ, Lavikainen P, Kettunen R, Sulkava R, et al. Serum total cholesterol levels and all-cause mortality in a home-dwelling elderly population: a six-year follow-up. *Scand J Prim Health Care.* (2010) 28:121–7. doi: 10.3109/02813432.2010.487371
29. Bathum L, Depont Christensen R, Engers Pedersen L, Lyngsie Pedersen P, Larsen J, Nexoe J. Association of lipoprotein levels with mortality in subjects aged 50 + without previous diabetes or cardiovascular disease: a population-based register study. *Scand J Prim Health Care.* (2013) 31:172–80. doi: 10.3109/02813432.2013.824157
30. Jacobs D, Blackburn H, Higgins M, Reed D, Iso H, McMillan G, et al. Report of the conference on low blood cholesterol: mortality associations. *Circulation.* (1992) 86:1046–60. doi: 10.1161/01.CIR.86.3.1046
31. Ranieri P, Rozzini R, Franzoni S, Barbisoni P, Trabucchi M. Serum cholesterol levels as a measure of frailty in elderly patients. *Exp Aging Res.* (1998) 24:169–79. doi: 10.1080/036107398244300
32. Burgess S, Davies NM, Thompson SG, Consortium EP-I. Instrumental variable analysis with a nonlinear exposure-outcome relationship. *Epidemiology.* (2014) 25:877–85. doi: 10.1097/EDE.0000000000000161
33. Mach F, Baigent C, Catapano AL, Koskinas KC, Casula M, Badimon L, et al. 2019 ESC/EAS Guidelines for the management of dyslipidaemias: lipid modification to reduce cardiovascular risk. *Eur Heart J.* (2020) 41:111–88. doi: 10.1093/eurheartj/ehz455

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers | Frontiers in Cardiovascular Medicine

TYPE Original Research
PUBLISHED 18 August 2022
DOI 10.3389/fcvm.2022.944003

OPEN ACCESS

EDITED BY
Chu-Huang Chen,
Texas Heart Institute, United States

REVIEWED BY
Tongyu Ma,
Franklin Pierce University, United States
Dick C. Chan,
University of Western Australia, Australia

*CORRESPONDENCE
Ling Liu
feliuling@csu.edu.cn

SPECIALTY SECTION
This article was submitted to Lipids in Cardiovascular Disease, a section of the journal Frontiers in Cardiovascular Medicine

RECEIVED 14 May 2022
ACCEPTED 19 July 2022
PUBLISHED 18 August 2022

CITATION
Xu J, Du X, Zhang S, Xiang Q, Zhu L and Liu L (2022) The accuracy of four formulas for LDL-C calculation at the fasting and postprandial states. *Front. Cardiovasc. Med.* 9:944003. doi: 10.3389/fcvm.2022.944003

The accuracy of four formulas for LDL-C calculation at the fasting and postprandial states

Jin Xu[1,2,3,4], Xiao Du[1,2,3,4], Shilan Zhang[1,2,3,4,5], Qunyan Xiang[1,2,3,4], Liyuan Zhu[1,2,3,4] and Ling Liu[1,2,3,4]*

[1]Department of Cardiovascular Medicine, The Second Xiangya Hospital, Central South University, Changsha, China, [2]Research Institute of Blood Lipid and Atherosclerosis, Central South University, Changsha, China, [3]Modern Cardiovascular Disease Clinical Technology Research Center of Hunan Province, Changsha, China, [4]Cardiovascular Disease Research Center of Hunan Province, Changsha, China, [5]Department of Gastroenterology, The Second Xiangya Hospital, Central South University, Changsha, China

Background: Elevated level of low-density lipoprotein cholesterol (LDL-C) is concerned as one of the main risk factors for cardiovascular disease, in both the fasting and postprandial states. This study aimed to compare the measured LDL-C with LDL-C calculated by the Friedewald, Martin–Hopkins, Vujovic, and Sampson formulas, and establish which formula could provide the most reliable LDL-C results for Chinese subjects, especially at the postprandial state.

Methods: Twenty-six subjects were enrolled in this study. The blood samples were collected from all the subjects before and after taking a daily breakfast. The calculated LDL-C results were compared with LDL-C measured by the vertical auto profile method, at both the fasting and postprandial states. The percentage difference between calculated and measured LDL-C (total error) and the number of results exceeding the total error goal of 12% were established.

Results: The calculated LDL-C_F levels showed no significant difference from LDL-C_{VAP} levels at the fasting state. The calculated LDL-C_S were significantly higher than LDL-C_{VAP} at the fasting state ($P < 0.05$), while the calculated LDL-C_S were very close to LDL-C_{VAP} levels after a daily meal. At the fasting state, the median total error of calculated LDL-C_F was 0 (quartile: −3.8 to 6.0), followed by LDL-C_S, LDL-C_{MH}, and LDL-C_V. At the postprandial states, the median total errors of LDL-C_S were the smallest, 1.0 (−7.5, 8.5) and −0.3 (−10.1, 10.9) at 2 and 4 h, respectively. The calculated LDL-C_F levels showed the highest correlation to LDL-C_{VAP} and accuracy in evaluating fasting LDL-C levels, while the Sampson formula showed the highest accuracy at the postprandial state.

Conclusion: The Friedewald formula was recommended to calculate fasting LDL-C, while the Sampson formula seemed to be a better choice to calculate postprandial LDL-C levels in Chinese subjects.

KEYWORDS

LDL-C, postprandial, Friedewald formula, Vujovic formula, Martin–Hopkins formula, Sampson formula, vertical auto profile method

10.3389/fcvm.2022.944003

Background

Cardiovascular disease (CVD) has been the leading cause of death worldwide (1). The elevated level of low-density lipoprotein cholesterol (LDL-C) is concerned as one of the main risk factors of CVD, especially for atherosclerotic CVD (2). In clinical practice, it is the main laboratory parameter used for cardiovascular risk assessment and the primary target for cholesterol control (3). Therefore, it is crucial to ensure reliable measurement of LDL-C levels.

There are several methods to measure LDL-C levels, including ultracentrifugation, formula methods, direct method, and nuclear MR (NMR) method (4). Among them, the ultracentrifugation method is recommended as the reference method for LDL-C measurement. Due to complex operations and expensive equipment, ultracentrifugation is difficult to be widely used in clinical practice. Vertical auto profile (VAP) methodology, one of the ultracentrifugation methods, was used to measure lipid profiles as a reference method, commonly at the fasting state (5). However, a study involving the measurement of lipid profiles by VAP at the postprandial or non-fasting state is very rare worldwide (6, 7), and there was no similar study in China.

In comparison, the formula method is simpler and cheaper. The Friedewald method developed in 1972 is the main mathematical formula for LDL-C calculation (8). It uses a fixed coefficient, 2.2 (for mmol/l), to describe the relationship between triglyceride (TG) and very-low-density lipoprotein cholesterol (VLDL-C) (8). When the TG level was above 4.5 mmol/l, the accuracy of this formula will decline. Thus, other formulas were proposed. In 2003, the Vujovic formula, which uses 3.0 (for mmol/l) as a ratio of TG to VLDL-C, was proposed for LDL-C calculation (9). Then, the Martin–Hopkins formula was developed with an adjustable ratio based on TG and non-high-density lipoprotein cholesterol (non-HDL-C) levels (10). In 2020, Sampson and colleagues (11) proposed a new formula, which was proved to be suitable for samples with TG levels up to 9.0 mmol/l. Those novel formulas were proved to be more accurate than the Friedewald formula (12).

Recently, a variety of expert recommendations have supported non-fasting lipid assessment (13–15), as elevated non-fasting TG and LDL-C levels had been regarded as independent risk factors of atherosclerotic CVD (16, 17). We once reported that the direct measured LDL-C levels were significantly higher than calculated LDL-C levels by the Friedewald formula at both the fasting and non-fasting states in Chinese subjects (18). Three novel formula methods and the VAP method were not involved in this study. Thus, this study aimed to establish which formula method could provide the most reliable LDL-C results when compared with the VAP method for Chinese subjects, especially in the postprandial state.

Methods

Study subjects

There were 26 subjects (in-patient) included in this study in the Department of Cardiovascular Medicine of the Second Xiangya Hospital, Central South University. All the subjects were invited to fill out a questionnaire on medical history and use of medication before participation. Subjects with fasting TG levels above 4.5 mmol/l were excluded. No subject had a history of thyroid diseases, liver and kidney diseases, autoimmune diseases, cancer, or other severe medical illnesses. The study was approved by the Ethics Committee of the Second Xiangya Hospital of Central South University and informed consent was gained from all the participants.

Specimen collection

After at least 12 h of overnight fasting, venous blood samples were collected in all the subjects before (i.e., 0 h) and at 2 and 4 h after taking a daily breakfast according to their daily habits, such as steamed bread, rice porridge, or noodles (19). All the subjects were required to complete the meal in 15 min. All the blood samples were centrifuged at 4°C for 3,000 rpm for 15 min and stored at −80°C refrigerator until analysis.

Laboratory assays

Blood lipids were detected in two ways. First, all the blood samples were measured in a medical laboratory in Second Xiangya Hospital by a laboratory technician who had no knowledge of this study as described before (20). Serum levels of total cholesterol (TC) and TG were measured by automated enzymatic assays, and the concentration of HDL-C was measured by a direct method, i.e., the selective protection method. LDL-C level was measured directly by the chemical masking (CM) method (i.e., LDL-C_{CM}) regardless of TG level. Then, the VAP method was used to measure all the lipid profiles, including LDL-C (i.e., LDL-C_{VAP}), as a reference method (5). In brief, it simultaneously measures cholesterol concentrations of all the five lipoprotein classes in <1 h. After centrifugation, the contents of the centrifuge tube (separated layers of lipoproteins) were analyzed for cholesterol using the continuous flow VAP analyzer (5).

Low-density lipoprotein cholesterol calculation

For each sample, the LDL-C level was calculated using mathematical formulas with CM measured lipids as follows:

Friedewald (8): LDL-C_F = TC – HDL-C – TG/2.2 (mmol/l)

Vujovic (9): LDL-C_V = TC – HDL-C – TG/3 (mmol/l)

Martin–Hopkins (10): LDL-C_{MH} = TC – HDL-C – TG/adjustable factor (mg/dl)

Sampson (11): LDL-C_S = TC/0.948 – HDL-C/0.971 – (TG/8.56 + TG × non-HDL-C/2,140 – TG2/16,100) – 9.44 (mg/dl).

The factor of 0.026 was used to convert LDL-C from mg/dl into mmol/l, if necessary.

Statistical analysis

All the continuous levels were expressed as median (interquartile range) and qualitative variables were expressed as numbers and percentages. The measured TC, HDL-C, LDL-C, and the calculated LDL-C were tested to be distributed normally, while the measured TG was proven to be non-normal distribution. The parametric and non-parametric statistical tests were used for corresponding data, respectively. Differences between different groups were analyzed by *one-way* ANOVA, while differences among different time points within the same group were analyzed by repeated measure *one-way* ANOVA analysis. Categorical variables were compared using the *chi-squared* statistic tests. The Bland–Altman difference plots were used to compare calculated LDL-C levels and the LDL-C_{VAP}. Correlation between calculated LDL-C levels and the LDL-C_{VAP} was conducted with Pearson correlation analyses. For each sample, the total error (%) between the mathematically calculated LDL-C and LDL-C_{VAP} was estimated as follows: [(LDL-$C_{formula}$ – LDL-C_{VAP})/LDL-C_{VAP}] × 100%. The accuracy of estimation was defined as the total error ± 12% (21). All the statistical analyses were performed with SPSS version 25.0. All the *P*-levels were 2-tailed, and $P < 0.05$ was considered statistically significant. For differences between LDL-C_{VAP} and LDL-$C_{formula}$, $P < 0.01$ was considered statistically significant, as we replaced *post-hoc* analysis in the repeated measure one-way ANOVA analyses using one-way ANOVA analyses.

Results

Population characteristics

There were 26 subjects who participated in this study, including 17 (65.4%) men and 9 (34.6%) women. Their ages ranged from 46 to 73 years, with a median age of 62.5 years. Five of them got a body mass index (BMI) of over 28 kg/m^2 and the median BMI of all the subjects was 25.5 kg/m^2. The patients with coronary heart disease, hypertension, and diabetes accounted for 69.2, 69.2, and 30.8%, respectively. There were 42.3% of smokers and 46.2% of subjects taking statins (Table 1).

TABLE 1 Study population characteristics.

Parameters	*N* = 26
Male, *n* (%)	17 (65.4)
Age, y	62.5 (54.75, 66.5)
BMI, Kg/m^2	25.51 (23.2, 27.0)
Current smoking, *n* (%)	11 (42.3)
CHD, *n* (%)	18 (69.2)
Hypertension, *n* (%)	18 (69.2)
Diabetes, *n* (%)	8 (30.8)
History of statins, *n* (%)	12 (46.2)

Values are represented as median (interquartile range) and n (%) as appropriate.
BMI, body mass index; CHD, coronary heart disease.

Postprandial changes in serum levels of blood lipids measured by different methods

It was obvious that the levels of TC, TG, and LDL-C measured by CM were significantly higher than those measured by VAP, while HDL-C levels measured by the two methods were similar at both the fasting and postprandial states (Figure 1). No matter which method was used, both the TC and LDL-C levels decreased significantly after a daily meal compared to the fasting state ($P < 0.05$, Figures 1A,C), while TG showed tremendously increase at the postprandial time points ($P < 0.05$, Figure 1B). The levels of HDL-C kept stable after a daily meal no matter which method was used (Figure 1D).

The calculated levels of LDL-C were acquired by four different formulas with blood lipids measured by CM, and they showed a similar decrease after a daily meal and the postprandial changes reached a statistic difference ($P < 0.05$, Figure 2). It is worth noting that there was no significant difference between calculated LDL-C levels at 2 and 4 h, no matter which formula was used.

The calculated LDL-C levels *via* Friedewald, Martin–Hopkins, and Sampson formulas showed no significant difference when compared to LDL-C_{VAP} levels at both the fasting and postprandial states (Figures 2A,C,D). However, the calculated LDL-C_V levels were significantly higher than LDL-C_{VAP} levels at the fasting state ($P < 0.05$ Figure 2B), while the calculated LDL-C_V levels were very close to LDL-C_{VAP} levels after a daily meal (Figure 2B). The calculated LDL-C_F levels seem to be lower than LDL-C_{VAP} levels at the postprandial

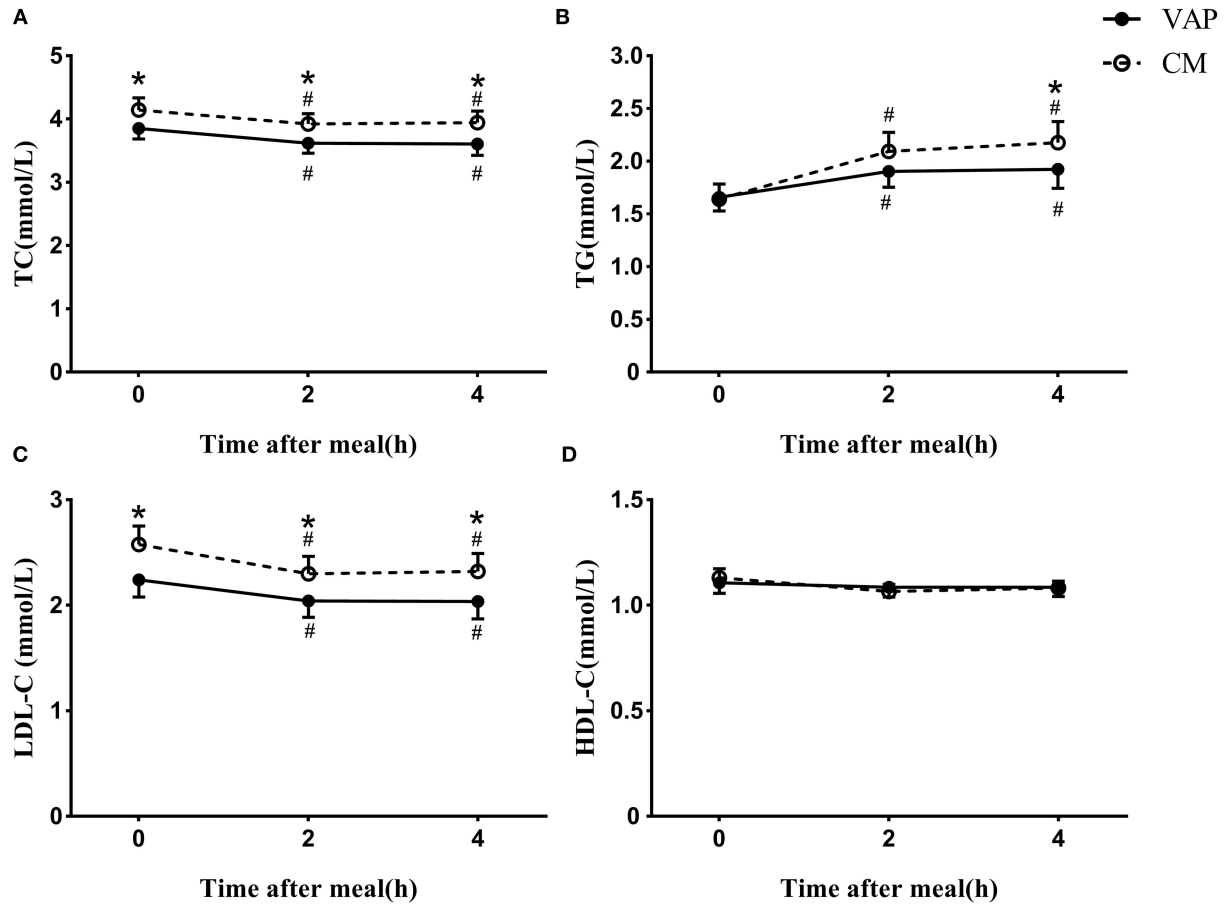


FIGURE 1
Changes in serum levels of blood lipids via VAP and chemical masking method after a daily meal. *$P < 0.05$ when compared with VAP measured values at the same time point. #$P < 0.05$ when compared with fasting value using the same measure method.

states, while the difference did not reach statistical significance ($P < 0.05$, Figure 2A).

The calculated LDL-C_F, LDL-C_S, and LDL-C_{MH} levels were all significantly lower than LDL-C_{CM} levels at the fasting and postprandial states ($P < 0.05$, Figures 2A,C,D). The LDL-C_V levels were lower than LDL-C_{CM} levels; however, the difference reached statistical significance only at 4 h postprandially (Figure 2B).

Consistency and correlation between estimated and measured low-density lipoprotein cholesterol

The Bland–Altman difference plots showed great consistency in estimated and measured LDL-C (Supplementary Figure 1). The measured LDL-C_{CM} also had a good consistency with LDL-C_{VAP} (Supplementary Figure 1). The Pearson correlation analyses showed a strong and positive correlation between LDL-C_{VAP} levels and the calculated LDL-C levels by four formulas, and the r levels ranged from 0.836 to 0.961 at the fasting and postprandial states ($P < 0.05$, Table 2). At the fasting state, the strongest correlation was found between LDL-C_{VAP} levels and LDL-C_F levels (r 0.870, $P < 0.05$). At the postprandial states, the strongest correlation was found between LDL-C_{VAP} levels and the LDL-C_V levels (2 h: r 0.961, 4 h: r 0.956, $P < 0.05$).

A positive correlation was also found between LDL-C_{VAP} and LDL-C_{CM} at the fasting and postprandial states (0 h: r 0.780, 2 h: r 0.883, 4 h: r 0.859, $P < 0.05$, Table 2). However, the correlation between LDL-C_{VAP} and LDL-C_{CM} was weaker than the correlation between LDL-C_{VAP} and the calculated LDL-C levels by four formulas at both the fasting and postprandial states (Table 2).

Distribution of the total error at the fasting and postprandial states

To determine the reliability of calculated LDL-C levels by different formulas, we calculated the total errors between

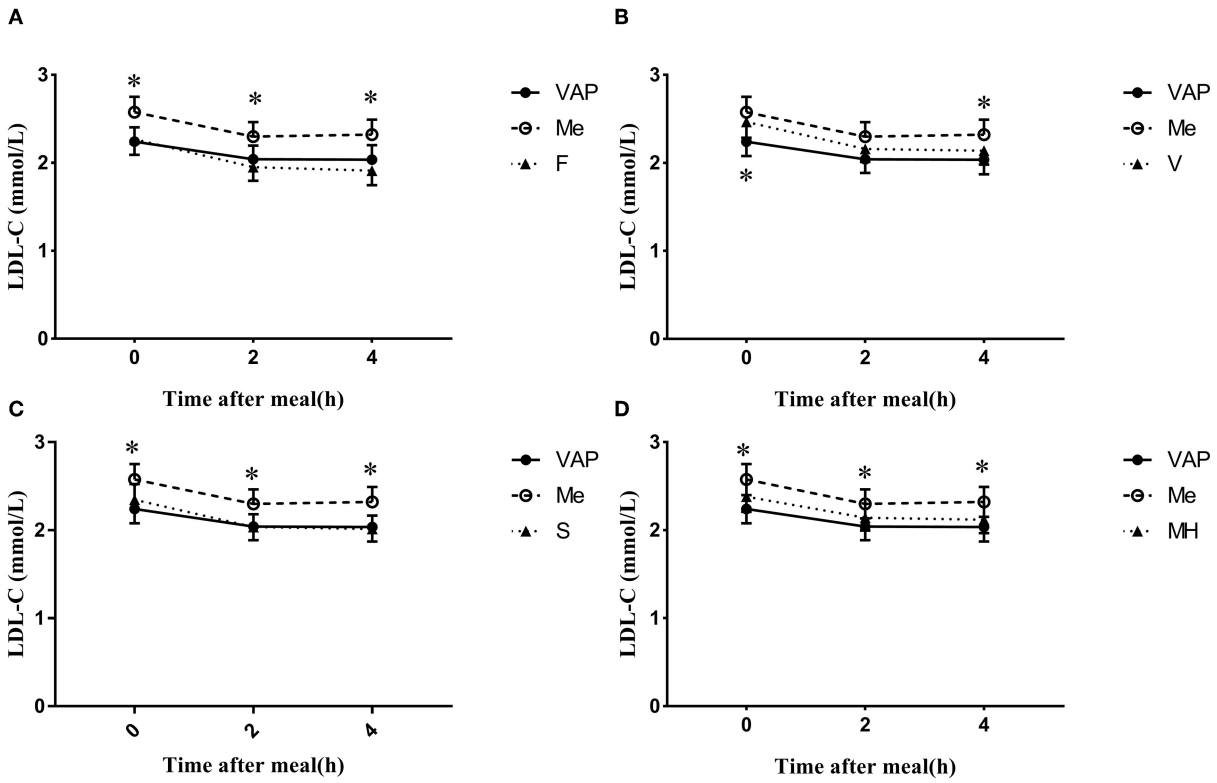


FIGURE 2
Changes in LDL-C levels via different calculated methods after a daily meal. *$P < 0.05$ when compared with calculated values at the same time point.

TABLE 2 Correlation in chemical masking method measured and formula estimated vs. VAP measured LDL-C.

	LDL0	LDL2	LDL4
Friedewald	0.870	0.920	0.913
Vujovic	0.85	0.961	0.956
Martin/Hopkins	0.837	0.927	0.902
Sampson	0.836	0.939	0.928
CM	0.780	0.883	0.859

calculated LDL-C and LDL-C_{VAP} levels. At the fasting state, the median total error of calculated LDL-C_F was 0 (quartile: −3.8 to 6.0; Figure 3A; Supplementary Table 1). The median total errors of calculated fasting LDL-C_V, LDL-C_{MH}, and LDL-C_S were 11.2 (3.2, 18.9), 7.0 (−2.5, 15.3), and 3.4 (−1.7, 10.0), respectively (Figures 3B–D; Supplementary Table 1).

The median total errors of LDL-C_F were −3.9 (−14.1, 2.4) and −9.9 (−15.3, 0) at 2 and 4 h, respectively (Figure 3A; Supplementary Table 1), which suggested that the Friedewald formula could underestimate LDL-C levels when compared with the VAP method at the postprandial state. The median total errors of LDL-C_V and LDL-C_{MH} ranged from 2.6 and 6.5 at the postprandial states (Figures 3B,C; Supplementary Table 1), which indicated that Vujovic and Martin–Hopkins formulas could overestimate LDL-C levels when compared with the VAP method at the postprandial state. The median total errors of postprandial LDL-C_S were small, i.e., 1.0 (−7.5, 8.5) and −0.3 (−10.1, 10.9) at 2 and 4 h, respectively (Figure 3D; Supplementary Table 1).

Percentage of the accuracy of estimated low-density lipoprotein cholesterol

The accuracy of calculated LDL-C levels by formulas was considered as the percentage of the total error between −12 and 12% when compared with LDL-C_{VAP} levels. The Friedewald formula showed the highest accuracy, 80.8%, at the fasting state, followed by Sampson, Martin–Hopkins, and Vujovic formulas (Figure 3E). The Sampson formula showed the highest accuracy, 80.8%, at 2 h postprandially, followed by Friedewald, Martin–Hopkins, and Vujovic formulas (Figure 3E). At 4 h after a daily meal, the Martin–Hopkins formula and Sampson formula showed higher accuracy than Vujovic and Friedewald formulas (Figure 3E).

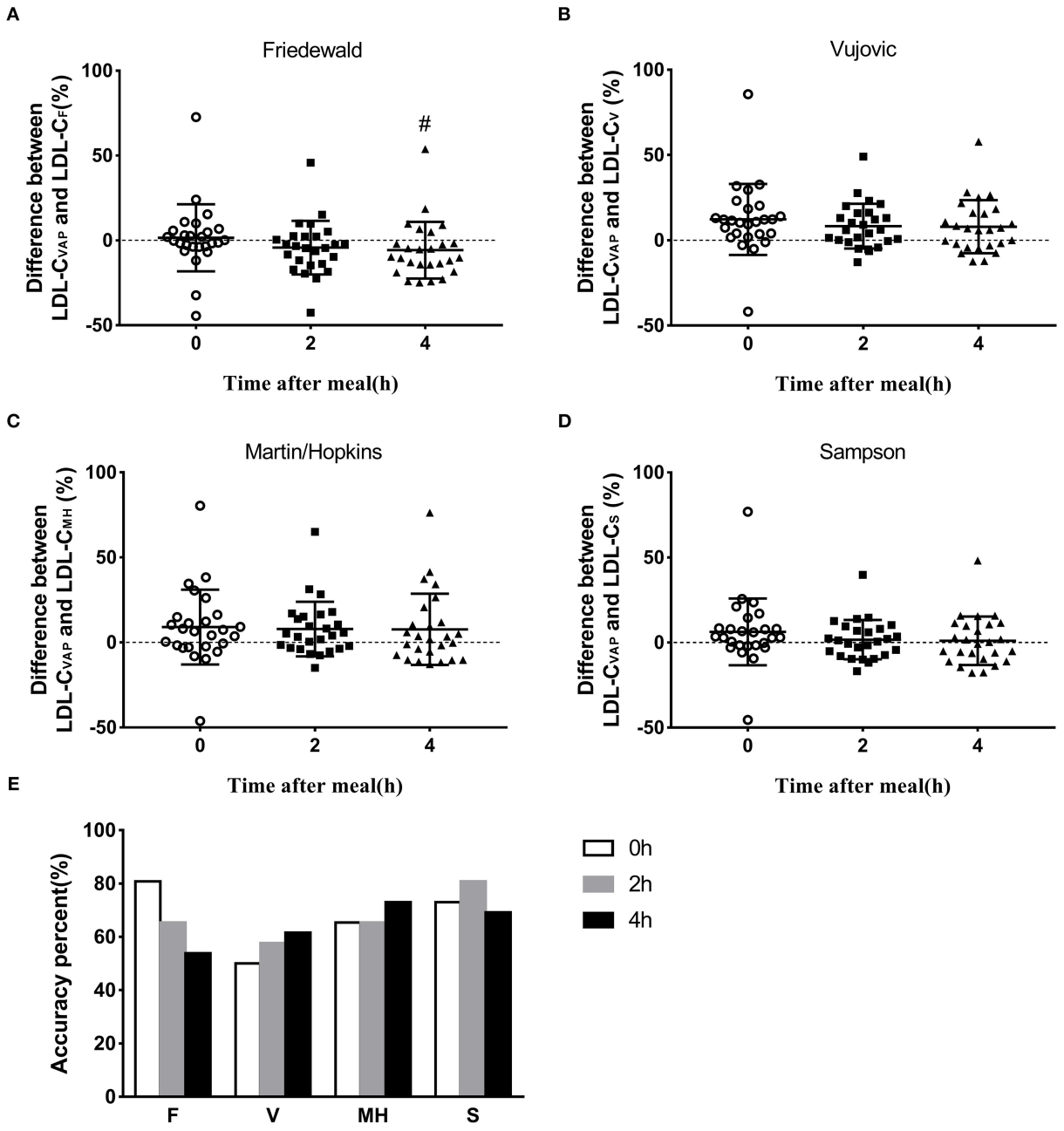


FIGURE 3
Accuracy of estimated LDL-C via different formulas. **(A–D)** Difference between LDL-C_{VAP} and LDL-$C_{formula}$ at fasting and postprandial states. **(E)** Accuracy percent (total error ±12%) of calculated value via four formulas at fasting and postprandial states. $^{\#}P < 0.05$ when compared with fasting state.

Discussion

This is the first study to compare the calculated LDL-C levels by different formulas to LDL-C_{VAP} levels at both the fasting and postprandial states in Chinese subjects. We found that the calculated LDL-C_F levels showed the highest correlation to LDL-C_{VAP} and accuracy in evaluating fasting LDL-C levels, while the Sampson formula showed the highest accuracy at the postprandial state. Therefore, the Friedewald and Sampson formulas seemed to be a better choice to calculate fasting and postprandial LDL-C levels, respectively, in Chinese subjects.

Similar to the postprandial change in LDL-C_{CM} levels, LDL-C_{VAP} levels significantly decreased at 2 and 4 h after a daily meal in this study, which was different from the results reported by Hu et al. (22) who measured lipid profiles by enzymatic- and NMR-based methods in 87 Chinese subjects and reported

that there was no significant reduction in LDL-C levels and LDL particles determined by NMR after a daily meal. However, they found cholesterol content in large LDL particles that significantly decreased at 2 and 4 h compared to the fasting one (22). American researchers compared lipid profiles detected by the VAP method between 10,135 fasting and 5,262 non-fasting (<8 h since last meal) subjects, and found significantly lower LDL-C levels and LDL particles in non-fasting subjects, although percent differences in these parameters were small (6). Chinese subjects showed a more obvious reduction in LDL-C levels at 2 and 4 h postprandially, for example, about 18% after a daily meal and 28% after a high-fat meal (18, 23). Moreover, considering that ultracentrifugation is the reference method for LDL-C measurement, and the VAP method is rapid ultracentrifugation, the reduction of LDL-C levels after a daily meal cannot be ignored, especially in Chinese subjects.

It was reported that LDL-C_{CM} levels were higher than LDL-C measured by NMR in Chinese subjects with different diseases (22). In this study, both the LDL-C_{VAP} levels and LDL-C levels calculated by formulas were lower than LDL-C_{CM} levels at both the fasting and postprandial states, which prompted us to pay more attention to the difference between LDL-C levels calculated by formulas and LDL-C_{VAP} levels.

The Friedewald formula is recommended to calculate LDL-C levels when TG levels are not very high (8). In this study, there was no subject with fasting TG $\geq$ 4.5 mmol/l, which may contribute to the strongest correlation between LDL-C_F and LDL-C_{VAP} at the fasting state. However, the TG/VLDL ratio varies with TG increasing at the fasting and non-fasting states, which decreases the accuracy of the Friedewald formula in LDL-C estimation. It is worth noting that the lowest accuracy was found in LDL-C_F at 4 h postprandially when TG reached the peak level. Therefore, other formulas were proposed for higher accuracy when TG increased, especially at the postprandial state.

Compared to the stable ratio of TG/VLDL-C in the Friedewald formula (2.2 for mmol/l or 5.0 for mg/dl), those in the Vujovic and Martin–Hopkins formulas were changed (9, 10). The ratio in the Vujovic formula was still fixed, but relatively greater, presenting as 3 for mmol/l or 6.85 for mg/dl (9). Its accuracy had been demonstrated in whole TC, TG, and LDL-C ranges (9). With TG levels increased, the postprandial correlation coefficients between LDL-C_V and LDL-C_{VAP} were stronger than the fasting state, and higher than those of the other three formulas. However, the accuracy of the Vujovic formula seemed to be relatively low, especially at the fasting state and 2 h postprandially, although it increased after a daily meal, and seemed to be better than Friedewald formula at 4 h after a daily meal. The low accuracy may be resulted from its overestimation of LDL-C compared to LDL-C_{VAP}.

The ratio of TG and VLDL-C in the Martin–Hopkins formula becomes complicated and dependent on TG and non-HDL-C levels, varying from 3.1 to 11.9 (for mg/dl) (10). With TG increasing and non-HDL-C decreasing, it elevates correspondently. The accuracy of Martin–Hopkins formula was moderate on the whole, but the difference in accuracy between the fasting and postprandial states was small, which was consistent with the findings of Sathiyakumar et al., which found that the Martin–Hopkins formula was less affected by diet than the Friedewald formula (7). However, the complexity of the Martin–Hopkins ratio could reduce the convenience in clinical practice to a certain extent. After all, clinicians cannot remember so many numbers.

Other than the Friedewald, Vujovic, and Martin–Hopkins formulas, the novel Sampson formula uses higher-order mathematical terms in the form of a bivariate quadratic equation that should better reflect the amount of TG in the core of the lipoproteins (24). The Sampson formula is based on the data of 8,656 American adults with a high frequency of hypertriglyceridemia, and it was confirmed to be suitable for LDL-C calculation of samples with TG over 9 mmol/l (11), which may contribute to the highest accuracy at the postprandial states after a daily meal. Actually, at the beginning of the establishment of the Sampson formula, a comparison was made between fasting and non-fasting samples, which suggested that this formula was also applicable to non-fasting samples (11).

The LDL particles could be divided into different subfractions according to their size. The size of LDL particles had been suggested as a reliable assessment of atherogenicity (25). The subfractions of LDL particles at the postprandial states were reported to be lower by a different degree than those in fasting states (6). This may contribute to the Friedewald and Sampson formulas being the best choice for fasting and postprandial states, respectively.

This study is associated with several limitations. First, the sample size in this study was small compared to other clinical studies (7). Second, there were 46.2% of subjects got a statin history which may cause variation with those without statin use. Third, we analyzed our subjects as a whole other than stratified analysis, which may make a more precise result.

Conclusion

In conclusion, among four formulas, the Friedewald formula was recommended to calculate fasting LDL-C, while the Sampson formula seemed to be a better choice to calculate postprandial LDL-C levels in Chinese subjects.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by Ethics Committee of The Second Xiangya Hospital

of Central South University. The patients/participants provided their written informed consent to participate in this study.

Author contributions

All authors have accepted responsibility for the entire content of this manuscript and approved its submission.

Funding

This study was supported by grants from the National Natural Science Foundation of China (Nos. 81270956 and 81470577 to LL), the Natural Science Foundation of Hunan Province (2022JJ70136), and the Project of the Health Commission of Hunan Province, China (No. 202203012938 to LL).

Acknowledgments

We would like to acknowledge professor Deng in Xiangya School of Public Health for providing help with statistical analyses.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcvm.2022.944003/full#supplementary-material

References

1. Reamy BV, Williams PM, Kuckel DP. Prevention of cardiovascular disease. *Prim Care.* (2018) 45:25–44. doi: 10.1016/j.pop.2017.11.003

2. Domanski MJ, Tian X, Wu CO, Reis JP, Dey AK, Gu Y, et al. Time course of LDL cholesterol exposure and cardiovascular disease event risk. *J Am Coll Cardiol.* (2020) 76:1507–16. doi: 10.1016/j.jacc.2020.07.059

3. Tomlinson B, Patil NG, Fok M, Lam CWK. Managing dyslipidemia in patients with Type 2 diabetes. *Expert Opin Pharmacother.* (2021) 22:2221–34. doi: 10.1080/14656566.2021.1912734

4. Wolska A, Remaley AT. Measuring LDL-cholesterol: what is the best way to do it? *Curr Opin Cardiol.* (2020) 35:405–11. doi: 10.1097/HCO.0000000000000740

5. Kulkarni KR. Cholesterol profile measurement by vertical auto profile method. *Clin Lab Med.* (2006) 26:787–802. doi: 10.1016/j.cll.2006.07.004

6. Farukhi ZM, Demler OV, Caulfield MP, Kulkarni K, Wohlgemuth J, Cobble M, et al. Comparison of non-fasting and fasting lipoprotein subfractions and size in 15,397 apparently healthy individuals: an analysis from the VITamin D and OmegA-3 TriaL. *J Clin Lipidol.* (2020) 14:241–51. doi: 10.1016/j.jacl.2020.02.005

7. Sathiyakumar V, Park J, Golozar A, Lazo M, Quispe R, Guallar E, et al. Fasting versus non-fasting and low-density lipoprotein cholesterol accuracy. *Circulation.* (2018) 137:10–9. doi: 10.1161/CIRCULATIONAHA.117.030677

8. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem.* (1972) 18:499–502. doi: 10.1093/clinchem/18.6.499

9. Vujovic A, Kotur-Stevuljevic J, Spasic S, Bujisic N, Martinovic J, Vujovic M, et al. Evaluation of different formulas for LDL-C calculation. *Lipids Health Dis.* (2010) 9:27. doi: 10.1186/1476-511X-9-27

10. Martin SS, Blaha MJ, Elshazly MB, Toth PP, Kwiterovich PO, Blumenthal RS, et al. Comparison of a novel method vs. the Friedewald equation for estimating low-density lipoprotein cholesterol levels from the standard lipid profile. *J Am Med Assoc.* (2013) 310:2061–8. doi: 10.1001/jama.2013.280532

11. Sampson M, Ling C, Sun Q, Harb R, Ashmaig M, Warnick R, et al. A new equation for calculation of low-density lipoprotein cholesterol in patients with normolipidemia and/or hypertriglyceridemia. *J Am Med Assoc Cardiol.* (2020) 5:540–8. doi: 10.1001/jamacardio.2020.0013

12. Piani F, Cicero AFG, Ventura F, Dormi A, Fogacci F, Patrono D, et al. Evaluation of twelve formulas for LDL-C estimation in a large, blinded, random Italian population. *Int J Cardiol.* (2021) 330:221–7. doi: 10.1016/j.ijcard.2021.02.009

13. Nordestgaard BG, Langsted A, Mora S, Kolovou G, Baum H, Bruckert E, et al. Fasting is not routinely required for determination of a lipid profile: clinical and laboratory implications including flagging at desirable concentration cut-points-a joint consensus statement from the European Atherosclerosis Society and European Federation of Clinical Chemistry and Laboratory Medicine. *Eur Heart J.* (2016) 37:1944–58. doi: 10.1093/eurheartj/ehw152

14. Kolovou GD, Mikhailidis DP, Kovar J, Lairon D, Nordestgaard BG, Ooi TC, et al. Assessment and clinical relevance of non-fasting and postprandial triglycerides: an expert panel statement. *Curr Vasc Pharmacol.* (2011) 9:258–70. doi: 10.2174/157021213146321611

15. Kolovou GD, Watts GF, Mikhailidis DP, Perez-Martinez P, Mora S, Bilianou H, et al. Postprandial hypertriglyceridaemia revisited in the era of non-fasting lipid profile testing: a 2019 expert panel statement, main text. *Curr Vasc Pharmacol.* (2019) 17:498–514. doi: 10.2174/1570161117666190507110519

16. Della Pepa G, Vetrani C, Vitale M, Bozzetto L, Costabile G, Cipriano P, et al. Effects of a diet naturally rich in polyphenols on lipid composition of postprandial lipoproteins in high cardiometabolic risk individuals: an ancillary analysis of a randomized controlled trial. *Eur J Clin Nutr.* (2020) 74:183–92. doi: 10.1038/s41430-019-0459-0

17. Kolovou G, Ooi TC. Postprandial lipaemia and vascular disease. *Curr Opin Cardiol.* (2013) 28:446–51. doi: 10.1097/HCO.0b013e3283606971

18. Lin QZ, Chen YQ, Guo LL, Xiang QY, Tian F, Wen T, et al. Comparison of non-fasting LDL-C levels calculated by Friedewald formula with those directly measured in Chinese patients with coronary heart disease after a daily breakfast. *Clin Chim Acta.* (2019) 495:399–405. doi: 10.1016/j.cca.2019.05.010

19. Xu J, Chen YQ, Zhao SP, Liu L. Determination of optimal cut-off points after a high-fat meal corresponding to fasting elevations of triglyceride and remnant cholesterol in Chinese subjects. *Lipids Health Dis.* (2019) 18:206. doi: 10.1186/s12944-019-1146-9

20. Xiang QY, Tian F, Lin QZ, Du X, Zhang SL, Gui YJ, et al. Comparison of remnant cholesterol levels estimated by calculated and measured LDL-C levels in Chinese patients with coronary heart disease. *Clin Chim Acta.* (2020) 500:75–80. doi: 10.1016/j.cca.2019.09.020

21. Bachorik PS, Ross JW. National Cholesterol Education Program recommendations for measurement of low-density lipoprotein cholesterol: executive summary. The National Cholesterol Education Program Working Group on lipoprotein measurement. *Clin Chem.* (1995) 41:1414–20. doi: 10.1093/clinchem/41.10.1414

22. Hu D, Mao L, Tang X, Chen J, Guo X, Luo Q, et al. Nuclear magnetic resonance reveals postprandial low-density lipoprotein cholesterol determined by enzymatic method could be a misleading indicator. *Clin Chim Acta.* (2021) 514:59–65. doi: 10.1016/j.cca.2020.12.013

23. Zhu LY, Wen XY, Xiang QY, Guo LL, Xu J, Zhao SP, et al. Comparison of the reductions in LDL-C and non-HDL-C induced by the red yeast rice extract Xuezhikang between fasting and non-fasting states in patients with coronary heart disease. *Front Cardiovasc Med.* (2021) 8:674446. doi: 10.3389/fcvm.2021.674446

24. Cwiklinska A, Wieczorek E, Gliwinska A, Marcinkowska M, Czaplinska M, Mickiewicz A, et al. Non-HDL-C/TG ratio indicates significant underestimation of calculated low-density lipoprotein cholesterol (LDL-C) better than TG level: a study on the reliability of mathematical formulas used for LDL-C estimation. *Clin Chem Lab Med.* (2021) 59:857–67. doi: 10.1515/cclm-2020-1366

25. Superko HR, Gadesam RR. Is it LDL particle size or number that correlates with risk for cardiovascular disease? *Curr Atheroscler Rep.* (2008) 10:377–85. doi: 10.1007/s11883-008-0059-2

frontiers | Frontiers in Cardiovascular Medicine

TYPE Review
PUBLISHED 22 August 2022
DOI 10.3389/fcvm.2022.956086

OPEN ACCESS

EDITED BY
Tatsuya Sawamura,
Shinshu University, Japan

REVIEWED BY
Jinxing Liu,
Fuwai Hospital, China
Hiroyuki Itabe,
Showa University, Japan

*CORRESPONDENCE
Feng Hua Ding
ruijindfh@126.com
Wei Feng Shen
rjshenweifeng@126.com

†These authors have contributed equally to this work

SPECIALTY SECTION
This article was submitted to Lipids in Cardiovascular Disease, a section of the journal Frontiers in Cardiovascular Medicine

RECEIVED 29 May 2022
ACCEPTED 01 August 2022
PUBLISHED 22 August 2022

CITATION
Shen Y, Wang XQ, Dai Y, Wang YX, Zhang RY, Lu L, Ding FH and Shen WF (2022) Diabetic dyslipidemia impairs coronary collateral formation: An update.
Front. Cardiovasc. Med. 9:956086.
doi: 10.3389/fcvm.2022.956086

Diabetic dyslipidemia impairs coronary collateral formation: An update

Ying Shen[1†], Xiao Qun Wang[1†], Yang Dai[1†], Yi Xuan Wang[1], Rui Yan Zhang[1,2], Lin Lu[1,3], Feng Hua Ding[1*] and Wei Feng Shen[1,3*]

[1]Department of Cardiovascular Medicine, School of Medicine, Ruijin Hospital, Shanghai Jiao Tong University, Shanghai, China, [2]Shanghai Clinical Research Center for Interventional Medicine, Shanghai, China, [3]Institute of Cardiovascular Disease, Shanghai Jiao Tong University School of Medicine, Shanghai, China

Coronary collateralization is substantially impaired in patients with type 2 diabetes and occlusive coronary artery disease, which leads to aggravated myocardial ischemia and a more dismal prognosis. In a diabetic setting, altered serum lipid profiles and profound glycoxidative modification of lipoprotein particles induce endothelial dysfunction, blunt endothelial progenitor cell response, and severely hamper growth and maturation of collateral vessels. The impact of dyslipidemia and lipid-lowering treatments on coronary collateral formation has become a topic of heightened interest. In this review, we summarized the association of triglyceride-based integrative indexes, hypercholesterolemia, increased Lp(a) with its glycoxidative modification, as well as quantity and quality abnormalities of high-density lipoprotein with impaired collateral formation. We also analyzed the influence of innovative lipid-modifying strategies on coronary collateral development. Therefore, clinical management of diabetic dyslipidemia should take into account of its effect on coronary collateralization in patients with occlusive coronary artery disease.

KEYWORDS

dyslipidemia, type 2 diabetes mellitus, coronary collateral circulation, coronary artery disease, lipid-lowering therapy

Abbreviations: AGE, advanced glycation end product; Apo, apolipoprotein; CAD, coronary artery disease; CEC, cholesterol efflux capacity; CETP, cholesterol ester transfer protein; CTRP, C1q tumor necrosis factor related protein; DPP4, dipeptidyl peptidase-4; eNOS, endothelial nitric oxide synthase; FGF, fibroblast growth factor; GLP-1, glucagon-like peptide-1; HDL, high-density lipoprotein; HDL-C, HDL cholesterol; HGF, hepatocyte growth factor; HIF, hypoxia-inducible factor; LCAT, lecithin cholesterol acyl transferase; LDL, low-density lipoprotein; LDL-C, low-density lipoprotein cholesterol; Lp(a), lipoprotein(a); MCP, monocyte chemotactic protein; NO, nitric oxide; oxLDL-C, oxidized low-density lipoprotein cholesterol; PCSK9, proprotein convertase subtilisin/kexin type 9; PON, paraoxonase; PUFA, polyunsaturated fatty acid; RAGE, receptor for advanced glycation end product; ROS, reactive oxide spices; sdLDL-C, small dense low-density lipoprotein cholesterol; SGLT2, sodium-glucose co-transporter-2; T2DM, type 2 diabetes mellitus; TG/HDL-C, triglyceride/high-density lipoprotein cholesterol; TRL, triglyceride-rich lipoprotein; TyG index, triglyceride-glucose index; VEGF, vascular endothelial growth factor; VEGFR, VEGF receptor; VLDL, very low density lipoprotein.

Introduction

Type 2 diabetes mellitus (T2DM) is increasingly prevalent worldwide, and cardiovascular disease represents the leading cause of morbidity and mortality in patients with T2DM (1). A cluster of lipid metabolic abnormalities, collectively referred to as diabetic dyslipidemia, have been well established as a major risk factor for adverse cardiovascular outcomes in diabetic patients. The pattern of diabetic dyslipidemia consists of hypertriglyceridemia associated with increased triglyceride-rich lipoproteins and their remnants, decreased high-density lipoprotein cholesterol (HDL-C), and elevated low-density lipoprotein cholesterol (LDL-C) levels with predominance of small dense LDL-C (sdLDL-C). Meanwhile, hyperglycemia and chronic inflammation in diabetic conditions promote glycation and oxidative modification of lipoprotein particles, leading to changes in conformation and function, altered interaction with membrane receptors and downstream signaling, and switch of the phenotype toward a more atheroprone state (2).

Coronary collaterals have been recognized as an important compensatory mechanism in salvage of ischemic myocardium, preservation of left ventricular function, and improvement of prognosis for patients with obstructive coronary artery disease (CAD) (3–5). During the development of coronary collaterals, two distinct processes, arteriogenesis and angiogenesis, are involved. The former pertains to the remodeling of preexisting arterial vessels through anatomic increase in lumen area and wall thickness. The latter is defined as growth of new capillaries that stem from the budding of preexisting capillary vessels (6). These processes are finely tuned by a variety of biomechanical and biochemical factors, including perfusion pressure, wall shear stress, systematic hypoxia, oxidative stress, inflammatory response and endothelial function.

Numerous clinical observations reveal substantially impaired collateral circulation in occlusive CAD patients with diabetes. Given the generally more severe atherosclerotic lesions and microcirculation dysfunction in diabetic patients, poor collateralization may provide a significant add-on effect to aggravate myocardial ischemia and contribute to a more dismal prognosis (7). A couple of underlying mechanisms for the poor collateral formation in diabetic patients have been identified. Chronic hyperglycemia and the engagement of advanced glycation end-products with their receptors (AGE-RAGE axis) adversely affects collateral development by inhibiting vessel growth and maturation (8). On the other hand, disturbed lipid metabolism also plays a critical role and is regarded as a hallmark of impaired angiogenesis (9, 10). Currently, the impact of dyslipidemia and lipid-lowering treatments on coronary collateral formation has become a topic of heightened interest. This review is the first to summarize the recent literature, in combination with our study findings, to elucidate the association of different components of diabetic dyslipidemia with coronary collateralization and highlight their potential clinical implications in T2DM patients with CAD. The relevant clinical studies investigating the association between lipid profiles and coronary collateralization are summarized in **Table 1**.

Impact of diabetic dyslipidemia on collateral formation

Hypertriglyceridemia

In patients with impaired glucose tolerance, blunt insulin sensitivity leads to compensatory hyperinsulinemia and increases secretion of triglyceride and triglyceride-rich lipoproteins. Hypertriglyceridemia confers an increased risk of CAD and adverse outcomes in patients with T2DM, by promoting release of excessive free fatty acids and stimulating production of proinflammatory cytokines, fibrinogen, and coagulation factors (11). Previous studies have shown that certain conditions with a cluster of risk components including hypertriglyceridemia (e.g., metabolic syndrome, overweight, or obesity) are more likely to be associated with endothelial dysfunction and reduced new vessel growth (10, 12), but the independent role of hypertriglyceridemia in coronary collateral formation remains difficult to be proven largely due to concomitant changes in other lipoproteins and relevant factors, particularly in patients with T2DM.

In recent years, several novel indexes by integrating triglyceride with some related metabolic measurements (such as HDL-C and glucose) have been proposed to better stratify the status of coronary collateralization. Triglyceride-glucose (TyG) index, calculated as log [fasting triglycerides (mg/dL) × fasting blood glucose (mg/dL)/2], has been suggested as a surrogate marker of insulin resistance (13). Elevated TyG index correlates well with high arterial stiffness and microvascular damage (14), which is associated with decreased coronary perfusion, reduced shear stress and arteriogenesis (15). In a large observational study, chronic total occlusion patients with poor coronary collaterals had higher TyG index compared to those with good collaterals. TyG was significantly associated with poor collateral formation even after adjusting for various confounders (16). Triglyceride to HDL-C ratio (TG/HDL-C ratio) and atherogenic index of plasma (logarithmic transformation of TG/HDL-C ratio) reflect the comprehensive situation of blood lipids and severity of insulin resistance (17). An observational study of elderly patients with acute myocardial infarction showed that an elevated TG/HDL ratio was independently associated with poor development of coronary collateral circulation (18). Of note, although a number of reports have suggested the prognostic role of atherogenic index of plasma beyond traditional risk factors (19, 20), further prospective studies are needed to examine if this index is applicable to predict coronary collateralization in type 2 diabetic patients with CAD.

TABLE 1 Summary of clinical studies investigating the association between lipid profiles and coronary collateralization.

Authors	Study population	Design	Number of patients	Parameter of lipid profiles	Main relevant results
Liu et al. (10)	Consecutive patients with CTO undergoing CAG	Observational study	1,653 patients (poor CC: 355; good CC: 1298)	TG	After multiple adjustment, the quartiles of TG (adjusted OR = 1.267, 95% CI 1.088–1.474, $P = 0.002$) remained an independent factor of poor CC.
Gao et al. (16)	Consecutive patients with acute coronary syndrome and CTO	Observational study	1,093 patients (poor CC: 775; good CC: 318)	TyG index	TyG index was significantly higher in patients with poor CC compared to those with good CC (9.3 ± 0.65 vs. 8.8 ± 0.53, $P < 0.001$). The proportion of poor CC increased stepwise from the lowest to the highest TyG index tertile (15.3% vs. 22.8 % vs. 49.2 %, $P < 0.001$). After adjusting for confounding factors, TyG index remained correlated with the occurrence of impaired CC (OR 1.59, 95% CI 1.07–2.36 and OR 5.72, 95% CI 3.83–8.54 for middle and highest tertile groups vs. lowest tertile group, all $P < 0.001$) The improvement of the AUC for assessing poor CC was most significant when adding TyG index to baseline model, with a best cut-off value of 9.105. The most significant enhancement in risk reclassification and discrimination was found after inclusion of TyG index into baseline model, with a NRI of 0.238 ($P < 0.001$) and an IDI of 0.103 ($P < 0.001$).
Liu et al. (18)	Consecutive patients (≥60 years) with ST-elevation MI undergoing primary PCI	Retrospective case-control study	346 patients (poor CC: 238; good CC: 108)	TG/HDL	TG/HDL ratio was significantly higher in patients with poorly developed CC than in those with well-developed CC (2.88 ± 2.52 vs. 1.81 ± 1.18, $P < 0.001$). In multivariate logistic regression analysis, higher TG/HDL ratio served as an independent positive predictor of poor development of CC (OR 1.789, 95% CI 1. 346–2.378, $P < 0.001$). The AUC of TG/HDL ratio for predicting poor CC was 0.716 (95% CI 0.654–0.778, $P < 0.001$) with an optimal cut-off value of 1.58, sensitivity of 55.7% and specificity of 71.9%.
Aras et al. (33)	Stable angina pectoris with CTO of one major coronary artery	Retrospective study	60 patients (poor CC: 31; good CC: 29)	Lp(a)	Lp (a) levels were significantly higher and vascular endothelial growth factor levels were significantly lower in patients with poor CC than in those with good CC (34 ± 19 vs. 20 ± 12 mg/dl, $P < 0.001$, and 2.5 ± 0.7 vs. 3.4 ± 0.8 ng/dl, $P < 0.001$, respectively). Poorly developed CC were more prevalent in patients with Lp (a) levels ≥30 mg/dl than in those with Lp (a) levels <30 mg/dL (72 vs. 37%, $P = 0.008$). A strong negative correlation was observed between Lp (a) and vascular endothelial growth factor ($r = -0.708$, $P < 0.0001$). High levels of Lp (a) negatively affected the development of CC (adjusted OR 0.92, 95% CI 0.88–0.96, $P = 0.009$).
Fan et al. (34)	Chronic stable coronary disease with at least one major coronary occlusion or a stenosis of ≥95% with TIMI grade 1	Observational study	654 patients (Rentrop score 0, 1, 2, and 3 in 44, 91, 232, and 287 patients, respectively)	Lp(a)	Lp(a) levels were significantly decreased across Rentrop score 0–3 (25.80 ± 24.72, 18.99 ± 17.83, 15.39 ± 15.80, and 8.40 ± 7.75 mg/dL, $P < 0.001$). In model 1, the risk of poor CC (Rentrop 0) was greater in the third Lp (a) tertile compared to the first Lp(a) tertile (OR 3.34, 95% CI 2.32–4.83, $P < 0.001$). In model 2, the risk of poor CC (Rentrop 0) was greater in Lp(a) >30 mg/dL group compared to Lp(a) <30 mg/dL group (OR 6.77, 95% CI 4.44–10.4, $P < 0.001$).
Shen et al. (35)	Consecutive stable CAD patients with CTO of at least one major epicardial coronary artery	Observational study	1284 patients (DM: 706; non-DM: 578; poor CC: 505; good CC: 779)	Lp(a) TC LDL-C Non-HDL HDL-C TG	For diabetic and non-diabetic patients, Lp(a), total cholesterol, LDL-C, and non-HDL-C levels were higher in patients with poor CC than in those with good CC, whereas HDL-C and TG levels were similar. After adjustment for potential confounding factors, tertiles of Lp(a), total cholesterol, LDL-C and non-HDL-C remained independent determinants for poor CC. A significant interaction between Lp(a) and total cholesterol, LDL-C or non-HDL-C was observed in diabetic patients (all P interaction <0.001) but not in non-diabetics. At high tertile of total cholesterol (≥5.35 mmol/L), LDL-C (≥3.36 mmol/L) and non-HDL-C (≥4.38 mmol/L), diabetic patients with high tertile of Lp(a) (≥30.23 mg/dL) had an increased risk of poor CC compared to those with low tertile of Lp(a) (<12.66 mg/dL) (adjusted OR = 4.300, 3.970 and 4.386, respectively, all $P < 0.001$).

(Continued)

TABLE 1 Continued

Authors	Study population	Design	Number of patients	Parameter of lipid profiles	Main relevant results
You et al. (36)	Consecutive acute MI undergoing interventional CAG	Observational study	409 patients (poor CC: 277: good CC 132)	Lp(a)	Patients with poor CC had a higher Lp (a) level than those with good CC (219.1 [98.0–506.9] vs. 122.0 [64.5–215.6] mg/L, $P < 0.001$). The AUC of Lp(a) for predicting poor CC was 0.647 (95% CI: 0.592–0.702) with the cut-off value of 199.0 mg/L, sensitivity of 55.7% and specificity of 71.9%. Regression analyses revealed that patients with high Lp(a) levels had a greater risk of poor CC than those with low Lp(a) levels (unadjusted OR 2.924, 95% CI 1.900–4.501; adjusted OR 2.929, 95% CI 1.863–4.604, both $P < 0.001$). Patients with Lp(a) ≥30 mg/dL also had a greater risk of poor CC than those with Lp(a) <30 mg/dL (unadjusted OR 3.394, 95% CI 2.042–5.640; adjusted OR 4.232, 95% CI 1.400–12.797, both $P < 0.001$).
Kadi et al. (38)	Consecutive patients with CTO of at least one major epicardial coronary artery	Case-control study	151 patients (poor CC: 49; good CC: 102)	HDL-C	Serum HDL-C was lower in poor CC group compared to good CC group (34.9 ± 8 mg/dL vs. 43.7 ± 9.4 mg/dL, $P < 0.001$). The proportion of patients with low HDL-C was significantly higher in the poor CC group compared with the good CC group ($P < 0.001$). Serum TG levels and percentage of MI history were higher in the poor CC group compared with good CC group ($P = 0.015$ and $P = 0.026$, respectively). There was a positive and strong correlation between Rentrop grade and serum HDL-C level ($r = 0.503$, $P < 0.001$). Multivariate regression analysis showed that reduced HDL-C level was an independent predictor for poor CC (OR 4.3, 95% CI 1.964–9.369, $P < 0.001$).
Hsu et al. (39)	Consecutive patients undergoing CAG	Case-control study	501 patients (poor CC: 311; good CC:190)	HDL-C	There was no significant difference in HDL-C and other variables between good and poor CC. Multivariate analysis showed only number of diseased vessels was a significant predictor of poor collateral development (OR 0.411, $p < 0.001$).
Lee et al. (44)	Consecutive patents undergoing CAG	Case-control study	226 patients (poor CC: 71; good CC:155)	CEC	CEC was higher in the good than in the poor CC group (22.0 ± 4.6% vs. 20.2 ± 4.7%, $P = 0.009$). In multivariable analyses, CEC was identified as an independent predictor of good CC after adjustment for age, sex, HDL-C (OR, 1.10, 95% CI 1.03–1.18, $P = 0.004$). It remained significant after additional adjustment for DM, acute coronary syndrome, and Gensini score (OR 1.09, 95% CI 1.02–1.17, $P = 0.011$).
Wang et al. (46)	Patients with stable angina and angiographic CTO of at least one major coronary artery	Case-control study	437 patients (DM: 102; non-DM: 355; poor CC: 210; good CC: 227)	CEC	Compared to good collateralization group, CEC in poor collateralization group was significantly higher in non-diabetic patients (17.54 ± 11.86% vs. 13.91 ± 9.07%, $P = 0.002$). In contrast, CEC was impaired in type 2 diabetes irrespective of collateralization status (14.66 ± 10.47% vs. 13.26 ± 8.64%, $P = 0.462$). CEC correlated closely with Rentrop score in non-diabetic subjects, whereas no such association was present for HDL-C or apolipoprotein A-I. After adjusting for conventional risk factors including apolipoprotein A-I in logistic regression analysis, elevated CEC was independently associated with higher risk of poor collateralization in non-diabetic but not in diabetic subjects.

AUC, area under the curve; CAD, coronary artery disease; CAG, coronary artery angiography; CC: coronary collaterals; CEC, cholesterol efflux capacity; CI, confidence interval; CTO: chronic total occlusion; DM, diabetes mellitus; HDL-C, high-density lipoprotein cholesterol; IDI, integrated discrimination improvement; LDL, low-density lipoprotein; Lp(a): lipoprotein (a); MI: myocardial infarction; NRI, net reclassification improvement; OR, odds ratio; PCI, percutaneous coronary intervention; TC, total cholesterol; TG, triglyceride; TyG index, triglyceride-glucose index.

Hypercholesterolemia

Chronic exposure to high levels of cholesterol and LDL-C results in functional and structural abnormalities of the vasculature, including endothelial dysfunction, subendothelial lipid deposition, plaque progression and compromised collateral vessel growth (21, 22). In diabetic dyslipidemia, hypercholesterolemia and a predominant increase in sdLDL particles play negative roles in coronary collateral formation.

Under hypercholesterolemia, angiogenesis and arteriogenesis in response to tissue hypoxia are markedly attenuated. Hypercholesterolemia decreases endothelial nitric oxide (NO) bioavailability and NO synthase (eNOS) expression and activity which are essential for endothelial progenitor cell (EPC) migration (23). In animal models, cholesterol at high concentration resulted in delayed native arteriolar growth caused by reduced early monocyte/macrophage influx and migration, and even mildly elevated cholesterol significantly decreased expression of fibroblast growth factor (FGF) receptor

1, vascular cell adhesion molecule-1, and macrophage scavenger receptor-1, mimicking relative changes in arteriogenesis and tissue perfusion (24). The extent of these alterations was related to the duration of hypercholesterolemia. In patients with hypercholesterolemia, the number and activity of circulating EPCs were decreased compared to normocholesterolemic subjects (25). Circulating EPCs have special cellular machinery that is resistant to various types of stress, which allow them to participate in tissue repair. Interestingly, hypercholesterolemia reduced arteriogenesis more dominantly than hyperglycemia or hyperinsulinemia (26).

The inhibitory effect of hypercholesterolemia on angiogenesis/arteriogenesis could be attributed to the negative effect of LDL-C on endothelial cell responsiveness to growth factors (25). T2DM is usually accompanied by oxidation or glycation of LDL, and glycoxidatively modified LDL poses more pro-atherogenic and antiangiogenic properties than native LDL (27). In addition, the predominance of sdLDL-C confers a threefold increased risk for CAD, owning to their greater propensity for endothelial penetration into arterial wall, lower binding affinity for LDL receptor, longer circulation time, and higher susceptibility to glycation, oxidative modification, and uptake by scavenger receptors (28). The prospective Framingham offspring study and large cohort studies suggest that sdLDL is superior to LDL-C and other biomarkers in predicting future cardiovascular events in stable CAD patients with T2DM or hypertriglyceridemia (29–31). Nevertheless, there is a paucity of clinical data regarding the impact of elevated LDL-C and sdLDL-C on collateral formation in T2DM patients with CAD.

Increased circulating lipoprotein(a)

Lipoprotein(a) is a genetically determined lipoprotein, which contains principally a cholesterol rich LDL particle, one molecule of apo B-100, and an apo (a). Noteworthy, Lp (a) is known to have atherothrombogenic property by inhibiting fibrinolysis system and promoting thrombus formation. In spite of a very skewed distribution, elevated circulating Lp(a) has emerged as an independent predictor of adverse outcomes for both general and higher risk populations, especially when LDL-C levels are elevated (32). In observational studies of patients with stable CAD, serum Lp(a) levels decreased stepwise across angiographic coronary collateral grade, and elevated Lp(a) predicted poor collateral development (33, 34). Intriguingly, a robust association between Lp(a) interactions with cholesterol-containing lipids and coronary collateral formation was suggested in patients with T2DM, which was non-linear and limited to high Lp(a) and LDL-C or non-HDL-C levels (35). At high tertiles of total cholesterol (≥5.35 mmol/L), LDL-C (≥3.36 mmol/L) and non-HDL-C (≥4.38 mmol/L), patients with high tertile of Lp(a) (≥30.23 mg/dL) had a significantly increased risk of poor collateralization compared with those with low tertile of Lp(a) (<12.66 mg/dL) (all $P < 0.001$). Furthermore, the additional inclusion of interaction of Lp(a) with total cholesterol, LDL-C and non-HDL-C provided better risk prediction of poor coronary collaterals. However, no interaction of Lp(a) with HDL-C and triglyceride on coronary collateralization was observed. In patients with acute myocardial infarction, increased Lp(a) in serum was closely correlated with poor coronary collaterals (36). Overexpression of Lp(a) in transgenic mice resulted in markedly reduced natural recovery of blood flow in hindlimb ischemia animal models in a dose-dependent manner. Lp(a) was found to stimulate the growth of vascular smooth muscle, which was reversed by intramuscular injection of hepatocyte growth factor (HGF) (37). Overall, these results highlight that Lp(a) may reflect coronary collateral status.

Lipoprotein(a) is highly concentrated in the arterial wall, carries cholesterol and binds oxidized phospholipids. Elevated circulating Lp(a) inhibits transforming growth factor-β activity and attenuates synthesis and/or release of vascular endothelial growth factor (VEGF) and decreases production of endothelium-derived NO, leading to impaired angiogenesis (33). Moreover, one of our ongoing studies indicates that circulating Lp(a) can also undergo glycation modification. As a characteristic protein of Lp(a), apo(a) is a large protein containing many kringle domains. Based on mass spectrometry results, the glycation modification sites of apo(a) are mainly concentrated on the kringle IV domain, whereas only a few glycation modification sites are distributed in other domains. Phenotypic experiments confirmed that glycated apo(a) and glycated apo(a)-kIV can consistently induce inflammatory factor expression and RAGE pathway activation. In a diabetic mouse model with hindlimb ischemia, intraperitoneal injection of glycated apo(a) and glycated apo(a)-kIV, respectively, resulted in a substantial inhibition of angiogenesis. Further studies have demonstrated that glycated apo(a) and glycated apo(a)-kIV promoted the expression of adhesion molecules, decreased the activities of eNOS and production of NO, and inhibited endothelial proliferation, migration, and tubular formation. Glycated apo(a) and glycated apo(a)-kIV induced endothelial dysfunction mainly through up-regulation of nuclear co-repressor NR0B1, which binds and inhibits the transcriptional activity of cardiovascular protective nuclear receptors such as LXR, NR4A1, and estrogen receptor.

Subnormal high-density lipoprotein cholesterol level and high-density lipoprotein dysfunction

Reduced HDL-C in serum is one of the typical manifestations of diabetic dyslipidemia. The relationship between serum levels of HDL-C and coronary collateral formation remains controversial. In patients with stable CAD,

one study showed that decreased HDL-C levels predicted poor coronary collateralization (38), but such results were not replicated in other studies (35, 39). These observations support a notion that HDL functionality rather than quantity alone may more reliably reflect its overall properties and has a better clinical relevance (40).

High-density lipoprotein particle is composed of an outer layer of apolipoproteins and phospholipids, surrounding a core of esterified cholesterol, and has pluripotent effects. It primarily mediates reverse cholesterol transport by carrying cholesterol from peripheral tissues to the liver for metabolism and excretion. In addition to its antioxidative, anti-inflammatory, antithrombotic and anti-apoptotic features, HDL itself has proangiogenic properties and regulates ischemia-induced angiogenesis in multiple ways (41). Cholesterol efflux capacity of HDL (HDL-CEC) is essential in maintaining cholesterol balance in endothelial cells, and it regulates angiogenesis *via* modulation of lipid rafts and VEGF receptor (VEGFR)-2 signaling (42). Large cohort studies and meta-analysis indicated that elevated HDL-CEC was associated with favorable clinical outcomes independent of circulating HDL-C levels (43). A case-control study reported a higher HDL-CEC in chronic total occlusion patients with good coronary collaterals compared to those with poor collaterals, and high HDL-CEC predicted the presence of good coronary collaterals (44). The degree of coronary collateralization from the contra-lateral vessel (usually *via* connections of the epicardial surface or intraventricular septum) was often visually estimated using the Rentrop grading system (45): 0 = no visible filling of any collateral channel; 1 = filling of side branches of the artery to be perfused by collateral vessels without visualization of epicardial segment; 2 = partially filling of the epicardial artery by collateral vessels; 3 = complete filling of the epicardial artery by collateral vessels. Patients were categorized into poor (grade 0 or 1) or good (grade 2 or 3) coronary collateralization group. This angiographic assessment of coronary collaterals is routinely applied in clinical practice. Wang et al. found that HDL-CEC correlated closely with angiographic Rentrop collateral score in non-diabetic patients, whereas HDL-CEC was impaired in patients with T2DM irrespective of collateralization status. Furthermore, this finding is supported by *in vitro* experimental results, showing that although HDL isolated from non-diabetics with poor collaterals had significantly greater potential in promoting endothelial tubular formation in Matrigel compared to HDL isolated from those with good collateralization, the proangiogenic capacity of HDL isolated from diabetic patients was markedly impaired which was not influenced by collateral conditions (46). These results imply that well-functioning HDL is biologically cardioprotective, contributing to coronary collateral formation. Nevertheless, the functional capacity of HDL is severely compromised in type 2 diabetic patients with CAD.

Glycation and oxidative modification are key underlying mechanisms that lead to HDL dysfunction and transforms the lipoprotein into a proinflammatory protein under diabetic conditions (47). Shen et al found that relative intensity of glycation of apoA-I (a predominant protein moiety in HDL) correlated positively, while HDL-associated paraoxonase (PON) 1 and PON3 activities inversely, with the severity of coronary atherosclerosis (48), and was related to decreased lecithin: cholesterol acyl transferase (LCAT) activity and plaque progression in type 2 diabetic patients undergoing percutaneous coronary intervention (49). Similarly, abundance of apo A-IV glycation was also correlated with the presence and severity of CAD in patients with T2DM. Glycosylated apo A-IV induces pro-inflammatory response *in vitro* and increases the expression of tumor necrosis factor (TNF) -α and adhesion molecules by the nuclear receptor NR4A3, thereby promoting atherosclerosis in apo E-/- mice (50). Recently, the deleterious effects of apo A-I and apo A-IV glycation on vessel growth in diabetes were assessed. In a diabetic hindlimb ischemia mouse model, blood reperfusion was determined by laser Doppler perfusion imaging after treatment with intraperitoneal injection of glycated apo A-I or glycated apo A-IV, and the gastrocnemius and soleus muscles were collected for pathological analysis and molecular biology evaluation. The results showed that both glycated apo A-I and glycated apo A-IV induced inflammatory reactions in endothelial cells and decreased new vessel growth. Further mechanistical studies revealed that glycated apo A-I skewed macrophage polarization toward M1 phenotype by activating SHP2, whereas glycated apo A-IV down-regulated cardiovascular protective nuclear receptor NR4A1 expression (51), all of which are recognized as important steps to the inhibition of angiogenesis. These findings point to a notion that HDL dysfunction and subnormal HDL-C levels act synergistically to decrease collateral formation in T2DM patients with CAD.

Clinical relevance

Since diabetic dyslipidemia hampers collateral vessel growth through inhibiting angiogenesis/arteriogenesis in its specific manner and coronary collateralization is of important clinical significance, the choice between the available management options for T2DM patients with CAD should account for its effect on collateral formation (**Figure 1**).

In terms of non-pharmacological intervention, intensive lifestyle modification (including living habitat change, exercise, and diet) exerts beneficial impact on homeostasis, lipid profiles and coronary collateral circulation (52), thus should be the main initial strategy. Cessation of cigarette smoking is proven to decrease inflammatory response, increase the number and function of EPCs, and improve VEGF activities, which are beneficial for new vessel growth (53). Regular physical

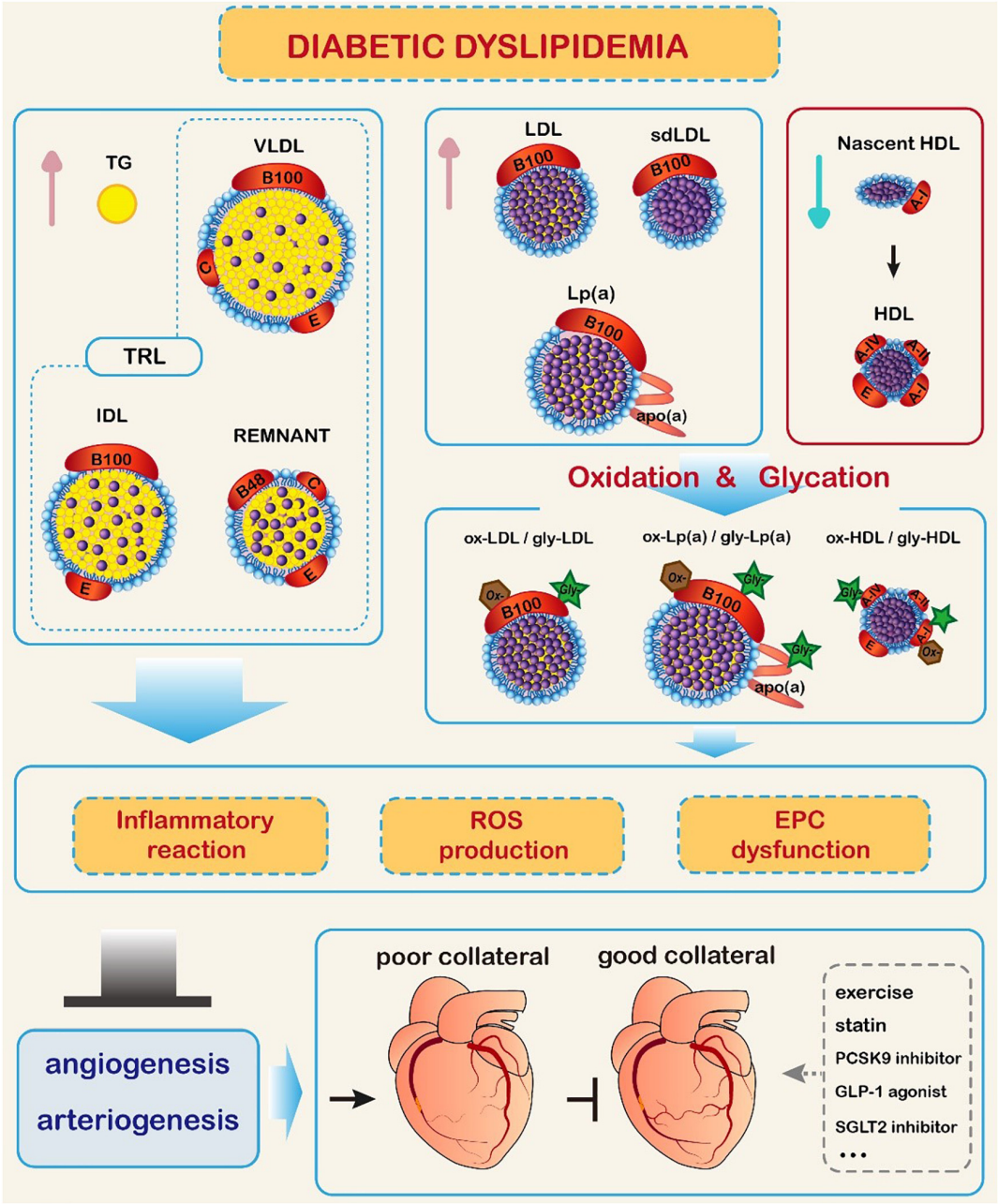


FIGURE 1
Impact of diabetic dyslipidemia on coronary collateral formation. In diabetic conditions, triglyceride (TG) and TG-rich lipoproteins (TRL) levels are increased, high-density lipoprotein cholesterol (HDL-C) level is reduced, and low-density lipoprotein cholesterol (LDL-C) level is elevated with predominance of small dense LDL-C (sdLDL-C). Meanwhile, glycation and oxidative modification of lipoprotein particles occur, promoting inflammatory reaction, production of reactive oxide species (ROS), and endothelial progenitor cell (EPC) dysfunction. These changes hamper collateral formation through inhibiting the process of angiogenesis and arteriogenesis. Exercise, lipid-lowering therapy, and antidiabetic agents may improve coronary collateral formation. ●Represents cholesterol esters. GLP-1: glucagon-like peptide-1; IDL: intermediate density lipoprotein; Lp(a): lipoprotein (a); PCSK9: proprotein convertase subtilisin/kexin type 9; SGLT2: sodium-glucose cotransporter 2; VLDL: very low-density lipoprotein.

exercise improves lipid profile (54), and augments myocardial oxygen demand and blood flow, acting as a driving force for arteriogenesis, which helps in coronary collateral formation in patients with stable CAD, exceeding the effect of any drug treatment (55). Similarly, optimal blood pressure control (especially diastolic blood pressure) is crucial in achieving maximal coronary collateral flow (56, 57). While dietary quality is important for overall health, the total daily caloric intake

per se should be a key determinant of hyperlipidemia in which a hypocaloric plan is favorable for reducing overweight and improving lipid profile and insulin sensitivity (58).

Hypertriglyceridemia should be treated to eliminate residual cardiovascular risk. Fibrates, a putative agonist ligand for peroxisome proliferator activated receptor-alpha, reduce the secretion of very-low-density lipoprotein (VLDL)-triglyceride, enhance removal of LDL, and increase HDL-C levels (59). High-dose of omega-3 polyunsaturated fatty acids (PUFA), mainly eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), is a worthwhile add-on treatment, especially in statin-treated patients with T2DM and CAD in whom triglyceride levels remain elevated (60). In addition, PUFA could attenuate inflammation, improve endothelial function, and decrease thrombus formation (61). The REDUCE-IT trial evaluated a highly purified EPA preparation (4 g/day) in patients with hypertriglyceridemia and high cardiovascular risk. The results were extraordinary, as there was a 25% relative reduction in cardiovascular events, total coronary revascularization as well as plaque burden (62). However, the STRENGTH trial failed to obtain similar favorable results by treatment with EPA/DHA preparation, and in contrast, it was associated with a slightly higher rate of atrial fibrillation (63). This discrepancy may be explained by different study design and various degrees of change in triglyceride-rich lipids as well as differential effects of EPA and DHA on membrane structure, inflammatory biomarkers, endothelial function, and tissue distribution (64). A recent study demonstrated a negative correlation between peri-coronary adipose tissue attenuation assessed by CT angiography and treatment with PUFA, suggesting a lower extent of coronary inflammation (65, 66). Adipokine C1q tumor-necrosis factor-related protein (CTRP) 1 has been shown to be involved in inflammatory reaction and disease development (67). Elevated circulating CTRP1 was associated with poor coronary collateralization in T2DM patients with stable angina pectoris. Notably, stimulation of EPCs with CTRP1 decreases both cord length and branch point number and VEGFR-2 levels (68). Whether the beneficial effect of fibrates alone or in combination with PUFA on collateral formation *via* affecting adipokines in T2DM patients with CAD merits further confirmation.

Cholesterol-lowering therapy is the mainstay in primary and secondary prevention of cardiovascular diseases (69, 70). Statins effectively decrease serum LDL-C and sdLDL-C while increasing HDL-C levels and reduce the susceptibility of apo B of LDL to undergo oxidation and glycation. They also display significant anti-inflammatory properties and improve endothelial function (so-called pleiotropic effects) (71). Robust evidence supports the fact that use of statins enhances angiogenesis as well as arteriogenesis independent of a cholesterol-lowering mechanism (72, 73). Collateral formation benefits from statin treatment in T2DM patients with CAD, due partly to reduced apoptosis and decreased release of soluble VEGFR-1 induced by proinflammatory cytokines (74, 75). Proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitors have been increasingly used in the management of dyslipidemia in individuals with T2DM (76, 77). The results of *post-hoc* subgroup analysis of randomized clinical trials indicated that alirocumab and evolocumab significantly reduced circulating LDL-C and Lp(a), and increased HDL-C, without affecting glycemic levels in patients with T2DM (78, 79). Current data concerning PCSK9 inhibitors on collateral formation are scarce. An *in vivo* study demonstrated a proangiogenic activity of evolocumab through promoting cell proliferation, migration, tubulogenesis, and VEGF secretion (80). Given their unambiguous lipid-lowering properties, such a specific role of PCSK9 inhibitors for neo-angiogenesis should be clinically attractive.

Several new hypoglycemic agents, such as glucagon-like peptide-1 (GLP-1) receptor agonists and sodium-glucose cotransporter-2 (SGLT2) inhibitors, have been shown to favorably affect lipoprotein metabolism (81, 82). Nevertheless, further studies are needed to examine if they can improve collateral formation especially for type 2 diabetic patients with CAD.

Conclusion

In type 2 diabetic patients with CAD, the role of hypertriglyceridemia in collateral formation is not clear likely due to the concomitant changes in other lipoproteins. Elevated circulating cholesterol and Lp(a) and their glycoxidative modification hamper the process of new vessel growth. Subnormal HDL-C levels and, more importantly, deficient HDL function may act synergistically to decrease collateral formation. The choice between the available management options should account for its effect on coronary collateralization. In the future, much more research needs to be done to focus on the benefits of innovative lipid-modifying strategies, including use of PCSK9 and new triglyceride- and Lp(a)-lowering treatment, anti-diabetic agents as well as therapeutic normalization of attenuated proangiogenic and antiatherogenic HDL function, in the improvement of coronary collateral formation and clinical outcome. Novel information as such should add new knowledge on coronary pathophysiology and provide useful guidance of patient care for clinicians.

Author contributions

YS and FD wrote the manuscript, substantially contributed to discussion of the content, and edited the manuscript. YD, XW, and YW researched data for the manuscript. RZ and LL substantially contributed to discussion of the content and

reviewed the manuscript. WS reviewed the manuscript before submission. All authors read and approved the final manuscript.

Funding

This study was supported by the National Natural Science Foundation of China (81870179, 81870357, 81970362, 81970293, 82170417, and 82170423), China Postdoctoral Science Foundation (2018M640408), Shanghai Municipal Education Commission-Gaofeng Clinical Medicine Grant Support (20181801), Technology Transfer Project of Shanghai Jiao Tong University School of Medicine (ZT202103), Medico-engineering Research Project of Shanghai Jiao Tong University (YG2021ZD04), and Shanghai Clinical Research Center for Interventional Medicine (19MC1910300).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Einarson TR, Acs A, Ludwig C, Panton UH. Economic burden of cardiovascular disease in type 2 diabetes: a systematic review. *Value Health.* (2018) 21:881–90. doi: 10.1016/j.jval.2017.12.019

2. Bahiru E, Hsiao R, Phillipson D, Watson KE. Mechanisms and treatment of dyslipidemia in diabetes. *Curr Cardiol Rep.* (2021) 23:26. doi: 10.1007/s11886-021-01455-w

3. Bigler MR, Seiler C. The human coronary collateral circulation, Its extracardiac anastomoses and their therapeutic promotion. *Int J Mol Sci.* (2019) 20:3726. doi: 10.3390/ijms20153726

4. Dai Y, Chang S, Wang S, Shen Y, Li C, Huang Z, et al. The preservation effect of coronary collateral circulation on left ventricular function in chronic total occlusion and its association with the expression of vascular endothelial growth factor A. *Adv Clin Exp Med.* (2020) 29:493–7. doi: 10.17219/acem/104535

5. Shen Y, Aihemaiti M, Shu XY, Yang CD, Chen JW, Dai Y, et al. Circulating chromogranin B is associated with left ventricular functional recovery after successful recanalization of chronic total occlusion. *Front Cardiovasc Med.* (2021) 8:756594. doi: 10.3389/fcvm.2021.756594

6. Zimarino M, D'Andreamatteo M, Waksman R, Epstein SE, De Caterina R. The dynamics of the coronary collateral circulation. *Nat Rev Cardiol.* (2014) 11:191–7. doi: 10.1038/nrcardio.2013.207

7. Yang ZK, Shen Y, Dai Y, Wang XQ, Hu J, Ding FH, et al. Impact of coronary collateralization on long-term clinical outcomes in type 2 diabetic patients after successful recanalization of chronic total occlusion. *Cardiovasc Diabetol.* (2020) 19:59. doi: 10.1186/s12933-020-01033-4

8. Shen Y, Ding FH, Dai Y, Wang XQ, Zhang RY, Lu L, et al. Reduced coronary collateralization in type 2 diabetic patients with chronic total occlusion. *Cardiovasc Diabetol.* (2018) 17:26. doi: 10.1186/s12933-018-0671-6

9. Balakrishnan S, Senthil Kumar B. Factors causing variability in formation of coronary collaterals during coronary artery disease. *Folia Morphol (Warsz).* (2021). doi: 10.5603/FM.a2021.0110

10. Liu T, Wu Z, Liu J, Lv Y, Li W. Metabolic syndrome and its components reduce coronary collateralization in chronic total occlusion: an observational study. *Cardiovasc Diabetol.* (2021) 20:104. doi: 10.1186/s12933-021-01297-4

11. Packard CJ, Boren J, Taskinen MR. Causes and consequences of hypertriglyceridemia. *Front Endocrinol (Lausanne).* (2020) 11:252. doi: 10.3389/fendo.2020.00252

12. di Somma M, Vliora M, Grillo E, Castro B, Dakou E, Schaafsma W, et al. Role of VEGFs in metabolic disorders. *Angiogenesis.* (2020) 23:119–30. doi: 10.1007/s10456-019-09700-1

13. Liu XC, He GD, Lo K, Huang YQ, Feng YQ. The triglyceride-glucose index, an insulin resistance marker, was non-linear associated with all-cause and cardiovascular mortality in the general population. *Front Cardiovasc Med.* (2021) 7:628109. doi: 10.3389/fcvm.2020.628109

14. Guo W, Zhu W, Wu J, Li X, Lu J, Qin P, et al. Triglyceride glucose index is associated with arterial stiffness and 10-year cardiovascular disease risk in a chinese population. *Front Cardiovasc Med.* (2021) 8:585776. doi: 10.3389/fcvm.2021.585776

15. Baykan AO, Gür M, Acele A, Şeker T, Quisi A, Yildirim A, et al. Coronary collateral development and arterial stiffness in patients with chronic coronary total occlusions. *Scand Cardiovasc J.* (2015) 49:228–34. doi: 10.3109/14017431.2015.1062130

16. Gao A, Liu J, Hu C, Liu Y, Zhu Y, Han H, et al. Association between the triglyceride glucose index and coronary collateralization in coronary artery disease patients with chronic total occlusion lesions. *Lipids Health Dis.* (2021) 20:140. doi: 10.1186/s12944-021-01574-x

17. Ouchi G, Komiya I, Taira S, Wakugami T, Ohya Y. Triglyceride/low-density-lipoprotein cholesterol ratio is the most valuable predictor for increased small, dense LDL in type 2 diabetes patients. *Lipids Health Dis.* (2022) 21:4. doi: 10.1186/s12944-021-01612-8

18. Liu GY, Meng XX, Zhang Z. Triglyceride to HDL-cholesterol ratio as an independent risk factor for the poor development of coronary collateral circulation in elderly patients with ST-segment elevation myocardial infarction and acute total occlusion. *Medicine (Baltimore).* (2018) 97:e12587. doi: 10.1097/MD.0000000000012587

19. Fu L, Zhou Y, Sun J, Zhu Z, Xing Z, Zhou S, et al. Atherogenic index of plasma is associated with major adverse cardiovascular events in patients with type 2 diabetes mellitus. *Cardiovasc Diabetol.* (2021) 20:201. doi: 10.1186/s12933-021-01393-5

20. Wu J, Zhou Q, Wei Z, Wei J, Cui M. Atherogenic index of plasma and coronary artery disease in the adult population: a meta-analysis. *Front Cardiovasc Med.* (2021) 8:817441. doi: 10.3389/fcvm.2021.817441

21. Bogachkov YY, Chen L, Le Master E, Fancher IS, Zhao Y, Aguilar V, et al. LDL induces cholesterol loading and inhibits endothelial proliferation and angiogenesis in Matrigels: correlation with impaired angiogenesis during wound healing. *Am J Physiol Cell Physiol.* (2020) 318:C762–76. doi: 10.1152/ajpcell.00495.2018

22. Deindl E, Quax PH. Arteriogenesis and therapeutic angiogenesis in its multiple aspects. *Cells.* (2020) 9:1439. doi: 10.3390/cells9061439

23. Duan J, Murohara T, Ikeda H, Katoh A, Shintani S, Sasaki K, et al. Hypercholesterolemia inhibits angiogenesis in response to hindlimb ischemia: nitric oxide-dependent mechanism. *Circulation.* (2000) 102(19 Suppl. 3):III370–6. doi: 10.1161/01.cir.102.suppl_3.iii-370

24. Tirziu D, Moodie KL, Zhuang ZW, Singer K, Helisch A, Dunn JF, et al. Delayed arteriogenesis in hypercholesterolemic Mice. *Circulation.* (2005) 112:2501–9. doi: 10.1161/CIRCULATIONAHA.105.542829

25. Luo Y, Yan QN, Wu WZ, Luo FY. Decreased count and dysfunction of circulating EPCs in postmenopausal hypercholesterolemic females via reducing

NO production. *Stem Cells Int.* (2018) 2018:2543847. doi: 10.1155/2018/2543847

26. van Weel V, de Vries M, Voshol PJ, Verloop RE, Eilers PHC, van Hinsbergh VWM, et al. Hypercholesterolemia reduces collateral artery growth more dominantly than hyperglycemia or insulin resistance in mice. *Arterioscler Thromb Vasc Biol.* (2006) 26:1383–90. doi: 10.1161/01.ATV.0000219234.78165.85

27. Lin FY, Tsao NW, Shih CM, Lin YW, Yeh JS, Chen JW, et al. The biphasic effects of oxidized-low density lipoprotein on the vasculogenic function of endothelial progenitor cells. *PLoS One.* (2015) 10:e0123971. doi: 10.1371/journal.pone.0123971

28. Jin X, Yang S, Lu J, Wu M. Small, dense low-density lipoprotein-cholesterol and atherosclerosis: relationship and therapeutic strategies. *Front Cardiovasc Med.* (2022) 8:804214. doi: 10.3389/fcvm.2021.804214

29. Ikezaki H, Lim E, Cupples LA, Liu CT, Asztalos BF, Schaefer EJ. Small dense low-density lipoprotein cholesterol is the most atherogenic lipoprotein parameter in the prospective Framingham offspring study. *J Am Heart Assoc.* (2021) 10:e019140. doi: 10.1161/JAHA.120.019140

30. Jin JL, Zhang HW, Cao YX, Liu HH, Hua Q, Li YF, et al. Association of small dense low-density lipoprotein with cardiovascular outcome in patients with coronary artery disease and diabetes: a prospective, observational cohort study. *Cardiovasc Diabetol.* (2020) 19:45. doi: 10.1186/s12933-020-01015-6

31. Huang J, Gu JX, Bao HZ, Li SS, Yao XQ, Yang M, et al. Elevated serum small dense low-density lipoprotein cholesterol may increase the risk and severity of coronary heart disease and predict cardiovascular events in patients with type 2 diabetes mellitus. *Dis Markers.* (2021) 2021:5597028. doi: 10.1155/2021/5597028

32. Zhang Y, Jin JL, Cao YX, Zhang HW, Guo YL, Wu NQ, et al. Lipoprotein (a) predicts recurrent worse outcomes in type 2 diabetes mellitus patients with prior cardiovascular events: a prospective, observational cohort study. *Cardiovasc Diabetol.* (2020) 19:111. doi: 10.1186/s12933-020-01083-8

33. Aras D, Geyik B, Topaloglu S, Ergun K, Ayaz S, Maden O, et al. Serum level of lipoprotein (a) is inversely associated with the development of coronary collateral circulation. *Coron Artery Dis.* (2006) 17:159–63. doi: 10.1097/00019501-200603000-00010

34. Fan Y, Hu JS, Guo F, Lu ZB, Xia H. Lipoprotein(a) as a predictor of poor collateral circulation in patients with chronic stable coronary heart disease. *Braz J Med Biol Res.* (2017) 50:e5979. doi: 10.1590/1414-431X20175979

35. Shen Y, Chen S, Dai Y, Wang XQ, Zhang RY, Yang ZK, et al. Lipoprotein (a) interactions with cholesterol-containing lipids on angiographic coronary collateralization in type 2 diabetic patients with chronic total occlusion. *Cardiovasc Diabetol.* (2019) 18:82. doi: 10.1186/s12933-019-0888-z

36. You XD, Jin JL, Zhang H, Guo N, Hou BJ, Guo YL, et al. Lipoprotein(a) as a marker for predicting coronary collateral circulation in patients with acute myocardial infarction. *Per Med.* (2020) 17:67–78. doi: 10.2217/pme-2018-0127

37. Morishita R, Sakaki M, Yamamoto K, Iguchi S, Aoki M, Yamasaki K, et al. Impairment of collateral formation in lipoprotein(a) transgenic mice: therapeutic angiogenesis induced by human hepatocyte growth factor gene. *Circulation.* (2002) 105:1491–6. doi: 10.1161/01.cir.0000012146.07240.fd

38. Kadi H, Ozyurt H, Ceyhan K, Koc F, Celik A, Burucu T. The relationship between high-density lipoprotein cholesterol and coronary collateral circulation in patients with coronary artery disease. *J Investig Med.* (2012) 60:808–12. doi: 10.2310/JIM.0b013e31824e980c

39. Hsu PC, Su HM, Juo SH, Yen HW, Voon WC, Lai WT, et al. Influence of high-density lipoprotein cholesterol on coronary collateral formation in a population with significant coronary artery disease. *BMC Res Notes.* (2013) 20:105. doi: 10.1186/1756-0500-6-105

40. Soria-Florido MT, Schröder H, Grau M, Fitó M, Lassale C. High density lipoprotein functionality and cardiovascular events and mortality: a systematic review and meta-analysis. *Atherosclerosis.* (2020) 302:36–42. doi: 10.1016/j.atherosclerosis.2020.04.015

41. Rohatgi A, Westerterp M, von Eckardstein A, Remaley A, Rye KA. HDL in the 21st century: a multifunctional roadmap for future HDL research. *Circulation.* (2021) 143:2293–309. doi: 10.1161/CIRCULATIONAHA.120.044221

42. Ebtehaj S, Gruppen EG, Bakker SJL, Dullaart RPF, Tietge UJF. HDL (high-density lipoprotein) cholesterol efflux capacity is associated with incident cardiovascular disease in the general population. *Arterioscler Thromb Vasc Biol.* (2019) 39:1874–83. doi: 10.1161/ATVBAHA.119.312645

43. Lee JJ, Chi G, Fitzgerald C, Kazmi SHA, Kalayci A, Korjian S, et al. Cholesterol efflux capacity and its association with adverse cardiovascular events: a systematic review and meta-analysis. *Front Cardiovasc Med.* (2021) 8:774418. doi: 10.3389/fcvm.2021.774418

44. Lee S, Park JM, Ann SJ, Kang M, Cheon EJ, An DB, et al. Cholesterol efflux and collateral circulation in chronic total coronary occlusion: effect-circ study. *J Am Heart Assoc.* (2021) 10:e019060. doi: 10.1161/JAHA.120.019060

45. Rentrop KP, Cohen M, Blanke H, Phillips RA. Changes in collateral channel filling immediately after controlled coronary artery occlusion by an angioplasty balloon in human subjects. *J Am Coll Cardiol.* (1985) 5:587–92. doi: 10.1016/s0735-1097(85)80380-6

46. Wang XQ, Shen Y, Chen JW, Shu XY, Li C, Yang CD, et al. Relationship of HDL-associated cholesterol efflux capacity to coronary collateralization in diabetic and non-diabetic patients with stable angina and chronic total occlusion. *Circulation* (2019) 140(Suppl. 1):A10762.

47. Ertek S. High-density lipoprotein (HDL) dysfunction and the future of HDL. *Curr Vasc Pharmacol.* (2018) 16:490–8. doi: 10.2174/1570161115666171116164612

48. Shen Y, Ding FH, Sun JT, Pu LJ, Zhang RY, Zhang Q, et al. Association of elevated apo A-I glycation and reduced paraoxonase 1, 3 activity, and their interaction with angiographic severity of coronary artery disease in patients with type 2 diabetes. *Cardiovasc Diabetol.* (2015) 14:52. doi: 10.1186/s12933-015-0221-4

49. Pu LJ, Lu L, Zhang RY, Du R, Shen Y, Zhang Q, et al. Glycation of apoprotein A-I is associated with coronary artery plaque progression in type 2 diabetic patients. *Diabetes Care.* (2013) 36:1312–20. doi: 10.2337/dc12-1411

50. Dai Y, Shen Y, Li QR, Ding FH, Wang XQ, Liu HJ, et al. Association of apolipoprotein A-IV glycation with CAD in T2DM patients: glycated apolipoprotein A-IV induces atherogenesis. *J Am Coll Cardiol.* (2017) 70:2006–19. doi: 10.1016/j.jacc.2017.08.053

51. Zhao Y, Bruemmer D. NR4A orphan nuclear receptors: transcriptional regulators of gene expression in metabolism and vascular biology. *Arterioscler Thromb Vasc Biol.* (2010) 30:1535–41. doi: 10.1161/ATVBAHA.109.191163

52. Möbius-Winkler S, Uhlemann M, Adams V, Sandri M, Erbs S, Lenk K, et al. Coronary collateral growth induced by physical exercise: results of the impact of intensive exercise training on coronary collateral circulation in patients with stable coronary artery disease (EXCITE) trial. *Circulation.* (2016) 133:1438–48; discussion 1448. doi: 10.1161/CIRCULATIONAHA.115.016442

53. Kida N, Nishigaki A, Kakita-Kobayashi M, Tsubokura H, Hashimoto Y, Yoshida A, et al. Exposure to cigarette smoke affects endometrial maturation including angiogenesis and decidualization. *Reprod Med Biol.* (2021) 20:108–18. doi: 10.1002/rmb2.12360

54. Kolwicz SC Jr. An "exercise" in cardiac metabolism. *Front Cardiovasc Med.* (2018) 5:66. doi: 10.3389/fcvm.2018.00066

55. Nickolay T, Nichols S, Ingle L, Hoye A. Exercise training as a mediator for enhancing coronary collateral circulation: a review of the evidence. *Curr Cardiol Rev.* (2020) 16:212–20. doi: 10.2174/1573403X15666190819144336

56. Shen Y, Ding FH, Wu F, Lu L, Zhang RY, Zhang Q, et al. Association of blood pressure and coronary collateralization in type 2 diabetic and nondiabetic patients with stable angina and chronic total occlusion. *J Hypertens.* (2015) 33:621–6; discussion 626. doi: 10.1097/HJH.0000000000000455

57. Shen Y, Dai Y, Wang XQ, Zhang RY, Lu L, Ding FH, et al. Searching for optimal blood pressure targets in type 2 diabetic patients with coronary artery disease. *Cardiovasc Diabetol.* (2019) 18:160. doi: 10.1186/s12933-019-0959-1

58. Ikezaki H, Furusyo N, Yokota Y, Ai M, Asztalos BF, Murata M, et al. Small dense low-density lipoprotein cholesterol and carotid intimal medial thickness progression. *J Atheroscler Thromb.* (2020) 27:1108–22. doi: 10.5551/jat.54130

59. Ida S, Kaneko R, Murata K. Efficacy and safety of pemafibrate administration in patients with dyslipidemia: a systematic review and meta-analysis. *Cardiovasc Diabetol.* (2019) 18:38. doi: 10.1186/s12933-019-0845-x

60. Lazarte J, Hegele RA. Dyslipidemia management in adults with diabetes. *Can J Diabetes.* (2020) 44:53–60. doi: 10.1016/j.jcjd.2019.07.003

61. Bhatt DL, Steg PG, Miller M, Brinton EA, Jacobson TA, Ketchum SB, et al. Cardiovascular risk reduction with icosapent ethyl for hypertriglyceridemia. *N Engl J Med.* (2019) 380:11–22. doi: 10.1056/NEJMoa1812792

62. Sheikh O, Vande Hei AG, Battisha A, Hammad T, Pham S, Chilton R. Cardiovascular, electrophysiologic, and hematologic effects of omega-3 fatty acids beyond reducing hypertriglyceridemia: as it pertains to the recently published REDUCE-IT trial. *Cardiovasc Diabetol.* (2019) 18:84. doi: 10.1186/s12933-019-0887-0

63. Doi T, Langsted A, Nordestgaard BG. A possible explanation for the contrasting results of REDUCE-IT vs. STRENGTH: cohort study mimicking trial designs. *Eur Heart J.* (2021) 42:4807–17. doi: 10.1093/eurheartj/ehab555

64. Pirillo A, Catapano AL. Omega-3 for cardiovascular diseases: where do we stand after REDUCE-IT and STRENGTH? *Circulation.* (2021) 144:183–5. doi: 10.1161/CIRCULATIONAHA.121.053144

65. Bittner DO, Goeller M, Dey D, Zopf Y, Achenbach S, Marwan M. High levels of eicosapentaenoic acid are associated with lower peri-coronary adipose tissue attenuation as measured by coronary CTA. *Atherosclerosis.* (2021) 316:73–8. doi: 10.1016/j.atherosclerosis.2020.10.006

66. Chait A, den Hartigh LJ. Adipose tissue distribution, inflammation and its metabolic consequences, including diabetes and cardiovascular disease. *Front Cardiovasc Med.* (2020) 7:22. doi: 10.3389/fcvm.2020.00022

67. Lu L, Zhang RY, Wang XQ, Liu ZH, Shen Y, Ding FH, et al. C1q/TNF-related protein-1: an adipokine marking and promoting atherosclerosis. *Eur Heart J.* (2016) 37:1762–71. doi: 10.1093/eurheartj/ehv649

68. Shen Y, Lu L, Liu ZH, Wu F, Zhu JZ, Sun Z, et al. Increased serum level of CTRP1 is associated with low coronary collateralization in stable angina patients with chronic total occlusion. *Int J Cardiol.* (2014) 174:203–6. doi: 10.1016/j.ijcard.2014.03.205

69. Rottura M, Scondotto G, Barbieri MA, Sorbara EE, Nasso C, Marino S, et al. Management of high cardiovascular risk in diabetic patients: focus on low density lipoprotein cholesterol and appropriate drug use in general practice. *Front Cardiovasc Med.* (2021) 8:749686. doi: 10.3389/fcvm.2021.749686

70. Tokgozoglu L, Orringer C, Ginsberg HN, Catapono AL. The year in cardiovascular medicine 2021: dyslipidemia. *Eur Heart J.* (2022) 43:807–17. doi: 10.1093/eurheartj/ehab875

71. Jansen-Chaparro S, López-Carmona MD, Cobos-Palacios L, Sanz-Cánovas J, Bernal-López MR, Gómez-Huelgas R. Statins and peripheral arterial disease: a narrative review. *Front Cardiovasc Med.* (2021) 8:777016. doi: 10.3389/fcvm.2021.777016

72. Dehnavi S, Kiani A, Sadeghi M, Biregani AF, Banach M, Atkin SL. Targeting AMPK by statins: a potential therapeutic approach. *Drugs.* (2021) 81:923–33. doi: 10.1007/s40265-021-01510-4

73. Annex BH, Cooke JP. New directions in therapeutic angiogenesis and arteriogenesis in peripheral arterial disease. *Circ Res.* (2021) 128:1944–57. doi: 10.1161/CIRCRESAHA.121.318266

74. Dincer I, Ongun A, Turhan S, Ozdol C, Ertas F, Erol C. Effect of statin treatment on coronary collateral development in patients with diabetes mellitus. *Am J Cardiol.* (2006) 97:772–4. doi: 10.1016/j.amjcard.2005.09.124

75. Gupta M, Tummala R, Ghosh RK, Blumenthal C, Philip K, Bandyopadhyay D, et al. An update on pharmacotherapies in diabetic dyslipidemia. *Prog Cardiovasc Dis.* (2019) 62:334–41. doi: 10.1016/j.pcad.2019.07.006

76. Felekos I, Karamasis GV, Pavlidis AN. PCSK9 inhibitors for the management of dyslipidemia in people with type 2 diabetes: how low is too low? *Curr Pharm Des.* (2021) 27:1008–14. doi: 10.2174/1381612826666200617170252

77. Bardolia C, Amin NS, Turgeon J. Emerging nlon-statin treatment options for lowering low-density ipoprotein cholesterol. *Front Cardiovasc Med.* (2021) 8:789931. doi: 10.3389/fcvm.2021.789931

78. Ray KK, Del Prato S, Müller-Wieland D, Cariou B, Colhoun HM, Tinahones FJ, et al. Alirocumab therapy in individuals with type 2 diabetes mellitus and atherosclerotic cardiovascular disease: analysis of the ODYSSEY DM-DYSLIPIDEMIA and DM-INSULIN studies. *Cardiovasc Diabetol.* (2019) 18:149. doi: 10.1186/s12933-019-0951-9

79. Lorenzatti AJ, Monsalvo ML, López JAG, Wang H, Rosenson RS. Effects of evolocumab in individuals with type 2 diabetes with and without atherogenic dyslipidemia: an analysis from BANTING and BERSON. *Cardiovasc Diabetol.* (2021) 20:94. doi: 10.1186/s12933-021-01287-6

80. Safaeian L, Vaseghi G, Jabari H, Dana N. Evolocumab, a proprotein convertase subtilisin/kexin type 9 inhibitor, promotes angiogenesis in vitro. *Can J Physiol Pharmacol.* (2019) 97:352–8. doi: 10.1139/cjpp-2018-0542

81. Berberich AJ, Hegele RA. Lipid effects of glucagon-like peptide 1 receptor analogs. *Curr Opin Lipidol.* (2021) 32:191–9. doi: 10.1097/MOL.0000000000000750

82. Szekeres Z, Toth K, Szabados E. The Effects of SGLT2 inhibitors on lipid metabolism. *Metabolites.* (2021) 11:87. doi: 10.3390/metabo11020087

frontiers | Frontiers in Cardiovascular Medicine

TYPE Mini Review
PUBLISHED 25 August 2022
DOI 10.3389/fcvm.2022.988698

OPEN ACCESS

EDITED BY
Tatsuya Sawamura,
Shinshu University, Japan

REVIEWED BY
Chiara Macchi,
University of Milan, Italy

*CORRESPONDENCE
Luca Liberale
luca.liberale@unige

SPECIALTY SECTION
This article was submitted to Lipids in Cardiovascular Disease, a section of the journal Frontiers in Cardiovascular Medicine

RECEIVED 07 July 2022
ACCEPTED 08 August 2022
PUBLISHED 25 August 2022

CITATION
Tirandi A, Montecucco F and Liberale L (2022) Physical activity to reduce PCSK9 levels.
Front. Cardiovasc. Med. 9:988698.
doi: 10.3389/fcvm.2022.988698

Physical activity to reduce PCSK9 levels

Amedeo Tirandi[1], Fabrizio Montecucco[1,2] and Luca Liberale[1,2]*

[1]First Clinic of Internal Medicine, Department of Internal Medicine, University of Genoa, Genoa, Italy, [2]IRCCS Ospedale Policlinico San Martino Genoa - Italian Cardiovascular Network, Genoa, Italy

The amount of physical activity (PA) people practice everyday has been reducing in the last decades. Sedentary subjects tend to have an impaired lipid plasma profile with a higher risk of atherosclerosis and related cardio- and cerebrovascular events. Regular PA helps in both primary and secondary cardiovascular prevention because of its beneficial effect on the whole metabolism. Several studies reported lower levels of plasma lipids in trained subjects, but the precise mechanisms by which PA modulates lipoproteins remain only partially described. Thereupon, proprotein convertase subtilisin/kexin type 9 (PCSK9) is a serin protease whose main function is to reduce the amount of low-density lipoprotein cholesterol (LDL-C) receptors, with the direct consequence of reducing LDL-C uptake by the liver and increasing its circulating pool. Accordingly, recently developed PCSK9 inhibitors improved cardiovascular prevention and are increasingly used to reach LDL-C goals in patients at high CV risk. Whether PA can modulate the levels of PCSK9 remains partially explored. Recent studies suggest PA as a negative modulator of such a deleterious CV mediator. Yet the level of evidence is limited. The aim of this review is to summarize the recent reports concerning the regulatory role of PA on PCSK9 plasma levels, highlighting the beneficial role of regular exercise on the prevention of atherosclerosis and overall CV health.

KEYWORDS

proprotein convertase subtilisin/kexin type 9, physical activity, exercise, inflammation, cardiovascular

Introduction

Regular exercise has been recommended to improve both quality and quantity of life in different clinical settings. The importance of fitness for a healthier life is particularly important nowadays, since the modern society tends to underestimate the time spent sitting in front of device screens, with almost two billion of physically inactive subjects worldwide (1). From being nomadic hunter gatherers, we became settled agriculturalists. Nowadays, we tend to spend even less time outdoor with many works are now available online and can be done at home using an internet connection. In this context, the recent severe acute respiratory syndrome coronavirus 2 (SARS-CoV2) pandemic did not help. Epidemiological data report that the total amount of physical activity (PA) has progressively reduced in the last decades (2), meanwhile the number of obese subjects almost tripled

worldwide (3). Obesity is a well-known cardiovascular risk factor and is associated with a high number of comorbidities involving most organs of our bodies.

However, physical activity might be aerobic or anaerobic. The aerobic training consists in exercises involving large group of muscles in a rhythmic and dynamic manner for a long time, while the anaerobic training in a more intense activity, exerted in a shorted time, and involving a restricted group of muscles (4). Even though, the aerobic physical exercise has always been regarded as healthier and therefore preferable, recent evidence shows that both aerobic and anaerobic physical activity may have beneficial effect on the cardiovascular system (4). Regular PA has protective effects against cardiovascular diseases and all-cause mortality (5) and atherosclerosis is negatively modulated by regular exercise (6). Even slight increase of daily amount of PA, in the magnitude of 30 min of light- to vigorous-intensity PA per day can significantly improve cardiovascular health (7). Greater results are obtained with regular high-intensity exercise training (8).

Our body has a greater ability to face PA and starvation periods rather than an excess of caloric intake and sedentarism. We have many hormonal pathways that can mobilize depots and increase circulating glucose and lipid levels, instead the machinery to reduce their levels is rather limited. As a result, the excess of caloric intake associated with low exercise training favor the development of metabolism impairment. The direct consequence is the slowly progression toward the so-called "X syndrome," better known as the metabolic syndrome. This syndrome is associated with several cardiovascular diseases (9) and sudden cardiac death (10). CV prevention therefore finds a cornerstone in strategies aiming at regulating lipid levels by reducing low-density lipoprotein cholesterol (LDL-C) and increasing high-density lipoprotein cholesterol (HDL-C). PA was shown to have beneficial effects on lipid profile (with sex-related differences), especially when coupled with better dietary habits (11, 12). When this is not enough to reach the target lipoprotein levels suggested by the guidelines, pharmacological approaches including statins and PCSK9 inhibitors are suggested. In consideration of the recent paradigm for cholesterol levels "the lower the better," the use of proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitors drugs such as alirocumab and evolocumab to reduce circulating LDL-C in patient at intermediate to very high risk is getting more and more common in the clinical practice.

PCSK9 is a serine protease whose main role is to favorite the catabolism of LDL-C receptor on the hepatocyte surface thereby reducing LDL-C internalization and increasing its circulating pool (13, 14). Reducing the PCSK9 plasma levels therefore leads to reduced circulating cholesterol levels with direct inhibitory effects on the atherosclerotic process. Yet, PCSK9 recently showed pleiotropic, non-LDL-C-mediated, effects that are nowadays known to mediate some of the anti-atherosclerotic effects of PCSK9 inhibitors (i.e., through inflammation) (15, 16). In fact, low grade inflammation is associated with PCSK9 transcription, especially in metabolic syndrome patients (17). Furthermore, inflammation favorite the expression of PCSK9 in the endothelial cells and vascular smooth muscle cells (18).

Whether PCSK9 mediates some of the beneficial effects of PA on plasma lipoproteins remains to be fully investigated. Recent evidence showed that PCSK9 plasma levels are regulated by regular exercise in both animal models and humans, as reported in Table 1. Although, the strength of this association and the possible pathways are not precisely described. The purpose of this review is to highlight the role of exercise training on PCSK9 plasma levels, as a prevention strategy against atherosclerosis. We searched PubMed and Web of Science for the following keywords: "proprotein convertase/subtilisin kexin type," "PCSK9" in combination with "exercise" and "physical activity." Additionally, a list of recent pre-clinical and clinical studies concerning this topic is reported as a table and discussed.

The effect of exercise training on low-density lipoprotein cholesterol

Exercise training consists in a structured and repetitive PA that is practiced regularly. As longtime known, the amount and the quality of exercises and trainings can determine different results ranging from beneficial to even negative results in different settings (29). As a result, one of the major pitfalls in comparing different trials is due to the different type, intensity, and duration of the exercise protocol. Concerning the effect of exercise on cholesterol, several preclinical (30–32) and clinical studies (33–35) found an amelioration of the lipid plasma profile *via* regular exercise, especially aerobic one.

The modulation of circulating LDL-C in trained animals and humans relates to the adaptation of the body toward a higher metabolic state. Accordingly, the effects of exercise can be seen even acutely. Immediately after PA, LDL-C plasma levels decreases (36, 37). After termination, LDL-C levels tend to return to their basal levels, yet with regular exercise they remain lower on the long-term (38). Concerning the abovementioned adaptations, a higher metabolic state associates with a reduction of PCSK9. Consequently, the reduced catabolism of LDL receptor, increases the uptake of circulating LDL thereby reducing circulating cholesterol levels. Accordingly, recent evidence showed that PA increases the amount of hepatic LDL-C receptor (19, 39). Table 1 summarizes the main evidence proving an effect of PA on PCSK9 levels. As most studies agree on a beneficial lowering effect, an augmentation of PCSK9 levels may coexist together with LDL-C reduction in well-trained people (27). Such mixed effect might be due to many limitations in the analysis and interpretation of the studies. Furthermore, the amount of evidence concerning this topic remains quite limited.

TABLE 1 Summary of recent pre-clinical and clinical studies evaluating the effect of exercise training on PCSK9.

Pre-clinical research

Author	Year	Model	Intervention	Findings
Wen et al. (19)	2013	Mouse	AET on treadmill and different types of diet lasting up to 8 weeks	Treadmill exercise increases hepatic PCSK9 mRNA while reducing circulating PCSK9. Reduced lipid levels in mice fed with high-fat diet
Ngo Sock et al. (20)	2014	Rat	Treadmill AET for 8 weeks	Exercise training has no effect on circulating PCSK9 even if it reestablishes the expression of sterol regulatory element binding protein 2
Farahnak et al. (21)	2018	Rat	AET *via* voluntary wheel running for 6 weeks	Increasing of intestinal LDL-R and PCSK9 transcripts in both intact and ovariectomized animals, indicating a possible role in the trans-intestinal cholesterol excretion
Li et al. (22)	2020	Rat	AET on treadmill for 8 weeks	Increase of hepatic LDL-R; inhibition of neointimal formation *via* PCSK9 and LOX-1 reduction
Wolf et al. (23)	2021	Rat	AET *via* voluntary wheel running for 10 months	Exercise favors the expression of PCSK9 in the muscles of normotensive rats without affecting circulating pool.

Clinical

Author	Year	Population	Intervention	Findings
Arsenault et al. (24)	2014	Obese, sedentary men aged between 30 and 65 years old	Moderate AET (160 min/week), more occupational PA, and diet	Modest reduction of PCSK9 levels after 1 year. PCSK9 is slightly associated with insulin resistance but not with LDL-C plasma levels
Kamani et al. (25)	2015	Hospital employees aged more than 18 years old	Use of stairs instead of elevator at workplace for up to 6 months	Reduction of circulating levels of PCSK9 up to 20% at 3rd month but similar levels to the baseline at 6th month
Boyer et al. (26)	2016	Men 39–80 years old undergoing coronary artery bypass graft	150 min/week of PA, and diet program for 1 year	Increment of PCSK9 in relation with fitness and visceral fat mobilization; no LDL-C modification
Sponder at al. (27)	2017	Subjects aged between 30 and 65 years old with at least one cardiovascular risk factor	Moderate-vigorous AET for 8 months, from 75 min/week of high-intensity to 150 min/week of moderate-intensity PA	Reduction of LDL-C levels with increased circulating PCSK9
Makela et al. (28)	2019	Sedentary, pre-diabetic, middle aged patients	AET for 60 min three times in a week for up to 3 months	Reduction of PCSK9 plasma levels even though low intensity PA seems not to influence PCSK9 levels

AET, aerobic exercise training; LDL-r, low-density lipoprotein cholesterol receptor; LOX-1, low-density lipoprotein receptor-1; mRNA, messenger ribonucleic acid; PCSK9, proprotein convertase subtilisin/kexin type 9.

Mediators of PCSK9 modulation in trained subjects

Trained people tend to show higher amount of lean mass and lower levels of visceral fat (40–42). As known today, visceral fat is not only a storage tissue, but it plays a crucial role in the pathophysiology of different diseases (e.g., atherosclerosis) by releasing several mediators with local and systemic effects (i.e., adipocytokines) (Figure 1).

The hypertrophic visceral adipose tissue of obese patients undergoes degenerative remodeling including hypoxia and macrophage infiltration, favoring the development of inflammation with direct effects on the quality and quantity of released adipocytokines. The reduction of atheroprotective

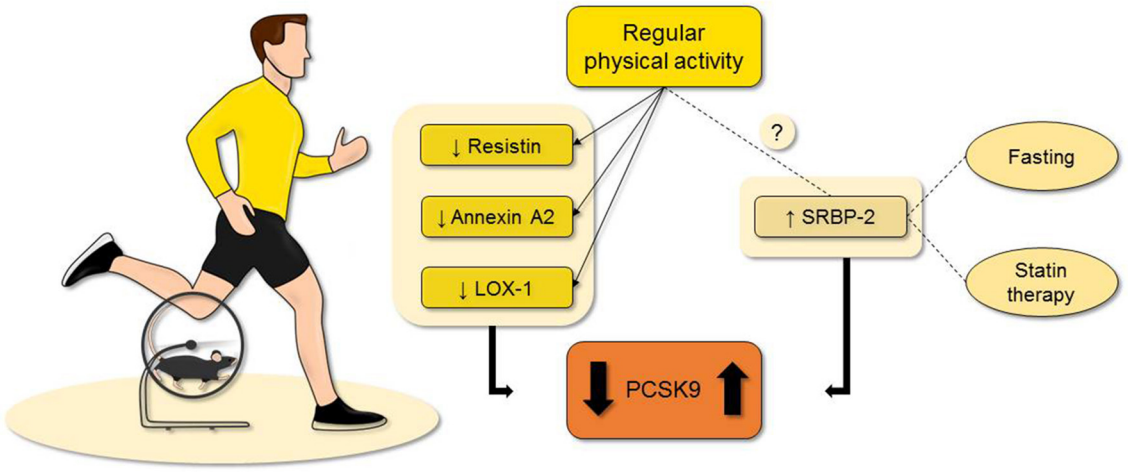


FIGURE 1
Evidence suggest that regular physical activity can regulate the PCSK9 plasma levels even though few evidence is available at the moment. As possible explanations, the reduction of resistin, annexin A2, and LOX-1 are reported with the most concrete results. Although, PCSK9 can be also augmented in trained subjects. This is probably related to a higher metabolism, a possible undiscovered mechanism, or confounding factors. While it is less known if exercise training affects the plasma levels of SREBP2, both fasting and statin treatment represent two confounding factors when evaluating the possible role of physical activity on PCSK9 levels. LOX-1, lectin-type oxidized low-density lipoprotein receptor-1; Proprotein convertase subtilisin/kexin type 9; SREBP2, sterol regulatory element-binding protein 2.

adiponectin (43) and the increased levels of resistin facilitate lipolysis with direct effects on vessel health (44). As exercise was shown to reduce resistin levels (45, 46), such adipokine can be a further link between exercise and PCSK9 since resistin was reported to increase the expression of PCSK9 in hepatocyte thereby modulating LDLR levels and indirectly atherogenesis (47). Furthermore, activation of adiponectin receptors was shown to regulate PCSK9 expression in experimental model of atherosclerosis with direct impact on the disease burden (48). Also, leptin interferes with PCSK9 expression *via* the activation of STAT3 and p38MAPK pathways (49, 50). Among, adipocytokines, fibroblast growth factor 21 is a peptide implied in fatty acids and glucose metabolism (51) under both physiological and pathological conditions including obesity and metabolic syndrome (52). Acute exercise was shown to increase the circulating levels of fibroblast growth factor 21 (53), which in turn impairs the expression of PCSK9 *via* the suppression of the hepatic sterol regulatory element binding protein 2 (54). Expressed by a variety of cell types including adipocytes, annexin A2 is an anionic phospholipid-binding proteins of the Ca^{2+} dependent family that was reported to reduce PCSK9 levels by favoring the modification of its catalytic subunit (55–57). Of interest, annexin A2 levels are elevated in people who regularly practice exercise (25), thereby indicating another possible molecular link.

Recently described as an important mediator of atherogenesis, lectin-type oxidized low-density lipoprotein receptor-1(LOX-1) may be another mediator of the effect of PA on PCSK9 levels. LOX-1 is a scavenger receptor with important function in oxidized LDL-C uptake by endothelial cells (58). PCSK9 and LOX-1 are both involved in the atherosclerotic process (59) and recent evidence showed that PCSK9 and LOX-1 influence each other in the vascular tissue (18, 60) and are co-related to the atheroma formation. Pre-clinical evidence showed that PA can reduce cholesterol accumulation in atherosclerotic plaques *via* the reduction of LOX-1 gene expression (61). Both PCSK9 and LOX-1 are reduced upon exercise (22).

Sterol regulatory element binding protein 2 (SREBP2) is a molecule implicated in the synthesis of cholesterol (62) as well as a transcriptional activator of PCSK9 (63, 64). For such reason, SREBP2 is often used to explain the paradoxical association of elevated PCSK9 levels and reduced LDL-C that is seen during fasting periods.

Even though it is not clear whether PA can modulate SREBP2 levels, regular exercise reduces the expression of several enzymes that regulates lipid metabolism in animal models (65, 66). The reduction of SREBP2 levels would end in lower PCSK9 plasma levels, as it happens during fasting (67, 68). Indeed, few days of fasting reduce the amount of circulating PCSK9 but with the unexpected results of increasing the quantity of circulating LDL-C (69).

Furthermore, regular PA can augment the circulating levels of interferon gamma, a cytokine with critical role in regulation of immune system, as showed in both pre-clinical (70) and clinical (71, 72) settings. Recent studies suggested a role for interferon gamma in PCSK9 regulation (73).

An excess of body fat is related to several detrimental is associated with higher risk of insulin resistance (74), and insulin resistance is one of the key element of the metabolic syndrome. Also, higher levels of insulin are associated with a higher expression of PCSK9 *via* sterol regulatory element binding protein 1c (SREBP-1c) (17). On the other hand, regular physical activity can improve insulin sensitivity (75–77).

Pitfalls in determining the effect of PA on PCSK9

First of all, the variations of LDL-C circulating levels can either be a consequence or a cause of PCSK9 variations. In fact, when PCSK9 modulate the amount of LDL-C also LDL-C can directly bind the PCSK9 molecule, causing a direct impairment of its functionality (78). Furthermore, many limitations reside in the evaluation of circulating levels of this mediator as it is not clear whether this is a fair counterpart of its hepatic levels. Also, as most studies investigate the levels of this molecule, they might not directly reflect its activity. Under this point of view, the presence of studies showing augmentation of PCSK9 along with reduction of LDL-C circulating levels might be explained by different PCSK9 functionality. Furthermore, most of the analytic method for detecting PCSK9 use antibodies binding to its mature form. However, the activity of PCSK9 resides in its catalytic processes (79).

As previously mentioned, another great limitation resides in the difficult standardization of exercise protocols. Also, with regard to clinical research, sample sizes are generally small, and some studies has non-negligible unbalanced gender and/or comorbidities differences. The interpretation of the relatively small number of recent clinical trials is also hampered by the fact that often they use holistic interventional program with prescription of both PA and diet. Lastly, the concomitant use of statin therapy can interfere with the interpretation of the results. In fact, statin treatment augments the amount of circulating PCSK9 (80–82), probably because they increase the levels of SREBP2 (83).

Conclusions

Regular exercise is known to ameliorate lipid profile and represent the cornerstone of cardiovascular prevention. Patient that practice regular exercise show a non-negligible reduced risk of developing atherosclerosis and possible cardiovascular events. The relationship between PCSK9 and PA can be a possible explanation with exercise training envisaging a non-pharmacological PCSK9 inhibitor. Indeed, the reduction of adiposity and molecules like LOX-1, annexin A2, fibroblast growth factor 21, and resistin secondary to exercise favor the reduction of PCSK9 in the bloodstream. Yet, at present, several limitations impact on the interpretation of the results including the difficult standardization of exercise protocols.

Author contributions

AT conceived and drafted the manuscript. FM and LL revised it for important intellectual content. All authors read and approved its final version.

Funding

LL received a research grant from the Swiss Heart Foundation.

Conflict of interest

Author LL is co-inventor on the International Patent WO/2020/226993 filed in April 2020; the patent relates to the use of antibodies which specifically bind interleukin-1α to reduce various sequelae of ischemia-reperfusion injury to the central nervous system.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Hallal PC, Andersen LB, Bull FC, Guthold R, Haskell W, Ekelund U, et al. Global physical activity levels: surveillance progress, pitfalls, and prospects. *Lancet.* (2012) 380:247–57. doi: 10.1016/S0140-6736(12)60646-1

2. Archer E, Lavie CJ, McDonald SM, Thomas DM, Hebert JR, Taverno Ross SE, et al. Maternal inactivity: 45-year trends in mothers' use of time. *Mayo Clin Proc.* (2013) 88:1368–77. doi: 10.1016/j.mayocp.2013.09.009

3. NCD Risk Factor Collaboration. Worldwide trends in body-mass index, underweight, overweight, and obesity from 1975 to 2016: a pooled analysis of 2416 population-based measurement studies in 128.9 million children, adolescents, and adults. *Lancet.* (2017) 390:2627–42. doi: 10.1016/S0140-6736(17)32129-3

4. Patel H, Alkhawam H, Madanieh R, Shah N, Kosmas CE, Vittorio TJ. Aerobic vs anaerobic exercise training effects on the cardiovascular system. *World J Cardiol.* (2017) 9:134–8. doi: 10.4330/wjc.v9.i2.134

5. Ekblom-Bak E, Halldin M, Vikstrom M, Stenling A, Gigante B, de Faire U, et al. Physical activity attenuates cardiovascular risk and mortality in men and women with and without the metabolic syndrome - a 20-year follow-up of a population-based cohort of 60-year-olds. *Eur J Prev Cardiol.* (2021) 28:1376–85. doi: 10.1177/2047487320916596

6. Lazaros G, Oikonomou E, Vogiatzi G, Christoforatou E, Tsalamandris S, Goliopoulou A, et al. The impact of sedentary behavior patterns on carotid atherosclerotic burden: implications from the corinthia epidemiological study. *Atherosclerosis.* (2019) 282:154–61. doi: 10.1016/j.atherosclerosis.2019.01.026

7. German C, Makarem N, Fanning J, Redline S, Elfassy T, McClain A, et al. Sleep, sedentary behavior, physical activity, and cardiovascular health: MESA. *Med Sci Sports Exerc.* (2021) 53:724–31. doi: 10.1249/MSS.0000000000002534

8. Karlsen T, Aamot IL, Haykowsky M, Rognmo O. High intensity interval training for maximizing health outcomes. *Prog Cardiovasc Dis.* (2017) 60:67–77. doi: 10.1016/j.pcad.2017.03.006

9. Tune JD, Goodwill AG, Sassoon DJ, Mather KJ. Cardiovascular consequences of metabolic syndrome. *Transl Res.* (2017) 183:57–70. doi: 10.1016/j.trsl.2017.01.001

10. Tirandi A, Carbone F, Montecucco F, Liberale L. The role of metabolic syndrome in sudden cardiac death risk: recent evidence and future directions. *Eur J Clin Invest.* (2022) 52:e13693. doi: 10.1111/eci.13693

11. Skoumas J, Pitsavos C, Panagiotakos DB, Chrysohoou C, Zeimbekis A, Papaioannou I, et al. Physical activity, high density lipoprotein cholesterol and other lipids levels, in men and women from the ATTICA study. *Lipids Health Dis.* (2003) 2:3. doi: 10.1186/1476-511X-2-3

12. Pitanga FJG, Griep RH, Almeida MDC, Fonseca M, Souza AR, Silva RC, et al. Association between leisure time physical activity and HDL-C in the elsa-brasil study participants: are there any gender differences in the dose-response effect? *Arq Bras Cardiol.* (2021) 117:494–500. doi: 10.36660/abc.20200438

13. Glerup S, Schulz R, Laufs U, Schluter KD. Physiological and therapeutic regulation of PCSK9 activity in cardiovascular disease. *Basic Res Cardiol.* (2017) 112:32. doi: 10.1007/s00395-017-0619-0

14. Luquero A, Badimon L, Borrell-Pages M. PCSK9 functions in atherosclerosis are not limited to plasmatic LDL-cholesterol regulation. *Front Cardiovasc Med.* (2021) 8:639727. doi: 10.3389/fcvm.2021.639727

15. Giunzioni I, Tavori H, Covarrubias R, Major AS, Ding L, Zhang Y, et al. Local effects of human PCSK9 on the atherosclerotic lesion. *J Pathol.* (2016) 238:52–62. doi: 10.1002/path.4630

16. Macchi C, Ferri N, Sirtori CR, Corsini A, Banach M, Ruscica M. Proprotein convertase subtilisin/kexin type 9: a view beyond the canonical cholesterol-lowering impact. *Am J Pathol.* (2021) 191:1385–97. doi: 10.1016/j.ajpath.2021.04.016

17. Ferri N, Ruscica M. Proprotein convertase subtilisin/kexin type 9 (PCSK9) and metabolic syndrome: insights on insulin resistance, inflammation, and atherogenic dyslipidemia. *Endocrine.* (2016) 54:588–601. doi: 10.1007/s12020-016-0939-0

18. Ding Z, Pothineni NVK, Goel A, Luscher TF, Mehta JL. PCSK9 and inflammation: role of shear stress, pro-inflammatory cytokines, and LOX-1. *Cardiovasc Res.* (2020) 116:908–15. doi: 10.1093/cvr/cvz313

19. Wen S, Jadhav KS, Williamson DL, Rideout TC. Treadmill exercise training modulates hepatic cholesterol metabolism and circulating PCSK9 concentration in high-fat-fed mice. *J Lipids.* (2013) 2013:908048. doi: 10.1155/2013/908048

20. Ngo Sock ET, Chapados NA, Lavoie JM. LDL receptor and Pcsk9 transcripts are decreased in liver of ovariectomized rats: effects of exercise training. *Horm Metab Res.* (2014) 46:550–5. doi: 10.1055/s-0034-1370910

21. Farahnak Z, Chapados N, Lavoie JM. Exercise training increased gene expression of LDL-R and PCSK9 in intestine: link to transintestinal cholesterol excretion. *Gen Physiol Biophys.* (2018) 37:309–17. doi: 10.4149/gpb_2017047

22. Li W, Park H, Guo E, Jo W, Sim KM, Lee SK. Aerobic exercise training inhibits neointimal formation via reduction of PCSK9 and LOX-1 in atherosclerosis. *Biomedicines.* (2020) 8:92. doi: 10.3390/biomedicines8040092

23. Wolf A, Kutsche HS, Atmanspacher F, Karadedeli MS, Schreckenberg R, Schluter KD. Untypical metabolic adaptations in spontaneously hypertensive rats to free running wheel activity includes uncoupling protein-3 (UCP-3) and proprotein convertase subtilisin/kexin type 9 (PCSK9) expression. *Front Physiol.* (2021) 12:598723. doi: 10.3389/fphys.2021.598723

24. Arsenault BJ, Pelletier-Beaumont E, Almeras N, Tremblay A, Poirier P, Bergeron J, et al. PCSK9 levels in abdominally obese men: association with cardiometabolic risk profile and effects of a one-year lifestyle modification program. *Atherosclerosis.* (2014) 236:321–6. doi: 10.1016/j.atherosclerosis.2014.07.010

25. Kamani CH, Gencer B, Montecucco F, Courvoisier D, Vuilleumier N, Meyer P, et al. Stairs instead of elevators at the workplace decreases PCSK9 levels in a healthy population. *Eur J Clin Invest.* (2015) 45:1017–24. doi: 10.1111/eci.12480

26. Boyer M, Levesque V, Poirier P, Marette A, Mathieu P, Despres JP, et al. Impact of a 1-year lifestyle modification program on plasma lipoprotein and PCSK9 concentrations in patients with coronary artery disease. *J Clin Lipidol.* (2016) 10:1353–61. doi: 10.1016/j.jacl.2016.08.014

27. Sponder M, Campean IA, Dalos D, Emich M, Fritzer-Szekeres M, Litschauer B, et al. Effect of long-term physical activity on PCSK9, high- and low-density lipoprotein cholesterol, and lipoprotein(a) levels: a prospective observational trial. *Pol Arch Intern Med.* (2017) 127:506–11. doi: 10.1093/eurheartj/ehx502.P1516

28. Makela KA, Leppaluoto J, Jokelainen J, Jamsa T, Keinanen-Kiukaanniemi S, Herzig KH. Effect of physical activity on plasma PCSK9 in subjects with high risk for type 2 diabetes. *Front Physiol.* (2019) 10:456. doi: 10.3389/fphys.2019.00456

29. Halbert JA, Silagy CA, Finucane P, Withers RT, Hamdorf PA. Exercise training and blood lipids in hyperlipidemic and normolipidemic adults: a meta-analysis of randomized, controlled trials. *Eur J Clin Nutr.* (1999) 53:514–22. doi: 10.1038/sj.ejcn.1600784

30. Heeren MV, De Sousa LE, Mostarda C, Moreira E, Machert H, Rigatto KV, et al. Exercise improves cardiovascular control in a model of dislipidemia and menopause. *Maturitas.* (2009) 62:200–4. doi: 10.1016/j.maturitas.2008.12.011

31. Meissner M, Lombardo E, Havinga R, Tietge UJ, Kuipers F, Groen AK. Voluntary wheel running increases bile acid as well as cholesterol excretion and decreases atherosclerosis in hypercholesterolemic mice. *Atherosclerosis.* (2011) 218:323–9. doi: 10.1016/j.atherosclerosis.2011.06.040

32. Donatto FF, Neves RX, Rosa FO, Camargo RG, Ribeiro H, Matos-Neto EM, et al. Resistance exercise modulates lipid plasma profile and cytokine content in the adipose tissue of tumour-bearing rats. *Cytokine.* (2013) 61:426–32. doi: 10.1016/j.cyto.2012.10.021

33. Knight S, Bermingham MA, Mahajan D. Regular non-vigorous physical activity and cholesterol levels in the elderly. *Gerontology.* (1999) 45:213–9. doi: 10.1159/000022090

34. Rigla M, Sanchez-Quesada JL, Ordonez-Llanos J, Prat T, Caixas A, Jorba O, et al. Effect of physical exercise on lipoprotein(a) and low-density lipoprotein modifications in type 1 and type 2 diabetic patients. *Metabolism.* (2000) 49:640–7. doi: 10.1016/S0026-0495(00)80041-4

35. Ruiz-Ramie JJ, Barber JL, Sarzynski MA. Effects of exercise on HDL functionality. *Curr Opin Lipidol.* (2019) 30:16–23. doi: 10.1097/MOL.0000000000000568

36. Ferguson MA, Alderson NL, Trost SG, Davis PG, Mosher PE, Durstine JL. Plasma lipid and lipoprotein responses during exercise. *Scand J Clin Lab Invest.* (2003) 63:73–9. doi: 10.1080/00365510310000510

37. Rahnama N, Younesian A, Mohammadion M, Bambaeichi E. A 90 minute soccer match decreases triglyceride and low density lipoprotein but not high-density lipoprotein and cholesterol levels. *J Res Med Sci.* (2009) 14:335–41.

38. Kujala UM, Ahotupa M, Vasankari T, Kaprio J, Tikkanen MJ. Low LDL oxidation in veteran endurance athletes. *Scand J Med Sci Sports.* (1996) 6:303–8. doi: 10.1111/j.1600-0838.1996.tb00475.x

39. Pinto PR, Rocco DD, Okuda LS, Machado-Lima A, Castilho G, da Silva KS, et al. Aerobic exercise training enhances the in vivo cholesterol trafficking from macrophages to the liver independently of changes in the expression of genes involved in lipid flux in macrophages and aorta. *Lipids Health Dis.* (2015) 14:109. doi: 10.1186/s12944-015-0093-3

40. Kay SJ, Fiatarone Singh MA. The influence of physical activity on abdominal fat: a systematic review of the literature. *Obes Rev.* (2006) 7:183–200. doi: 10.1111/j.1467-789X.2006.00250.x

41. Dahl-Petersen IK, Brage S, Bjerregaard P, Tolstrup JS, Jorgensen ME. Physical activity and abdominal fat distribution in greenland. *Med Sci Sports Exerc.* (2017) 49:2064–70. doi: 10.1249/MSS.0000000000001337

42. Jung HC, Jeon S, Lee NH, Kim K, Kang M, Lee S. Effects of exercise intervention on visceral fat in obese children and adolescents. *J Sports Med Phys Fitness.* (2019) 59:1045–57. doi: 10.23736/S0022-4707.18.08935-1

43. Neeland IJ, Ross R, Despres JP, Matsuzawa Y, Yamashita S, Shai I, et al. Visceral and ectopic fat, atherosclerosis, and cardiometabolic

disease: a position statement. *Lancet Diabetes Endocrinol.* (2019) 7:715–25. doi: 10.1016/S2213-8587(19)30084-1

44. Neeland IJ, Poirier P, Despres JP. Cardiovascular and metabolic heterogeneity of obesity: clinical challenges and implications for management. *Circulation.* (2018) 137:1391–406. doi: 10.1161/CIRCULATIONAHA.117.029617

45. Kadoglou NP, Perrea D, Iliadis F, Angelopoulou N, Liapis C, Alevizos M. Exercise reduces resistin and inflammatory cytokines in patients with type 2 diabetes. *Diabetes Care.* (2007) 30:719–21. doi: 10.2337/dc06-1149

46. Jung SH, Park HS, Kim KS, Choi WH, Ahn CW, Kim BT, et al. Effect of weight loss on some serum cytokines in human obesity: increase in IL-10 after weight loss. *J Nutr Biochem.* (2008) 19:371–5. doi: 10.1016/j.jnutbio.2007.05.007

47. Melone M, Wilsie L, Palyha O, Strack A, Rashid S. Discovery of a new role of human resistin in hepatocyte low-density lipoprotein receptor suppression mediated in part by proprotein convertase subtilisin/kexin type 9. *J Am Coll Cardiol.* (2012) 59:1697–705. doi: 10.1016/j.jacc.2011.11.064

48. Sun L, Yang X, Li Q, Zeng P, Liu Y, Liu L, et al. Activation of adiponectin receptor regulates proprotein convertase subtilisin/kexin type 9 expression and inhibits lesions in ApoE-deficient mice. *Arterioscler Thromb Vasc Biol.* (2017) 37:1290–300. doi: 10.1161/ATVBAHA.117.309630

49. Du Y, Li S, Cui CJ, Zhang Y, Yang SH, Li JJ. Leptin decreases the expression of low-density lipoprotein receptor via PCSK9 pathway: linking dyslipidemia with obesity. *J Transl Med.* (2016) 14:276. doi: 10.1186/s12967-016-1032-4

50. Macchi C, Greco MF, Botta M, Sperandeo P, Dongiovanni P, Valenti L, et al. Leptin, resistin, and proprotein convertase subtilisin/kexin type 9: the role of STAT3. *Am J Pathol.* (2020) 190:2226–36. doi: 10.1016/j.ajpath.2020.07.016

51. Fisher FM, Maratos-Flier E. Understanding the physiology of FGF21. *Annu Rev Physiol.* (2016) 78:223–41. doi: 10.1146/annurev-physiol-021115-105339

52. Xie T, Leung PS. Fibroblast growth factor 21: a regulator of metabolic disease and health span. *Am J Physiol Endocrinol Metab.* (2017) 313:E292–302. doi: 10.1152/ajpendo.00101.2017

53. Khalafi M, Alamdari KA, Symonds ME, Nobari H, Carlos-Vivas J. Impact of acute exercise on immediate and following early post-exercise FGF-21 concentration in adults: systematic review and meta-analysis. *Hormones.* (2021) 20:23–33. doi: 10.1007/s42000-020-00245-3

54. Huang Z, Xu A, Cheung BMY. The potential role of fibroblast growth factor 21 in lipid metabolism and hypertension. *Curr Hypertens Rep.* (2017) 19:28. doi: 10.1007/s11906-017-0730-5

55. Ly K, Saavedra YG, Canuel M, Routhier S, Desjardins R, Hamelin J, et al. Annexin A2 reduces PCSK9 protein levels via a translational mechanism and interacts with the M1 and M2 domains of PCSK9. *J Biol Chem.* (2014) 289:17732–46. doi: 10.1074/jbc.M113.541094

56. Mayer G, Poirier S, Seidah NG. Annexin A2 is a C-terminal PCSK9-binding protein that regulates endogenous low density lipoprotein receptor levels. *J Biol Chem.* (2008) 283:31791–801. doi: 10.1074/jbc.M805971200

57. Seidah NG, Poirier S, Denis M, Parker R, Miao B, Mapelli C, et al. Annexin A2 is a natural extrahepatic inhibitor of the PCSK9-induced LDL receptor degradation. *PLoS ONE.* (2012) 7:e41865. doi: 10.1371/journal.pone.0041865

58. Pirillo A, Norata GD, Catapano AL. LOX-1, OxLDL, and atherosclerosis. *Mediators Inflamm.* (2013) 2013:152786. doi: 10.1155/2013/152786

59. Tam J, Thankam F, Agrawal DK, Radwan MM. Critical role of LOX-1-PCSK9 axis in the pathogenesis of atheroma formation and its instability. *Heart Lung Circ.* (2021) 30:1456–66. doi: 10.1016/j.hlc.2021.05.085

60. Ding Z, Liu S, Wang X, Deng X, Fan Y, Shahanawaz J, et al. Cross-talk between LOX-1 and PCSK9 in vascular tissues. *Cardiovasc Res.* (2015) 107:556–67. doi: 10.1093/cvr/cvv178

61. Pinto PR, da Silva KS, Iborra RT, Okuda LS, Gomes-Kjerulf D, Ferreira GS, et al. Exercise training favorably modulates gene and protein expression that regulate arterial cholesterol content in CETP transgenic mice. *Front Physiol.* (2018) 9:502. doi: 10.3389/fphys.2018.00502

62. Weber LW, Boll M, Stampfl A. Maintaining cholesterol homeostasis: sterol regulatory element-binding proteins. *World J Gastroenterol.* (2004) 10:3081–7. doi: 10.3748/wjg.v10.i21.3081

63. Horton JD, Shah NA, Warrington JA, Anderson NN, Park SW, Brown MS, et al. Combined analysis of oligonucleotide microarray data from transgenic and knockout mice identifies direct SREBP target genes. *Proc Natl Acad Sci USA.* (2003) 100:12027–32. doi: 10.1073/pnas.1534923100

64. Jeong HJ, Lee HS, Kim KS, Kim YK, Yoon D, Park SW. Sterol-dependent regulation of proprotein convertase subtilisin/kexin type 9 expression by sterol-regulatory element binding protein-2. *J Lipid Res.* (2008) 49:399–409. doi: 10.1194/jlr.M700443-JLR200

65. Griffiths MA, Fiebig R, Gore MT, Baker DH, Esser K, Oscai L, et al. Exercise down-regulates hepatic lipogenic enzymes in food-deprived and refed rats. *J Nutr.* (1996) 126:1959–71.

66. Fiebig RG, Hollander JM, Ji LL. Exercise down-regulates hepatic fatty acid synthase in streptozotocin-treated rats. *J Nutr.* (2001) 131:2252–9. doi: 10.1093/jn/131.9.2252

67. Horton JD, Bashmakov Y, Shimomura I, Shimano H. Regulation of sterol regulatory element binding proteins in livers of fasted and refed mice. *Proc Natl Acad Sci USA.* (1998) 95:5987–92. doi: 10.1073/pnas.95.11.5987

68. Persson L, Cao G, Stahle L, Sjoberg BG, Troutt JS, Konrad RJ, et al. Circulating proprotein convertase subtilisin kexin type 9 has a diurnal rhythm synchronous with cholesterol synthesis and is reduced by fasting in humans. *Arterioscler Thromb Vasc Biol.* (2010) 30:2666–72. doi: 10.1161/ATVBAHA.110.214130

69. Browning JD, Horton JD. Fasting reduces plasma proprotein convertase, subtilisin/kexin type 9 and cholesterol biosynthesis in humans. *J Lipid Res.* (2010) 51:3359–63. doi: 10.1194/jlr.P009860

70. Heidarianpour A, Vahidian Rezazadeh M, Zamani A. Effect of moderate exercise on serum interferon-gamma and interleukin-17 levels in the morphine withdrawal period. *Int J High Risk Behav Addict.* (2016) 5:e26907. doi: 10.5812/ijhrba.26907

71. Vijayaraghava A, K R. Alteration of interferon gamma (IFN-gamma) in human plasma with graded physical activity. *J Clin Diagn Res.* (2014) 8:BC05–7. doi: 10.7860/JCDR/2014/9502.4440

72. Zamani A, Salehi I, Alahgholi-Hajibehzad M. Moderate exercise enhances the production of interferon-gamma and interleukin-12 in peripheral blood mononuclear cells. *Immune Netw.* (2017) 17:186–91. doi: 10.4110/in.2017.17.3.186

73. Pirault J, Polyzos KA, Petri MH, Ketelhuth DFJ, Back M, Hansson GK. The inflammatory cytokine interferon-gamma inhibits sortilin-1 expression in hepatocytes via the JAK/STAT pathway. *Eur J Immunol.* (2017) 47:1918–24. doi: 10.1002/eji.201646768

74. Patel P, Abate N. Body fat distribution and insulin resistance. *Nutrients.* (2013) 5:2019–27. doi: 10.3390/nu5062019

75. Yamanouchi K, Nakajima H, Shinozaki T, Chikada K, Kato K, Oshida Y, et al. Effects of daily physical activity on insulin action in the elderly. *J Appl Physiol.* (1992) 73:2241–5. doi: 10.1152/jappl.1992.73.6.2241

76. Macias-Cervantes MH, Malacara JM, Garay-Sevilla ME, Diaz-Cisneros FJ. Effect of recreational physical activity on insulin levels in Mexican/Hispanic children. *Eur J Pediatr.* (2009) 168:1195–202. doi: 10.1007/s00431-008-0907-7

77. Cai C, Zhang Z, McDonald S, Strom C, Skow RJ, May LE, et al. Leisure-Time physical activity before and during pregnancy is associated with improved insulin resistance in late pregnancy. *Int J Environ Res Public Health.* (2021) 18:4413. doi: 10.3390/ijerph18094413

78. Kosenko T, Golder M, Leblond G, Weng W, Lagace TA. Low density lipoprotein binds to proprotein convertase subtilisin/kexin type-9 (PCSK9) in human plasma and inhibits PCSK9-mediated low density lipoprotein receptor degradation. *J Biol Chem.* (2013) 288:8279–88. doi: 10.1074/jbc.M112.421370

79. Farnier M. The role of proprotein convertase subtilisin/kexin type 9 in hyperlipidemia: focus on therapeutic implications. *Am J Cardiovasc Drugs.* (2011) 11:145–52. doi: 10.2165/11590330-000000000-00000

80. Careskey HE, Davis RA, Alborn WE, Troutt JS, Cao G, Konrad RJ. Atorvastatin increases human serum levels of proprotein convertase subtilisin/kexin type 9. *J Lipid Res.* (2008) 49:394–8. doi: 10.1194/jlr.M700437-JLR200

81. Mayne J, Dewpura T, Raymond A, Cousins M, Chaplin A, Lahey KA, et al. Plasma PCSK9 levels are significantly modified by statins and fibrates in humans. *Lipids Health Dis.* (2008) 7:22. doi: 10.1186/1476-511X-7-22

82. Sahebkar A, Simental-Mendia LE, Guerrero-Romero F, Golledge J, Watts GF. Effect of statin therapy on plasma proprotein convertase subtilisin kexin 9 (PCSK9) concentrations: a systematic review and meta-analysis of clinical trials. *Diabetes Obes Metab.* (2015) 17:1042–55. doi: 10.1111/dom.12536

83. Roglans N, Verd JC, Peris C, Alegret M, Vazquez M, Adzet T, et al. High doses of atorvastatin and simvastatin induce key enzymes involved in VLDL production. *Lipids.* (2002) 37:445–54. doi: 10.1007/s11745-002-0916-0

frontiers | Frontiers in Cardiovascular Medicine

TYPE Mini Review
PUBLISHED 29 August 2022
DOI 10.3389/fcvm.2022.957897

OPEN ACCESS

EDITED BY
Tatsuya Sawamura,
Shinshu University, Japan

REVIEWED BY
Masayuki Yoshida,
Tokyo Medical and Dental University, Japan

*CORRESPONDENCE
Takuro Miyazaki
taku@pharm.showa-u.ac.jp

SPECIALTY SECTION
This article was submitted to Lipids in Cardiovascular Disease, a section of the journal Frontiers in Cardiovascular Medicine

RECEIVED 31 May 2022
ACCEPTED 11 August 2022
PUBLISHED 29 August 2022

CITATION
Miyazaki T (2022) Pinocytotic engulfment of lipoproteins by macrophages.
Front. Cardiovasc. Med. 9:957897.
doi: 10.3389/fcvm.2022.957897

Pinocytotic engulfment of lipoproteins by macrophages

Takuro Miyazaki*

Department of Biochemistry, Showa University School of Medicine, Tokyo, Japan

Atherosclerosis is a major cause of acute coronary syndrome and stroke. Foam cell formation in macrophages is involved in controlling plaque stability and the pathogenesis of atherosclerosis. Accordingly, many studies have examined the processes of lipid incorporation, such as scavenger receptor-mediated uptake of oxidized low-density lipoprotein, in cells. In addition to receptor-mediated machinery, growing evidence has suggested that pinocytosis, which is a receptor-independent endocytic pathway, is associated with foam cell formation when a sufficient number of lipoproteins is accumulated around cells. Pinocytotic engulfment of nanoparticles is initiated by plasma membrane ruffling in a phosphatidylinositol-3 kinase-dependent manner. Subsequent to pinosome closure, the majority of pinosomes are internalized through endocytic processes, and they can be recycled into the plasma membrane. These pinocytotic processes are modulated by small GTPases and their cytoskeletal rearrangement. Moreover, pinocytotic abilities may vary between immunological subsets in cells. Accordingly, macrophages may show diverse pinocytotic abilities depending on the surrounding microenvironment. This review summarizes the current understanding of pinocytotic engulfment of lipoprotein in macrophages, and discusses how this endocytic process is governed under hypercholesterolemic conditions.

KEYWORDS

CWC22, calpain-6, Rac1, Cdc42, Akt, liver X receptors, macrophage-colony stimulating factor

Introduction

Ischemic heart disease is the leading cause of death globally. Atherosclerosis is a major risk factor for coronary disease because expansion of such eccentric thickening of the arterial wall can lead to thromboembolism and thrombotic occlusion. Numerous basic and epidemiological studies have shown that an abnormality of cholesterol handling, excessive adaptive response to vascular insults, and inflammation burden are associated with the pathogenesis of atherosclerosis (1, 2). Among these events, cholesterol accumulation in macrophages is an essential process to expand atherosclerotic plaques and is a major factor for defining plaque stability (3, 4). Several endocytic pathways for internalizing low-density lipoprotein (LDL) cholesterol have been reported. Scavenger receptors, such as scavenger receptor-A, CD36, and lectin-like oxidized LDL receptor 1 (5, 6), in macrophages recognize oxidized LDL, but not native LDL, to internalize LDL through the conventional endocytic process. In addition to the receptor-mediated pathways, macrophages enable the incorporation

of nanoparticles through pinocytosis. Pinocytosis is a fundamental cellular process that engulfs extracellular fluids. Pinocytosis is categorized into micropinocytosis and macropinocytosis, which are endocytic processes that engulf small particles (typically $< 0.1\,\mu m$) and large particles (typically $> 0.2\,\mu m$), respectively (7). Such pinocytotic engulfment in macrophages, which occurs spontaneously and can be further modified by environmental factors, are related to the innate immune system to monitor surrounding antigens and microbial-associated molecules. Growing evidence has suggested that pinocytosis is mediated through incorporation of native LDL, thereby inducing formation of foamy macrophages (8). This mini review summarizes the recent understanding of internalization and trafficking of pinosomes and their regulatory mechanisms.

Membrane regulation and endocytic trafficking during pinocytotic incorporation of LDL in macrophages

The pinocytotic deposition of native LDL is sufficient to convert cultured macrophages into foam cells (9). Pinocytotic uptake is independent of the degree of LDL oxidation and does not saturate (7), and the molecular mechanisms underlying pinocytotic LDL uptake are distinct from those of receptor-mediated pathways. Indeed, targeted deletion of the macrophage scavenger receptors scavenger receptor-A and CD36 does not inhibit pinocytotic LDL uptake in macrophage-colony stimulating factor (M-CSF)-differentiated macrophages (10). Generally, macropinocytotic internalization of nanoparticles is divided into plasma membrane ruffling and pinosome closure, which are regulated by submembranous actin organization. Small GTPases have central roles in this cytoskeletal regulation. Ras-GTP drives the activation of phosphoinositide 3-kinases (PI3Ks), which generates patches of PtdIns(3,4,5)P3 (11). The Rho family GTPase Rac and actin-nucleation-promoting factors, such as SCAR/WAVE complex, enable binding to these patches, which nucleates actin filaments. In addition to actin-regulating factors, phospholipase Cγ can be activated by PtdIns(3,4,5)P3, thereby generating diacylglycerol and subsequent activation of protein kinase C, which is involved in the positive regulation of pinocytosis (12). Kruth and colleagues investigated mechanisms underlying pinocytosis using pharmacological approaches in M-CSF-differentiated macrophages, and found that pinocytosis was inhibited by a broad-range PI3K inhibitors (10). In contrast, inhibition of the class I PI3K isoforms β, γ, or δ did not affect micropinocytosis in M-CSF-stimulated macrophages. Similarly, macrophages from mice expressing dominant-negative class I PI3K β, γ, or δ isoforms had no inhibitory effects. Therefore, PI3Ks, excluding class I isoforms, drive macropinocytosis in macrophages. Pharmacological screening of signaling pathways has shown that dynamin, microtubules, actin, and vacuolar type H(+)-ATPase appear to be associated with pinocytotic uptake (10). Accordingly, phosphoinositide metabolism accompanied by cytoskeletal regulation are indispensable even for pinocytotic LDL uptake in macrophages (Figure 1).

In addition to the molecules noted above, the contribution of Rho GTPases to pinocytotic regulation has been well–documented. Anzinger et al. reported that the Rho GTPase inhibitor toxin B substantially inhibited pinocytotic LDL uptake in M-CSF-differentiated human macrophages (13). Using time-lapse microscopy, they found that this inhibitor almost completely inhibited macropinocytosis, although cholesterol deposition in cells was not completely inhibited. Their findings suggest the contribution of another endocytic process, such as micropinocytosis, to LDL cholesterol uptake. In contrast, pharmacological inhibition of Rac1 failed to inhibit pinocytotic LDL uptake in M-CSF-differentiated macrophages (10). Our previous study showed that expression of the Rho GTPases RhoA and Rac1 in bone marrow cells was imperceptible, while it was dramatically induced during the differentiation of cells into macrophages in the presence of M-CSF (14). Treatment of bone marrow cells with tumor necrosis factor (TNF)-α suppressed maturation of *Rac1* mRNA and potentiated macropinocytosis. Deficiency of calpain-6, which is a non-proteolytic isoform of the calpain protease family, in TNF-α-stimulated murine macrophages showed interrupted macropinocytosis concomitantly with the normalization of *Rac1* splicing. Macropinocytosis in calpain-6-deficient macrophages was restored by small interfering RNA-based silencing of *Rac1*. Therefore, aberrant *Rac1* mRNA regulation exerts inhibitory actions in pinocytotic LDL uptake in TNF-α-stimulated macrophages. While the mechanisms by which Rac1 or its splice variants interrupt LDL uptake are unclear, Rac1-mediated regulation of the endosomal recycling pathway may contribute to the inhibitory actions. Indeed, pinosomes in Rac1-expressing macrophages frequently express the recycling endosome marker Rab11. Moreover, macropinosome velocity in cells is decelerated by pharmacological inhibition of Rac1. Therefore, pinocytotic LDL uptake is likely to be due to Rac1-dependent vesicle trafficking rather than Rac1-dependent membrane ruffling. Rho GTPases, such as RhoD, Rac1, Cdc42, TCL, and TC10, are thought to be essential factors for endocytic trafficking (15). Indeed, overexpression of dominant active forms of Rac1 accelerate macropinocytosis activity in rat fibroblasts (16). However, notably, prolonged Rac1 activity impairs the maturation of Rab21-positive pinosomes in macrophages (17), suggesting that optimal Rac1 regulation can maximize pinocytosis. However, Ding et al. showed positive regulation of pinocytotic LDL uptake by Cdc42, which is a small GTPase, in human and murine macrophages (18). Indeed, the loss of Akt3 in murine and human M-CSF-differentiated macrophages upregulates with-no-lysine kinase 1 and subsequently activates

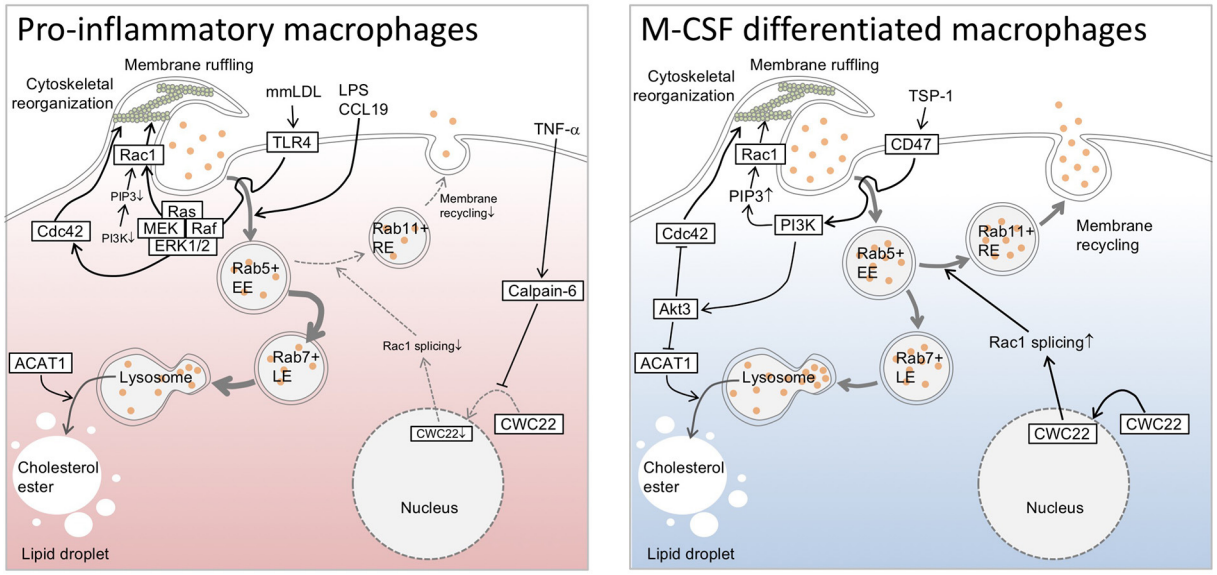


FIGURE 1
Overview of pinocytotic deposition of low-density lipoprotein cholesterol in macrophages. Phosphoinositide 3-kinase drives pinocytotic plasma membrane ruffling. Small GTPases are associated with trafficking and recycling of pinosomes, as well as with pinocytotic plasma membrane regulation, and have diverse actions in pinocytotic low-density lipoprotein cholesterol uptake in macrophages. While pinocytosis appears to be optimized in an alternative (M2) macrophage subset, certain elements, such as Toll-like receptor 4 (TLR4)-mediated signaling, enable the restoration of pinocytosis, even in an inflammatory (M1) subset. TLR4 driver lipopolysaccharide (LPS) can polarize macrophage differentiation toward M1 subset, and activates pinocytotic activation through unknown mechanisms. Chemokine (C-C motif) ligand 19 reportedly possesses similar pinocytotic effects in the cells. In the case of mmLDL-differentiated macrophages, cytoskeletal rearrangement appears to be driven through TLR4/Ras/Raf/ERK/MEK axis independently of PI3K signaling. Furthermore, pro-inflammatory cytokine TNF-α upregulates calpain-6, a non-proteolytic isoform of calpain protease family, to inhibit CWC22-mediated Rac1 splicing. This interferes with endosomal recycling pathway, and in turn increases lysosomal processing of endosome-derived lipoprotein cholesterol to generate cytosolic lipid droplets. In contrast to M1 subset, macrophage-colony stimulating factor (M-CSF)-differentiated M2 macrophages exhibits phosphatidylinositol 3-kinase (PI3K)-dependent cytoskeletal rearrangement and membrane ruffling. Similarly, thrombospndin-1 enables to elicit CD47-mediated activation of PI3K and subsequent pinocytotic uptake of native LDL. In this case, it is likely that Akt3 negatively regulates Cdc42 and acyl-CoA cholesterol acyltransferase 1 to decelerate pinocytotic cholesterol deposition in the cells. ACAT1, acyl-CoA cholesterol acyltransferase 1; CCL19, Chemokine (C-C motif) ligand 19; EE, early endosome; LE, late endosome; LPS, lipopolysaccharide; M-CSF, macrophage-colony stimulating factor; mmLDL, minimally modified low density lipoprotein; PI3K, phosphatidylinositol 3-kinase; PIP3, phosphatidyl inositol 3-phosphate; Rac1, Rac family small GTPase 1; RE, recycling endosome; TLR4, Toll-like receptor 4; TNF-α, tumor necrosis factor-α; TSP-1, thrombospndin-1.

serum and glucocorticoid-inducible kinase 1. Serum and glucocorticoid-inducible kinase 1 promotes expression of the Rho family GTPase Cdc42, thereby accelerating cytoskeletal rearrangement and pinocytosis. Similarly, Akt3-dependent negative regulation of pinocytosis is detectable in murine peritoneal macrophages. This regulation is accompanied by limited receptor-dependent uptake of acetylated LDL and downregulation of acyl-CoA cholesterol acyltransferase (19). Acyl-CoA cholesterol acyltransferase converts free cholesterol into cholesterol ester to form cytosolic lipid droplets in peritoneal macropahges (20). Therefore, Akt3 has a protective role in foam cell formation in macrophages and atherogenesis. How Akt3 simultaneously downregulates receptor-dependent and receptor-independent pathways is currently unclear, but Akt3 might interfere with a common signaling pathway, such as endocytic vesicle trafficking or the exocytotic process. This possibility is consistent with a previous study, which showed that overexpression of dominant-negative Cdc42 counteracted macropinocytosis in vascular endothelial cells (21). Collectively, small GTPases are associated with vesicle trafficking and pinocytotic plasma membrane regulation (Figure 1), and have diverse actions regarding pinocytotic LDL uptake in macrophages. Therefore, defining pinocytotic activity by expression levels of small GTPases alone is difficult.

Regulatory mechanisms underlying pinocytotic LDL incorporation

Macrophages can be divided into a variety of subpopulations, which comprise pro-inflammatory, immunosuppressive, and tissue-repairing types, and they are dynamically interconverted depending on the tissue

microenvironment (22). During bacterial infection, monocyte-macrophages can be activated by inflammatory elements, such as Toll-like receptor (TLR) ligands and interferon-γ, which facilitate the skewing of macrophages into the M1 subset (classically activated macrophages). M1 macrophages produce inflammatory cytokines, such as TNF-α, interleukin (IL)-6, and IL-12, reactive oxygen species, and reactive nitrogen species, and induce a Th1-type immune response (23). Furthermore, M1 macrophages exert strong antibacterial or antiviral activity and antitumor effects. In contrast to the M1 subset, macrophages activated by IL-4 and IL-13 produced by Th2 cells, basophils, mast cells, and innate lymphoid cells are converted into M2 macrophages (alternatively activated macrophages) (23). This activation exerts host defense against parasites, tissue repair, angiogenesis, and tumor growth, and becomes immunosuppressive. M2 macrophages strongly express arginase and mannose receptors (24). Tumor-associated macrophages that are infiltrated into tumor tissue are thought to be converted from M1 to M2 subsets, thereby accelerating tumor progression (25). Tumor-associated macrophages showing low IL-12 expression levels and high IL-10 expression levels, possess weak antitumor activity, and drive matrix remodeling and angiogenesis (26). In addition to the progression of cancer, aberrant regulation of macrophage subsets can be involved in various diseases. Therefore, phenotypic regulation of macrophage subsets can be regarded as a therapeutic target. Arteriosclerosis, which is a Th1-dominant vascular disorder, can be treated by skewing M1 to M2 macrophages (27). In contrast, bronchial asthma, which is a Th2-dominant disease, might be targeted by the opposite strategy (28).

M2 macrophages show constitutive pinocytotic activity and can be further stimulated with related cytokines such as M-CSF. Notably, the majority of the above-mentioned observations on pinocytotic LDL uptake were from investigations using M-CSF-differentiated human monocyte-derived macrophages. Redka et al. showed that M-CSF/IL-4-differentiated M2 macrophages had robust micropinocytosis activity, while that in granulocyte M-CSF/interferon-γ/lipopolysaccharide-differentiated M1 macrophages was negligible (29). They found that Rho GTPase expression levels in M1 macrophages were lower than those in the M2 subset. In addition to small GTPases, insufficient PI3K activity is likely responsible for modest pinocytotic ability in the M1 subset (29). Calcium-sensing receptors appear to be necessary for sustaining small GTPases and PI3K in M2 macrophages. However, notably, M1 macrophages show robust pinocytotic activity when the cells are stimulated with certain pro-inflammatory substances. Indeed, acute treatment of pro-inflammatory macrophages with lipopolysaccharide or CC chemokine ligand 19 markedly accelerates macropinosome formation in human monocyte-derived macrophages (29). Moreover, minimally oxidized low-density lipoprotein (mmLDL), which is an alternative TLR4 ligand, and cholesteryl ester hydroperoxide, an active component of mmLDL, induce TLR4-dependent macropinocytotic incorporation of oxidized LDL and native LDL (30). In this case, mmLDL elicits an association of spleen tyrosine kinase with a TLR4 complex, TLR4 phosphorylation, activation of the Vav1-Ras-Raf-MEK-ERK1/2 axis, phosphorylation of paxillin, and activation of Rac, Cdc42, and Rho in murine peritoneal macrophages. These findings suggest that mmLDL-induced TLR4 signaling normalizes the disparity of small GTPases. Collectively, the status of small GTPases is likely to depend on types of extracellular stimuli rather than synchronizing with the phenotypic status of M1/M2 subsets. Accordingly, the master regulator of pinocytosis is currently unclear.

While the regulatory mechanisms underlying pinocytosis are poorly understood, several studies have focused on the upstream modulator of this process (Figure 1). Agonists of liver X receptors (LXRs) are involved in the downregulation of pinocytotic uptake of native LDL in M-CSF-differentiated macrophages (31). LXRs are ligand-activated transcription factors involved in the control of lipid metabolism and inflammation. Because targeting LXRs in bone marrow cells facilitates atherosclerosis (32), LXRs may interfere with pinocytotic cholesterol deposition in macrophages and subsequent pathogenesis of atherosclerosis. Csányi et al. found that thrombospndin-1 (TSP-1) and its cytoskeletal regulation potentiated pinocytosis (33). Indeed, treatment of TSP-1 with human and murine M-CSF-differentiated macrophages stimulated membrane ruffle formation and pericellular solute internalization by macropinocytosis. The TSP1 cognate receptor CD47, NADPH oxidase 1 (Nox1) signaling, PI3K, and myotubularin-related protein 6 appear to be associated with TSP1-induced macropinocytosis. Our previous study showed that CWC22, which is an essential loading factor of exon junction complex, was associated with pinocytotic incorporation of native LDL in murine bone marrow-derived macrophages (14). CWC22 in macrophages shows nuclear localization in human mild atherosclerotic lesions, while it shows cytosolic localization in advanced atherosclerotic lesions. Macrophages in advanced lesions simultaneously express calpain-6, which potentiates formation of a calpain-6/CWC22 complex in the cytoplasm and inhibits nuclear localization of CWC22. Because CWC22 has a direct role in *Rac1* mRNA splicing, calpain-6 counteracts CWC22-mediated maturation of *Rac1* mRNA. Indeed, calpain-6-deficient macrophages show low Rac1 protein expression levels and insufficient macropinocytosis activity. Knockdown of *Cwc22* in calpain-6-deficient macrophages leads to the recovery macropinocytosis, concomitantly with restoring *Rac1* mRNA maturation. As noted above, Rac1 facilitates the dynamics of recycling endosomes. Therefore, CWC22 and related splicing factors are thought to be negative regulators of the pinocytotic process.

Does pinocytosis drive atherogenesis?

While fluid phase pinocytosis is a constitutive process in macrophages and other cells under the physiological conditions, this process is reportedly potentiated in former cell type when they are localized in atherosclerotic lesions. *In vivo* experiments using *Apoe*$^{-/-}$ hypercholesterolemic mice have indicated that fluorescent nanobeads, which is similar in size to LDL and were injected into blood circulation, were massively accumulated in CD68-positive macrophages in atherosclerotic lesions, but not in non-atheroma regions in arteries (34). We also investigated the uptake of nanobeads using *Ldlr*$^{-/-}$ hypercholesterolemic mice. As a result, similar deposition of nanoparticle was reproduced in foamy macrophages in atherosclerotic lesions (14). These observations suggest that macrophages in atherosclerotic lesions preferentially engulf environmental LDL-like particles under hypercholesterolemia. Interestingly, targeting calpain-6 suppressed nanobeads incorporation in foamy macrophages as well as atherogenesis in hypercholesterolemic mice without altering plasma lipid profiles. Notably, overexpression of calpain-6 can upregulate pinocytotic incorporation of LDL in bone marrow-derived macrophages without modifying receptor-mediated uptake of oxidative LDL and phagocytic uptake of aggregated LDL. Considering that calpain-6 is exclusively expressed in macrophages in atherosclerotic lesions, it is interpreted that the pinocytotic incorporation of LDL in lesional macrophages, at least of their calpain-6-mediated portion, may be responsible for the pathogenesis of atherosclerosis. Nevertheless, since calpain-6 contribute to the other atherogenic processes in macrophages such as cellular motility (14), the pathophysiological importance of this endocytic process in the pathogenesis of atherosclerosis has not been fully determined.

Discussion

As noted above, the contribution of pinocytosis to the pathogenesis of atherosclerosis is currently sketchy. This is because of the lack of responsible regulatory element(s) of this processes. Cell-based experiments suggest the dominant roles of small GTPases in pinocytotic membrane ruffling, while they also contribute to the other fundamental processes of cells, including mitosis and cell motility. Identification of the pinocytotic master regulator enables to perform intervention study in animal models to determine the pathogenic significance of this processes. It was reported that targeted deletion of scavenger receptors, CD36, and scavenger receptor-A in hypercholesterolemic mice inhibits the pathogenesis of atherosclerosis without altering oxidized LDL uptake in macrophages (35). Therefore, pinocytotic uptake of LDL in the cells is worthy to be further investigated. To identify the master regulator of pinocytotic LDL uptake, comprehensive studies evaluating membrane regulation, vesicle trafficking, exocytosis/efflux, and subsequent cholesterol deposition are necessary.

Author contributions

TM conceived and designed the review, appraised the literature, and wrote the manuscript.

Funding

This study was supported in part by the Japan Society for the Promotion of Science KAKENHI grants [grant nos.: JP19K08590 and JP22H03520 (to TM)], a research grant from Bristol-Myers Squibb (to TM), a research grant from the Mochida Memorial Foundation for Medical and Pharmaceutical Research (to TM), and a research grant from the Naito Memorial Foundation (to TM).

Acknowledgments

We thank Ellen Knapp, PhD, from Edanz (https://jp.edanz.com/ac) for editing a draft of this manuscript.

Conflict of interest

The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Miyazaki T, Miyazaki A. Dysregulation of calpain proteolytic systems underlies degenerative vascular disorders. *J Atheroscler Thromb.* (2018) 25:1–15. doi: 10.5551/jat.RV17008

2. Ross R. Atherosclerosis–an inflammatory disease. *N Engl J Med.* (1999) 340:115–26. doi: 10.1056/NEJM199901143400207

3. Miyazaki T, Miyazaki A. Impact of dysfunctional protein catabolism on macrophage cholesterol handling. *Curr Med Chem.* (2019) 26:1631–43. doi: 10.2174/0929867325666180326165234

4. Miyazaki T, Miyazaki A. Emerging roles of calpain proteolytic systems in macrophage cholesterol handling. *Cell Mol Life Sci.* (2017) 74:3011–21. doi: 10.1007/s00018-017-2528-7

5. Tian K, Ogura S, Little PJ, Xu SA-O, Sawamura T. Targeting LOX-1 in atherosclerosis and vasculopathy: current knowledge and future perspectives. *Ann N Y Acad Sci.* (2019) 1443:34–53. doi: 10.1111/nyas.13984

6. Chistiakov DA, Bobryshev YV, Orekhov AN. Macrophage-mediated cholesterol handling in atherosclerosis. *J Cell Mol Med.* (2016) 20:17–28. doi: 10.1111/jcmm.12689

7. Kruth HS. Fluid-phase pinocytosis of LDL by macrophages: a novel target to reduce macrophage cholesterol accumulation in atherosclerotic lesions. *Curr Pharm Des.* (2013) 19:5865–72. doi: 10.2174/1381612811319330005

8. Kruth HS. Receptor-independent fluid-phase pinocytosis mechanisms for induction of foam cell formation with native low-density lipoprotein particles. *Curr Opin Lipidol.* (2011) 22:386–93. doi: 10.1097/MOL.0b013e32834adadb

9. Kruth HS, Jones NL, Huang W, Zhao B, Ishii I, Chang J, et al. Macropinocytosis is the endocytic pathway that mediates macrophage foam cell formation with native low density lipoprotein. *J Biol Chem.* (2005) 280:2352–60. doi: 10.1074/jbc.M407167200

10. Barthwal MJ, Anzinger JJ, Xu Q, Bohnacker T, Wymann MP, Kruth HS. Fluid-phase pinocytosis of native low density lipoprotein promotes murine M-CSF differentiated macrophage foam cell formation. *PLoS ONE.* (2013) 8:e58054. doi: 10.1371/journal.pone.0058054

11. Hoeller O, Bolourani P, Clark J, Stephens LR, Hawkins PT, Weiner OD, et al. Two distinct functions for PI3-kinases in macropinocytosis. *J Cell Sci.* (2013) 126:4296–307. doi: 10.1242/jcs.134015

12. Singla B, Ghoshal P, Lin H, Wei Q, Dong Z, Csányi G. PKCδ-Mediated Nox2 activation promotes fluid-phase pinocytosis of antigens by immature dendritic cells. *Front Immunol.* (2018) 9:537. doi: 10.3389/fimmu.2018.00537

13. Anzinger JJ, Chang J, Xu Q, Buono C, Li Y, Leyva FJ, et al. Native low-density lipoprotein uptake by macrophage colony-stimulating factor-differentiated human macrophages is mediated by macropinocytosis and micropinocytosis. *Arterioscler Thromb Vasc Biol.* (2010) 30:2022–31. doi: 10.1161/ATVBAHA.110.210849

14. Miyazaki T, Tonami K, Hata S, Aiuchi T, Ohnishi K, Lei XF, et al. Calpain-6 confers atherogenicity to macrophages by dysregulating pre-mRNA splicing. *J Clin Invest.* (2016) 126:3417–32. doi: 10.1172/JCI85880

15. Phuyal S, Farhan H. Multifaceted Rho GTPase Signaling at the Endomembranes. *Front Cell Dev Biol.* (2019) 7:127. doi: 10.3389/fcell.2019.00127

16. Ahram M, Sameni M, Qiu RG, Linebaugh B, Kirn D, Sloane BF. Rac1-induced endocytosis is associated with intracellular proteolysis during migration through a three-dimensional matrix. *Exp Cell Res.* (2000) 260:292–303. doi: 10.1006/excr.2000.5031

17. Fujii M, Kawai K, Egami Y, Araki N. Dissecting the roles of Rac1 activation and deactivation in macropinocytosis using microscopic photo-manipulation. *Sci Rep.* (2013) 3:2385. doi: 10.1038/srep02385

18. Ding L, Zhang L, Kim M, Byzova T, Podrez E. Akt3 kinase suppresses pinocytosis of low-density lipoprotein by macrophages via a novel WNK/SGK1/Cdc42 protein pathway. *J Biol Chem.* (2017) 292:9283–93. doi: 10.1074/jbc.M116.773739

19. Ding L, Biswas S, Morton RE, Smith JD, Hay N, Byzova TV, et al. Akt3 deficiency in macrophages promotes foam cell formation and atherosclerosis in mice. *Cell Metab.* (2012) 15:861–71. doi: 10.1016/j.cmet.2012.04.020

20. Huang L-H, Melton EM, Li H, Sohn P, Rogers MA, Mulligan-Kehoe MJ, et al. Myeloid Acyl-CoA:cholesterol acyltransferase 1 deficiency reduces lesion macrophage content and suppresses atherosclerosis progression. *J Biol Chem.* (2016) 291:6232–44. doi: 10.1074/jbc.M116.713818

21. Tkachenko E, Lutgens E, Stan RV, Simons M. Fibroblast growth factor 2 endocytosis in endothelial cells proceed via syndecan-4-dependent activation of Rac1 and a Cdc42-dependent macropinocytic pathway. *J Cell Sci.* (2004) 117:3189–99. doi: 10.1242/jcs.01190

22. Murray PJ, Allen JE, Biswas SK, Fisher EA, Gilroy DW, Goerdt S, et al. Macrophage activation and polarization: nomenclature and experimental guidelines. *Immunity.* (2014) 41:14–20. doi: 10.1016/j.immuni.2014.06.008

23. Shapouri-Moghaddam A, Mohammadian S, Vazini H, Taghadosi M, Esmaeili SA, Mardani F, et al. Macrophage plasticity, polarization, and function in health and disease. *J Cell Physiol.* (2018) 233:6425–40. doi: 10.1002/jcp.26429

24. Gensel JC, Zhang B. Macrophage activation and its role in repair and pathology after spinal cord injury. *Brain Res.* (2015) 1619:1–11. doi: 10.1016/j.brainres.2014.12.045

25. Miyazaki T, Akasu R, Miyazaki A. Calpain-associated proteolytic regulation of the stromal microenvironment in cancer. *Curr Pharm Des.* (2021) 27:3128–38. doi: 10.2174/1381612827666210311143053

26. Yamaguchi T, Fushida S, Yamamoto Y, Tsukada T, Kinoshita J, Oyama K, et al. Tumor-associated macrophages of the M2 phenotype contribute to progression in gastric cancer with peritoneal dissemination. *Gastric Cancer.* (2016) 19:1052–65. doi: 10.1007/s10120-015-0579-8

27. de Gaetano M, Crean D, Barry M, Belton O. M1- and M2-type macrophage responses are predictive of adverse outcomes in human atherosclerosis. *Front Immunol.* (2016) 7:275. doi: 10.3389/fimmu.2016.00275

28. Ogulur I, Pat Y, Ardicli O, Barletta E, Cevhertas L, Fernandez-Santamaria R, et al. Advances and highlights in biomarkers of allergic diseases. *Allergy.* (2021) 76:3659–86. doi: 10.1111/all.15089

29. Redka DS, Gütschow M, Grinstein S, Canton J. Differential ability of proinflammatory and anti-inflammatory macrophages to perform macropinocytosis. *Mol Biol Cell.* (2018) 29:53–65. doi: 10.1091/mbc.E17-06-0419

30. Choi S-H, Harkewicz R, Lee JH, Boullier A, Almazan F, Li AC, et al. Lipoprotein accumulation in macrophages via toll-like receptor-4-dependent fluid phase uptake. *Circ Res.* (2009) 104:1355–63. doi: 10.1161/CIRCRESAHA.108.192880

31. Buono C, Li Y, Waldo SW, Kruth HS. Liver X receptors inhibit human monocyte-derived macrophage foam cell formation by inhibiting fluid-phase pinocytosis of LDL. *J Lipid Res.* (2007) 48:2411–8. doi: 10.1194/jlr.M700170-JLR200

32. Tangirala RK, Bischoff ED, Joseph SB, Wagner BL, Walczak R, Laffitte BA, et al. Identification of macrophage liver X receptors as inhibitors of atherosclerosis. *Proc Natl Acad Sci U S A.* (2002) 99:11896–901. doi: 10.1073/pnas.182199799

33. Csányi G, Feck DM, Ghoshal P, Singla B, Lin H, Nagarajan S, et al. CD47 and Nox1 mediate dynamic fluid-phase macropinocytosis of native LDL. *Antioxid Redox Signal.* (2017) 26:886–901. doi: 10.1089/ars.2016.6834

34. Buono C, Anzinger JJ, Amar M, Kruth HS. Fluorescent pegylated nanoparticles demonstrate fluid-phase pinocytosis by macrophages in mouse atherosclerotic lesions. *J Clin Invest.* (2009) 119:1373–81. doi: 10.1172/JCI35548

35. Manning-Tobin JJ, Moore KJ, Seimon TA, Bell SA, Sharuk M, Alvarez-Leite JI, et al. Loss of SR-A and CD36 activity reduces atherosclerotic lesion complexity without abrogating foam cell formation in hyperlipidemic mice. *Arterioscler Thromb Vasc Biol.* (2009) 29:19–26. doi: 10.1161/ATVBAHA.108.176644

frontiers | Frontiers in Cardiovascular Medicine

TYPE Original Research
PUBLISHED 30 August 2022
DOI 10.3389/fcvm.2022.903390

OPEN ACCESS

EDITED BY
Alexander Akhmedov,
University of Zurich, Switzerland

REVIEWED BY
Yuling Zhang,
Sun Yat-sen Memorial Hospital, China
Aleksandra Klisic,
Primary Health Care Center Podgorica, Montenegro

*CORRESPONDENCE
Yan Zhang
drzhy1108@163.com
Yong Huo
huoyong@263.net.cn

SPECIALTY SECTION
This article was submitted to Lipids in Cardiovascular Disease, a section of the journal Frontiers in Cardiovascular Medicine

RECEIVED 24 March 2022
ACCEPTED 09 August 2022
PUBLISHED 30 August 2022

CITATION
Liu B, Fan F, Zheng B, Yang Y, Jia J, Sun P, Jiang Y, Li K, Liu J, Chen C, Li J, Zhang Y and Huo Y (2022) Association of remnant cholesterol and lipid parameters with new-onset carotid plaque in Chinese population. *Front. Cardiovasc. Med.* 9:903390. doi: 10.3389/fcvm.2022.903390

Association of remnant cholesterol and lipid parameters with new-onset carotid plaque in Chinese population

Bo Liu[1], Fangfang Fan[1,2], Bo Zheng[1,2], Ying Yang[1,3], Jia Jia[1,2], Pengfei Sun[1], Yimeng Jiang[1], Kaiyin Li[1], Jiahui Liu[1], Chuyun Chen[1], Jianping Li[1,2], Yan Zhang[1,2]* and Yong Huo[1,2]*

[1]Department of Cardiology, Peking University First Hospital, Beijing, China, [2]Institute of Cardiovascular Disease, Peking University First Hospital, Beijing, China, [3]Echocardiography Core Lab, Institute of Cardiovascular Disease, Peking University First Hospital, Beijing, China

Background: Remnant lipoprotein cholesterol (RC) is an independent risk factor for cardiovascular disease (CVD). However, the relationships of remnant cholesterol and other conventional lipid parameters with new-onset carotid plaque are not fully understood in the Chinese community-based population.

Materials and methods: A total of 872 plaque-free participants (51.39 ± 4.96 years old) with no history of CVD were included in this study. The plasma concentrations of RC were calculated by subtracting low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C) from total cholesterol (TC). Multivariate regression models were used to evaluate and compare the associations between RC and other lipid parameters and new-onset carotid plaque.

Results: After a mean 6.77-year follow-up, the incidence of new-onset carotid plaque was 188 (21.56%). RC was significantly associated with new-onset carotid plaque [Odd ratio (OR) = 1.57 per 1 mmol/L increase, 95% confidence interval (CI): 1.03–2.41, $p = 0.038$]. The highest tertile of RC (T3 group) had the highest risk of new-onset carotid plaque ($OR = 2.53$, 95% CI: 1.63–3.95). Similar results were seen for increased other lipid parameters, but decreased HDL-C levels. When adding another lipid parameter into the adjusted model with RC simultaneously, only RC remained significantly associated with new-onset carotid plaque after adjusting for other lipid parameters (all p value < 0.005). Furthermore, RC was strongly associated with new-onset carotid plaque in participants with lower baseline LDL-C levels.

Conclusion: Increased RC levels were superior to other conventional lipid parameters to be associated with new-onset carotid plaque in the Chinese community-based population. Furthermore, RC should be considered in participants with lower LDL-C levels for the purpose of early atherosclerosis prevention.

KEYWORDS

remnant cholesterol, carotid plaque, lipids, atherosclerosis, population study

Introduction

There is considerable residual risk of arteriosclerotic cardiovascular disease (ASCVD) after reduction of low-density lipoprotein cholesterol (LDL-C) to the recommended concentration achieved by statin regimens, and even after managing other modifiable risk factors, such as hypertension (1–3). Over the past many years, numerous clinical studies have focused on high levels of triglyceride-rich lipoproteins cholesterol, which indicates increased concentrations of potentially remnant cholesterol and may help to explain the residual risk (4–6).

Remnant cholesterol is the cholesterol content of triglyceride-rich lipoproteins, and is composed of VLDL and IDL in the fasting state, and chylomicron remnants in the non-fasting state (2). When there is an excess of remnant lipoproteins in the plasma, remnants can carry large amounts of cholesterol and have the same potential ability as LDL to penetrate and become trapped in the intima of the arterial wall, resulting in the formation of foam cells, atherosclerosis, and low-grade inflammation (7–12).

The presence of new-onset carotid plaque frequently serves as a risk predictor in the assessment of CVD/Stroke risk, and carotid plaque formation is a surrogate marker of a high-risk of carotid atherosclerotic disease (13–15). The relationship between remnant lipoproteins cholesterol and cardiovascular events has been demonstrated for decades (4, 8, 16–20). However, few studies have focused on comparing the differences between RC and other conventional lipid parameters in atherosclerotic disease, even other surrogate markers, such as carotid plaque formation (21–24). In other words, the lack of development in the evidence base for the associations between RC and conventional lipid parameters and the risk of new-onset carotid plaque has been more important, especially in the Chinese community-based population with no history of cardiovascular disease (25).

The present study aimed to longitudinally evaluate the relationships between RC and other conventional lipid parameters and new-onset carotid plaque, and further assess the comparisons of RC and other parameters in relation to new-onset carotid plaque when both lipids were put into the model simultaneously.

Materials and methods

Study population

All participants included in this study were enrolled from a community-based atherosclerosis cohort set up in 2011 in Beijing, China. Detailed descriptions of the study procedures have been described previously (26). Initially, a total of 4,431 participants aged ≥ 40 years underwent the baseline survey in 2012 and responded on-site during the follow-up visit in 2018. For the present study, 1,960 participants with carotid plaque-free status at baseline were selected, and then 988 participants with quantitative carotid artery measurements at the follow-up visit were included. After stepwise exclusion, 116 participants included using lipid-lowering medications ($n = 80$), history of cardiovascular disease ($n = 33$), and missing data for lipid profiles ($n = 3$). Ultimately, this analysis included 872 eligible participants with a mean 6.77-year follow-up (**Supplementary Figure 1**). This study was approved by the ethics committee of Peking University First Hospital, and confirmed to the provisions of the Declaration of Helsinki. All participants signed informed consent.

Data collection

Baseline and follow-up data were collected by trained researcher staff according to standard operating procedures. All participants were interviewed using a standard questionnaire that was specifically designed for the present study, to obtain information on demographic characteristics, education, occupation, lifestyle, personal, and medical history. Current smoking means smoking at least one cigarette per day for at least 6 months. Current drinking means drinking alcohol at least once per week for at least 6 months. The body mass index (BMI) was calculated by the following formula: BMI = weight (kg)/height (m^2). The peripheral systolic (SBP) and diastolic blood pressure (DBP) readings used the mean value of these three successful measurements using a standard method (26).

A venous blood sample was obtained from the forearm of each participant after an overnight fast (at least 12 h) at the baseline survey. Subsequently, the Roche C8000 Automatic Analyzer was used to examine all biochemistry parameters in serum, including fasting blood glucose (FBG), 2-h glucose in the standard 75-g oral glucose tolerance test (OGTT), total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C), which were also directly measured by a chemical method; serum creatinine (Scr, μmol/L) levels were measured enzymatically. Non-HDL-C was calculated by subtracting HDL-C from TC. RC was calculated by subtracting LDL-C and HDL-C from TC, as done previously (21, 27, 28). In addition, the estimated glomerular filtration rate (eGFR) was determined by the CKD-EPI equation (26).

Hypertension was defined as any self-reported history, SBP ≥ 140 mmHg or DBP ≥ 90 mmHg, or taking anti-hypertensive medication. Diabetes mellitus was defined as any self-reported history of diabetes, use of hypoglycemic medication, FBG ≥ 7.0 mmol/L, and/or OGTT ≥ 11.1 mmol/L.

Carotid ultrasonography

All participants underwent carotid ultrasonography by trained and certified sonographers both at the baseline survey in 2012 using the high-resolution B-mode ultrasound system (GE Vivid 7, 8∼10 MHz linear-array vascular transducer; Milwaukee, WI, United States) and at the follow-up visit in 2018 using a Terason Echo Ultrasound System (Burlington, MA, United States). Briefly, carotid ultrasound was performed according to standard scanning and reading protocols at the baseline survey and follow-up visit. Intima-media thickness (IMT) was detected as the distance between the lumen-intima and the media-adventitia ultrasound interfaces. Carotid IMT (cIMT) was defined as the mean IMT measured at 1 cm lengths of the far wall of the bilateral distal common carotid artery. Carotid plaque was defined as focal structures encroaching into the arterial lumen of at least 0.5 mm or 50% of the surrounding cIMT value, or demonstrating a thickness > 1.5 mm as measured from the intima–lumen interface to the media-adventitia interface at any level of the bilateral common carotid artery, internal carotid artery, and/or bifurcation (29).

Statistical analysis

Descriptive statistics were expressed as the mean ± standard deviation (SD) or median (interquartile range) for continuous variables and number (percentage) for dichotomous variables. Normally distributed continuous variables were compared using Student's *t*-test, whereas Kruskal-Wallis test was used for variables with a skewed distribution. Pearson's χ^2-test or Fisher's exact test was applied to all categorical variables as appropriate. Univariate and multivariate regression models were used to evaluate the relationships between baseline lipid parameters (both as a continuous and categorical variable) and new-onset carotid plaque, after adjusting for sex and age (Model 1), and further adjusting for BMI, current smoking, current drinking, estimated glomerular filtration rate, diabetes mellitus, hypertension, and the use of antihypertensive and hypoglycemic medications (Model 2). Regarding possible collinearity, the variance inflation factor (VIF) was calculated for the included variables in each multivariable regression model (**Supplementary Table 1**). We further assessed the comparisons of RC and other conventional lipid parameters in relation to new-onset carotid plaque when both lipid parameters were put into the model simultaneously. In addition, we conducted

TABLE 1 Baseline characteristics stratified by remnant lipoprotein cholesterol (RC) tertiles.

	Total	Remnant cholesterol, mmol/L			*P-value*
		Tertile 1 (< 0.42)	Tertile 2 (0.42- < 0.64)	Tertile 3 (≥ 0.64)	
N	872	290	290	292	
Age, year	51.39 ± 4.96	50.86 ± 5.01	51.01 ± 5.11	52.30 ± 4.62	< 0.001
Female, N (%)	642 (73.62%)	227 (78.28%)	213 (73.45%)	202 (69.18%)	0.045
BMI, kg/m^2	25.62 ± 3.31	24.35 ± 3.12	25.84 ± 3.25	26.67 ± 3.15	< 0.001
Total cholesterol, mmol/L	5.27 ± 0.90	4.83 ± 0.71	5.21 ± 0.80	5.76 ± 0.92	< 0.001
Triglycerides, mmol/L	1.22 (0.88, 1.77)	0.79 (0.63, 1.02)	1.23 (1.00, 1.55)	2.07 (1.54, 2.71)	< 0.001
HDL-C, mmol/L	1.49 ± 0.40	1.74 ± 0.41	1.46 ± 0.33	1.26 ± 0.29	< 0.001
LDL-C, mmol/L	3.20 ± 0.74	2.79 ± 0.57	3.23 ± 0.65	3.57 ± 0.78	< 0.001
Non-HDL-C, mmol/L	3.78 ± 0.91	3.09 ± 0.60	3.76 ± 0.66	4.50 ± 0.83	< 0.001
Remnant cholesterol, mmol/L	0.52 (0.37–0.70)	0.32 (0.24–0.37)	0.52 (0.47–0.58)	0.81 (0.70–0.97)	< 0.001
FBG, mmol/L	5.84 ± 1.49	5.64 ± 1.43	5.71 ± 1.05	6.17 ± 1.83	< 0.001
eGFR, mL/min/1.73°m^2	100.23 ± 9.42	101.75 ± 8.82	101.03 ± 9.27	97.92 ± 9.73	< 0.001
Current drinking, N (%)	196 (22.48%)	57 (19.66%)	69 (23.79%)	70 (23.97%)	0.370
Current smoking, N (%)	133 (15.25%)	33 (11.38%)	41 (14.14%)	59 (20.21%)	0.010
Disease, N (%)					
Hypertension	235 (26.95%)	57 (19.66%)	67 (23.10%)	111 (38.01%)	< 0.001
Diabetes mellitus	113 (12.96%)	30 (10.34%)	28 (9.66%)	55 (18.84%)	0.001
Treatment, N (%)					
Antihypertensive	114 (13.07%)	35 (12.07%)	33 (11.38%)	46 (15.75%)	0.242
Hypoglycemic	34 (3.90%)	12 (4.15%)	7 (2.41%)	15 (5.14%)	0.229

Data are shown as mean ± standard deviation (SD) or median (IQR, Q1-Q3) for continuous variables and number (percentage) for dichotomous variables.
BMI, body mass index; RC, remnant cholesterol; TC, total cholesterol; TG, triglycerides; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; Non-HDL-C, non-high-density lipoprotein cholesterol; FBG, fasting blood glucose; eGFR, estimated glomerular filtration rate.

threshold effect analysis for lipid parameters if the relationships were non-linear (**Supplementary Table 2**), and investigated the modification of baseline LDL-C levels for the effect of RC on new-onset carotid plaque. In this study, a *P*-value of < 0.05 (two-sided) was considered statistically significant for all tests. All statistical analyses were performed using Empower(R) (X&Y solutions, Inc., Boston, MA, United States) and R software.[1]

Results

Baseline patient characteristics

Table 1 shows the baseline characteristics of eligible participants, both overall and stratified by RC tertiles. Among the 872 subjects, 73.62% were female, with an average age of 51.39 ± 4.96 years old and a mean (SD) BMI of 25.62 ± 3.31 kg/m^2. Those with hypertension and diabetes accounted for 26.95% (235), and 12.96% (113), respectively. The mean (SD) baseline lipid parameters were 5.27 ± 0.90 mmol/L for TC, 3.20 ± 0.74 mmol/L for LDL-C, 1.49 ± 0.40 mmol/L for HDL-C, and 3.78 ± 0.91 mmol/L for non-HDL-C, respectively. The median (interquartile range, IQR) RC was 0.52 (0.37, 0.70)°mmol/, and TG was 1.22 (0.88, 1.77)°mmol/L. The participants with higher RC (the top tertile) had higher levels of BMI, TC, LDL-C, TG, non-HDL-C, FBG, lower levels of HDL-C, and a higher prevalence of hypertension, diabetes mellitus ($p < 0.05$). There was no significant difference between the different RC tertiles for current drinking, or the use of anti-hypertensive and hypoglycemic medication.

Associations of remnant lipoprotein cholesterol and other lipid parameters with new-onset carotid plaque when considered individually

Of the 872 eligible plaque-free participants at baseline in this study, 188 (21.56%) individuals developed new-onset carotid plaque after a mean 6.77-year follow-up. As shown in **Figure 1**, there was mainly positive association between lipid parameters and new-onset carotid plaque, except for a negative linear association with HDL-C. **Table 2** demonstrates the associations of RC and other conventional lipid parameters with new-onset carotid plaque. RC (per 1 mmol/L increase) was significantly associated with increases of 65% (95% CI:

1 www.R-project.org

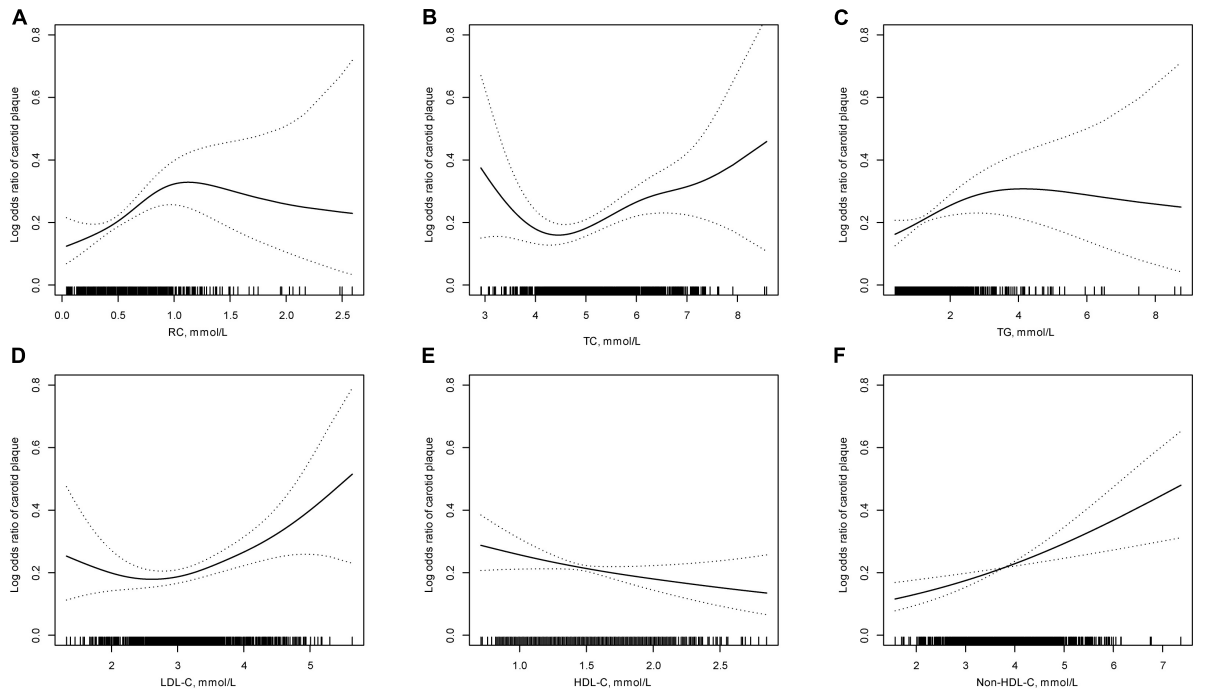


FIGURE 1
The relationship between lipid parameters and new-onset carotid plaque*. **(A)** Remnant lipoprotein cholesterol (RC); **(B)** total cholesterol (TC); **(C)** triglycerides (TG); **(D)** low-density lipoprotein cholesterol (LDL-C); **(E)** high-density lipoprotein cholesterol (HDL-C); and **(F)** Non-HDL-C. *Adjusted for: sex, age, body mass index, current drinking, current smoking, estimated glomerular filtration rate, diabetes mellitus, hypertension, antihypertensive, and hypoglycemic drugs.

TABLE 2 Logistic regressions for the effects of baseline lipid parameters and new-onset carotid plaque.

Lipid parameters	N (%)	OR (95% CI) *P-value*		
		Crude	Adjusted model 1	Adjusted model 2
RC, per 1 mmol/L increase	188 (21.56%)	1.65 (1.10–2.48) 0.016	1.52 (1.02–2.28) 0.042	1.57 (1.03–2.41) 0.038
Tertiles of RC				
T1 (< 0.42)	46 (15.86%)	Ref.	Ref.	Ref.
T2 (0.42–< 0.64)	50 (17.24%)	1.11 (0.71–1.71) 0.655	1.07 (0.69–1.67) 0.764	1.23 (0.78–1.95) 0.376
T3 (≥ 0.64)	92 (31.51%)	2.44 (1.64–3.64) < 0.001	2.18 (1.45–3.28) < 0.001	2.53 (1.63–3.95) < 0.001
p for trend		< 0.001	< 0.001	< 0.001
TC, per 1 mmol/L increase	188 (21.56%)	1.28 (1.08–1.54) 0.006	1.30 (1.08–1.56) 0.006	1.28 (1.06–1.55) 0.011
Tertiles of TC				
T1 (< 4.87)	50 (17.30%)	Ref.	Ref.	Ref.
T2 (4.87–< 5.60)	59 (20.21%)	1.21 (0.80–1.84) 0.370	1.25 (0.81–1.91) 0.313	1.23 (0.79–1.90) 0.355
T3 (≥ 5.60)	79 (27.15%)	1.78 (1.19–2.66) 0.005	1.81 (1.19–2.76) 0.005	1.81 (1.18–2.78) 0.007
p for trend		0.004	0.005	0.006
TG, per 1 mmol/L increase	188 (21.56%)	1.15 (1.01–1.31) 0.040	1.12 (0.97–1.28) 0.115	1.12 (0.97–1.30) 0.128
Tertiles of TG				
T1 (< 0.99)	45 (15.68%)	Ref.	Ref.	Ref.
T2 (0.99–< 1.55)	64 (21.92%)	1.51 (0.99–2.30) 0.056	1.49 (0.97–2.29) 0.070	1.55 (0.99–2.41) 0.053
T3 (≥ 1.55)	79 (26.96%)	1.99 (1.32–2.99) 0.001	1.79 (1.18–2.72) 0.006	1.92 (1.22–3.00) 0.004
p for trend		0.001	0.007	0.005
HDL-C, per 1 mmol/L increase	188 (21.56%)	0.63 (0.41–0.96) 0.032	0.73 (0.46–1.15) 0.175	0.64 (0.39–1.05) 0.079
Tertiles of HDL-C				
T1 (< 1.28)	69 (23.88%)	Ref.	Ref.	Ref.
T2 (1.28–< 1.60)	69 (23.79%)	1.00 (0.68–1.46) 0.982	1.03 (0.69–1.55) 0.867	0.96 (0.64–1.46) 0.863
T3 (≥ 1.60)	50 (17.06%)	0.66 (0.44–0.99) 0.042	0.74 (0.48–1.15) 0.183	0.65 (0.41–1.05) 0.077
p for trend		0.046	0.188	0.0780
LDL-C, per 1 mmol/L increase	188 (21.56%)	1.43 (1.15–1.78) 0.001	1.40 (1.12–1.76) 0.003	1.40 (1.11–1.77) 0.004
Tertiles of LDL-C				
T1 (< 2.85)	50 (17.42%)	Ref.	Ref.	Ref.
T2 (2.85–< 3.46)	58 (19.80%)	1.17 (0.77–1.78) 0.463	1.10 (0.72–1.69) 0.666	1.14 (0.73–1.76) 0.567
T3 (≥ 3.46)	80 (27.40%)	1.79 (1.20–2.67) 0.004	1.70 (1.13–2.57) 0.012	1.75 (1.14–2.67) 0.010
p for trend		0.004	0.010	0.009
Non-HDL-C, per 1 mmol/L increase	188 (21.56%)	1.39 (1.16–1.65) < 0.001	1.35 (1.12–1.61) 0.001	1.36 (1.13–1.65) 0.001
Tertiles of Non-HDL-C				
T1 (< 3.37)	49 (16.96%)	Ref.	Ref.	Ref.
T2 (3.37–< 4.10)	57 (19.52%)	1.19 (0.78–1.81) 0.424	1.09 (0.71–1.68) 0.696	1.14 (0.73–1.77) 0.571
T3 (≥ 4.10)	82 (28.18%)	1.92 (1.29–2.87) 0.001	1.81 (1.20–2.73) 0.005	1.89 (1.23–2.89) 0.004
p for trend		0.001	0.003	0.003

Model 1: adjusted for age and sex. Model 2: adjusted for age, sex, body mass index, current drinking, current smoking, estimated glomerular filtration rate, diabetes mellitus, hypertension, antihypertensive, and hypoglycemic drugs.
OR, odds ratio; CI, confidence interval; Ref., reference value; RC, remnant cholesterol; TC, total cholesterol; TG, triglycerides; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; Non-HDL-C, non-high-density lipoprotein cholesterol.

1.10–2.48; $p = 0.016$) for the risk of new-onset carotid plaque. In the adjusted multivariable regression models, increased RC was strongly associated with new-onset carotid plaque ($OR = 1.57$ per 1 mmol/L increase; 95% CI: 1.03–2.41; $p = 0.038$). Similar results appeared in lipid parameters as categorical variables in tertiles, and showed a gradient relationship except for HDL-C (p for trend < 0.05).

Associations of remnant lipoprotein cholesterol and other lipid parameters with new-onset carotid plaque when considered simultaneously

When RC and another conventional lipid parameter were put into the multivariable regression model simultaneously,

TABLE 3 Comparisons of remnant lipoprotein cholesterol (RC) and another lipid parameter in relation to new-onset carotid plaque.

Comparisons	OR (95% CI) *P-value*		OR (95% CI) *P-value*
Comparison I† (when considered RC and TC simultaneously)			
RC, mmol/L		**TC, mmol/L**	
T1 (< 0.42)	**Ref.**	**T1 (< 4.87)**	**Ref.**
T2 (0.42–< 0.64)	1.16 (0.73–1.86) 0.525	T2 (4.87–< 5.60)	1.12 (0.72–1.75) 0.611
T3 (≥ 0.64)	2.26 (1.40–3.65) < 0.001	T3 (≥ 5.60)	1.33 (0.83–2.12) 0.230
Comparison II† (when considered RC and TG simultaneously)			
RC, mmol/L		**TG, mmol/L**	
T1 (< 0.42)	**Ref.**	**T1 (< 0.99)**	**Ref.**
T2 (0.42–< 0.64)	1.16 (0.70–1.94) 0.564	T2 (0.99–< 1.55)	1.26 (0.77–2.07) 0.363
T3 (≥ 0.64)	2.55 (1.41–4.61) 0.002	T3 (≥ 1.55)	1.01 (0.55–1.85) 0.963
Comparison III† (when considered RC and HDL-C simultaneously)			
RC, mmol/L		**HDL-C, mmol/L**	
T1 (< 0.42)	**Ref.**	**T1 (< 1.28)**	**Ref.**
T2 (0.42–< 0.64)	1.21 (0.75–1.94) 0.428	T2 (1.28–< 1.60)	1.22 (0.79–1.88) 0.377
T3 (≥ 0.64)	2.54 (1.55–4.15) < 0.001	T3 (≥ 1.60)	1.02 (0.60–1.74) 0.929
Comparison IV† (when considered RC and LDL-C simultaneously)			
RC-mmol/L		**LDL-C, mmol/L**	
T1 (< 0.42)	**Ref.**	**T1 (< 2.85)**	**Ref.**
T2 (0.42–< 0.64)	1.14 (0.71–1.84) 0.578	T2 (2.85–< 3.46)	1.07 (0.68–1.67) 0.772
T3 (≥ 0.64)	2.25 (1.39–3.63) < 0.001	T3 (≥ 3.46)	1.33 (0.84–2.11) 0.230
Comparison V† (when considered RC and Non-HDL-C simultaneously)			
RC, mmol/L		**Non-HDL-C, mmol/L**	
T1 (< 0.42)	**Ref.**	**T1 (< 3.37)**	**Ref.**
T2 (0.42–< 0.64)	1.19 (0.72–1.96) 0.494	T2 (3.37–< 4.10)	0.94 (0.59–1.52) 0.811
T3 (≥ 0.64)	2.28 (1.31–3.97) 0.004	T3 (≥ 4.10)	1.17 (0.68–2.00) 0.569

†RC and other lipid parameters were simultaneously added into the multivariable regression model. The model was adjusted for age, sex, body mass index, current drinking, current smoking, estimated glomerular filtration rate, diabetes mellitus, hypertension, antihypertensive, and hypoglycemic drugs.
OR, odds ratio; CI, confidence interval; Ref., reference value; RC, remnant cholesterol; TC, total cholesterol; TG, triglycerides; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; Non-HDL-C, non-high-density lipoprotein cholesterol.

only RC remained significantly associated with new-onset carotid plaque, even after adjusting for other lipid parameters respectively in different comparisons. Compared with the bottom tertile (T1), the effect of higher RC (the top tertile) for new-onset carotid plaque was increased by 2.26 (95% CI: 1.40–3.65) after adjusting for TC, 2.55 (95% CI: 1.41–4.16) after adjusting for TG, 2.54 (95% CI: 1.55–4.15) after adjusting for HDL-C, 2.25 (95% CI: 1.39–3.63) after adjusting for LDL-C, and 2.28 (95% CI: 1.31–3.97) after adjusting for non-HDL-C, respectively (Table 3).

Association of remnant lipoprotein cholesterol for new-onset carotid plaque modified by baseline low-density lipoprotein cholesterol levels

Furthermore, we investigated the modification of baseline LDL-C levels for the effect of RC on new-onset carotid plaque in participants with baseline LDL-C levels. After adjusting for possible covariates, Figure 2 displays the smooth curves showing the relationships between RC and new-onset carotid plaque stratified by baseline LDL-C. Table 4 shows that baseline LDL-C levels modified the association of RC for new-onset carotid plaque, with an increased OR to 1.95 (95% CI: 1.06–3.56) in participants with lower baseline LDL-C levels (p for interaction = 0.044).

Discussion

The major findings of this study are that conventional lipid parameters, especially RC, were superiorly associated with new-onset carotid plaque, independent of other lipids, in Chinese community-based population with no history of cardiovascular disease. Additionally, among participants with lower baseline LDL-C levels, RC should be considered an important biomarker to assess carotid artery atherosclerosis risk.

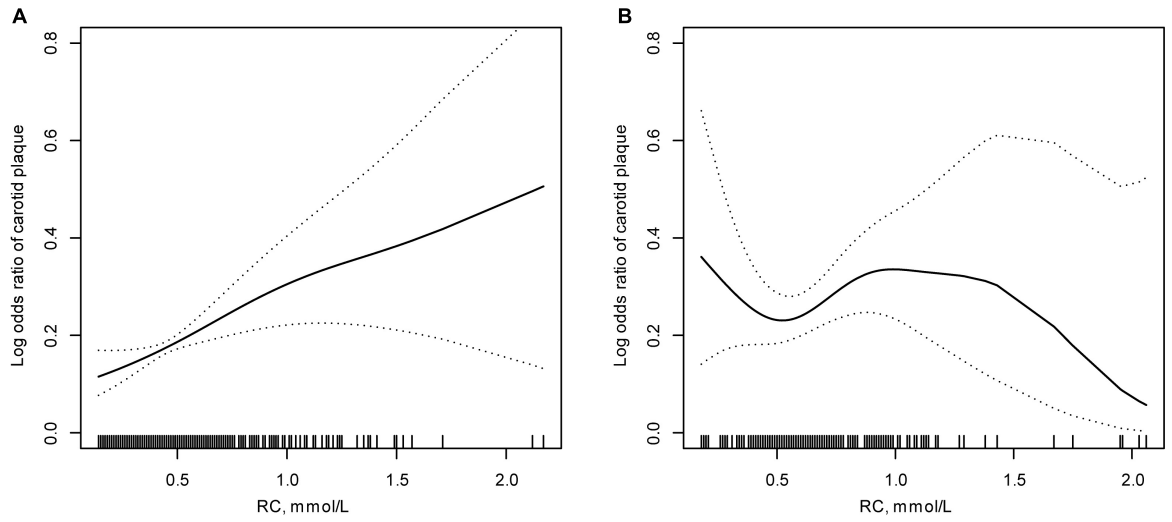


FIGURE 2
Effect of new-onset carotid plaque based on remnant lipoprotein cholesterol (RC) modified by low-density lipoprotein cholesterol (LDL-C) levels*. **(A)** Baseline LDL-C < 3.4 mmol/L; **(B)** baseline LDL-C ≥ 3.4 mmol/L. *Adjusted for: sex, age, body mass index, current drinking, current smoking, estimated glomerular filtration rate, diabetes mellitus, hypertension, antihypertensive, and hypoglycemic drugs.

Previous studies have already investigated the relationship between remnant lipoprotein cholesterol and cardiovascular diseases (8, 16–20, 30–34). Remnant cholesterol was considered a risk factor for various cardiovascular events. Varbo and colleagues found that elevated RC could causes ischemic heart disease, independent of reduced HDL-C (8). Remnant-like particle (RLP) cholesterol has also similarly been shown to be an independent risk factor for cardiovascular disease among 1,567 women from the Framingham Heart Study (30), and in elderly Japanese coronary artery disease (CHD) patients (31). In addition, some prospective studies have been presented supporting the prognostic value of remnant lipoprotein for cardiovascular disease, the results from the Jackson Heart Study and Framingham Offspring Cohort Study demonstrated that RC was positively associated with incident CHD events, but the association was not significant after adjustments for HDL-C and LDL-C (16). Some studies have reported the significant association between remnant lipoprotein cholesterol and the risk of coronary events in CHD or ACS patients with or without diabetes (32–36).

However, few studies have focused on carotid atherosclerosis assessed by carotid plaque. Masson et al. conducted a cross-sectional study and concluded that higher RC was associated with the presence of carotid atherosclerotic plaque (21). In the present study, a superior independent association of increased RC levels with new-onset carotid plaque compared to other conventional lipid parameters was demonstrated.

Several potential mechanisms may account for the effect of elevated levels of RC on new-onset carotid plaque. Like LDL-C passing the endothelial layer and trapping into the arterial intima, this would lead to the accumulation of cholesterol, the occurrence of atherosclerosis and cardiovascular events (3). Unlike LDL, remnant lipoprotein cholesterol could be taken up directly (no need to be modified: oxidation) by macrophages to cause foam cell formation and atherosclerotic plaque formation (37). Additionally, it has been shown that RC is an indicator of endothelial vasomotor dysfunction (38) that can upregulate the expression of pro-inflammatory factors (facilitate monocyte movement into the arterial wall), adhesion molecules (promote the formation of thrombus) (39), and coagulation factors (enhance the aggregation of platelets) (40). Elevated RC was causally associated with low-grade inflammation at a whole-body level, with 37% higher C-reactive protein levels for 1-mmol/L higher levels of RC (12), and related to carotid macrophage content, a marker for plaque instability (24). Taken together, the direct and indirect roles (pro-inflammatory and pro-atherothrombotic) of remnant lipoprotein cholesterol could partially explain increased risk of new-onset carotid plaque.

TABLE 4 Association of remnant lipoprotein cholesterol (RC) for new-onset carotid plaque modified by baseline low-density lipoprotein cholesterol (LDL-C) levels.

Variables	N (%)	OR (95% CI)	*P-value*	*p* interaction
LDL-C, mmol/L				
< 3.4	105 (18.72%)	1.95 (1.06–3.56)	0.031	0.044
≥ 3.4	83 (26.69%)	0.97 (0.40–2.34)	0.952	

Model adjusted for age, sex, body mass index, current drinking, current smoking, estimated glomerular filtration rate, diabetes mellitus, hypertension, antihypertensive, and hypoglycemic drugs.
OR, odds ratio; CI, confidence interval; LDL-C, low-density lipoprotein cholesterol.

In addition, Nakamura et al. reported that RC was superior to non-HDL-C for predicting cardiovascular events with LDL-C levels < 2.6 mmol/L treated with statins in patients with coronary artery disease (41). Consistently, our study demonstrated that increased RC levels were more strongly associated with the risk of new-onset carotid plaque when comparing two lipid parameters in the same model simultaneously. Studies have reported that increased RC can explain part of the residual risk of cardiovascular disease with lower or well-controlled levels of LDL-C goal (4, 42). Lin et al. found that higher RC concentrations were significantly associated with coronary atherosclerotic burden, even with an optimal level of LDL-C (23). In another study, the investigator reported that subjects with higher baseline RC had a higher risk of major adverse cardiovascular events (MACEs) than those at lower concentrations, especially lower LDL-C levels, with a highest HR of 2.69 ($p = 0.001$) (20). Similarly, our study found that the stronger association of RC with the risk of new-onset carotid plaque was demonstrated in participants with lower baseline LDL-C levels (< 3.4 mmol/L), which indicated that RC remained a residual risk factor for ASCVD for new-onset carotid plaque when LDL-C achieved to goal (< 3.4 mmol/L). A similar study demonstrated that the high RC/low LDL-C group, was associated with increased ASCVD risk (43).

The present study, to the best of our knowledge, is the first to evaluate the associations between RC and new-onset carotid plaque, and to compare RC and other lipid parameters in relation to new-onset carotid plaque in the Chinese population. Additionally, different baseline LDL-C levels modified the association of RC for carotid plaque. There are several limitations that need to be addressed. First, all participants were from a community-based cohort, and therefore external generalizability is limited. Second, the use of fasting samples may underestimate the contribution of chylomicron, due to VLDL are the dominant constituents of circulating remnants (44), and calculated RC cannot be as accurate as direct measurement, while it's easier to calculate RC by other conventional lipid parameters to save costs, and the association was remarkably consistent (27, 45–47). Third, data such as inflammatory biomarkers, dietary habits, fatty liver, vascular ultrasound in other territories, etc., were not collected at baseline, which may affect atherosclerosis formation. Finally, carotid plaque formation is a marker for carotid artery damage to evaluate the risk of cardiovascular events, and the need to observe the risk of MACEs during continuous follow-up should be considered.

In conclusion, remnant cholesterol was superior and independent of other conventional lipid parameters, and was significantly associated with new-onset carotid plaque when considered simultaneously. Remnant cholesterol could be helpful to predict carotid artery damage in participants with lower baseline LDL-C levels for the purpose of early atherosclerosis prevention.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by Peking University First Hospital Ethics Committee. The patients/participants provided their written informed consent to participate in this study.

Author contributions

YZ and YH were responsible for the study concept and design. BZ, YY, and JL helped with the design and coordination of the study. PS, YJ, KL, JhL, and CC collected and rechecked the data. FF, JJ, and BL analyzed and interpreted the data. BL drafted the manuscript. FF and YZ revised the manuscript. All authors reviewed and approved the manuscript and agreed to be accountable for all aspects of the work.

Funding

This study was supported by grant from National Key Research and Development Program of China (2021YFC2500500 and 2021YFC2500503), Projects of National Natural Science Foundation of China (Grants 81703288 and 82170452), UMHS-PUHSC Joint Institute for Translational and Clinical Research and the Fundamental Research Funds for the Central Universities (BMU20110177 and BMU20160530), Chinese Cardiovascular Association-Access Fund (2019-CCA-ACCESS-112), Key Laboratory of Molecular Cardiovascular Sciences (Peking University), and National Health Commission Key Laboratory of Cardiovascular Molecular Biology and Regulatory Peptides.

Acknowledgments

We thank the staff and participants of the present study for their important contributions. We are especially grateful to the site managers of Gucheng and Pingguoyuan Community Health Centers for their supports.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcvm.2022.903390/full#supplementary-material

References

1. Varbo A, Nordestgaard BG. Remnant lipoproteins. *Curr Opin Lipidol.* (2017) 28:300–7. doi: 10.1097/MOL.0000000000000429

2. Nordestgaard BG, Varbo A. Triglycerides and cardiovascular disease. *Lancet.* (2014) 384:626–35. doi: 10.1016/S0140-6736(14)61177-6

3. Nordestgaard BG. Triglyceride-rich lipoproteins and atherosclerotic cardiovascular disease: new insights from epidemiology. *Genet Biol Circ Res.* (2016) 118:547–63. doi: 10.1161/CIRCRESAHA.115.306249

4. Jepsen AM, Langsted A, Varbo A, Bang LE, Kamstrup PR, Nordestgaard BG. Increased remnant cholesterol explains part of residual risk of all-cause mortality in 5414 patients with ischemic heart disease. *Clin Chem.* (2016) 62:593–604. doi: 10.1373/clinchem.2015.253757

5. Schwartz GG, Abt M, Bao W, DeMicco D, Kallend D, Miller M, et al. Fasting triglycerides predict recurrent ischemic events in patients with acute coronary syndrome treated with statins. *J Am Coll Cardiol.* (2015) 65:2267–75. doi: 10.1016/j.jacc.2015.03.544

6. Sampson UK, Fazio S, Linton MF. Residual cardiovascular risk despite optimal ldl cholesterol reduction with statins: the evidence, etiology, and therapeutic challenges. *Curr Atheros Rep.* (2012) 14:1–10. doi: 10.1007/s11883-011-0219-7

7. Twickler TB, Dallinga-Thie GM, Cohn JS, Chapman MJ. Elevated remnant-like particle cholesterol concentration: a characteristic feature of the atherogenic lipoprotein phenotype. *Circulation.* (2004) 109:1918–25. doi: 10.1161/01.CIR.0000125278.58527.F3

8. Varbo A, Benn M, Tybjaerg-Hansen A, Jorgensen AB, Frikke-Schmidt R, Nordestgaard BG. Remnant cholesterol as a causal risk factor for ischemic heart disease. *J Am Coll Cardiol.* (2013) 61:427–36. doi: 10.1016/j.jacc.2012.08.1026

9. Varbo A, Benn M, Nordestgaard BG. Remnant cholesterol as a cause of ischemic heart disease: evidence, definition, measurement, atherogenicity, high risk patients, and present and future treatment. *Pharmacol Ther.* (2014) 141:358–67. doi: 10.1016/j.pharmthera.2013.11.008

10. Nordestgaard BG, Benn M, Schnohr P, Tybjaerg-Hansen A. Nonfasting triglycerides and risk of myocardial infarction, ischemic heart disease, and death in men and women. *JAMA.* (2007) 298:299–308. doi: 10.1001/jama.298.3.299

11. Izumida T, Nakamura Y, Hino Y, Ishikawa S. Combined effect of small dense low-density lipoprotein cholesterol (Sdldl-C) and remnant-like particle cholesterol (Rlp-C) on low-grade inflammation. *J Atheroscler Thromb.* (2020) 27:319–30. doi: 10.5551/jat.49528

12. Varbo A, Benn M, Tybjaerg-Hansen A, Nordestgaard BG. Elevated remnant cholesterol causes both low-grade inflammation and ischemic heart disease, whereas elevated low-density lipoprotein cholesterol causes ischemic heart disease without inflammation. *Circulation.* (2013) 128:1298–309. doi: 10.1161/CIRCULATIONAHA.113.003008

13. Mitchell C, Korcarz CE, Gepner AD, Kaufman JD, Post W, Tracy R, et al. Ultrasound carotid plaque features, cardiovascular disease risk factors and events: the multi-ethnic study of atherosclerosis. *Atherosclerosis.* (2018) 276:195–202. doi: 10.1016/j.atherosclerosis.2018.06.005

14. Hollander M, Bots ML, Del Sol AI, Koudstaal PJ, Witteman JC, Grobbee DE, et al. Carotid plaques increase the risk of stroke and subtypes of cerebral infarction in asymptomatic elderly: the Rotterdam study. *Circulation.* (2002) 105:2872–7. doi: 10.1161/01.cir.0000018650.58984.75

15. Yang Y, Fan F, Kou M, Yang Y, Cheng G, Jia J, et al. Brachial-ankle pulse wave velocity is associated with the risk of new carotid plaque formation: data from a Chinese community-based cohort. *Sci Rep.* (2018) 8:7037. doi: 10.1038/s41598-018-25579-2

16. Joshi PH, Khokhar AA, Massaro JM, Lirette ST, Griswold ME, Martin SS, et al. Remnant lipoprotein cholesterol and incident coronary heart disease: the Jackson heart and Framingham offspring cohort studies. *J Am Heart Assoc.* (2016) 5:2765. doi: 10.1161/JAHA.115.002765

17. Varbo A, Nordestgaard BG. Remnant cholesterol and triglyceride-rich lipoproteins in atherosclerosis progression and cardiovascular disease. *Arterioscler Thromb Vasc Biol.* (2016) 36:2133–5. doi: 10.1161/ATVBAHA.116.308305

18. Saeed A, Feofanova EV, Yu B, Sun W, Virani SS, Nambi V, et al. Remnant-like particle cholesterol, low-density lipoprotein triglycerides, and incident cardiovascular disease. *J Am Coll Cardiol.* (2018) 72:156–69. doi: 10.1016/j.jacc.2018.04.050

19. Jorgensen AB, Frikke-Schmidt R, West AS, Grande P, Nordestgaard BG, Tybjaerg-Hansen A. Genetically elevated non-fasting triglycerides and calculated remnant cholesterol as causal risk factors for myocardial infarction. *Eur Heart J.* (2013) 34:1826–33. doi: 10.1093/eurheartj/ehs431

20. Castaner O, Pinto X, Subirana I, Amor AJ, Ros E, Hernaez A, et al. Remnant cholesterol, not ldl cholesterol, is associated with incident cardiovascular disease. *J Am Coll Cardiol.* (2020) 76:2712–24. doi: 10.1016/j.jacc.2020.10.008

21. Masson W, Lobo M, Molinero G, Siniawski D. Discordant lipid pattern and carotid atherosclerotic plaque. *Import Remnant Choles Arq Bras Cardiol.* (2017) 108:526–32. doi: 10.5935/abc.20170069

22. Gao Y, Lou Y, Liu Y, Wu S, Xi Z, Wang X, et al. The relationship between residual cholesterol risk and plaque characteristics in patients with acute coronary syndrome: insights from an optical coherence tomography study. *Atherosclerosis.* (2021) 317:10–5. doi: 10.1016/j.atherosclerosis.2020.11.033

23. Lin A, Nerlekar N, Rajagopalan A, Yuvaraj J, Modi R, Mirzaee S, et al. Remnant cholesterol and coronary atherosclerotic plaque burden assessed by computed tomography coronary angiography. *Atherosclerosis.* (2019) 284:24–30. doi: 10.1016/j.atherosclerosis.2019.02.019

24. Zambon A, Puato M, Faggin E, Grego F, Rattazzi M, Pauletto P. Lipoprotein remnants and dense Ldl are associated with features of unstable carotid plaque: a flag for non-Hdl-C. *Atherosclerosis.* (2013) 230:106–9. doi: 10.1016/j.atherosclerosis.2013.06.024

25. Joint committee issued Chinese guideline for the management of dyslipidemia in adults. [2016 Chinese guideline for the management of dyslipidemia in adults]. *Zhonghua Xin Xue Guan Bing Za Zhi.* (2016) 44:833–53. doi: 10.3760/cma.j.issn.0253-3758.2016.10.005

26. Fan F, Qi L, Jia J, Xu X, Liu Y, Yang Y, et al. Noninvasive central systolic blood pressure is more strongly related to kidney function decline than peripheral systolic blood pressure in a Chinese community-based population. *Hypertension.* (2016) 67:1166–72. doi: 10.1161/HYPERTENSIONAHA.115.07019

27. Varbo A, Freiberg JJ, Nordestgaard BG. Extreme nonfasting remnant cholesterol vs extreme Ldl cholesterol as contributors to cardiovascular disease and all-cause mortality in 90000 individuals from the general population. *Clin Chem.* (2015) 61:533–43. doi: 10.1373/clinchem.2014.234146

28. Varbo A, Benn M, Smith GD, Timpson NJ, Tybjaerg-Hansen A, Nordestgaard BG. Remnant cholesterol, low-density lipoprotein cholesterol, and blood pressure as mediators from obesity to ischemic heart disease. *Circ Res.* (2015) 116:665–73. doi: 10.1161/CIRCRESAHA.116.304846

29. Touboul PJ, Hennerici MG, Meairs S, Adams H, Amarenco P, Bornstein N, et al. Mannheim carotid intima-media thickness and plaque consensus (2004-2006-2011). An update on behalf of the advisory board of the 3rd, 4th and 5th watching the risk symposia, at the 13th, 15th and 20th European stroke conferences, Mannheim, Germany, 2004, Brussels, Belgium, 2006, and Hamburg, Germany, 2011. *Cerebrovascular Dis (Basel Switzerland).* (2012) 34:290–6. doi: 10.1159/000343145

30. McNamara JR, Shah PK, Nakajima K, Cupples LA, Wilson PW, Ordovas JM, et al. Remnant-like particle (Rlp) cholesterol is an independent cardiovascular disease risk factor in women: results from the Framingham heart study. *Atherosclerosis.* (2001) 154:229–36. doi: 10.1016/s0021-9150(00)00484-6

31. Inoue T, Uchida T, Kamishirado H, Takayanagi K, Hayashi T, Morooka S, et al. Remnant-like lipoprotein particles as risk factors for coronary artery disease in elderly patients. *Horm Metab Res.* (2004) 36:298–302. doi: 10.1055/s-2004-814486

32. Kugiyama K, Doi H, Takazoe K, Kawano H, Soejima H, Mizuno Y, et al. Remnant lipoprotein levels in fasting serum predict coronary events in patients with coronary artery disease. *Circulation.* (1999) 99:2858–60. doi: 10.1161/01.cir.99.22.2858

33. Fukushima H, Sugiyama S, Honda O, Koide S, Nakamura S, Sakamoto T, et al. Prognostic value of remnant-like lipoprotein particle levels in patients with coronary artery disease and type ii diabetes mellitus. *J Am Coll Cardiol.* (2004) 43:2219–24. doi: 10.1016/j.jacc.2003.09.074

34. Nguyen SV, Nakamura T, Uematsu M, Fujioka D, Watanabe K, Watanabe Y, et al. Remnant lipoproteinemia predicts cardiovascular events in patients with type 2 diabetes and chronic kidney disease. *J Cardiol.* (2017) 69:529–35. doi: 10.1016/j.jjcc.2016.04.011

35. Shao Q, Yang Z, Wang Y, Li Q, Han K, Liang J, et al. Elevated remnant cholesterol is associated with adverse cardiovascular outcomes in patients with acute coronary syndrome. *J Atheroscler Thromb.* (2022) 2022:63397. doi: 10.5551/jat.63397

36. Chen Y, Li G, Guo X, Ouyang N, Li Z, Ye N, et al. The effects of calculated remnant-like particle cholesterol on incident cardiovascular disease: insights from a general Chinese population. *J Clin Med.* (2021) 10:15. doi: 10.3390/jcm10153388

37. Nakajima K, Nakano T, Tanaka A. The oxidative modification hypothesis of atherosclerosis: the comparison of atherogenic effects on oxidized Ldl and remnant lipoproteins in plasma. *Clin Chim Acta.* (2006) 367:36–47. doi: 10.1016/j.cca.2005.12.013

38. Kugiyama K, Doi H, Motoyama T, Soejima H, Misumi K, Kawano H, et al. Association of remnant lipoprotein levels with impairment of endothelium-dependent vasomotor function in human coronary arteries. *Circulation.* (1998) 97:2519–26. doi: 10.1161/01.cir.97.25.2519

39. Doi H, Kugiyama K, Oka H, Sugiyama S, Ogata N, Koide SI, et al. Remnant lipoproteins induce proatherothrombogenic molecules in endothelial cells through a redox-sensitive mechanism. *Circulation.* (2000) 102:670–6. doi: 10.1161/01.cir.102.6.670

40. Saniabadi AR, Umemura K, Shimoyama M, Adachi M, Nakano M, Nakashima M. Aggregation of human blood platelets by remnant like lipoprotein particles of plasma chylomicrons and very low density lipoproteins. *Thromb Haemost.* (1997) 77:996–1001.

41. Nakamura T, Obata JE, Hirano M, Kitta Y, Fujioka D, Saito Y, et al. Predictive value of remnant lipoprotein for cardiovascular events in patients with coronary artery disease after achievement of Ldl-cholesterol goals. *Atherosclerosis.* (2011) 218:163–7. doi: 10.1016/j.atherosclerosis.2011.04.040

42. Fujihara Y, Nakamura T, Horikoshi T, Obata JE, Fujioka D, Watanabe Y, et al. Remnant lipoproteins are residual risk factor for future cardiovascular events in patients with stable coronary artery disease and on-statin low-density lipoprotein cholesterol levels <70 Mg/Dl. *Circ J Offi J Japanese Circ Soc.* (2019) 83:1302–8. doi: 10.1253/circj.CJ-19-0047

43. Quispe R, Martin SS, Michos ED, Lamba I, Blumenthal RS, Saeed A, et al. Remnant cholesterol predicts cardiovascular disease beyond ldl and apob: a primary prevention study. *Eur Heart J.* (2021) 2021:432. doi: 10.1093/eurheartj/ehab432

44. Wang T, Nakajima K, Leary ET, Warnick GR, Cohn JS, Hopkins PN, et al. Ratio of remnant-like particle-cholesterol to serum total triglycerides is an effective alternative to ultracentrifugal and electrophoretic methods in the diagnosis of familial type III hyperlipoproteinemia. *Clin Chem.* (1999) 45:1981–7.

45. Zhao Q, Zhang TY, Cheng YJ, Ma Y, Xu YK, Yang JQ, et al. Prognostic impact of estimated remnant-like particle cholesterol in patients with differing glycometabolic status: an observational cohort study from china. *Lipids Health Dis.* (2020) 19:179. doi: 10.1186/s12944-020-01355-y

46. Chen J, Kuang J, Tang X, Mao L, Guo X, Luo Q, et al. Comparison of calculated remnant lipoprotein cholesterol levels with levels directly measured by nuclear magnetic resonance. *Lipids Health Dis.* (2020) 19:132. doi: 10.1186/s12944-020-01311-w

47. Varbo A, Nordestgaard BG. Directly measured vs. calculated remnant cholesterol identifies additional overlooked individuals in the general population at higher risk of myocardial infarction. *Eur Heart J.* (2021) 2021:293. doi: 10.1093/eurheartj/ehab293

frontiers | Frontiers in Cardiovascular Medicine

TYPE Review
PUBLISHED 04 October 2022
DOI 10.3389/fcvm.2022.993633

OPEN ACCESS

EDITED BY
Kailash Gulshan,
Cleveland State University,
United States

REVIEWED BY
Chia-Feng Liu,
Cleveland Clinic, United States
Babunageswararao Kanuri,
The Ohio State University,
United States

*CORRESPONDENCE
Chu-Huang Chen
cchen@texasheart.org

SPECIALTY SECTION
This article was submitted to Lipids in Cardiovascular Disease, a section of the journal Frontiers in Cardiovascular Medicine

RECEIVED 13 July 2022
ACCEPTED 12 September 2022
PUBLISHED 04 October 2022

CITATION
Lee H-C, Akhmedov A and Chen C-H (2022) Spotlight on very-low-density lipoprotein as a driver of cardiometabolic disorders: Implications for disease progression and mechanistic insights. *Front. Cardiovasc. Med.* 9:993633. doi: 10.3389/fcvm.2022.993633

Spotlight on very-low-density lipoprotein as a driver of cardiometabolic disorders: Implications for disease progression and mechanistic insights

Hsiang-Chun Lee[1,2,3,4,5], Alexander Akhmedov[6] and Chu-Huang Chen[7]*

[1]Department of Internal Medicine, Division of Cardiology, Kaohsiung Medical University Hospital, Kaohsiung Medical University, Kaohsiung, Taiwan, [2]Department of Internal Medicine, School of Medicine, College of Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan, [3]Lipid Science and Aging Research Center, College of Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan, [4]Institute/Center of Medical Science and Technology, National Sun Yat-sen University, Kaohsiung, Taiwan, [5]Graduate Institute of Animal Vaccine Technology, National Pingtung University of Science and Technology, Pingtung, Taiwan, [6]Center for Molecular Cardiology, University of Zurich, Schlieren, Switzerland, [7]Vascular and Medicinal Research, Texas Heart Institute, Houston, TX, United States

Very-low-density lipoprotein (VLDL) is the only lipoprotein containing apolipoprotein B that is secreted from the liver, where VLDL is assembled from apolipoproteins, cholesterol, and triglycerides. The primary function of VLDL is to transport cholesterol and other lipids to organs and cells for utilization. Apart from its role in normal biologic processes, VLDL is also known to contribute to the development of atherosclerotic cardiovascular disease. Large VLDL particles, which are subclassified according to their size by nuclear magnetic resonance spectrometry, are significantly correlated not only with atherosclerosis, but also with insulin resistance and diabetes incidence. VLDL can also be subclassified according to surface electrical charge by using anion-exchange chromatography. The most electronegative VLDL subclass is highly cytotoxic to endothelial cells and may contribute to coronary heart disease. In addition, electronegative VLDL contributes to the development of atrial remodeling, especially in patients with metabolic syndrome, which is an established risk factor for atrial fibrillation. In this review, we focus on the VLDL subclasses that are associated with apolipoprotein alterations and are involved in cardiometabolic disease. The postprandial enhancement of VLDL's pathogenicity is a critical medical issue, especially in patients with metabolic syndrome. Therefore, the significance of the postprandial modification of VLDL's chemical and functional properties is extensively discussed.

KEYWORDS

very-low-density lipoprotein, cardiovascular disease, triglycerides, metabolic syndrome, apolipoproteins, cardiometabolic disorders

Introduction

Composition of very-low-density lipoprotein

Very-low-density lipoprotein (VLDL) is a precursor to intermediate-density lipoprotein (IDL), which subsequently forms low-density lipoprotein (LDL). Density-gradient ultracentrifugation is the standard method used to isolate VLDL and other major lipoproteins, including chylomicrons, IDL, LDL, and high-density lipoprotein (HDL) from serum or plasma (1, 2). The lipid core of VLDL consists of triglycerides (TGs, 50–70% of particle mass), cholesterol ester (10–25%), and fatty acids (<10%). The major core protein of VLDL is apolipoprotein (apo)B100; other proteins include apoCI, apoCII, apoCIII, and apoE. These surface apolipoproteins also serve as ligands for cell-surface receptors and coordinators for lipolysis (3).

The physiologic functions of very-low-density lipoprotein – More than a cargo carrier for lipids

VLDL functions as a cargo carrier, transporting cholesterol, TGs, and proteins to peripheral cells for essential bioactivities. In the liver, TGs and cholesterol are incorporated with apoB100, which affects the lipid abundance and size of secreted VLDL (4). After VLDL is secreted, it is hydrolyzed by lipoprotein lipase (LPL), which is present in the capillary endothelium or associated with VLDL receptors, and transformed into VLDL remnant and IDL. HDL then takes up apoCII from VLDL remnant and IDL, and cholesterol ester transfer protein (CETP) exchanges their TGs and phospholipids with cholesterol. IDL can be taken up by the liver via the LDL receptor or after being transformed into LDL upon losing apoE and TGs (3). VLDL is a TG-rich lipoprotein, and its assembly and metabolism are affected by insulin resistance and long-term nutrient excess (5). VLDL also modulates nitric oxide signaling, which is essential for vascular smooth muscle relaxation and blood pressure control (6). In addition, VLDL enhances phospholipase D activity by increasing cytosolic calcium levels and stimulates aldosterone synthesis in the adrenal gland (7). Therefore, VLDL does not only serve as a lipid cargo carrier, but it also modulates lipid-related blood pressure regulation.

The classification of very-low-density lipoprotein by particle size

The diameter of VLDL particles can be measured using nuclear magnetic resonance (NMR) spectrometry. To classify VLDL subfractions by particle diameter, most studies have used a simplified classification system with different categories of average diameter. The quantitative analysis of serum or plasma lipoprotein subfractions requires high reproducibility. Such reproducibility has been examined by pooling quality control plasma lipoprotein samples and comparing NMR results among 11 spectrometers and 5 laboratories. In total, 16 subclasses were identified: 6 for VLDL, 6 for LDL, and 4 for HDL (8). However, a consensus has not been reached with respect to standard diameter ranges for classifying VLDL subfractions. For instance, in the study by Garvey et al. (9), three categories were defined as follows: large VLDL (>60nm), intermediate VLDL (35–60 nm), and small VLDL (<35 nm). In the study by Phillips et al. (10), the categories were defined as follows: large VLDL (including chylomicrons, if present, >60 nm), medium VLDL (42–60 nm), and small VLDL (<42 nm). Wang et al. (11) used six categories of VLDL as follows: largest (including chylomicrons, ± 75 nm), very large (average diameter, 64.0 nm), large (53.6 nm), medium (44.5 nm), small (36.8 nm), and very small (31.3 nm) VLDL.

The classification of very-low-density lipoprotein by particle charge

In 1988, Avogaro et al. (12) first characterized LDL on the basis of surface electrical charge rather than particle size by using anion-exchange chromatography to separate LDL into LDL(+) and LDL(–). In addition, Yang et al. (13) and Chen et al. (14) divided LDL into five subfractions according to electrical charge, called L1-L5. Similarly, Chen et al. also used the same method of anion-exchange chromatography to separate VLDL into five subfractions, called V1-V5 (15) (**Table 1**).

Immunochemical isolation of very-low-density lipoprotein according to apolipoprotein content

Apolipoproteins are chemically unique, maintaining the structural integrity and functional specificity of different lipoprotein particles in lipid transport processes. Therefore, lipoproteins can be classified immunochemically according to their apolipoprotein composition (16). The two major classes of apolipoprotein-based families are apoA-containing and apoB-containing lipoproteins. VLDL, along with IDL and LDL, is an apoB-containing lipoprotein family. The apoB-containing lipoproteins can be divided into several subfamilies, including cholesterol ester-rich lipoprotein (LP-B) and TG-rich lipoproteins (16).

TABLE 1 VLDL subclassified by size and electrical charge and the effects of VLDL subclasses on atherosclerotic CVD, MetS, and other conditions.

Classification	Patients	Fasting/postprandial	Effects	References
NMR-based VLDL subclasses and atherosclerotic CVD				
Large, medium, and small VLDL particles	Adults with incident coronary artery calcium (n = 6814; age, 45–85 years)	Overnight fasting (12 h)	Large VLDL was positively associated with incident coronary artery calcification in a model adjusted for scanner type, age, gender, and race	Zeb et al. (25)
Large, medium, and small VLDL particles	Healthy postmenopausal women (n = 286; mean age, 61.7 years)	Fasting (12 h)	Large VLDL was positively associated ($p < 0.05$) with higher coronary artery calcification after adjusting for age, systolic blood pressure, current smoking status, LDL cholesterol, HDL cholesterol, and triglycerides	Mackey et al. (26)
NMR-based VLDL subclasses and MetS or other conditions				
Large VLDL, medium VLDL, and small VLDL	Irish adults (n = 1834, middle-aged)	Overnight fasting	Metabolically healthy patients with smaller (below median) VLDL size	Phillips et al. (10)
Largest VLDL (including chylomicrons) and five different VLDL subclasses	Finnish men with or without glucose intolerance (n = 9399; mean age, 56.8 ± 6.9)	Overnight fasting	The concentrations of all lipid components in the VLDL subclasses were increased as glucose tolerance decreased	Wang et al. (11)
Large, intermediate, and small VLDL particles	Patients with or without diabetes (n = 148; mean age, 36.8 ± 11.8 years)	Overnight fasting	Progressive insulin resistance was associated with increased VLDL size and an increase in large VLDL particle concentrations	Garvey et al. (9)
Large, medium, and small VLDL particles	Healthy women (n = 26,836; age ≥ 45 years)	75.8% without-diabetes and 78.6% with diabetes were fasting	Large VLDL imparted a higher risk for incident type 2 diabetes mellitus than did small particles	Mora et al. (27)
	Women with type 1 diabetes mellitus (n = 112; mean age, 44.9 ± 7.8 years)	Overnight fasting (10–12 h)	Medium VLDL was associated with previous pre-eclampsia	Amor et al. (28)
Six VLDL subfractions (V1-V6, increasing density)	Adults, free of clinically detectable CVD (n = 6814; age, 44–84 years)	Fasting (12 h)	Several VLDL subfractions (V1-V4) were associated with abdominal body composition and intra-muscle fat infiltration	Marron et al. (91)
Anion-exchange chromatography–based VLDL subclasses and MetS				
VLDL subfractions with increasing negative charge (V1-V5)	Patients with or without MetS (n = 26)	Overnight fasting	V5, a highly negatively charged VLDL subfraction, directly damaged the endothelium	Chen et al. (15)
LDL and VLDL subfractions with increasing negative charge (L1-L5, V1–V5)	Asymptomatic individuals (n = 33; age, 32–64 years)	Fasting	Combined electronegativity of L5 and V5 plasma concentration was significantly correlated with coronary heart disease risk	Shen et al. (31)
Most electronegatively charged VLDL subfraction (VLDL-χ)	Patients with or without MetS (n = 167; age, 23–74 years)	Overnight fasting and postprandial	Plasma concentration of VLDL-χ (%) at 2 h postprandial was positively correlated with atrial enlargement in patients with MetS	Lee et al. (71)

CVD, cardiovascular disease; LDL, low-density lipoprotein; NMR, nuclear magnetic resonance; VLDL, very-low-density lipoprotein.

Pathogenic very-low-density lipoprotein

The physiologic basis for the differences in composition, structure, and function among VLDL particles is important because these differences can strongly influence the atherogenic properties of VLDL. Moreover, abnormal VLDL can adversely affect vascular or cardiac cells (see below), which has important implications. In this review, we present a summary of the emerging evidence for VLDL in promoting cardiometabolic diseases and highlight how the subclassification of VLDL can be used to distinguish VLDL particles that are pathogenic from those that are physiologically necessary.

Independent of low-density lipoprotein, very-low-density lipoprotein is associated with cardiometabolic disorders

Cholesterols carried by both low-density lipoprotein and very-low-density lipoprotein are associated with atherosclerosis

Plasma LDL-cholesterol (LDL-C) alone is not sufficient to predict all non-atherosclerotic and atherosclerotic cardiovascular disease (ASCVD). Aside from LDL-C, VLDL cholesterol (VLDL-C) is also known to contribute to the development of ASCVD. Plasma VLDL-C is the primary component of non–HDL-cholesterol (HDL-C) (17) and is a predictor of ASCVD independent of LDL cholesterol (LDL-C) (18–20).

Prenner et al. (18) used cardiac electron beam computed tomography scanning to assess coronary artery calcification, which is an independent predictor of CVD risk, in a population of high-risk patients with type 2 diabetes. Their results showed that VLDL-C is an independent risk factor for coronary artery calcification, particularly in women. Furthermore, this association was independent of circulatory TG levels (18). In patients with type 2 diabetes who previously underwent coronary stent implantation, an elevated VLDL-C level >0.52 mmol/L was independently associated with in-stent restenosis (hazard ratio = 3.01) (21). Iannuzzi et al. (22) used ultrasound to measure carotid intima–media thickness in postmenopausal women and showed that VLDL-C was the lipoprotein most strongly associated with subclinical atherosclerosis. In addition, evidence from clinical studies has consistently indicated a causal role for TG-rich lipoproteins such as VLDL in ASCVD. An updated consensus statement regarding the current understanding of the role of TG-rich lipoproteins and their remnants in ASCVD has been published recently (5).

The size of very-low-density lipoprotein affects its atherogenicity

Large VLDL particles have a greater association with the incidence of atherosclerosis than do smaller VLDL particles (**Table 1**). The size-based subclassification of lipoproteins is performed by the NMR analyzer, which uses characteristic signals of lipoprotein subclasses with different sizes as the basis for quantification. A set of purified standards is required for converting signal amplitudes to specific particle concentrations (23). The standards for VLDL are isolated by using a combination of ultracentrifugation and agarose gel filtration, and the size distribution is determined by using electron microscopy (2).

VLDL circulates in the blood for about 4 h before it is converted to IDL and then LDL (24). Lipolytic remodeling is responsible for the down-sizing of the largest VLDL particles and their conversion to IDL and LDL. Unlike small LDL, large VLDL was associated with an increased risk of incident coronary artery calcification and calcium score progression during follow-up (25). Likewise, in relatively healthy postmenopausal women, large VLDL was positively associated with coronary artery calcification, suggesting that the measurement of lipoprotein subclasses may improve the prediction of coronary artery disease beyond using the conventional lipid panel (26).

In addition, the size of VLDL was shown to be correlated with insulin resistance and diabetes mellitus (11, 27) (**Table 1**). In a prospective study by Mora et al. (27) of 26,836 initially healthy women followed for 13 years, large VLDL particles were found to predict type 2 diabetes. Likewise, Wang et al. (11) reported in a population study of 9399 Finnish men that abnormal glucose tolerance and new onset type 2 diabetes were associated with an increase in VLDL particles, with the exception of very small VLDL. Conversely, a lower number of large VLDL particles was shown to be the most significant predictor of metabolic health in adults, regardless of body mass index and obesity status (10). Garvey et al. (9) described the effects of insulin resistance and type 2 diabetes on the particle size and concentration of lipoprotein subclasses. Their results showed that progressive insulin resistance was associated with increased VLDL size. Compared with individuals who have normal insulin sensitivity, patients with insulin resistance or diabetes showed increased concentrations of large VLDL particles, but no change in medium VLDL or small VLDL particle concentrations. For patients with type 1 diabetes, medium VLDL particle concentration was independently associated with previous pre-eclampsia during pregnancy after adjusting for age and statin use (28).

The charge-based electronegativity of very-low-density lipoprotein determines its atherogenicity

Lipoprotein particles can be separated according to charge by using anion-exchange chromatography. L5, which is the most electronegatively charged subfraction of LDL, induces endothelial apoptosis through the lectin-like oxidized LDL receptor-1 (LOX-1) in the absence of the LDL receptor (LDLR) (29). Similarly, the most electronegative subfraction of VLDL, V5, was shown to induce endothelial apoptosis and was the subfraction most rapidly internalized into endothelial cells (15). In addition, patients with metabolic syndrome (MetS) were found to have increased levels of electronegative VLDL. VLDL isolated from patients with MetS induced brain inflammation with glial cell activation

TABLE 2 Clinical studies showing altered VLDL apolipoproteins in patients with metabolic and atherogenic diseases.

Apolipoprotein	Study type	Patients	Fasting/ postprandial	Effects	References
ApoCI	Human	Cross-sectional studies (age, 56–80 years)	Fasting and postprandial (4 h)	ApoC1 positively correlated with carotid atherosclerosis	(35–37)
ApoCIII	Human	Ludwigshafen Risk and Cardiovascular Health Study (LURIC; $n = 3041$)	Not specified	Seven common variants of *APOC3* (rs734104, rs4520, rs5142, rs5141, rs5130, rs5128, and rs4225) were associated with modestly raised apoC-III and elevated VLDL/TG but were not associated with CAD	(92)
	Human	Middle-aged patients ($n = 688$; average age, 66 years; 52% women)	Fasting	ApoCII, apoCIII, and apoE were associated with composite CVD (fatal and non-fatal myocardial infarction, ischemic stroke, and sudden cardiac death)	(39)
ApoAV	Human	Patients with non-alcoholic fatty liver disease ($n = 17$) vs. healthy liver ($n = 6$)	Fasting	ApoA5 mRNA level was associated with hepatosteatosis	(46)
ApoE	Human	Two independent cohorts: women ($n = 322$; age, 30–55 years) and men ($n = 418$; age, 40–75 years)	Not specified	Increased apoE content in VLDL and LDL with apoCIII were associated with a lower risk of CHD	(33)
Angiopoietin-like protein (ANGPTL)-3	Human and mice	Humans and mice (e.g., *Angptl3*$^{-/-}$, *Ldlr*$^{-/-}$, *Lipg*$^{-/-}$) with hyperlipidemia	Fasting	ANGPTL-3 inhibition reduces the content and size of lipids in VLDL	(54)

CAD, coronary artery disease; CHD, coronary heart disease; CVD, cardiovascular disease; LDL, low-density lipoprotein; TG, triglyceride; VLDL, very-low-density lipoprotein.

in mice, suggesting that electronegative VLDL can promote cognitive dysfunction (30). Furthermore, Shen et al. (31) further confirmed that the most electronegative human plasma LDL (i.e., L5) and VLDL (i.e., V5) are highly atherogenic. In their study, the combined electronegativity of L5 and plasma concentration of V5 was significantly correlated with coronary heart disease risk in an age-adjusted analyses of asymptomatic individuals. Moreover, when human aortic endothelial cells were treated with L5 + V5 and L1 + V1, L5 + V5 induced significantly greater senescence-associated–β-galactosidase activity than did L1 + V1. In *ApoE*$^{-/-}$ mice, aortic lipid accumulation and cellular senescence were associated with the electronegativity of LDL and VLDL (31).

Altered apolipoprotein content in very-low-density lipoprotein affects its atherogenicity

By 1972, the primary structures, including protein and DNA sequences, had been determined for almost all apolipoproteins (AI, AIV, B, CI, CII, CIII, D, E, I, and J) (16). VLDL particles containing apoE, apoCI, apoCIII, and apoAV have been shown to affect VLDL metabolism, site utilization, and atherogenicity. In the following sections, each lipoprotein is briefly described.

ApoE

Emerging evidence supports that the compositional change of apolipoproteins in VLDL affects its atherogenicity (**Table 2**). VLDL is one of several major lipoproteins containing apoE, which is a specific ligand for cysteine-binding repeats of the VLDL receptor (VLDLR). VLDLR is widely expressed throughout the body, including the heart, skeletal muscle, adipose tissue, and brain, and it has an important role in the uptake and metabolism of apoE-containing TG-rich lipoproteins. ApoE is a polymorphic protein arising from three alleles at a single gene locus (32). The enrichment of apoE content in VLDL has been shown to protect against coronary heart disease (33).

ApoCI

Primarily associated with HDL in the fasting state, apoCI transiently attaches to the surface of TG-rich lipoproteins such

as chylomicrons and VLDL postprandially. ApoCI modulates several enzymes involved in lipoprotein metabolism and can reduce the uptake of VLDL by inhibiting its binding to VLDLR (34). The increased intima-media thickness of the common carotid artery indicates early atherosclerosis and was found to be associated with apoCI content in postprandial TG-rich lipoproteins (35, 36). In addition, the number of apoCI molecules per VLDL particle in the fasting state was associated with the plaque size of carotid atherosclerosis (37). ApoCI was also shown to be correlated with cholesterol enrichment in VLDL particles and the delayed clearance of TG-rich lipoproteins (37). In hypercholesterolemic rabbits, the constitutive expression of human apoCI provided protection against serious atherosclerosis (38). This benefit was found to be related to the inhibition of plasma cholesteryl ester transfer protein (CETP) activity (38). These findings support that apoCI enrichment attenuates the atherogenicity of VLDL particles.

ApoCIII

ApoCIII has been suggested to be a central regulator of TG-rich lipoprotein metabolism (39). A direct association of apoCIII with atherosclerosis was revealed by clinical genetic studies and studies showing that loss-of-function mutations in *APOC3* are associated with low TG levels (40) and a reduced incidence of ischemic CVD (41). Increased plasma levels of apoCIII are associated with increased levels of VLDL, IDL particles, and TGs (42). In human monocytic THP-1 cells, apoCIII activated protein kinase C alpha (PKCα) and transforming protein RhoA, which resulted in β1-integrin activation and promoted endothelial cell adhesion. These results suggested that apoCIII not only modulates lipoprotein metabolism, but may also contribute to atherosclerosis development (43). The antisense apoCIII inhibitor volanesorsen, which reduces apoCIII levels by >75% and plasma TGs levels, inhibits apoCIII synthesis in the liver (44). However, the indication for the clinical use of volanesorsen is limited to patients with familial chylomicronaemia syndrome for preventing pancreatitis; therefore, its effect on reducing CVD remains undetermined (39).

ApoAV

In contrast to *APOC3*, genotype combinations of common *APOA5* variants (c.-1131 T > C, S19 W, and c.*31C > T) are associated with elevated TG levels and increased CHD risk (45). In addition, patients with non-alcoholic fatty liver disease have elevated apoAV expression, which promotes hepatic TG storage in lipid droplets but decreases VLDL secretion by the liver (46). ApoAV also accelerates TG-rich lipoprotein uptake by the liver (47). However, the mechanism by which apoAV regulates circulatory VLDL metabolism remains largely unknown.

Mechanisms of modified very-low-density lipoprotein in cardiometabolic disorders

Overproduction of TGs in the liver and non-alcoholic fatty liver disease

A key feature of large VLDL is the overproduction of TGs in the liver, which may occur for several years before the onset of type 2 diabetes (27). In the liver, the biogenesis of VLDLs and the assembly of apolipoproteins are complex and highly regulated processes (4). A major source of TG synthesis is the endoplasmic reticulum (ER) lumen, where TGs are assembled with apoB100 to form lipid-poor primordial VLDL particles. This process is facilitated by microsomal triglyceride transfer protein (MTP) (4), which transfers both neutral and polar lipids to form VLDL particles (**Figure 1**). Whether and how MTP is modulated in patients with insulin resistance and diabetes remain unclear.

Because of its large size (average diameter >60 nm), VLDL is shifted from the ER membrane to the cis Golgi for cargo selection and vesicle formation. However, the utilization of vesicular carrier proteins for VLDL remains an ongoing subject of investigation (4). It has been suggested that VLDL exits the hepatic ER in a specialized vesicle (i.e., the VLDL transport vesicle), which can accommodate a particle diameter of up to 100–200 nm (48).

Patients with non-alcoholic fatty liver disease have increased hepatic stearol-CoA desaturase (SCD)-1 activity, which converts saturated fatty acids to monosaturated fatty acids that serve as a major substrate for the synthesis of *de novo* TGs and other lipids (49). How the abundance of TGs and the degree of TG desaturation are controlled or regulated during VLDL synthesis remain undetermined.

Hepatic apoAIV expression, which is regulated by nuclear transcription factor cAMP-responsive element-binding protein H (CREBH), is correlated with hepatic TG content in patients with chronic liver steatosis (50). CREBH activation plays key roles in hepatic steatosis by upregulating apoAIV during VLDL assembly in the ER and promotes the assembly of large and TG-enriched VLDL particles (50) (**Figure 1**). In addition to its expression in the liver, apoAIV is predominantly expressed in human enterocytes to facilitate intestinal chylomicron assembly and is highly upregulated after a fatty meal (51).

Regulation of lipolysis

The utilization of VLDL and the breakdown of TGs in organs require the key enzyme lipoprotein lipase (LPL) to generate free fatty acids. The inhibition of

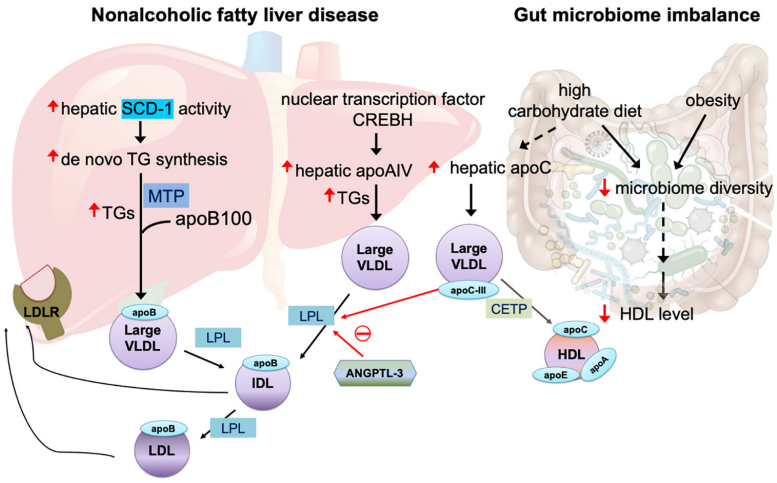


FIGURE 1
Mechanisms of large very-low-density lipoprotein (VLDL) in non-alcoholic fatty liver disease and gut microbiome imbalance. The overproduction of triglycerides (TGs) is related to increased activity of hepatic stearol-CoA desaturase (SCD)-1, which converts saturated fatty acids to monosaturated fatty acids that serve as the substrate for the synthesis of *de novo* TGs. The assembly of TGs with apolipoprotein (apo)B100 is facilitated by microsomal triglyceride transfer protein (MTP). In non-alcoholic fatty liver disease, the nuclear transcription factor cAMP-responsive element-binding protein H (CREBH) is upregulated, in turn increasing expression of hepatic apoAIV, which promotes the assembly of TG-rich, large VLDL. Angiopoietin-like protein family 3 (ANGPTL3) inhibits the enzyme activity of lipoprotein lipase (LPL), which is essential for breakdown of TGs in VLDL utilization. Both intermediate-density lipoprotein (IDL) and LDL particles are recognized by LDL receptor (LDLR) expressed in the liver. LPL activity is also inhibited by apoCIII. Large VLDL promotes plasma CETP-induced remodeling of TG-rich HDL. A high-carbohydrate diet and obesity impair microbiome diversity, which is related to reduced plasma HDL levels and increased hepatic apoCIII production that in turn inhibit LPL activity and enhance the abundance of large VLDL in the circulation.

lipolysis increases the size of circulating VLDL. Several members of the angiopoietin-like protein (ANGPTL) family regulate the activity of LPL. ANGPTL3, ANGPTL4, and ANGPTL8 are upregulated in patients with type 2 diabetes and obesity (52). In a group of patients who received RNA inhibition therapy with antisense oligonucleotides targeting *ANGPTL3*, protein levels of ANGPTL3 were reduced by as much as 84.5% from baseline 6 weeks after injection, while levels of TGs were reduced by 63.1%, VLDL cholesterol by 60.0%, and apoCIII by 58.8% (53). In mice, ANGPTL3 inhibition reduced TG content in the liver and retarded atherosclerosis progression (53). Endothelial lipase, which reduces LDL-C via an LDLR-independent mechanism, is essential for phospholipid reduction in VLDL and LDL (54). In $LDLR^{-/-}$ mice, ANGPTL3 inhibition caused a marked reduction in the TG content of VLDL. Furthermore, in $ApoE^{-/-}$ mice, ANGPTL3 inhibition promoted VLDL clearance with the involvement of multiple remnant receptors (54). However, in the liver, ANGPTL3 did not perturbate apoB lipidation and hepatic VLDL assembly (54). These findings suggest that ANGPTL3 governs VLDL catabolism and largely affects VLDL lipid content and size. On the other hand, endothelial lipase exerts anti-atherogenic effects by enhancing the catabolism of β-VLDLs (55), which are cholesterol-rich chylomicron and VLDL remnants that accumulate in the plasma of patients with type III dysbetalipoproteinemia (56). In elderly patients, the removal of TG-rich lipoprotein remnants is delayed, but TG breakdown is unchanged. Whether VLDL receptor function is impaired and whether ANGPTL3 is involved in aging-related, delayed VLDL removal remain unknown.

Interaction of very-low-density lipoprotein with high-density lipoprotein

The reverse-remnant cholesterol transport mechanism, which is the acquisition of VLDL surface components by HDL during LPL-mediated lipolysis, plays an important role in VLDL catabolism (57). HDL affects the lipolysis of VLDL TGs and the release of surface lipids, free cholesterol, phospholipids, and exchangeable apoE, apoCII, and apoCIII from VLDL during lipolysis (58). HDL can also be classified into subpopulations according to size, apolipoprotein content, charge, mass, and density. Although subpopulations of both large and small HDL particles increased VLDL TG lipolysis efficiency and surface material removal from VLDL, the small, protein-enriched HDL particles exhibited a greater effect on this process and promoted a more efficient release of surface components, thereby affecting the properties of the generated remnants. Loss of apoC proteins from VLDL during lipolysis promoted the metabolism of

apoB-containing lipoprotein because both apoCII and apoCIII inhibit the binding of apoB lipoproteins to the LDLR (58).

Increased TG content has been suggested to decrease the stability of HDL, VLDL, and LDL via several mechanisms. First, TGs have a direct destabilizing effect on lipoprotein particles from the CETP-induced remodeling of TG-rich HDL. Second, TGs have indirect effects that enhance spontaneous and enzymatic hydrolysis and oxidation. Third, products of the aforementioned processes, particularly free fatty acids, further augment lipoprotein destabilization and fusion. TGs are also involved in the substantial release of proteins from lipoproteins. Finally, the combination of destabilized LDL and VLDL enhances their retention in the arterial wall, triggering atherosclerosis (59).

Genetic variants associated with very-low-density lipoprotein particles

Genetic variants have been associated with lipoprotein subclasses. Among those, the common variant rs73059724 resulted in small VLDL particles with fewer phospholipids (60). The variant rs73059724 is located on chromosome 19 and is associated with the promoter and intron of *HIF3A*, which regulates the cellular uptake of cholesterol esters and VLDL by promoting hypoxic conditions. In addition, *HIF3A* hypermethylation is associated with increased adiposity in Asian infants and children (61, 62). These findings suggest that HIF3A may regulate VLDL particle size. Furthermore, DNA methylation at *HIF3A* may explain the prenatal influences on adiposity. In another recent genetic study, Li-Gao et al. (63) investigated postprandial metabolomics and found that the *ANKRD55* locus led by the rs458741:C variant was strongly associated with extremely large VLDL, body composition, and the incidence of diabetes. This finding illuminates the strong genetic linkage between VLDL modification and insulin resistance.

Gut microbiome imbalance

Vojinovic et al. (64) showed in a prospective population-based cohort of 2309 individuals that 32 microbial families and genera in gut microbiota were associated with size-defined subfractions of VLDL, HDL, serum lipid values, and glycolysis-related metabolites. Among the 32, 18 microbial families and genera were significantly associated with VLDL particles of various sizes (extra small, small, medium, large, very large, and extremely large) (64). Another recent study showed that, in healthy individuals, low microbiota diversity was associated with obesity, abdominal obesity, and low HDL-C level (65). These reports suggest that gut microbiota imbalance may be involved in the alteration of VLDL particle size. Thus, the source of altered VLDL particles is presumably the intestines, although the real origin of altered VLDL particles may be diet. In animals and humans, a high-carbohydrate diet results in the elevation of large TG-enriched VLDL particles, along with the enrichment of apoC proteins. Carbohydrate intake increases hepatic secretory rates of VLDL TGs without changing the secretion of apoB, which together lead to large and dense VLDL particles (66).

Very-low-density lipoprotein particles in the non-fasting state carry a risk for atherosclerosis and atrial fibrillation

Very-low-density lipoprotein particle changes in fasting and postprandial states

Postprandial hypertriglyceridemia is a hallmark of dyslipidemia in patients with type 2 diabetes. Recently, it has been suggested that postprandial dyslipidemia is equally as important as the estimation of lipids in the fasting state, particularly for patients with type 2 diabetes (67). Mora et al. (27) characterized lipoprotein particles according to size in fasting and non-fasting states by using NMR, noting similar results between LDL and HDL particles. However, compared with fasting VLDL, non-fasting large VLDL particles carried much higher risk for diabetes. In the Copenhagen General Population Study, in which NMR spectrometry was used to analyze the lipids of 9293 individuals, the results showed that VLDL and IDL particles contained one-third of plasma cholesterol in the non-fasting state (68). Postprandial TGs are carried by primarily chylomicron and VLDL remnants, which are ligands of the VLDL receptor involved in macrophage foam cell formation during the development of atherosclerosis (69).

Correlation of postprandial very-low-density lipoprotein rather than fasting very-low-density lipoprotein with atrial cardiopathy

VLDL utilization serves as the major energy source for the heart. Under physiologic conditions, approximately 70% of the heart's energy is derived from fatty acid oxidation (70). Lee et al. (71) showed that postprandial VLDL is independently correlated with atrial enlargement, indicating that postprandial VLDL is a risk factor for atrial fibrillation (**Table 1**). In a prospective study of individuals with MetS ($n = 87$) and without MetS ($n = 80$), they found that negatively-charged VLDL (2-h postprandial VLDL-χ, concentration in %), waist and hip

circumferences, body mass index, and blood pressure were positively correlated with left atrial diameter. After adjusting for obesity and blood pressure, 2-h postprandial VLDL-χ, but not fasting VLDL, was independently correlated with left atrial diameter. Each 1% increase in VLDL-χ correlated with an incremental left atrial diameter increase of 0.23 cm. Nakajima et al. (72) showed that postprandial VLDL has a higher affinity to the VLDL receptor, with better internalization into cells than non-postprandial VLDL. With these findings in mind, postprandial modified VLDL has been suggested as a therapeutic target for atrial remodeling in patients with MetS (54).

VLDL composition, especially in the postprandial state, is influenced by meals and eating habits. Guerrero et al. (73) described the effects of a sucrose-enriched diet on elevated levels of VLDL-cholesterol and TGs, insulin resistance, and hepatic steatosis in male Wistar rats. In addition, Drorna et al. (74) reviewed the available evidence for the impact of high-fructose intake on health. In healthy individuals, the consumption of up to 1.5 g fructose/kg body weight per day for 4 weeks resulted in increased plasma TG concentrations (74). In addition to elevating TG levels, high fructose intake can induce hepatic steatosis, insulin resistance, and hyperuricemia (74). It is very likely that high fructose intake can alter VLDL particles with respect to size and TG richness. After a single high-fat meal, postprandial changes in TGs and VLDL can be significant in men with abdominal obesity compared with non-obese men (75). However, no such difference was observed between obese and non-obese women (75), suggesting sex-based differences in postprandial VLDL secretion during the reproductive stage.

Therapeutic implications

Nutritional intervention

In patients with existing cardiometabolic risks, 8-week nutritional intervention with a high polyphenol diet can significantly reduce the postprandial lipid content of large VLDL after a high-fat test meal (76). Another study showed that the consumption of a diet composed of fruit, avocado, whole grains, and trout for 8 weeks can reduce fasting insulin and VLDL and lower the postprandial increase in TGs and VLDL (77). With respect to the intake of fish, notable differences were seen in the NMR lipoprotein profile of the three main n-3 fatty acid subtypes: eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), and a-linolenic acid (ALA). Only a high intake of EPA significantly reduced VLDL particles and VLDL TGs (78). In addition, the reduction of apoCIII expression is believed to be the mechanism underlying the TG-lowering effects of omega-3 carboxylic acids, which contain 50–60% EPA and 15–25% DHA, as well as other active omega-3 free fatty acids (79). Fasting *per se* is beneficial for VLDL modification. In a study of 40 relatively healthy, middle-aged individuals, long-term fasting improved the postprandial lipid profile, especially with respect to the concentrations of large VLDL particles, which are significantly decreased after 7 and 14 days of fasting (80). Nevertheless, the impact of nutritional intervention on clinical cardiovascular outcomes warrants long-term observation and follow-up.

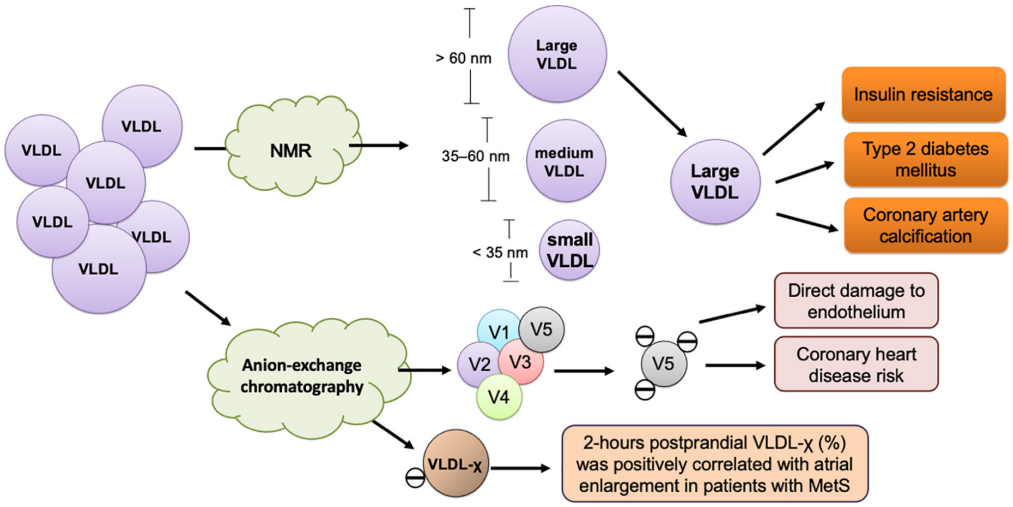


FIGURE 2
Size- and charge-defined subfractions of VLDL and their association with cardiometabolic diseases. The size-defined classification of VLDL according to particle diameter is performed using nuclear magnetic resonance (NMR) spectrometry. Large VLDL, which has a diameter larger than 60 nm, is associated with insulin resistance, type 2 diabetes mellitus, and coronary artery calcification. VLDL-χ or V5, the most negatively-charged subfraction of VLDL, is isolated and measured using anion-exchange chromatography. VLDL-χ or V5 causes direct damage to the endothelium and associated with coronary heart disease risk and atrial myopathy in metabolic syndrome (MetS).

Potential of other very-low-density lipoprotein-targeted therapies

In addition to nutritional intervention, synbiotic and probiotic supplements that improve gut microbiome imbalance have shown potential for decreasing serum VLDL-C levels (81). In addition, several oral anti-diabetic drugs have been identified that promote beneficial effects on VLDL metabolism. Pioglitazone, a PPAR-γ activator, was shown to facilitate LPL activity and promote the clearance of VLDL (82). Furthermore, glucagon-like peptide 1 (GLP-1) agonist reduced TG levels in the liver and the VLDL secretion rate (83).

Commonly used lipid-lowering drugs, although not specifically VLDL-targeted, have also been shown to help reduce VLDL. HMG-CoA reductase inhibitors (i.e., statins) reduce one-third of VLDL-TGs and more than 40% of apoCIII levels (84). In addition, peroxisome proliferator-activated receptor-α (PPAR-α) agonists (i.e., fibrates), which are prescribed primarily for managing hypertriglyceridemia, reduce VLDL-apoCIII levels, as well (84). Similar to selective estrogen receptor modulators, the first selective PPAR-α modulator (SPPARMα) LY-518674, which targets the receptor–cofactor binding profile of the PPARα ligand, modulates tissue- and gene-selective responses. In clinical phase II/III trials, this SPPARMα agonist reduced TG and apoCIII levels by about 50% (85). Proprotein convertase subtilisin-kexin type 9 (PCSK9) inhibitors, which reduce the degradation of LDL receptors and promote LDL uptake in the liver, also upregulate VLDL receptors and reduce VLDL levels. PCSK9 inhibitors have also been shown to preferentially modify the size and apolipoprotein composition of VLDL particles (86).

Several lipid-lowering agents are under development, including CETP inhibitor (87), microsomal triglyceride transfer protein (MTTP) inhibitor (88), and antisense oligonucleotides targeting the genes encoding apoB100 (88) and apoCIII (89). These therapeutics are currently being tested in clinical trials. Monoclonal antibody targeting ANGPTL3 has been shown to robustly reduce VLDL levels but at the expense of elevating LDL levels (90). In addition, ARO-ANG3 is an siRNA-based medication that inhibits the hepatic translation of *ANGPTL3* mRNA [102]. These new medications have the potential to produce favorable effects on VLDL structure and metabolism.

Concluding remarks

Independent of LDL-C, VLDL's atherogenic properties are associated with TG abundance, which largely affects particle size, apolipoprotein content alteration, electrical charge, and lipid composition, especially in the postprandial state (**Figure 2**). With adverse modification, VLDL facilitates ectopic lipid accumulation, which has been observed in the liver, heart, and skeletal muscles. To elucidate the pathogenic roles of VLDL in cardiovascular diseases, the issues of modification, in both fasting and postprandial states, should be taken into consideration. To improve adversely modified VLDL, nutritional intervention, especially through the reduction of fructose content in food, should be widely recommended, especially for patients with insulin resistance and cardiometabolic risks. However, interpreting data from only the size-based, charge-based, or apolipoprotein-based classified VLDL does not provide complete knowledge or information about lipids in health and diseases. To obtain a more comprehensive understanding of the lipid transport and metabolism process, methodologies are needed that can reflect the complex immunochemical and functional properties of all apolipoprotein-containing lipoproteins in the blood.

Author contributions

H-CL contributed to the conceptualization of the study, participated in funding acquisition, and wrote the manuscript. AA and C-HC reviewed and edited the manuscript. All authors have approved the submitted version and agreed to be personally accountable for their own contributions.

Funding

This study was supported by Kaohsiung Medical University Hospital (KMUH109-9R11 and KMUH110-0R11), Taiwan Ministry of Science and Technology Grants (MOST 109-2314-B-037-111-MY3), KMU Global Networking Talent Plan (110KMUOR01), and National Health Research Institutes NHRI (NHRI-EX107-10724SC, NHRI-EX108-10724SC, NHRI-EX109-10724SC, and NHRI-EX110-10724SC).

Acknowledgments

We thank Ms. Yi-Lin Shiao for her artwork shown in the schematic figure, and Nicole Stancel, Ph.D., ELS(D), of Scientific Publications at the Texas Heart Institute, for her contributions to the editing of this manuscript.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Niimi, M, Yan H, Chen Y, Wang Y, Fan J. Isolation and analysis of plasma lipoproteins by ultracentrifugation. *J Vis Exp.* (2021). doi: 10.3791/61790

2. Chapman MJ, Goldstein S, Lagrange D, Laplaud PM. A density gradient ultracentrifugal procedure for the isolation of the major lipoprotein classes from human serum. *J Lipid Res.* (1981) 22:339–58.

3. Huang JK, Lee HC. Emerging evidence of pathological roles of very-low-density lipoprotein (VLDL). *Int J Mol Sci.* (2022) 23:4300.

4. Tiwari S, Siddiqi SA. Intracellular trafficking and secretion of VLDL. *Arterioscl Thromb Vasc Biol.* (2012) 32:1079–86.

5. Ginsberg HN, Packard CJ, Chapman MJ, Boren J, Aguilar-Salinas CA, Averna M, et al. Triglyceride-rich lipoproteins and their remnants: metabolic insights, role in atherosclerotic cardiovascular disease, and emerging therapeutic strategies-a consensus statement from the European Atherosclerosis Society. *Eur Heart J.* (2021) 42:4791–806. doi: 10.1093/eurheartj/ehab551

6. Magnifico MC, Oberkersch RE, Mollo A, Giambelli L, Grooten Y, Sarti P, et al. VLDL induced modulation of nitric oxide signalling and cell redox homeostasis in HUVEC. *Oxid Med Cell Longev.* (2017) 2017:2697364. doi: 10.1155/2017/2697364

7. Tsai YY, Rainey WE, Bollag WB. Very low-density lipoprotein (VLDL)-induced signals mediating aldosterone production. *J Endocrinol.* (2017) 232:R115–29.

8. Jimenez B, Holmes E, Heude C, Tolson RF, Harvey N, Lodge SL, et al. Quantitative lipoprotein subclass and low molecular weight metabolite analysis in human serum and plasma by (1)H NMR spectroscopy in a multilaboratory trial. *Anal Chem.* (2018) 90:11962–71. doi: 10.1021/acs.analchem.8b02412

9. Garvey WT, Kwon S, Zheng D, Shaughnessy S, Wallace P, Hutto A, et al. Effects of insulin resistance and type 2 diabetes on lipoprotein subclass particle size and concentration determined by nuclear magnetic resonance. *Diabetes.* (2003) 52:453–62.

10. Phillips CM, Perry IJ. Lipoprotein particle subclass profiles among metabolically healthy and unhealthy obese and non-obese adults: does size matter? *Atherosclerosis.* (2015) 242:399–406. doi: 10.1016/j.atherosclerosis.2015.07.040

11. Wang J, Stancakova A, Soininen P, Kangas AJ, Paananen J, Kuusisto J, et al. Lipoprotein subclass profiles in individuals with varying degrees of glucose tolerance: a population-based study of 9399 Finnish men. *J Intern Med.* (2012) 272:562–72. doi: 10.1111/j.1365-2796.2012.02562.x

12. Avogaro P, Bon GB, Cazzolato G. Presence of a modified low density lipoprotein in humans. *Arteriosclerosis.* (1988) 8:79–87.

13. Yang CY, Raya JL, Chen HH, Chen CH, Abe Y, Pownall HJ, et al. Isolation, characterization, and functional assessment of oxidatively modified subfractions of circulating low-density lipoproteins. *Arterioscler Thromb Vasc Biol.* (2003) 23:1083–90. doi: 10.1161/01.ATV.0000071350.78872.C4

14. Chen CH, Jiang T, Yang JH, Jiang W, Lu J, Marathe GK, et al. Low-density lipoprotein in hypercholesterolemic human plasma induces vascular endothelial cell apoptosis by inhibiting fibroblast growth factor 2 transcription. *Circulation.* (2003) 107:2102–8. doi: 10.1161/01.CIR.0000065220.70220.F7

15. Chen CH, Lu J, Chen SH, Huang RY, Yilmaz HR, Dong J, et al. Effects of electronegative VLDL on endothelium damage in metabolic syndrome. *Diabetes Care.* (2012) 35:648–53. doi: 10.2337/dc11-1623

16. Alaupovic P. Significance of apolipoproteins for structure, function, and classification of plasma lipoproteins. *Methods Enzymol.* (1996) 263:32–60.

17. Johannesen CDL, Mortensen MB, Langsted A, Nordestgaard BG. Apolipoprotein B and Non-HDL cholesterol better reflect residual risk than LDL cholesterol in statin-treated patients. *J Am Coll Cardiol.* (2021) 77:1439–50.

18. Prenner SB, Mulvey CK, Ferguson JF, Rickels MR, Bhatt AB, Reilly MP. Very low density lipoprotein cholesterol associates with coronary artery calcification in type 2 diabetes beyond circulating levels of triglycerides. *Atherosclerosis.* (2014) 236:244–50. doi: 10.1016/j.atherosclerosis.2014.07.008

19. Ren J, Grundy SM, Liu J, Wang W, Wang M, Sun J, et al. Long-term coronary heart disease risk associated with very-low-density lipoprotein cholesterol in Chinese: the results of a 15-Year Chinese Multi-Provincial Cohort Study (CMCS). *Atherosclerosis.* (2010) 211:327–32. doi: 10.1016/j.atherosclerosis.2010.02.020

20. Liu J, Sempos CT, Donahue RP, Dorn J, Trevisan M, Grundy SM. Non-high-density lipoprotein and very-low-density lipoprotein cholesterol and their risk predictive values in coronary heart disease. *Am J Cardiol.* (2006) 98:1363–8.

21. Qin Z, Zheng FW, Zeng C, Zhou K, Geng Y, Wang JL, et al. Elevated levels of very low-density lipoprotein cholesterol independently associated with in-stent restenosis in diabetic patients after drug-eluting stent implantation. *Chin Med J.* (2017) 130:2326–32. doi: 10.4103/0366-6999.213575

22. Iannuzzi A, Giallauria F, Gentile M, Rubba P, Covetti G, Bresciani A, et al. Association between Non-HDL-C/HDL-C ratio and carotid intima-media thickness in post-menopausal women. *J Clin Med.* (2021) 11:78. doi: 10.3390/jcm11010078

23. Petersen M, Dyrby M, Toubro S, Engelsen SB, Norgaard L, Pedersen HT, et al. Quantification of lipoprotein subclasses by proton nuclear magnetic resonance-based partial least-squares regression models. *Clin Chem.* (2005) 51:1457–61. doi: 10.1373/clinchem.2004.046748

24. Nakajima K, Nakano T, Tokita Y, Nagamine T, Inazu A, Kobayashi J, et al. Postprandial lipoprotein metabolism: VLDL vs chylomicrons. *Clin Chim Acta.* (2011) 412:1306–18.

25. Zeb I, Jorgensen NW, Blumenthal RS, Burke GL, Lloyd-Jones D, Blaha MJ, et al. Association of inflammatory markers and lipoprotein particle subclasses with progression of coronary artery calcium: the multi-ethnic study of atherosclerosis. *Atherosclerosis.* (2021) 339:27–34. doi: 10.1016/j.atherosclerosis.2021.11.003

26. Mackey RH, Kuller LH, Sutton-Tyrrell K, Evans RW, Holubkov R, Matthews KA. Lipoprotein subclasses and coronary artery calcium in postmenopausal women from the healthy women study. *Am J Cardiol.* (2002) 90:71i–6i. doi: 10.1016/s0002-9149(02)02636-x

27. Mora S, Otvos JD, Rosenson RS, Pradhan A, Buring JE, Ridker PM. Lipoprotein particle size and concentration by nuclear magnetic resonance and incident type 2 diabetes in women. *Diabetes.* (2010) 59:1153–60.

28. Amor AJ, Vinagre I, Valverde M, Urquizu X, Meler E, Lopez E, et al. Nuclear magnetic resonance lipoproteins are associated with carotid atherosclerosis in type 1 diabetes and pre-eclampsia. *Diabetes Metab Res Rev.* (2021) 37:e3362. doi: 10.1002/dmrr.3362

29. Lu J, Yang JH, Burns AR, Chen HH, Tang D, Walterscheid JP, et al. Mediation of electronegative low-density lipoprotein signaling by LOX-1: a possible mechanism of endothelial apoptosis. *Circ Res.* (2009) 104:619–27.

30. Lin YS, Liu CK, Lee HC, Chou MC, Ke LY, Chen CH, et al. Electronegative very-low-density lipoprotein induces brain inflammation and cognitive dysfunction in mice. *Sci Rep.* (2021) 11:6013. doi: 10.1038/s41598-021-85502-0

31. Shen MY, Hsu JF, Chen FY, Lu J, Chang CM, Madjid M, et al. Combined LDL and VLDL electronegativity correlates with coronary heart disease risk in asymptomatic individuals. *J Clin Med.* (2019) 8:1193. doi: 10.3390/jcm8081193

32. Mahley RW, Rall SC Jr. Apolipoprotein E: far more than a lipid transport protein. *Annu Rev Genomics Hum Genet.* (2000) 1:507–37.

33. Mendivil CO, Rimm EB, Furtado J, Sacks FM. Apolipoprotein E in VLDL and LDL with apolipoprotein C-III is associated with a lower risk of coronary heart disease. *J Am Heart Assoc.* (2013) 2:e000130. doi: 10.1161/JAHA.113.000130

34. Fuior EV, Gafencu AV. Apolipoprotein C1: its pleiotropic effects in lipid metabolism and beyond. *Int J Mol Sci.* (2019) 20:53. doi: 10.3390/ijms20235939

35. Hamsten A, Silveira A, Boquist S, Tang R, Bond MG, de Faire U, et al. The apolipoprotein CI content of triglyceride-rich lipoproteins independently predicts early atherosclerosis in healthy middle-aged men. *J Am Coll Cardiol.* (2005) 45:1013–7. doi: 10.1016/j.jacc.2004.12.049

36. Hansen JB, Fernandez JA, Noto AT, Deguchi H, Bjorkegren J, Mathiesen EB. The apolipoprotein C-I content of very-low-density lipoproteins is associated with fasting triglycerides, postprandial lipemia, and carotid atherosclerosis. *J Lipids.* (2011) 2011:271062. doi: 10.1155/2011/271062

37. Noto AT, Mathiesen EB, Brox J, Bjorkegren J, Hansen JB. The ApoC-I content of VLDL particles is associated with plaque size in persons with carotid atherosclerosis. *Lipids.* (2008) 43:673–9. doi: 10.1007/s11745-008-3193-2

38. Gautier T, Deckert V, Aires V, Le Guern N, Proukhnitzky L, Patoli D, et al. Human apolipoprotein C1 transgenesis reduces atherogenesis in hypercholesterolemic rabbits. *Atherosclerosis.* (2021) 320:10–8. doi: 10.1016/j.atherosclerosis.2021.01.011

39. Pechlaner R, Tsimikas S, Yin X, Willeit P, Baig F, Santer P, et al. Very-low-density lipoprotein–associated apolipoproteins predict cardiovascular events and are lowered by inhibition of APOC-III. *J. Am. Coll. Cardiol.* (2017) 69:789–800. doi: 10.1016/j.jacc.2016.11.065

40. Wulff AB, Nordestgaard BG, Tybjaerg-Hansen A. APOC3 loss-of-function mutations, remnant cholesterol, low-density lipoprotein cholesterol, and cardiovascular risk: mediation- and meta-analyses of 137 895 individuals. *Arterioscl Thromb Vasc Biol.* (2018) 38:660–8. doi: 10.1161/ATVBAHA.117.310473

41. Jorgensen AB, Frikke-Schmidt R, Nordestgaard BG, Tybjaerg-Hansen A. Loss-of-function mutations in APOC3 and risk of ischemic vascular disease. *N Engl J Med.* (2014) 371:32–41.

42. van Capelleveen JC, Bernelot Moens SJ, Yang X, Kastelein JJP, Wareham NJ, Zwinderman AH, et al. Apolipoprotein CIII levels and incident coronary artery disease risk: the EPIC-Norfolk prospective population study. *Arterioscler Thromb Vasc Biol.* (2017):doi: <PMID<PMID:NOPMID</PMID<

43. Kawakami A, Aikawa M, Libby P, Alcaide P, Luscinskas FW, Sacks FM. Apolipoprotein CIII in apolipoprotein B lipoproteins enhances the adhesion of human monocytic cells to endothelial cells. *Circulation.* (2006) 113:691–700. doi: 10.1161/CIRCULATIONAHA.105.591743

44. Graham MJ, Lee RG, Bell TA III, Fu W, Mullick AE, Alexander VJ, et al. Antisense oligonucleotide inhibition of apolipoprotein C-III reduces plasma triglycerides in rodents, nonhuman primates, and humans. *Circ Res.* (2013) 112:1479–90. doi: 10.1161/CIRCRESAHA.111.300367

45. Dai W, Zhang Z, Yao C, Zhao S. Emerging evidences for the opposite role of apolipoprotein C3 and apolipoprotein A5 in lipid metabolism and coronary artery disease. *Lipids Health Dis.* (2019) 18:220. doi: 10.1186/s12944-019-1166-5

46. Feng Q, Baker SS, Liu W, Arbizu RA, Aljomah G, Khatib M, et al. Increased apolipoprotein A5 expression in human and rat non-alcoholic fatty livers. *Pathology.* (2015) 47:341–8. doi: 10.1097/PAT.0000000000000251

47. Zheng XY, Zhao SP, Yan H. The role of apolipoprotein A5 in obesity and the metabolic syndrome. *Biol Rev Camb Philos Soc.* (2013) 88:490–8.

48. Gusarova V, Brodsky JL, Fisher EA. Apolipoprotein B100 exit from the endoplasmic reticulum (ER) is COPII-dependent, and its lipidation to very low density lipoprotein occurs post-ER. *J Biol Chem.* (2003) 278:48051–8. doi: 10.1074/jbc.M306898200

49. Kotronen A, Seppanen-Laakso T, Westerbacka J, Kiviluoto T, Arola J, Ruskeepaa AL, et al. Hepatic stearoyl-CoA desaturase (SCD)-1 activity and diacylglycerol but not ceramide concentrations are increased in the nonalcoholic human fatty liver. *Diabetes.* (2009) 58:203–8.

50. Cheng D, Xu X, Simon T, Boudyguina E, Deng Z, VerHague M, et al. Very low density lipoprotein assembly is required for cAMP-responsive element-binding protein H processing and hepatic apolipoprotein A-IV expression. *J Biol Chem.* (2016) 291:23793–803. doi: 10.1074/jbc.M116.749283

51. Black DD. Development and physiological regulation of intestinal lipid absorption. I. Development of intestinal lipid absorption: cellular events in chylomicron assembly and secretion. *Am J Physiol Gastrointest Liver Physiol.* (2007) 293:G519–24. doi: 10.1152/ajpgi.00189.2007

52. Abu-Farha M, Al-Khairi I, Cherian P, Chandy B, Sriraman D, Alhubail A, et al. Increased ANGPTL3, 4 and ANGPTL8/betatrophin expression levels in obesity and T2D. *Lipids Health Dis.* (2016) 15:181. doi: 10.1186/s12944-016-0337-x

53. Graham MJ, Lee RG, Brandt TA, Tai L-J, Fu W, Peralta R, et al. Cardiovascular and metabolic effects of ANGPTL3 antisense oligonucleotides. *N Engl J Med.* (2017) 377:222-32.

54. Adam RC, Mintah IJ, Alexa-Braun CA, Shihanian LM, Lee JS, Banerjee P, et al. Angiopoietin-like protein 3 governs LDL-cholesterol levels through endothelial lipase-dependent VLDL clearance. *J Lipid Res.* (2020) 61:1271–86. doi: 10.1194/jlr.RA120000888

55. Yan H, Niimi M, Wang C, Chen Y, Zhou H, Matsuhisa F, et al. Endothelial lipase exerts its anti-atherogenic effect through increased catabolism of beta-VLDLs. *J Atheroscler Thromb.* (2021) 28:157–68. doi: 10.5551/jat.55244

56. Hopkins PN, Wu LL, Schumacher MC, Emi M, Hegele RM, Hunt SC, et al. Type III dyslipoproteinemia in patients heterozygous for familial hypercholesterolemia and apolipoprotein E2. Evidence for a gene-gene interaction. *Arterioscler Thromb.* (1991) 11:1137–46. doi: 10.1161/01.atv.11.5.1137

57. Kontush A. HDL and reverse remnant-cholesterol transport (RRT): relevance to cardiovascular disease. *Trends Mol Med.* (2020) 26:1086–100.

58. Wieczorek E, Cwiklinska A, Kuchta A, Kortas-Stempak B, Gliwinska A, Jankowski M. The differential effects of HDL subpopulations on lipoprotein lipase (LPL)-mediated VLDL catabolism. *Biomedicines.* (2021) 9:1839. doi: 10.3390/biomedicines9121839

59. Jayaraman S, Baveghems C, Chavez OR, Rivas-Urbina A, Sanchez-Quesada JL, Gursky O. Effects of triacylglycerol on the structural remodeling of human plasma very low- and low-density lipoproteins. *Biochim Biophys Acta Mol Cell Biol Lipids.* (2019) 1864:1061–71.

60. Davis JP, Huyghe JR, Locke AE, Jackson AU, Sim X, Stringham HM, et al. Common, low-frequency, and rare genetic variants associated with lipoprotein subclasses and triglyceride measures in Finnish men from the METSIM study. *PLoS Genet.* (2017) 13:e1007079. doi: 10.1371/journal.pgen.1007079

61. Wang S, Song J, Yang Y, Zhang Y, Wang H, Ma J. HIF3A DNA methylation is associated with childhood obesity and ALT. *PLoS One.* (2015) 10:e0145944. doi: 10.1371/journal.pone.0145944

62. Pan H, Lin X, Wu Y, Chen L, Teh AL, Soh SE, et al. HIF3A association with adiposity: the story begins before birth. *Epigenomics.* (2015) 7:937–50. doi: 10.2217/epi.15.45

63. Li-Gao R, Hughes DA, van Klinken JB, de Mutsert R, Rosendaal FR, Mook-Kanamori DO, et al. Genetic studies of metabolomics change after a liquid meal illuminate novel pathways for glucose and lipid metabolism. *Diabetes.* (2021) 70:2932–46. doi: 10.2337/db21-0397

64. Vojinovic D, Radjabzadeh D, Kurilshikov A, Amin N, Wijmenga C, Franke L, et al. Relationship between gut microbiota and circulating metabolites in population-based cohorts. *Nat Commun.* (2019) 10:5813. doi: 10.1038/s41467-019-13721-1

65. Kashtanova DA, Klimenko NS, Tkacheva ON, Strazhesko ID, Metelskaya VA, Gomyranova NV, et al. Subfractional spectrum of serum lipoproteins and gut microbiota composition in healthy individuals. *Microorganisms.* (2021) 9:1461. doi: 10.3390/microorganisms9071461

66. Witztum JL, Schonfeld G. Carbohydrate diet-induced changes in very low density lipoprotein composition and structure. *Diabetes.* (1978) 27:1215–29.

67. Chahal J, Gupta S, Chawla SPS, Grewal H. Comparative study on fasting and postprandial lipid profile in type 2 diabetes mellitus. *J Family Med Prim Care.* (2021) 10:1288–93.

68. Balling M, Langsted A, Afzal S, Varbo A, Davey Smith G, Nordestgaard BG. A third of nonfasting plasma cholesterol is in remnant lipoproteins: lipoprotein subclass profiling in 9293 individuals. *Atherosclerosis.* (2019) 286:97–104.

69. Takahashi S. Triglyceride rich lipoprotein -LPL-VLDL receptor and Lp(a)-VLDL receptor pathways for macrophage foam cell formation. *J Atheroscler Thromb.* (2017) 24:552–9. doi: 10.5551/jat.RV17004

70. Niu YG, Evans RD. Very-low-density lipoprotein: complex particles in cardiac energy metabolism. *J Lipids.* (2011) 2011:189876.

71. Lee HC, Shin SJ, Huang JK, Lin MY, Lin YH, Ke LY, et al. The role of postprandial very-low-density lipoprotein in the development of atrial remodeling in metabolic syndrome. *Lipids Health Dis.* (2020) 19:210. doi: 10.1186/s12944-020-01386-5

72. Nakajima K, Tokita Y, Tanaka A, Takahashi S. The VLDL receptor plays a key role in the metabolism of postprandial remnant lipoproteins. *Clin Chim Acta.* (2019) 495:382–93. doi: 10.1016/j.cca.2019.05.004

73. Plazas Guerrero CG, Acosta Cota SJ, Castro Sanchez FH, Vergara Jimenez MJ, Rios Burgueno ER, Sarmiento Sanchez JI, et al. Evaluation of sucrose-enriched diet consumption in the development of risk factors associated to type 2 diabetes, atherosclerosis and non-alcoholic fatty liver disease in a murine model. *Int J Environ Health Res.* (2021) 31:651–69. doi: 10.1080/09603123.2019.1680817

74. Dornas WC, de Lima WG, Pedrosa ML, Silva ME. Health implications of high-fructose intake and current research. *Adv Nutr.* (2015) 6:729–37.

75. Sabaka P, Kruzliak P, Gaspar L, Caprnda M, Bendzala M, Balaz D, et al. Postprandial changes of lipoprotein profile: effect of abdominal obesity. *Lipids Health Dis.* (2013) 12:179.

76. Della Pepa G, Vetrani C, Vitale M, Bozzetto L, Costabile G, Cipriano P, et al. Effects of a diet naturally rich in polyphenols on lipid composition of postprandial lipoproteins in high cardiometabolic risk individuals: an ancillary analysis of a

randomized controlled trial. *Eur J Clin Nutr.* (2020) 74:183–92. doi: 10.1038/s41430-019-0459-0

77. Munoz-Perez DM, Gonzalez-Correa CH, Astudillo-Munoz EY, Porras-Hurtado GL, Sanchez-Giraldo M, Lopez-Miranda J, et al. Alternative foods in cardio-healthy dietary models that improve postprandial lipemia and insulinemia in obese people. *Nutrients.* (2021) 13:2225. doi: 10.3390/nu13072225

78. Amigo N, Akinkuolie AO, Chiuve SE, Correig X, Cook NR, Mora S. Habitual fish consumption, n-3 fatty acids, and nuclear magnetic resonance lipoprotein subfractions in women. *J Am Heart Assoc.* (2020) 9:e014963. doi: 10.1161/JAHA.119.014963

79. Morton AM, Furtado JD, Lee J, Amerine W, Davidson MH, Sacks FM. The effect of omega-3 carboxylic acids on apolipoprotein CIII-containing lipoproteins in severe hypertriglyceridemia. *J Clin Lipidol.* (2016) 10:1442–51.e4. doi: 10.1016/j.jacl.2016.09.005

80. Grundler F, Plonne D, Mesnage R, Muller D, Sirtori CR, Ruscica M, et al. Long-term fasting improves lipoprotein-associated atherogenic risk in humans. *Eur J Nutr.* (2021) 60:4031–44. doi: 10.1007/s00394-021-02578-0

81. Shakeri H, Hadaegh H, Abedi F, Tajabadi-Ebrahimi M, Mazroii N, Ghandi Y, et al. Consumption of synbiotic bread decreases triacylglycerol and VLDL levels while increasing HDL levels in serum from patients with type-2 diabetes. *Lipids.* (2014) 49:695–701. doi: 10.1007/s11745-014-3901-z

82. Rodriguez V, Newman JD, Schwartzbard AZ. Towards more specific treatment for diabetic dyslipidemia. *Curr Opin Lipidol.* (2018) 29:307.

83. Patel VJ, Joharapurkar AA, Shah GB, Jain MR. Effect of GLP-1 based therapies on diabetic dyslipidemia. *Curr Diabetes Rev.* (2014) 10:238–50.

84. Ooi EM, Ng TW, Watts GF, Chan DC, Barrett PHR. Effect of fenofibrate and atorvastatin on VLDL apoE metabolism in men with the metabolic syndrome. *J Lipid Res.* (2012) 53:2443–9. doi: 10.1194/jlr.P029223

85. Fruchart JC, Santos RD, Aguilar-Salinas C, Aikawa M, Al Rasadi K, Amarenco P, et al. The selective peroxisome proliferator-activated receptor alpha modulator (SPPARMalpha) paradigm: conceptual framework and therapeutic potential : a consensus statement from the International Atherosclerosis Society (IAS) and the Residual Risk Reduction Initiative (R3i) Foundation. *Cardiovasc Diabetol.* (2019) 18:71. doi: 10.1186/s12933-019-0864-7

86. Hollstein T, Vogt A, Grenkowitz T, Stojakovic T, März W, Laufs U, et al. Treatment with PCSK9 inhibitors reduces atherogenic VLDL remnants in a real-world study. *Vasc Pharmacol.* (2019) 116:8–15. doi: 10.1016/j.vph.2019.03.002

87. Group HTRC. Effects of anacetrapib in patients with atherosclerotic vascular disease. *N Engl J Med.* (2017) 377:1217–27.

88. Butt WZ, Yee JK. The role of non-statin lipid-lowering medications in youth with hypercholesterolemia. *Curr Atheroscler Rep.* (2022) 24:379–89. doi: 10.1007/s11883-022-01013-x

89. Zhang J, de Albuquerque Rocha N, McCullough PA. Contribution of ApoCIII to diabetic dyslipidemia and treatment with volanesorsen. *Rev Cardiovasc Med.* (2018) 19:13–9.

90. Ahmad Z, Pordy R, Rader DJ, Gaudet D, Ali S, Gonzaga-Jauregui C, et al. Inhibition of angiopoietin-like protein 3 with evinacumab in subjects with high and severe hypertriglyceridemia. *J Am Coll Cardiol.* (2021) 78:193–5. doi: 10.1016/j.jacc.2021.04.091

91. Marron MM, Allison M, Kanaya AM, Larsen B, Wood AC, Herrington D, et al. Associations between lipoprotein subfractions and area and density of abdominal muscle and intermuscular adipose tissue: the multi-ethnic study of atherosclerosis. *Front Physiol.* (2021) 12:713048. doi: 10.3389/fphys.2021.713048

92. Silbernagel G, Scharnagl H, Kleber ME, Hoffmann MM, Delgado G, Stojakovic T, et al. Common APOC3 variants are associated with circulating ApoC-III and VLDL cholesterol but not with total apolipoprotein B and coronary artery disease. *Atherosclerosis.* (2020) 311:84–90. doi: 10.1016/j.atherosclerosis.2020.08.017

frontiers | Frontiers in Cardiovascular Medicine

TYPE Review
PUBLISHED 06 October 2022
DOI 10.3389/fcvm.2022.989428

OPEN ACCESS

EDITED BY
Tatsuya Sawamura,
Shinshu University, Japan

REVIEWED BY
Masatsune Ogura,
Eastern Chiba Medical Center, Japan
Fabrizia Bonacina,
University of Milan, Italy

*CORRESPONDENCE
Elena Osto
elena.osto@uzh.ch

SPECIALTY SECTION
This article was submitted to Atherosclerosis and Vascular Medicine, a section of the journal Frontiers in Cardiovascular Medicine

RECEIVED 08 July 2022
ACCEPTED 16 September 2022
PUBLISHED 06 October 2022

CITATION
Dietrich E, Jomard A and Osto E (2022) Crosstalk between high-density lipoproteins and endothelial cells in health and disease: Insights into sex-dependent modulation. *Front. Cardiovasc. Med.* 9:989428. doi: 10.3389/fcvm.2022.989428

Crosstalk between high-density lipoproteins and endothelial cells in health and disease: Insights into sex-dependent modulation

Elisa Dietrich [1], Anne Jomard [1] and Elena Osto [1,2]*

[1]Institute for Clinical Chemistry, University of Zurich and University Hospital Zurich, Zurich, Switzerland, [2]Department of Cardiology, Heart Center, University Hospital Zurich, Zurich, Switzerland

Atherosclerotic cardiovascular disease is the leading cause of death worldwide. Intense research in vascular biology has advanced our knowledge of molecular mechanisms of its onset and progression until complications; however, several aspects of the patho-physiology of atherosclerosis remain to be further elucidated. Endothelial cell homeostasis is fundamental to prevent atherosclerosis as the appearance of endothelial cell dysfunction is considered the first pro-atherosclerotic vascular modification. Physiologically, high density lipoproteins (HDLs) exert protective actions for vessels and in particular for ECs. Indeed, HDLs promote endothelial-dependent vasorelaxation, contribute to the regulation of vascular lipid metabolism, and have immune-modulatory, anti-inflammatory and anti-oxidative properties. Sex- and gender-dependent differences are increasingly recognized as important, although not fully elucidated, factors in cardiovascular health and disease patho-physiology. In this review, we highlight the importance of sex hormones and sex-specific gene expression in the regulation of HDL and EC cross-talk and their contribution to cardiovascular disease.

KEYWORDS

HDL, endothelial cells, sex differences, cardiovascular disease, HDL-endothelial crosstalk

Introduction

The relationship between high-density lipoproteins (HDLs) and cardiovascular disease (CVD) is a topic of intense investigation since decades (1).

Epidemiological studies have shown a correlation between low levels of HDL-cholesterol (HDL-C) and increased incidence of CVD (2). Indeed, a U-shape correlation has been recently reported whereby both low (<50 mg/dl in women and <40 mg/dl in men; 0.8 and 1.3 mmol/L, respectively) and high (>80 to 90 mg/dl; >2.3 mmol/L) levels

of HDL have been associated to increased all-cause and CV mortality in both men and women without previous CVD (3–5).

Increasing evidence suggests that rather than cholesterol levels present on HDL, HDL particle number, lipid and protein composition play a key protective role in reducing CVD risk (6–8). HDL particle composition directly influences HDL vaso-protective functions (i.e. reverse cholesterol transport (RCT), nitric oxide (NO) production from endothelial cells (ECs), anti-oxidative and anti-inflammatory properties).

ECs are a physical barrier between blood and body tissues, which act as gatekeepers of cardiovascular homeostasis. Indeed, EC-released vasoactive substances (in particular NO) regulate hemostasis, control vascular permeability and modulate both acute and chronic immune responses to injuries (9). In light of its strong vasodilatory, anti-inflammatory and anti-oxidative properties, NO plays a central role in the maintenance of vascular health (10). Reduction in NO bioavailability is the hallmark of endothelial cell dysfunction (ECD), which in turn favors atherosclerosis (11).

Sex-related inter-individual variability (hormonal levels, hormone therapies, gene expression profiles etc.) can influence CVD risk by acting on both HDLs and ECs (12–14).

Increasing evidence suggests that sexual hormone levels—in particular testosterone and estradiol—and sex-specific cellular gene expression profile can influence not only HDL-C levels but also HDL subclasses and function. Indeed, men display reduced levels of HDL-C and a more pro-atherogenic phenotype compared to women (15–17).

Furthermore, estrogens are well-recognized EC protective molecules, able to stimulate NO production, EC growth and wound healing mechanisms (18, 19). Of note, differences in gene expression profile between female and male ECs appear to influence EC susceptibility to insults, with the activation in female ECs of more efficient stress-response mechanisms compared to male ECs (20, 21). These differences could explain, at least in part, why pre-menopausal women have lesser CVD risk than age-matched men and could give useful hints for personalized therapy development.

In this Review, we mainly focused on the influence of sex-specific factors on both HDL and EC function and how sex-dependent differences modulating HDL-EC cross- talk may contribute to the CV protection of pre-menopausal women compared to age-matched men (22–24). Indeed, sex closely interacts with gender in the development of atherosclerosis therefore, although not systematically addressed, some gender-specific aspects (i.e., pertaining to the socio-economic and cultural sphere) have been also mentioned in case of their known influence on HDL and EC function and potential CV patho-physiological impact (23–26).

HDL-targeting drugs: The failure of cholesteryl ester transfer protein inhibitors

The concept that HDL is the "good cholesterol" first originated from the Framingham Heart Study, which showed strong inverse association between HDL-C and coronary heart disease (CHD) (27). However, this concept has been challenged by results of following clinical trials in which cholesteryl ester transfer protein (CETP) inhibitors, despite raising HDL-C levels, failed to reduce CV morbidity and mortality. These results suggested that beyond the simple increase of HDL-C plasma levels, the modulation of HDL composition could be more important to achieve cardiovascular benefits (28–30). CETP is a plasma protein that transfers cholesteryl ester from HDL to apolipoprotein B (ApoB)-containing lipoproteins in exchange for triglyceride (TG). The inhibition of CETP leads to higher cholesterol levels in HDLs. Indeed, species lacking CETP and patients with CETP deficiency are characterized by increased HDL-C levels and reduced risk for CVD (31–34). In the Investigation of Lipid Level Management to Understand its Impact in Atherosclerotic Events (ILLUMINATE) trial, the CETP inhibitor Torcetrapib increased HDL-C levels as expected, but this increase was not paralleled by decreased CHD and the trial was stopped due to elevated risk of cardiac and death events (35).

In line with the notion that HDL-C alone may not be a reliable marker of the cardio-protective quality of HDLs, it has been recently shown in a sex-mixed pool of patients that CETP inhibitors, Torcetrapib and Evacetrapib, not only increased HDL-C but also enhanced the concomitant content of apoC3/apoE in HDLs. These two proteins rendered HDLs dysfunctional and were associated with higher CHD (36). Different CETP inhibitors, such as Dalcetrapib and Anacetrapib slightly reduced CHD risk, although this effect could have been influenced by the concomitant reduction in non-HDL-C in treated patients (37–42).

Genetic polymorphisms associated with increased HDL-C levels also did not influence the risk score for myocardial infarction (1, 43). Population studies carried out in Copenhagen highlighted a dramatic enhancement of CHD risk in women with CETP deficiency, in spite of the elevated HDL-C levels (44). Furthermore, as result of rare genetic variants on scavenger receptor BI (SR-BI) gene and reduced ability of HDLs to deliver cholesterol to the liver, the consequent increased HDL-C levels were linked to higher rather than lower risk of CHD risk in both men and women (45, 46). Indeed, the increased cholesterol in HDL in these specific circumstances was linked to an impaired HDL-mediated RCT. Taken together; this evidence questioned the rationale of using CETP inhibitors as treatment for CVD and highlighted the need for a better characterization of the

complexity of HDL, in particular focusing on HDL composition as key determinant of function in health and disease.

Sex- and age-related differences in HDL measurements

HDL-C is commonly used as a predictor marker for CVD risk, as reported in SCORE (Systematic Coronary Risk Evaluation) risk charts and the ASCVD Pooled Cohort Equations (47, 48). So far, reference values for lipid profiles, including HDL, used in clinical practice are the same for both men and women, despite growing evidence on the influence of sex differences in the discriminative performance of CVD risk scores. Indeed, pre-menopausal women have higher HDL-C levels and lower risk of CVD compared to men (Figure 1) (12, 49–51). Phases of menstrual cycle may influence lipid profile. While post-prandial serum TG were higher in women during the follicular compared to the luteal phase of menstrual cycle, HDL and ApoB levels were stable in both phases (54). In another study, a significant decrease in the mean levels of TC, LDL-C, TC/HDL-C, LDL/HDL and TG/HDL was observed in the luteal compared to the follicular phase of menstrual cycle (55). Some studies suggested assessing female parameters during the follicular phase of menstrual cycle could help to minimize differences due to sexual hormones fluctuations (55, 56).

Patient age is another important factor influencing sex-dependent differences, since HDL-C levels can vary during individual lifetime. Healthy pre-pubertal children had high levels of HDL-C independently from their sex (56). HDL-C levels then drastically decreased in boys after puberty (∼45 mg/dL/1.16 mmol/L), while remaining higher in girls (∼55 mg/dL/1.42 mmol/L) (57). These differences were lost in post-menopausal women, independently from menopausal age (58, 59).

Hormonal therapies can also alter lipid parameters. Transgender men (i.e., female to male) displayed a clear reduction in HDL compared to women, but higher levels than cis-gender men (25, 60).

These sex- and age-dependent differences need to be taken into account when HDL-C levels are used as a CVD prognostic marker. Moreover, when considering the relationship between high HDL-C levels and increased all cause and CV mortality, relevant factors to be evaluated are sex differences together with the presence of CVD and other comorbidities. In fact, the increased cardiovascular risk associated with high HDL-C initially reported in a sex-mixed pool of patients without previous cardiovascular conditions by the CANHEART Study and others (3, 5) has not been confirmed afterwards in women with hypertension (61). Another study analyzed six community-based cohorts and showed that in men the inverse linear association between HDL-C and CHD events has a broader span compared to women. For HDL-C values >90 mg/dL (>2.33 mmol/L) in men and HDL-C values >75 mg/dL (>1.94 mmol/L) in women, the association between HDL-C and CHD events reached a plateau with no further reductions in CHD risk (62).

All-cause mortality in healthy, smoking, middle-aged (50–59 years) and older (>60 years) Finnish men was positively associated with HDL-C in the middle-aged group, while there was a U-shaped pattern in older men. Of note, the middle-aged group had a higher reported alcohol intake than the older individuals. Moreover, alcohol- and violence-related mortality was strongly positively associated with HDL-C specifically in the middle age group (63). Thus, alcohol may have influenced the association of HDL-C and mortality through its HDL raising effect and being a risk factor for behavioral-related non-natural as well as alcohol-related deaths beyond coronary disease, such as cancer, cardiomyopathy, stroke (5, 63).

Insights into sex-dependent and independent differences in HDL structure and composition

HDLs are heterogeneous lipoproteins formed by a cholesterol ester and TG enriched hydrophobic core and a surface lipid bilayer containing mainly free cholesterol, phospholipids and various proteins (6, 64). The biogenesis of HDLs starts from the synthesis and secretion of apolipoprotein-AI (ApoA-I) in the liver and intestine (65). The interaction between secreted ApoA-I and cell membrane protein ATP-binding cassette transporter A1 (ABCA1), expressed by hepatocytes and enterocytes (66), allows the acquisition of lipids and formation of nascent HDLs. Nascent HDLs are converted into mature particles *via* cholesterol esterification performed by lecithin-cholesterol-acyl transferase (LCAT) (67). Endothelial lipase and hepatic lipase are involved in the lipolysis of phospholipids and TGs in HDLs, leading to smaller HDL particles (68). Phospholipid transfer protein further exchanges lipids between HDLs (69). HDL clearance is orchestrated by SR-BI and CETP, which regulate the transfer of cholesteryl-ester from HDLs to the liver and the exchange of ApoB-containing lipoproteins with TGs (70).

Women have increased HDL-C and ApoA-I levels and lower ApoB compared to men. These sex-related differences in plasma lipoproteins start to be evident during puberty, in concomitance with the increase in testosterone in males and estrogens in females (Figure 1) (52).

Estrogens increased ApoA-I expression in the liver and HDL-C levels in pre-menopausal women by modulating the expression of SR-BI and hepatic lipase (71–73). On the contrary, testosterone administration enhanced hepatic lipase activity, increasing HDL catabolism (Figure 1) (52). Androgen therapy was also associated with an unfavorable shift toward an atherogenic lipid profile characterized by reduced ApoA-I and

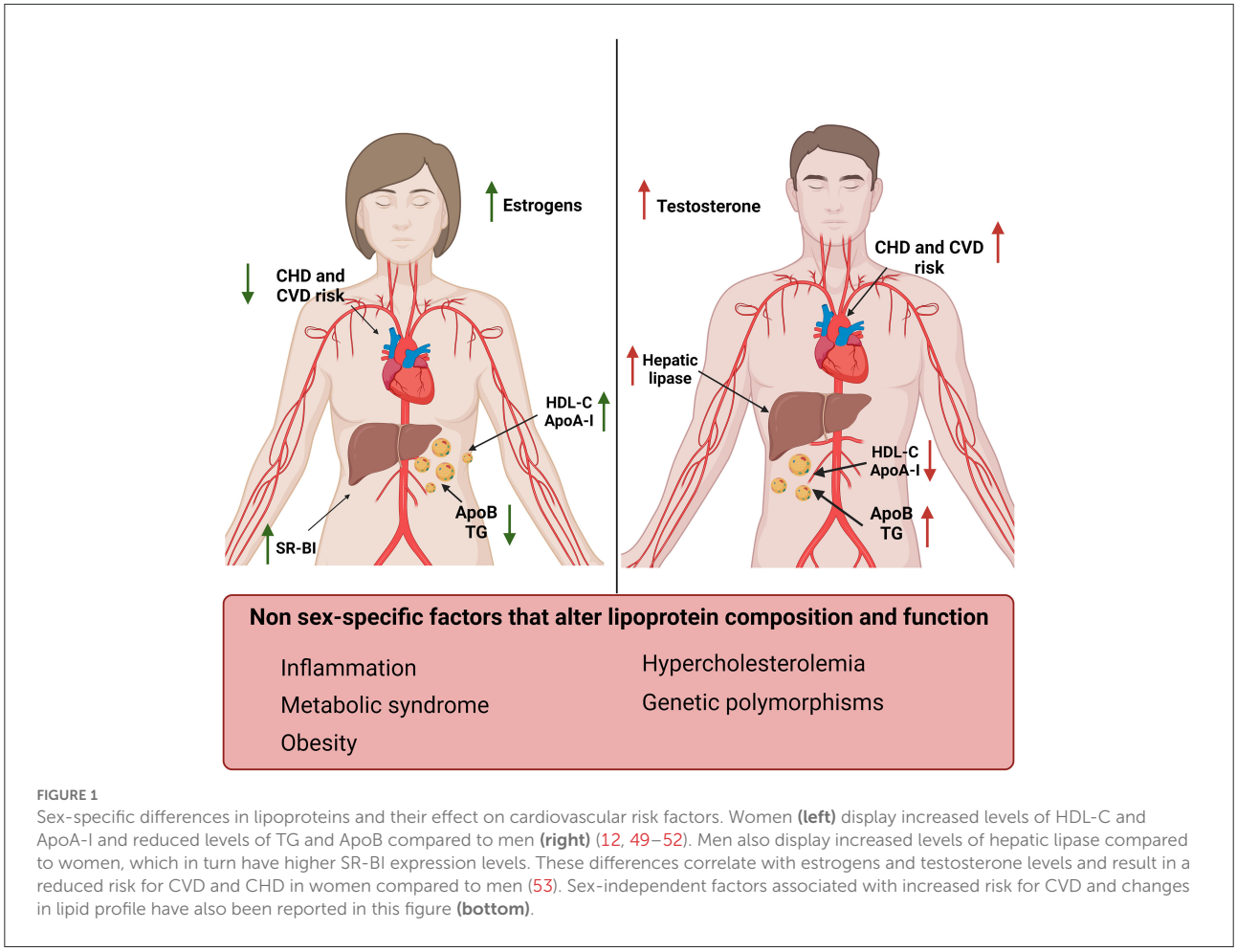


FIGURE 1
Sex-specific differences in lipoproteins and their effect on cardiovascular risk factors. Women **(left)** display increased levels of HDL-C and ApoA-I and reduced levels of TG and ApoB compared to men **(right)** (12, 49–52). Men also display increased levels of hepatic lipase compared to women, which in turn have higher SR-BI expression levels. These differences correlate with estrogens and testosterone levels and result in a reduced risk for CVD and CHD in women compared to men (53). Sex-independent factors associated with increased risk for CVD and changes in lipid profile have also been reported in this figure **(bottom)**.

increased apo-B levels in men (74). Suppression of androgens in men, in fact, leaded to an increase in HDL-C, ApoA-I and reduced ApoB levels (75). It has also been shown that hyperandrogenism, which is a common feature of polycystic ovary syndrome, was associated with lower HDL-C levels and dyslipidemia (76, 77).

Differences in lipid profile have also been associated with sex-specific gene expression profile. The KDM6A gene encodes for a histone-demethylase protein highly expressed in the female liver and its expression levels positively correlated with HDL-C (78, 79). In turn, KDM6A silencing in hepatocytes lead to downregulation of genes regulating HDL-C levels (13).

Single nucleotide polymorphisms (SNPs) on the CETP gene have been associated with higher HDL-C and ApoA-I levels (80, 81). TaqIB is the most common SNP variant of the CETP gene and the TaqIB genotype can be expressed as either dominant B1B1 homozygote, B1B2 heterozygote or recessive B2B2. In particular, B2B2 carriers had higher HDL-C plasma levels and 20% lower risk of CHD vs. the B1B1 carriers (82). Of note, the increase in HDL-C levels in CETP-TaqIB, B2B2 carriers seemed to be independent from sexual hormones (81) and was lost in obesity and type 2 diabetes (T2D). Indeed, other CETP SNP variants in both sexes were not associated with HDL-C levels nor with metabolic syndrome and obesity (83). A 16% increase in HDL-C levels has been reported in men with B2 TaqIB variant affected by T2D compared with those homozygous for the B1 allele (83).

ApoE, encoded by APOE gene, is the major ligand for clearance of TG-rich lipoproteins and has anti-atherogenic function (84, 85). APOE-e2 polymorphism has a sex-specific effect on lipid profile and has been associated with high HDL-C levels in woman and increased TG levels in men (86). There are no sex-differences reported for ApoE isoform 4 in the context of CVD risk, while the ApoE4 allele seems to confer a memory advantage in midlife men and an increased risk of Alzheimer in women (87, 88).

HDL-associated LCAT increased mass concentration and higher LCAT activity have been correlated with CHD risk in women but not in men (89, 90). However, mechanisms of the sex-specific association of LCAT and CV risk need further investigation given the conflicting results so far available, for instance in patients with sickle cell anemia and proteinuria

where a less pronounced reduction of LCAT activity in women compared to men has been considered protective against accelerated kidney disease progression in this patient population (91). Moreover, LCAT deficiency led to the development of spontaneous atherosclerotic lesions similarly in aged male and female mice (92) and a female specific protection against diet-induced obesity and insulin resistance has been described in mice with combined LCAT and LDL receptor deficiency (93).

Inflammation decreases HDL-C levels and altered HDL composition in a sex-independent manner (Figure 1) (94, 95). Changes in the HDL-associated lipids include a decrease in cholesterol ester and an increase in free cholesterol, TG, free fatty acids and ceramide-enriched lipoproteins. Dysfunctional HDLs show marked alterations in protein composition and become pro-atherogenic. These changes include an increase of serum amyloid A (SAA), a decrease apoA-I but also variations in enzymes and transfer proteins, such as LCAT, CETP, PON-1, and apolipoprotein-M (apoM) (94).

Central adiposity directly correlates with CVD risk (96, 97). Increase in central adiposity was able to alter HDL subclasses distribution, but overall HDL-C levels seemed not affected by this parameter (98). Obesity also affects HDL composition, function and subclasses distribution (99, 100). Obesity induces, most prominently in women compared to men, a pro-atherogenic dyslipidemia characterized by increased LDL and TG and reduced HDL-C, ApoA-I and ApoA-II levels. We and others showed that in morbidly severe obese patients bariatric surgery restores HDL endothelial-protective properties by modulating HDL composition (101–104). Bariatric surgery improves CV morbidity and mortality regardless of sex and gender (105, 106). Indeed, in a small patient cohort, we also showed after Roux-en-Y gastric bypass similar benefits on HDL endothelial protective function for both sexes (103). Circulating HDL-C levels increased in our patients after RYGB in agreement with other studies (107) however concentrations usually remains well below cut offs (80–90 mg/dL; 2.06–2.33 mmol/L) that are associated with higher CVD risk (53, 108). Finally, it is worth to consider that, in the context of obesity and bariatric surgery, gender-dependent differences (e.g., differences between women and men in the perception of their body weight in relation to esthetic, health and therapeutic perspective) are very important and need to be appraised when evaluating study results and identifying gaps of existing knowledge (106).

Insight into sex-dependent regulation of EC function

Women before menopause have lower risk of developing CHD and endothelial-protective properties of estrogens can be important contributors (Figure 1) (109). Aging-driven reduction of flow-mediated dilation appears at the age of menopause, more than a decade later than in men, in concomitance with the loss of circulating estrogens (110). Chronic treatment with estrogens improved endothelial-dependent vasodilation in both ovariectomized animal models and post-menopausal women (111–113). Moreover, clinical studies suggested that estradiol treatment was able to revert endothelial dysfunction in post-menopausal women with atherosclerotic, non-stenotic arteries by preventing acetylcholine-induced coronary vasospasm (114). At present however, controversies still exist on the cardio-protective effect of hormonal replacement therapy in post-menopausal women (115).

At the molecular level, estrogens can stimulate ECs primarily through estrogen receptors (ERα and Erβ, GPER1) on EC surface. Activation of ERs increases eNOS activity and NO production, thus promoting EC-mediated vasodilation (Figure 2) (116, 123). EC glycocalyx protects ECs from second insults after trauma hemorrhagic shock (T/SH) (117). Recently, it has been shown that estrogen administration after T/SH protects the EC glycocalyx from degradation by regulating tPA and PAI-1 levels, making ECs more resistant to additional damages (124). Furthermore, estradiol signaling attenuated endothelial inflammatory response by reducing cytokine and chemokine release (such as monocytes chemoattractant protein 1 (MCP-1) and IL-8) as well as EC adhesion molecule expression (125, 126). Prolonged exposure to estradiol also created a new homeostatic status in which immune cells were potentiated and ECs were less sensible to pro-inflammatory stimuli and apoptosis (Figure 2) (109).

While the EC protective role of estrogens have been well established, the effect of androgens on ECs is still under debate. Monocytes binding to aortic ECs seemed to be higher in male than female rabbits with hypercholesterolemia, suggesting a correlation between androgen levels and EC inflammation. However, this sex-dependent difference was also evident in non-hypercholesterolemic rabbits (127). Testosterone has also been associated with impaired vascular function in women (122, 128). This could be due to the fact that, although testosterone production is 10 times higher in men compared to women, women may be more sensitive to this hormone (129). Indeed, several studies pointed out an important sex-independent role of androgen receptors in regulating EC viability, proliferation, and angiogenesis/repair likely *via* upregulation of the VEGF-A, cyclin A, and cyclin D1 expression (Figure 2) (121). Of note, abundance of testosterone in male mice may favor its conversion into estradiol mediated by aromatase P450, causing hyper-activation of ERs promoting atherosclerosis (130, 131).

An overall marker of early atherosclerosis is the transformation of macrophages into foam cells through intracellular lipid accumulation. Treatment with testosterone promoted foam cell formation in men (but not in women) by increasing lipid loading, thus contributing to the development of atherosclerosis (Figure 2) (132).

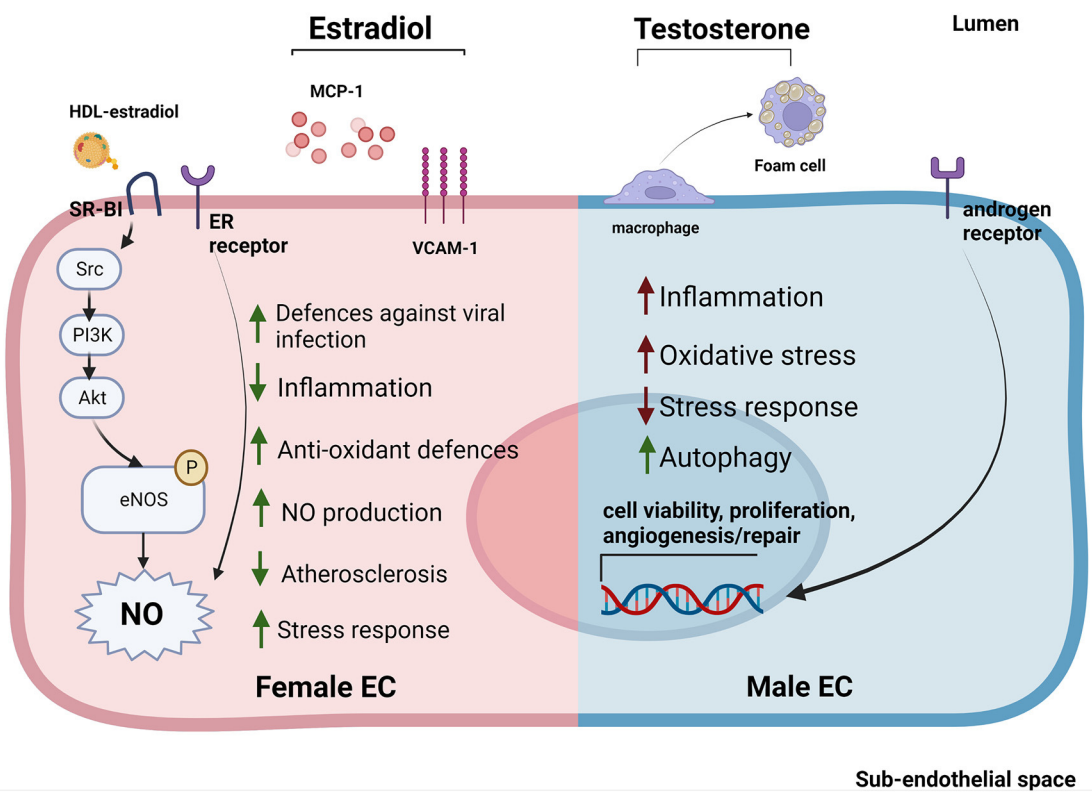


FIGURE 2
Sex-specific gene expression patterns and sexual hormones influence EC response to stimuli and EC-HDL cross talk. ECs derived from female donors **(left)** display reduced sensibility to stress and inflammation thanks to the activation of sex-specific pathways involved in stress response. Estradiol is able to reduce inflammation by reducing MCP-1 release and VCAM-1 expression. Furthermore, estradiol bound to HDLs is able to strongly activate SR-BI pathway increasing the NO production (53, 114, 116, 117). On the contrary, ECs derived from male donors **(right)** display a stronger susceptibility to inflammation, increased levels of oxidative stress and autophagy (118–120). Testosterone also contributes to create a pro-inflammatory environment by favoring the transformation of macrophages into foam cells (121). Estrogens stimulate NO production in both sexes through the activation of ER receptors (114). In contrast, androgen receptors are able to increase the expression of genes involved in cell viability, proliferation and angiogenesis/repair both in men and women ECs (122).

Interestingly, transgender men treated with testosterone for 12 weeks displayed increased leukocytes-endothelium interactions, expression of adhesion molecules on EC surface, pro-inflammatory cytokine release, decreased HDL-C levels and dyslipidemia (23, 26). Furthermore, the levels of polymorphonucleate adhesion to ECs in transgender men were similar to diabetic men with silent myocardial ischemia, which highlight the need of a closer monitoring of cardiovascular risk in these patients (26). Progesterone, instead, protected ECs after cerebrovascular occlusion in male rats (133) and has been associated with increased NO production in women (134). However, administration of synthetic progesterone analogs, such as medroxyprogesterone, correlated with increased risk of coronary disease and stroke in women under hormonal replacement therapy (115, 135). Indeed, it has been shown that estrogen or progesterone and its synthetic analogs differently affect plasma lipoproteins, in particular, estrogen increases whereas progesterone and its synthetic analogs decrease HDL-C concentrations (136). Accordingly, while 17beta-estradiol had no effects, progesterone and three synthetic analogs suppressed ApoA-I-mediated cellular cholesterol release from human fibroblasts resulting in generation of less HDL particles (137).

Sex is a key variable in vascular biology and in particular, EC function is influenced by sexual hormones, but also by chromosomes resulting in sex-specific differences in gene expression profile. Human umbilical vein endothelial cells (HUVECs) derived from females and males were found intrinsically different independently from their exposure to sexual hormones, implicating a role for genomic sexual dimorphisms in CV system (14, 138). Transcriptomic performed in HUVECs in boy-girl twins or in non-twin adult ECs showed that sex-differences were present either at birth and maintained throughout life or acquired over life (118). As expected, sex differences in adult EC transcriptome involved many genes influenced by estrogens or androgens. Interestingly, androgen and estrogen receptors were not differentially expressed in

adult ECs. Intriguingly, half of the genes showing sex-specific differences in HUVECs were sex chromosomal genes. Moreover, coronary artery disease targets (derived using multiple genome-wide association studies) were also enriched in the gene set showing sex difference in HUVECs, making possible to speculate about sex differences in CAD rooted in differential gene expression in ECs already at birth (118). Gene hallmark analysis showed increased expression of genes involved in endothelial to mesenchymal transition, NF-kB pathway and hypoxia in females, while increased expression in MYC targets, oxidative phosphorylation and mTOR pathway were reported in males (118). Other studies reported target-specific differences comparing male and female non-twin HUVECs, which may contribute to sex differences between males and females in endothelial function. Higher cell proliferation, migratory properties and endothelial NO synthase expression were observed in female HUVECs, while in the male cells beclin-1 and the LC3-II/LC3-I ratio, two widely accepted markers of autophagy, were higher (119). Notably, cellular size, shape as well as mRNA and protein expression of estrogen and androgen receptors were similar among sexes (119). Proteomic analysis of the secretome of serum-deprived HUVECs isolated from healthy female and male newborns revealed higher expression of proteins involved in cellular response to stress (e.g., several members of Annexin and Heat Shock Protein families) and apoptosis (e.g., PTX3) in male cells (120). These results are in agreement with reports obtained in different cells (e.g., neurons or cardiomyocytes) challenged with stressor stimuli and overall suggest lower resistance to oxidative stress and higher propensity of male cells to undergo apoptosis. On the contrary, female neurons/cardiomyocytes may be more resistant to oxidative stress with a pro-autophagy predisposition (139, 140); the latter characteristic will need to be further investigated in ECs as the above mentioned study on HUVEC transcriptome in boy-girls twins shows a male and not a female pro-autophagy gene signature (118).

Female HUVECs showed a stronger transcriptional response after shear stress exposure compared to male cells involving, for instance, upregulation of genes such as eNOS, heme-oxygenase 1 (HO-1) downregulation of NADPH oxidase 4 (Nox 4), endothelin-1 (ET-1) or vascular cell adhesion molecule 1 (VCAM-1), the latter downregulated by 22.2-fold in female vs only 3.5-fold in males (141).

Regarding EC energy supply, similar baseline ratios of glycolysis vs. mitochondrial respiration were observed in HUVECs obtained from male/female twins, but female cells performed better under starvation or under VEGF stimulation with higher ATP and metabolite levels compared to male cells, suggesting a more flexible modulation of energy production in females (120, 142).

Further studies will need to elucidate whether the described higher adaptability of female ECs to stress may confer them protection against CVD risk. Conversely, a stronger transcriptional response in female ECs might, in specific cases, favor disease onset and progression (e.g., in the context of the higher prevalence of autoimmune diseases in the female population) (143–145).

Collectively, increasing evidence highlights the presence of sex dependent differences in ECs at different stages of life. However, there are very few studies in adult ECs (i.e., HAECs) compared to the studies in HUVECs, which makes difficult to adequately investigate or compare changes in EC gene expression acquired later in life. Moreover, it is important to consider sex as a crucial biological variable not only in cardiovascular clinical research but also in experimental studies on EC biology to increase the quality and translational value of results.

Insights into sex-dependent differences in HDL and EC crosstalk

Lifestyle and CVD: Sex-dependent differences

Smoking, alcohol consumption, diet and exercise are modifiable CVD risk factors (Table 1).

The number of smoked cigarettes positively correlated with increased CHD risk in both sexes. In fact, smoking induced endothelial dysfunction and damage, increasing lipid oxidation, decreasing HDL, and promoting inflammation, and a pro-thrombotic state (146, 147). Furthermore, a worse lipid profile characterized by increased ApoB and reduced ApoA-I and ApoA-II was reported in smokers compared to non-smokers independently from their sex (148). Interestingly, smoking was reported as a stronger risk factor for CVD in women than in men accordingly to the Finnmark Study (149). This could be partially attributed to the ability of smoking to alter estradiol metabolism leading to the formation of inactive catechols (150, 151), thus inhibiting estradiol vaso-protective properties. Moreover, exposure to passive smoking from birth was associated with reduced HDL-C levels in adolescent girls but not in boys (152). The anti-estrogenic effect of smoking positively correlates with increased CHD risk and strong reduction in HDL-C levels in young compared to older women and men (150, 153). The evidence that ex-smokers had higher HDL-C levels compared to smokers of both sexes further confirm these results (154). Furthermore, the Copenhagen City Heart Study reported that smoking women had 9.4 higher risk of myocardial infarction compared to non-smoking women, while the risk score was only 2.9 times higher in smoking men compared to non-smokers (155). On the contrary, reduced levels of endothelial progenitor cells (EPCs) have been reported in men compared to women. Smoking further decreased EPCs in men, while no difference was found between smoking and non-smoking women. In this case,

TABLE 1 Comparative description of the effect of lifestyle habits on HDLs and ECs in men and women.

	Men	Women
Smoking	Increases CHD risk (147, 148) Induces EC dysfunction (147, 148) Reduces HDL number and functionality [147, 148] Promotes inflammation (147, 148) Reduces EPCs number (157)	Increases CHD risk (147, 148) Induces EC dysfunction (147, 148) Reduces HDL number and functionality (147, 148) Promotes inflammation (147, 148) Alters estrogen metabolism (151, 152) Increases risk of CVD and MI (152)
Alcohol	Increases HDL-C levels (162) Prevents EC activation and inflammation (170)	Increases HDL-C levels (162) Reduces stroke risk (169) Increases overall mortality (169) Prevents EC activation and inflammation (170)
Diet	Mediterranean diet: reduces small dense LDL and increases medium LDL, reduces EC inflammation and oxidative stress (174, 175)	Mediterranean diet: reduces medium dense LDL and increases small LDL, reduces EC inflammation and oxidative stress. Increases eNOS activity and reduces CVD risk. (174, 175, 192) Dairy diet: Reduces insulin sensitivity (176, 177)
Physical activity	Prevents EC dysfunction and atherosclerosis (194, 195) Increases HDL-C levels (198, 199)	Prevents EC dysfunction and atherosclerosis (194, 195) Increases HDL-C levels (198, 199)

CHD, Coronary Heart Disease; EC, Endothelial Cells; HDL, High-Density Lipoproteins; HDL-C, HDL-Cholesterol; CVD, Cardiovascular Disease; MI, Myocardial Infarction; LDL, Low-Density Lipoproteins; eNOS, endothelial Nitric Oxide Synthase; EPCs, Endothelial Precursor Cells.

sex-differences on the effect of smoking were mostly attributed to a protective effect of estradiol on EPCs (156).

Low to moderate alcohol consumption did not affect CVD risk in both sexes (157). The CoLaus Study reported no differences in expression of HDL-related genes (i.e., ABCA1, APOE5, CETP, hepatic lipase and lipoprotein lipase) based on alcohol consume in a sex-mixed pool of Caucasian patients (158). CHD risk could perhaps vary depending on the ethnicity of the patients. The Atherosclerosis Risk in Communities (ARIC) study reported reduced CHD in whites but increased disease in black alcohol consumer men independently from levels of alcohol consumed (159). This was partially attributed to different hepatic gene variants and expression levels (i.e., CETP, hepatic lipase, LPL, and PON1) between these two ethnicities (159, 160). Meta-analysis data suggested that HDL-C levels increased an average of 0.06 mmol/L per 23 g/day of alcohol consumed (161). The increase of HDL-C levels in alcohol consumers have been attributed to enhanced HDL production (hepatic and extra-hepatic), decreased CETP activity and lower HDL-C clearance (162). However, this increase is strongly influenced by alcohol-gene interactions (163). As an example, men and post-menopausal women carrying the homodimeric γ2 variant of the ADHIC gene had a slower rate of alcohol clearance, which was associated with elevated HDL-C levels (164, 165). It is worth specifying that sex differences in alcohol consumption are difficult to detect, since generally women can tolerate lesser amount of alcohol due to their sex-specific absorption, body fat/water ratio, reduced levels of enzymes involved in alcohol metabolism and glomerular filtration rate (166). Furthermore, studies in the general population indicated that among all alcohol consumers/abusers, only 1/3 were women (167). Nevertheless, moderate alcohol consumption was associated with lower risk of stroke in women compare to men but also with a 10% increased risk of overall mortality (168).

Alcohol has also a direct effect on ECs. Indeed, moderate levels of alcohol were able to prevent endothelial activation and pro-inflammatory cytokine release in human coronary artery ECs stimulated with the pro-inflammatory SAA (169). Furthermore, a reduction in monocyte adhesion to TNF-α-stimulated ECs was reported in moderate alcohol consumer men compared to non-consumers (170). On the other hand, heavy alcohol consumption (both measured and self-reported) was associated with increased circulating vascular adhesion molecules (i.e., E-selectin, intracellular adhesion molecule 1 (ICAM-1) and VCAM-1) and reduced flow-mediated dilation in sex-mixed cohorts of patients, independently of alcoholic liver disease (171, 172).

Diet also affects women and men in a different manner. Mediterranean diet was able to reduce total cholesterol, LDL-C, ApoB and ApoA-1 plasma levels in both sexes. However, men under Mediterranean diet experienced a reduction in small dense LDL and an increase in medium LDL, while the opposite trend was observed in women (173, 174). Furthermore, a comparison between red meat and dairy diet highlighted a reduction in insulin sensitivity in women following the dairy diet. Instead, no differences between the two diets were reported in men (175, 176). ABCA1 is one of the most sex-influenced gene in the liver and its expression is higher in females (177). Indeed,

estrogen levels and dietary components were able to regulate ABCA1 expression in macrophages, leukocytes and liver in human and rodents, increasing ApoE-positive HDL particles and improving cholesterol efflux (178–181). Among all the genetic variants, ABCA1/R230C was associated with low HDL-C (182). It has been shown that dietary macronutrient proportions regulated the effect of ABCA1/R230C in premenopausal women by directly interacting with ABCA1 gene (183). In particular, metabolically unfavorable pattern was found in ABCA1/R230C premenopausal women following high carbohydrates and low fat diet, while the opposite pattern was found in women following high fat and low carbohydrates diet (183).

On the contrary, lowering dietary fat intake was able to restore HDL functionality (in particular HDL-CEC) in hypercholesterolemic female pigs by reducing cholesterol plasma levels (184).

There is some evidence that diet could directly affect EC function. Transitory disruption of endothelial function and reduction in vasorelaxation have been reported after acute administration of high-fat meal, in concomitance with increased triglyceride-rich lipoproteins in plasma (185, 186). On the contrary, chronic consumption of low-fat diets (i.e., Mediterranean diet) was associated with improved endothelial function and reduced markers of endothelial activation in plasma in men (187–189), most likely through changes in cholesterol metabolism and the presence of oleate and decosahexanoic acid, which were able to reduce pro-inflammatory molecule expression and monocyte adhesion in ECs *in vitro* (190). Same results were shown also in women. Indeed, a pilot study demonstrated that specific components of Mediterranean diet (i.e., legumes, red meat, and overall proteins) were associated with reduced inflammation and oxidative stress, increased eNOS activity and reduced CVD risk in a cohort of ethnically mixed women (191).

Physical activity is well known as protective factor against CVD (192). Evidence showed that physical activity was able to slow down EC dysfunction and atherosclerosis progression in both sexes (193, 194). However, how physical activity differently influence CVD risk in women and men is still under debate. A systematic review focused on physical activity and stroke incidence claiming that, among all the analyzed studies, 35% of them reported a strong association between physical activity and stroke incidence in women, while the same correlation in men was evident only in few studies (195). Even so, the Framingham Study reported that physical activity conferred protection against stroke in men, but not in women (196).

The correlation between physical activity and reduction in CVD risk could be partially attributed to increased HDL-C levels in physically active individuals compared to sedentary ones. However, the increase in HDL-C seemed to be significant only when a threshold volume of physical activity was reached (197, 198). Even if the threshold level has not been accurately and systematically estimated yet, epidemiological and cross-sectional studies suggested that the threshold value was 1,500 kcal/week in men and 1,200 kcal/week in women independently from their menopausal status (199–201).

Reverse cholesterol transport

The best-known property of HDL is RCT, which consists in the ability of HDL to accept excess cholesterol from peripheral cells, in particular macrophages, and transport it to the liver for excretion or re-utilization. RCT is considered the most important anti-atherogenic function of HDLs. Components of cholesterol efflux include the passive diffusion of cholesterol from cells as well as the active cellular cholesterol transfer by ABCA1, ABCG1, and SR-BI. In this context, ECs may represent a potential barrier to HDL in reaching macrophages within the vessel wall. However, HDL and lipid-free ApoA-I are able to cross intact aortic EC monolayers from the apical to the basolateral compartment in a transcytosis process, involving ABCG1 and SR-BI (202). Contrary to other cells that form the atherosclerotic plaque (smooth muscle cells and macrophages), ECs do not accumulate cholesterol (203) and have a strong ability to efflux cellular cholesterol to HDLs independently of ABCA1, ABCG1, and SR-BI expression or activity (204). On the other hand, it has been shown that in conditions of hyperlipidemia ECs can metabolize LDLs into cholesterol crystals, which accumulate intracellularly and confer a foam cell-like morphology to ECs (205).

HDLs derived from healthy normolipidemic men and women had different RCT capacities due to the activation of sex-specific mechanisms for cholesterol efflux (206). The rs1799837 (APOA1) and rs1800588 (LIPC) SNP variants represented the major determinants of HDL cholesterol efflux capacity in women, while rs2230806 (ABCA1) and rs5082 (APOAII) variants were key determinants in men (207). Furthermore, serum isolated from women displayed an enrichment in large-HDL-particles (L-HDL-P) and increased capacity to mediate cholesterol efflux through SR-BI receptors. On the other hand, serum isolated from men showed increased preβ-HDL particles and cholesterol efflux through ABCA1 receptors (206). Both low and high HDL-C levels were associated with reduced free cholesterol transfer on HDLs in both sexes, especially in women, in patients with acute myocardial infarction and Tangier disease (208).

These findings not only suggest that HDL composition differs in men compared to women, but also that these differences in HDL pool may have an impact in their ability to stimulate cholesterol efflux. Estradiol levels, in fact, positively correlated with HDL cholesterol efflux capacity (HDL-CEC) from macrophages in pre-menopausal women and were associated with increased concentration of L-HDL-P and lower concentration of small-HDL-particles (S-HDL-P) (209). However, HDL-CEC decreased in transgender women (men

to women) under estradiol hormone therapy, suggesting that reduction of testosterone and increase in estradiol may act synergistically in reducing HDL-CEC (24). This hypothesis is in line with the evidence that testosterone deprivation in men increased HDL-C levels but not HDL-CEC, while estradiol treatment had the opposite effect (210). The correlation between androgen levels and CVD in men is controversial. Low levels of androgens were associated to increased CVD in older men (211). On the other hand, testosterone administration in hypogonadal men can blunt EC-mediated vasorelaxation (212, 213). Thus, age and type of androgen used are important factors to be considered.

Inflammation

Increased expression levels of specific adhesion molecules—such as VCAM-1 and ICAM-1 and E-selectin—are a well-recognized marker of EC inflammation and oxidative stress. HDL particle concentration is inversely correlated with the expression of cellular adhesion molecules, as well as inflammatory mediators C-reactive protein (CRP) and TNF-α. The underlying mechanism can be partly attributed to HDL-associated sphingosine 1 phosphate (S1P). S1P signaling through S1P receptor has been shown to protect against TNF-α-induced monocyte binding to ECs preventing the activation of NF-kB and c-Jun pathways as well as reducing the secretion of pro-inflammatory chemokines (214).

OxLDLs can induce MCP-1, which is involved in the recruitment of monocytes into the sub-endothelial space and their differentiation into foam cells. It is an important inflammatory process in the initial stages of atherosclerosis (215). *In vitro* and *in vivo* experimental studies suggested that HDL associated-PON1 inhibited LDL-oxidation by catalyzing the breakdown of oxidized phospholipids, thus abolishing the production of pro-inflammatory cytokines (MCP-1, IL-8 and macrophage colony stimulating factor) from ECs (216–220).

HDLs are also able to transfer microRNA to ECs (221). It was shown that HDL-transferred microRNA-223 directly targeted ICAM-1 gene at 3'UTR sites suppressing gene expression and function in HUVECs stimulated with TNF-α thus reducing leukocyte adhesion. Regulation of TNFα-induced ICAM-1 expression by HDLs was not found in fibroblasts, suggesting a specific miR-223 delivery on ECs (222–224).

HDL-induced NO production also plays an important role in the reduction of EC inflammation. The induction of PI3K-Akt-eNOS signaling mediated by the binding of ApoA-1 and SR-B1 up-regulates cyclooxygenase (COX-2) expression and prostaglandin I2 (PGI_2) release in ECs (225). PGI_2 is a potent inhibitor of inflammation, which limits immune cell proliferation as well as inhibits platelet aggregation, thus affecting smooth muscle relaxation and vasodilation (226).

HDL-C levels also correlated with risk of infection. Similarly to what was showed regarding all-cause mortality risk (3, 4), both low and high levels of HDL-C were associated with increased risk of infection (227). The increased risk of infection associated with low levels of HDL-C could be in part due to the loss of leucopoietic control and immune cell modulation from HDLs (228, 229). The mechanism in case of high levels of HDL-C is less clear, but particular genetic mutations associated with increased HDL levels may also affect disease susceptibility. For instance, in case of LIPC and SCARB1 (encoding for hepatic lipase and SR-BI, respectively), whose mutations were associated with increased risk of CAD (46, 230). Interestingly, several SNP variants located in the promoter and intron 1 of LIPC gene were associated with changes in HDL-C levels in women but not in men (231). SCARB1 rs5888 SNP variant, instead, has been associated with a greater reduction in total cholesterol, LDL-C and ApoB in women treated with atorvastatin compared to men in patients with acute coronary syndrome (232).

Peri-menopausal and menopausal women had increased levels of TNF-α, CRP, TG and LDL compared to their pre-menopausal counterparts, which may underline a reduction on HDL functionality driven by the collapse in estrogen levels (233).

HDL anti-inflammatory properties are impaired or lost in chronic inflammatory conditions. Concomitantly with a decrease of HDL particle levels also changes in the structure and function of HDLs are observed. Patients with chronic inflammatory disorders, including rheumatoid arthritis, systemic lupus erythematosus, and psoriasis, which are associated with an increased risk of atherosclerotic CVD, exhibit a consistent decrease in HDL particles and ApoA-1 levels and HDL vaso-protective properties in a sex-independent manner (234–236). However, in other diseases as in the antiphospholipid syndrome, studies reporting a strong reduction in HDL functionality were conducted comparing only affected and healthy women (237). Thus, it will be important to further elucidate with sex-matched comparisons whether or not men and women HDLs are similarly affected also in those immune-inflammatory diseases.

Interestingly, it has been recently shown that HDL-C and ApoA-I levels measured before SARS-CoV-2 infection negatively correlated with COVID-19 mortality and hospitalization, independently of age, sex, comorbidities, or statin treatment (238–240). Furthermore, HDL cargo was profoundly altered in severe COVID-19 patients, with increased abundance of SAA-1 and−2, SFTPB, ApoF, and inter-alpha-trypsin inhibitor heavy chain H4 (241). These findings are corroborated by the evidence that treatment with reconstituted HDLs in COVID-19 patients reduced SAA-1, SFTPB, and ApoF in HDLs (242). Of note, men with COVID-19 were more prone to develop into the severe condition and die compared to women (Figure 2) (243, 244). Indeed, no striking differences were found between pre- and post-menopausal women, suggesting that reduction in female mortality may be independent from

estrogen levels (243, 244). *In vitro* experiments conducted on ECs exposed to SARS-CoV-2 S1 spike protein showed a significant increase in the overall inflammatory status in cells treated with androgens (245). Recent findings showed that male sex clinical-biological characteristics, rather than male gender-related differences (i.e., pertaining to the socio-economic sphere, such as education) were independently associated with intensive care unit admission, invasive ventilation, and/or death in COVID-19 (246).

Anti-apoptotic and anti-oxidative properties

EC homeostasis relies on the balance between pro- and anti-apoptotic stimuli coming from bloodstream and neighboring cells (247, 248). Once the balance is disrupted, (pro)-apoptotic ECs favor platelet aggregation and coagulation, creating a pro-atherogenic environment (249, 250). OxLDL can promote EC apoptosis by increasing intracellular Ca^{2+} levels (251–253), favoring the onset and progression of CVD (114). In contrast to LDLs, HDLs protect ECs from apoptosis by preserving mitochondrial integrity and inhibiting the activation of the caspase-downstream cascade (254–256). Indeed, HDLs isolated from a mixed sex pool of healthy donors was able to reduce EC apoptosis both *in vivo* and *in vitro*, while sex-matched HDLs isolated from CAD patients showed opposite results (257).

In particular, HDL particles containing ApoA-I seemed to be more cytoprotective than other HDL subclasses (258, 259).

Moreover EPCs can quickly differentiate into mature ECs to rescue vascular integrity in conditions of high cell turnover (260). HDLs can promote endothelial repair by increasing EPC number and function in male mice (261, 262).

In addition to their anti-apoptotic properties, HDLs can also reduce oxidative stress. PON-1 is an accessory protein of HDL that, in coordination with ApoA-I, protect lipoproteins, ECs and intimal macrophages from oxidative insults by hydrolyzing lipo-lactones (263–266). High levels of oxidative stress can increase HDL lipid peroxide loading, decreasing their protective activity against LDL oxidation (267, 268). Significant differences in HDL peroxide levels between men and women have been reported as a readout of sex-specific reduction in HDL anti-oxidative functions in men (269).

Decreased HDL anti-oxidative properties driven by high glycemic burden have also been reported in both type 1 and type 2 diabetic men and post-menopausal women (270, 271). In this context, it has been shown that PON-1 activity was more strongly impaired in T2D women compared to men (272).

Furthermore, women with hypertension, metabolic syndrome or peri-menopause not only had higher levels of oxLDLs compared to healthy middle-age or pre-menopausal women, but also reduced defenses against oxLDLs. However, the contribution of estrogens in this context is still unclear (273–275).

HDLs can serve as carriers for other molecules, for instance estrogens. It has been shown that the binding of estrogens with HDLs increases their anti-oxidative properties due to estrogen esterification performed by LCAT (276). Indeed, LCAT was able to esterify HDL-bounded E2. Esterified E2 was then transferred from HDL to LDL thanks to CETP (276). Incubation experiments demonstrated that E2 esterification and further association with LDL was able to increase LDL resistance to oxidation (276, 277). On the contrary, hyperandrogenism is associated with increased oxidative stress and reduced HDL anti-oxidative functionality in women (278, 279). To the best of our knowledge HDLs have not been reported as carriers for androgens.

HDL-mediated endothelial NO-production

Several mechanisms account for the endothelial NO-stimulating capacity of HDLs. In cultured ECs, HDLs directly activate the production and release of NO by binding of ApoA-I to SR-BI, leading to increased intracellular ceramide levels and phosphorylation of endothelial NO-synthase (eNOS) (280). Cholesterol efflux from ECs to HDLs *via* ABCA1 also promotes NO synthesis by modulating cholesterol-binding protein caveolin-1 and eNOS (281). Mechanistically, the binding of HDLs to SR-B1 leads to tyrosine kinase Src-mediated activation of phosphoinositide 3-kinase (PI3K), which in turn stimulates Akt and Erk pathways. The activation of Akt directly stimulates eNOS by phosphorylation at Ser-1179 (282) (see Figure 2).

Estradiol is able to induce rapid arterial vasodilation by stimulating eNOS activity by acting *via* ERs (18). HDLs isolated from female mice and healthy women (but not male mice or men) were able to enhance NO production. In addition to ERs, estradiol bound to HDLs was also able to enhance eNOS activity through the activation of SR-BI receptors (Figure 2) (283).

Specific lipid and protein components of HDLs have a strong impact on NO production. S1P is a lipid carried mainly on apoM-containing HDLs (>50% of circulating S1P). S1P-apoM HDLs are involved in persistent activation of Akt/eNOS pathway, thus leading to NO dependent vasodilation through S1P receptor stimulation (284–286). Decreased levels of ApoM/HDL correlated with increased CVD risk and have been reported in type 2 diabetes in both sexes and in women (but not in men) with type 1 diabetes (287–289).

Reduced HDL-associated PON-1 activity leads to the activation of endothelial lectin-like oxidized LDL receptor (LOX-1) and PKCαII, thus inhibiting the activity of eNOS (290). Inflammation and metabolic syndromes can also drastically

affect the ability of HDLs to stimulate NO production (291, 292), thus promoting atherosclerosis (293).

Conclusions

Sex hormones and sex-specific gene expression are important although still incompletely understood determinants in the regulation of HDL and EC cross talk and their contribution to cardiovascular health and disease. Despite increasing evidence pointing out that sex-origin of cultured cells and in particular ECs can strongly affect scientific results (294–296), most of the articles do not report any information about the sex of the cells or of the animals used in their experiments. The use of sex-mixed cohorts of patients or imbalanced number of women and men during clinical trials also represents a potential source of bias. As highlighted in this Review, it is of foremost importance to always consider the influence of sex as biological variable in each step of research and to clearly report and analyze this information.

Author contributions

ED, AJ, and EO wrote the manuscript. ED designed the figures under BioRender common creative license. All authors contributed to the article and approved the submitted version.

Funding

This research was funded by the Swiss National Science Foundation (SNSF), grant number PRIMA: PR00P3_179861/1 and the Swiss Life Foundation, the Alfred and Annemarie von Sick Grants for Translational and Clinical Research Cardiology and Oncology, the Heubergstiftung and the Swiss Heart Foundation, Switzerland all to EO.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Voight BF, Peloso GM, Orho-Melander M, Frikke-Schmidt R, Barbalic M, Jensen MK, et al. Plasma HDL cholesterol and risk of myocardial infarction: a mendelian randomisation study. *Lancet.* (2012) 380:572–80. doi: 10.1016/S0140-6736(12)60312-2

2. Casula M, Colpani O, Xie S, Catapano AL, Baragetti A. HDL in atherosclerotic cardiovascular disease: in search of a role. *Cells.* (2021) 10:1869. doi: 10.3390/cells10081869

3. Madsen CM, Varbo A, Nordestgaard BG. Extreme high high-density lipoprotein cholesterol is paradoxically associated with high mortality in men and women: two prospective cohort studies. *Eur Heart J.* (2017) 38:2478–86. doi: 10.1093/eurheartj/ehx163

4. Yu Y, Li M, Huang X, Zhou W, Wang T, Zhu L, et al. A U-shaped association between the LDL-cholesterol to HDL-cholesterol ratio and all-cause mortality in elderly hypertensive patients: a prospective cohort study. *Lipids Health Dis.* (2020) 19:238. doi: 10.1186/s12944-020-01413-5

5. Ko DT, Alter DA, Guo H, Koh M, Lau G, Austin PC, et al. High-density lipoprotein cholesterol and cause-specific mortality in individuals without previous cardiovascular conditions: the CANHEART study. *J Am Coll Cardiol.* (2016) 68:2073–83. doi: 10.1016/j.jacc.2016.08.038

6. Kontush A, Lhomme M, Chapman MJ. Unraveling the complexities of the HDL lipidome. *J Lipid Res.* (2013) 54:2950–63. doi: 10.1194/jlr.R036095

7. Birner-Gruenberger R, Schittmayer M, Holzer M, Marsche G. Understanding high-density lipoprotein function in disease: recent advances in proteomics unravel the complexity of its composition and biology. *Prog Lipid Res.* (2014) 56:36–46. doi: 10.1016/j.plipres.2014.07.003

8. Kontush A, Lhomme M, Calabresi L, Chapman MJ, Davidson WS, Davidson WS. Structure of HDL: particle subclasses and molecular components. *Handb Exp Pharmacol.* (2015) 224:3–15. doi: 10.1007/978-3-319-09665-0_1

9. Sturtzel C. Endothelial cells. *Adv Exp Med Biol.* (2017) 1003:71–91. doi: 10.1007/978-3-319-57613-8_4

10. Cyr AR, Huckaby LV, Shiva SS, Zuckerbraun BS. Nitric oxide and endothelial dysfunction. *Crit Care Clin.* (2020) 36:307–21. doi: 10.1016/j.ccc.2019.12.009

11. Gimbrone MA, García-Cardeña G. Endothelial cell dysfunction and the pathobiology of atherosclerosis. *Circ Res.* (2016) 118:620–36. doi: 10.1161/CIRCRESAHA.115.306301

12. Davis CE, Williams DH, Oganov RG, Tao SC, Rywik SL, Stein Y, et al. Sex difference in high density lipoprotein cholesterol in six countries. *Am J Epidemiol.* (1996) 143:1100–6. doi: 10.1093/oxfordjournals.aje.a008686

13. García-Calzón S, Perfilyev A, de Mello VD, Pihlajamäki J, Ling C. Sex differences in the methylome and transcriptome of the human liver and circulating HDL-cholesterol levels. *J Clin Endocrinol Metab.* (2018) 103:4395–408. doi: 10.1210/jc.2018-00423

14. Huxley VH, Kemp SS, Schramm C, Sieveking S, Bingaman S, Yu Y, et al. Sex differences influencing micro- and macrovascular endothelial phenotype in vitro. *J Physiol.* (2018) 596:3929–49. doi: 10.1113/JP276048

15. Agirbasli M, Agaoglu NB, Orak N, Caglioz H, Ocek T, Karabag T, et al. Sex hormones, insulin resistance and high-density lipoprotein cholesterol levels in children. *Horm Res Paediatr.* (2010) 73:166–74. doi: 10.1159/000284357

16. Morrison JA, Barton BA, Biro FM, Sprecher DL. Sex hormones and the changes in adolescent male lipids: longitudinal studies in a biracial cohort. *J Pediatr.* (2003) 142:637–42. doi: 10.1067/mpd.2003.246

17. El Khoudary SR, Brooks MM, Thurston RC, Matthews KA. Lipoprotein subclasses and endogenous sex hormones in women at midlife. *J Lipid Res.* (2014) 55:1498–504. doi: 10.1194/jlr.P049064

18. Mendelsohn ME. Protective effects of estrogen on the cardiovascular system. *Am J Cardiol.* (2002) 89:12E–7E. doi: 10.1016/S0002-9149(02)02405-0

19. Mendelsohn ME. Mechanisms of estrogen action in the cardiovascular system. *J Steroid Biochem Mol Biol.* (2000) 74:337–43. doi: 10.1016/S0960-0760(00)00110-2

20. James BD, Allen JB. Sex-specific response to combinations of shear stress and substrate stiffness by endothelial cells in vitro. *Adv Healthc Mater.* (2021) 10:e2100735. doi: 10.1002/adhm.202100735

21. Boscaro C, Trenti A, Baggio C, Scapin C, Trevisi L, Cignarella A, et al. Sex differences in the pro-angiogenic response of human endothelial cells: focus on PFKFB3 and FAK activation. *Front Pharmacol.* (2020) 11:587221. doi: 10.3389/fphar.2020.587221

22. Polderman TJC, Kreukels BPC, Irwig MS, Beach L, Chan YM, Derks EM, et al. The biological contributions to gender identity and gender diversity: bringing data to the table. *Behav Genet.* (2018) 48:95–108. doi: 10.1007/s10519-018-9889-z

23. Maraka S, Singh Ospina N, Rodriguez-Gutierrez R, Davidge-Pitts CJ, Nippoldt TB, Prokop LJ, et al. Sex steroids and cardiovascular outcomes in transgender individuals: a systematic review and meta-analysis. *J Clin Endocrinol Metab.* (2017) 102:3914–23. doi: 10.1210/jc.2017-01643

24. van Velzen DM, Adorni MP, Zimetti F, Strazzella A, Simsek S, Sirtori CR, et al. The effect of transgender hormonal treatment on high density lipoprotein cholesterol efflux capacity. *Atherosclerosis.* (2021) 323:44–53. doi: 10.1016/j.atherosclerosis.2021.03.008

25. Humble RM, Greene DN, Schmidt RL, Winston McPherson G, Rongitsch J, Imborek KL, et al. Reference intervals for clinical chemistry analytes for transgender men and women on stable hormone therapy. *J Appl Lab Med.* (2022). doi: 10.1093/jalm/jfac025

26. Iannantuoni F, Salazar JD, Martinez de. Marañon A, Bañuls C, López-Domènech S, Rocha M, et al. Testosterone administration increases leukocyte-endothelium interactions and inflammation in transgender men. *Fertil Steril.* (2021) 115:483–9. doi: 10.1016/j.fertnstert.2020.08.002

27. Kannel WB, Dawber TR, Friedman GD, Glennon WE, McNamara. PMRISK factors in coronary heart diseasean evaluation of several serum lipids as predictors of coronary heart disease, the framingham. study. *Ann Intern Med.* (1964) 61:888–99. doi: 10.7326/0003-4819-61-5-888

28. Cheung MC, Brown BG, Wolf AC, Albers JJ. Altered particle size distribution of apolipoprotein A-I-containing lipoproteins in subjects with coronary artery disease. *J Lipid Res.* (1991) 32:383–94. doi: 10.1016/S0022-2275(20)42061-9

29. Robert J, Osto E, von Eckardstein A. The endothelium is both a target and a barrier of HDL's protective functions. *Cells.* (2021) 10:1041. doi: 10.3390/cells10051041

30. Asztalos BF, Cupples LA, Demissie S, Horvath KV, Cox CE, Batista MC, et al. High-density lipoprotein subpopulation profile and coronary heart disease prevalence in male participants of the Framingham Offspring Study. *Arterioscler Thromb Vasc Biol.* (2004) 24:2181–7. doi: 10.1161/01.ATV.0000146325.93749.a8

31. Smith JD, Breslow JL. The emergence of mouse models of atherosclerosis and their relevance to clinical research. *J Intern Med.* (1997) 242:99–109. doi: 10.1046/j.1365-2796.1997.00197.x

32. Calabresi L, Gomaraschi M, Franceschini G. Endothelial protection by high-density lipoproteins: from bench to bedside. *Arterioscler Thromb Vasc Biol.* (2003) 23:1724–31. doi: 10.1161/01.ATV.0000094961.74697.54

33. Inazu A, Brown ML, Hesler CB, Agellon LB, Koizumi J, Takata K, et al. Increased high-density lipoprotein levels caused by a common cholesteryl-ester transfer protein gene mutation. *N Engl J Med.* (1990) 323:1234–8. doi: 10.1056/NEJM199011013231803

34. Barter PJ, Brewer HB. Jr, Chapman MJ, Hennekens CH, Rader DJ, Tall AR Cholesteryl ester transfer protein: a novel target for raising HDL and inhibiting atherosclerosis Arterioscler. *Thromb Vasc Biol.* (2003) 23:160–7. doi: 10.1161/01.ATV.0000054658.91146.64

35. Barter PJ, Caulfield M, Eriksson M, Grundy SM, Kastelein JJ, Komajda M, et al. Effects of torcetrapib in patients at high risk for coronary events. *N Engl J Med.* (2007) 357:2109–22. doi: 10.1056/NEJMoa0706628

36. Furtado JD, Ruotolo G, Nicholls SJ, Dullea R, Carvajal-Gonzalez S, Sacks FM. Pharmacological inhibition of CETP (Cholesteryl Ester Transfer Protein) increases HDL (High-Density Lipoprotein) that contains ApoC3 and other HDL subspecies associated with higher risk of coronary heart disease. *Arterioscler Thromb Vasc Biol.* (2022) 42:227–37. doi: 10.1161/ATVBAHA.121.317181

37. Schwartz GG, Olsson AG, Barter PJ. Dalcetrapib in patients with an acute coronary syndrome. *N Engl J Med.* (2013) 368:869–70. doi: 10.1056/NEJMc1300057

38. Tardif JC, Rhéaume E, Lemieux Perreault LP, Grégoire JC, Feroz Zada Y, Asselin G, et al. Pharmacogenomic determinants of the cardiovascular effects of dalcetrapib. *Circ Cardiovasc Genet.* (2015) 8:372–82. doi: 10.1161/CIRCGENETICS.114.000663

39. Miyares MA. Anacetrapib and dalcetrapib: two novel cholesteryl ester transfer protein inhibitors. *Ann Pharmacother.* (2011) 45:84–94. doi: 10.1345/aph.1P446

40. Bowman L, Chen F, Sammons E, Hopewell JC, Wallendszus K, Stevens W, et al. Randomized Evaluation of the Effects of Anacetrapib through Lipid-modification (REVEAL)-A large-scale, randomized, placebo-controlled trial of the clinical effects of anacetrapib among people with established vascular disease: Trial design, recruitment, and baseline characteristics. *Am Heart J.* (2017) 187:182–90. doi: 10.1016/j.ahj.2017.02.021

41. Group THT-RC, Committee W, Sammons E, Hopewell JC, Chen F, Stevens W, et al. Long-term safety and efficacy of anacetrapib in patients with atherosclerotic vascular disease. *Eur Heart J.* (2021) 43:1416–24.

42. Krishna R, Gheyas F, Liu Y, Hagen DR, Walker B, Chawla A, et al. Chronic administration of anacetrapib is associated with accumulation in adipose and slow elimination. *Clin Pharmacol Ther.* (2017) 102:832–40. doi: 10.1002/cpt.700

43. Palmer TM, Lawlor DA, Harbord RM, Sheehan NA, Tobias JH, Timpson NJ, et al. Using multiple genetic variants as instrumental variables for modifiable risk factors. *Stat Methods Med Res.* (2012) 21:223–42. doi: 10.1177/0962280210394459

44. Agerholm-Larsen B, Tybjaerg-Hansen A, Schnohr P, Steffensen R, Nordestgaard BG. Common cholesteryl ester transfer protein mutations, decreased HDL cholesterol, and possible decreased risk of ischemic heart disease: The Copenhagen City Heart Study. *Circulation.* (2000) 102:2197–203. doi: 10.1161/01.CIR.102.18.2197

45. Vergeer M, Korporaal SJ, Franssen R, Meurs I, Out R, Hovingh GK, et al. Genetic variant of the scavenger receptor BI in humans. *N Engl J Med.* (2011) 364:136–45. doi: 10.1056/NEJMoa0907687

46. Zanoni P, Khetarpal SA, Larach DB, Hancock-Cerutti WF, Millar JS, Cuchel M, et al. Rare variant in scavenger receptor BI raises HDL cholesterol and increases risk of coronary heart disease. *Science.* (2016) 351:1166–71. doi: 10.1126/science.aad3517

47. Arnett DK, Blumenthal RS, Albert MA, Buroker AB, Goldberger ZD, Hahn EJ, et al. (2019). ACC/AHA guideline on the primary prevention of cardiovascular disease: executive summary: a report of the american college of cardiology/american heart association task force on clinical practice guidelines. *J Am Coll Cardiol.* (2019) 74:1376–414.

48. Conroy RM, Pyörälä K, Fitzgerald AP, Sans S, Menotti A, De Backer G, et al. Estimation of ten-year risk of fatal cardiovascular disease in Europe: the SCORE project. *Eur Heart J.* (2003) 24:987–1003. doi: 10.1016/S0195-668X(03)00114-3

49. Kim HJ, Park HA, Cho YG, Kang JH, Kim KW, Kang JH, et al. Gender difference in the level of HDL cholesterol in Korean adults. *Korean J Fam Med.* (2011) 32:173–81. doi: 10.4082/kjfm.2011.32.3.173

50. Klingel SL, Roke K, Hidalgo B, Aslibekyan S, Straka RJ, An P, et al. Sex differences in blood HDL-c, the total cholesterol/HDL-c ratio, and palmitoleic acid are not associated with variants in common candidate genes. *Lipids.* (2017) 52:969–80. doi: 10.1007/s11745-017-4307-5

51. Yi S-W, Park H-B, Jung M-H, Yi J-J, Ohrr H. High-density lipoprotein cholesterol and cardiovascular mortality: a prospective cohort study among 15.8 million adults. *Eur J Prev Cardiol.* (2021) 29:844–54. doi: 10.1093/eurjpc/zwab230

52. Bagatell CJ, Bremner WJ. Androgen and progestagen effects on plasma lipids. *Prog Cardiovasc Dis.* (1995) 38:255–71. doi: 10.1016/S0033-0620(95)80016-6

53. Jomard A, Liberale L, Doytcheva P, Reiner MF, Müller D, Visentin M, et al. Effects of acute administration of trimethylamine N-oxide on endothelial function: a translational study. *Sci Rep.* (2022) 12:8664. doi: 10.1038/s41598-022-12720-5

54. Tzeravini E, Tentolouris A, Eleftheriadou I, Chaviaras N, Kolovou G, Apostolidou-Kiouti F, et al. Comparison of postprandial serum triglyceride and apolipoprotein B concentrations between the two phases of menstrual cycle in healthy women. *Curr Vasc Pharmacol.* (2021) 19:411–22. doi: 10.2174/1573406416666200611105113

55. Vashishta S, Gahlot S, Goyal R. Effect of menstrual cycle phases on plasma lipid and lipoprotein levels in regularly menstruating women. *J Clin Diagn Res.* (2017) 11:Cc05–cc7. doi: 10.7860/JCDR/2017/26031.9799

56. López-Simón L, de Oya M, Lasunción MA, Riestra P, Benavente M, de Oya I, et al. Genetic determinants of plasma HDL-cholesterol levels in prepubertal children. *Clin Chim Acta.* (2009) 403:203–6. doi: 10.1016/j.cca.2009.03.002

57. Cho KH, Kim JR. Rapid decrease in HDL-C in the puberty period of boys associated with an elevation of blood pressure and dyslipidemia in Korean teenagers: an explanation of why and when men have lower HDL-C levels than women. *Med Sci (Basel).* (2021) 9:35. doi: 10.3390/medsci9020035

58. Inaraja V, Thuissard I, Andreu-Vazquez C, Jodar E. Lipid profile changes during the menopausal transition. *Menopause.* (2020) 27:780–7. doi: 10.1097/GME.0000000000001532

59. Collins P, HDL-C. in post-menopausal women: an important therapeutic target. *Int J Cardiol.* (2008) 124:275–82. doi: 10.1016/j.ijcard.2007.06.009

60. Jacobson TA, Ito MK, Maki KC, Orringer CE, Bays HE, Jones PH, et al. National Lipid Association recommendations for patient-centered management of dyslipidemia: part 1 - executive summary. *J Clin Lipidol.* (2014) 8:473–88. doi: 10.1016/j.jacl.2014.07.007

61. Trimarco V, Izzo R, Morisco C, Mone P, Maria Virginia M, Falco A, et al. High HDL (high-density lipoprotein) cholesterol increases cardiovascular risk in hypertensive patients. *Hypertension*. 2022:101161hypertensionaha12219912. doi: 10.1161/HYPERTENSIONAHA.122.19912

62. Wilkins JT, Ning H, Stone NJ, Criqui MH, Zhao L, Greenland P, et al. Coronary heart disease risks associated with high levels of HDL cholesterol. *J Am Heart Assoc.* (2014) 3:e000519. doi: 10.1161/JAHA.113.000519

63. Paunio M, Heinonen OP, Virtamo J, Klag MJ, Manninen V, Albanes D, et al. HDL cholesterol and mortality in Finnish men with special reference to alcohol intake. *Circulation.* (1994) 90:2909–18. doi: 10.1161/01.CIR.90.6.2909

64. Rached FH, Chapman MJ, Kontush A. HDL particle subpopulations: focus on biological function. *Biofactors.* (2015) 41:67–77. doi: 10.1002/biof.1202

65. Zannis VI, Fotakis P, Koukos G, Kardassis D, Ehnholm C, Jauhiainen M, et al. HDL biogenesis, remodeling, and catabolism. *Handb Exp Pharmacol.* (2015) 224:53–111. doi: 10.1007/978-3-319-09665-0_2

66. Parks JS, Chung S Fau - Shelness GS, Shelness GS. Hepatic ABC transporters and triglyceride metabolism. *Curr Opin Lipidol.* (2012) 23:1473-6535. doi: 10.1097/MOL.0b013e328352dd1a

67. Norum KR, Remaley AT, Miettinen HE, Strøm EH, Balbo BEP, Sampaio C, et al. Lecithin:cholesterol acyltransferase: symposium on 50 years of biomedical research from its discovery to latest findings. *J Lipid Res.* (2020). 61:1539-7262. doi: 10.1194/jlr.S120000720

68. Duong M, Psaltis M, Rader DJ, Marchadier D, Barter PJ, Rye K-A. Evidence that hepatic lipase and endothelial lipase have different substrate specificities for high-density lipoprotein phospholipids. *Biochemistry.* (2003) 42:13778–85. doi: 10.1021/bi034990n

69. Albers JJ, Vuletic S, Cheung MC. Role of plasma phospholipid transfer protein in lipid and lipoprotein metabolism. *Biochim Biophys Acta Mol Cell Biol Lipids.* (2012) 1821:345–57. doi: 10.1016/j.bbalip.2011.06.013

70. Schaefer EJ, Anthanont P, Asztalos BF. High-density lipoprotein metabolism, composition, function, and deficiency. *Curr Opin Lipidol.* (2014) 25:194–9. doi: 10.1097/MOL.0000000000000074

71. Lamon-Fava S, Micherone D. Regulation of apoA-I gene expression: mechanism of action of estrogen and genistein. *J Lipid Res.* (2004) 45:106–12. doi: 10.1194/jlr.M300179-JLR200

72. Lopez D, Sanchez MD, Shea-Eaton W, McLean MP. Estrogen activates the high-density lipoprotein receptor gene via binding to estrogen response elements and interaction with sterol regulatory element binding protein-1A. *Endocrinology.* (2002) 143:2155–68. doi: 10.1210/endo.143.6.8855

73. Jones DR, Schmidt RJ, Pickard RT, Foxworthy PS, Eacho PI. Estrogen receptor-mediated repression of human hepatic lipase gene transcription. *J Lipid Res.* (2002) 43:383–91. doi: 10.1016/S0022-2275(20)30144-9

74. 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) 38:253–9. doi: 10.1136/bjsm.2003.000199

75. Goldberg RB, Rabin D, Alexander AN, Doelle GC, Getz GS. Suppression of plasma testosterone leads to an increase in serum total and high density lipoprotein cholesterol and apoproteins A-I and B. *J Clin Endocrinol Metab.* (1985) 60:203–7. doi: 10.1210/jcem-60-1-203

76. Wekker V, van Dammen L, Koning A, Heida KY, Painter RC, Limpens J, et al. Long-term cardiometabolic disease risk in women with PCOS: a systematic review and meta-analysis. *Hum Reprod Update.* (2020) 26:942–60. doi: 10.1093/humupd/dmaa029

77. Spałkowska M, Mrozińska S, Gałuszka-Bednarczyk A, Gosztyła K, Przywara A, Guzik J, et al. The PCOS patients differ in lipid profile according to their phenotypes. *Exp Clin Endocrinol Diabetes.* (2018) 126:437–44. doi: 10.1055/s-0043-121264

78. Hall E, Volkov P, Dayeh T, Esguerra JL, Salö S, Eliasson L, et al. Sex differences in the genome-wide DNA methylation pattern and impact on gene expression, microRNA levels and insulin secretion in human pancreatic islets. *Genome Biol.* (2014) 15:522. doi: 10.1186/s13059-014-0522-z

79. Mayne BT, Bianco-Miotto T, Buckberry S, Breen J, Clifton V, Shoubridge C, et al. Large scale gene expression meta-analysis reveals tissue-specific, sex-biased gene expression in humans. *Front Genet.* (2016) 7:183. doi: 10.3389/fgene.2016.00183

80. Russo GT, Horvath KV, Di Benedetto A, Giandalia A, Cucinotta D, Asztalos B. Influence of menopause and cholesteryl ester transfer protein (CETP) TaqIB polymorphism on lipid profile and HDL subpopulations distribution in women with and without type 2 diabetes. *Atherosclerosis.* (2010) 210:294–301. doi: 10.1016/j.atherosclerosis.2009.11.011

81. Lamon-Fava S, Asztalos BF, Howard TD, Reboussin DM, Horvath KV, Schaefer EJ, et al. Association of polymorphisms in genes involved in lipoprotein metabolism with plasma concentrations of remnant lipoproteins and HDL subpopulations before and after hormone therapy in postmenopausal women. *Clin Endocrinol (Oxf).* (2010) 72:169–75. doi: 10.1111/j.1365-2265.2009.03644.x

82. Boekholdt SM, Sacks FM, Jukema JW, Shepherd J, Freeman DJ, McMahon AD, et al. Cholesteryl ester transfer protein TaqIB variant, high-density lipoprotein cholesterol levels, cardiovascular risk, and efficacy of pravastatin treatment: individual patient meta-analysis of 13,677 subjects. *Circulation.* (2005) 111:278–87. doi: 10.1161/01.CIR.0000153341.46271.40

83. Heilbronn LK, Noakes M, Clifton PM. Association between HDL-cholesterol and the Taq1B polymorphism in the cholesterol ester transfer protein gene in obese women. *Atherosclerosis.* (2002) 162:419–24. doi: 10.1016/S0021-9150(01)00733-X

84. Rader DJ. Molecular regulation of HDL metabolism and function: implications for novel therapies. *J Clin Invest.* (2006) 116:3090–100. doi: 10.1172/JCI30163

85. Reilly M, Rader DJ. Apolipoprotein E and coronary disease: a puzzling paradox. *PLoS Med.* (2006) 3:e258. doi: 10.1371/journal.pmed.0030258

86. Mosher MJ, Lange LA, Howard BV, Lee ET, Best LG, Fabsitz RR, et al. Sex-specific interaction between APOE genotype and carbohydrate intake affects plasma HDL-C levels: the Strong Heart Family Study. *Genes Nutr.* (2008) 3:87–97. doi: 10.1007/s12263-008-0075-4

87. Zokaei N, Giehl K, Sillence A, Neville MJ, Karpe F, Nobre AC, et al. Sex and APOE: A memory advantage in male APOE ε4 carriers in midlife. *Cortex.* (2017) 88:98–105. doi: 10.1016/j.cortex.2016.12.016

88. Altmann A, Tian L, Henderson VW, Greicius MD. Sex modifies the APOE-related risk of developing Alzheimer disease. *Ann Neurol.* (2014) 75:563–73. doi: 10.1002/ana.24135

89. Tanaka S, Yasuda T, Ishida T, Fujioka Y, Tsujino T, Miki T, et al. Increased serum cholesterol esterification rates predict coronary heart disease and sudden death in a general population. *Arterioscler Thromb Vasc Biol.* (2013) 33:1098–104. doi: 10.1161/ATVBAHA.113.301297

90. Holleboom AG, Kuivenhoven JA, Vergeer M, Hovingh GK, van Miert JN, Wareham NJ, et al. Plasma levels of lecithin:cholesterol acyltransferase and risk of future coronary artery disease in apparently healthy men and women: a prospective case-control analysis nested in the EPIC-Norfolk population study. *J Lipid Res.* (2010) 51:416–21. doi: 10.1194/P900038-JLR200

91. Emokpae MA, Uwumarongie OH, Osadolor HB. Sex dimorphism in serum lecithin: cholesterol acyltransferase and lipoprotein lipase activities in adult sickle cell anaemia patients with proteinuria. *Indian J Clin Biochem.* (2011) 26:57–61. doi: 10.1007/s12291-010-0096-9

92. Guo M, Liu Z, Xu Y, Ma P, Huang W, Gao M, et al. Spontaneous atherosclerosis in aged LCAT-deficient hamsters with enhanced oxidative stress-brief report. *Arterioscler Thromb Vasc Biol.* (2020) 40:2829–36. doi: 10.1161/ATVBAHA.120.315265

93. Li L, Hossain MA, Sadat S, Hager L, Liu L, Tam L, et al. Lecithin cholesterol acyltransferase null mice are protected from diet-induced obesity and insulin resistance in a gender-specific manner through multiple pathways. *J Biol Chem.* (2011) 286:17809–20. doi: 10.1074/jbc.M110.180893

94. Khovidhunkit WKMM, Memon RR, Shigenaga JK, Moser AH, Feingold KR, Grunfeld C. Effects of infection and inflammation on lipid and lipoprotein metabolism: mechanisms and consequences to the host. *J Lipid Res.* (2004) 45:1169–96. doi: 10.1194/jlr.R300019-JLR200

95. Memon RA, Holleran WM, Moser AH, Seki T, Uchida Y, Fuller J, et al. Endotoxin and cytokines increase hepatic sphingolipid biosynthesis and produce lipoproteins enriched in ceramides and sphingomyelin. *Arterioscler Thromb Vasc Biol.* (1998) 18:1257–65. doi: 10.1161/01.ATV.18.8.1257

96. Prasad DS, Kabir Z, Dash AK, Das BC. Abdominal obesity, an independent cardiovascular risk factor in Indian subcontinent: a clinico epidemiological evidence summary. *J Cardiovasc Dis Res.* (2011) 2:199–205. doi: 10.4103/0975-3583.89803

97. Selvaraj S, Martinez EE, Aguilar FG, Kim KY, Peng J, Sha J, et al. Association of central adiposity with adverse cardiac mechanics: findings from the hypertension genetic epidemiology network study. *Circ Cardiovasc Imaging.* (2016) 9:e004396. doi: 10.1161/CIRCIMAGING.115.004396

98. Woudberg NJ, Lecour S, Goedecke JH. HDL subclass distribution shifts with increasing central adiposity. *J Obes.* (2019) 2019:2107178. doi: 10.1155/2019/2107178

99. Stadler JT, Lackner S, Mörkl S, Trakaki A, Scharnagl H, Borenich A, et al. Obesity affects HDL metabolism, composition and subclass distribution. *Biomedicines*. (2021) 9. doi: 10.3390/biomedicines9030242

100. Talbot CPJ, Plat J, Joris PJ, Konings M, Kusters Y, Schalkwijk CG, et al. HDL cholesterol efflux capacity and cholesteryl ester transfer are associated with body mass, but are not changed by diet-induced weight loss: A randomized trial in abdominally obese men. *Atherosclerosis*. (2018) 274:23–8. doi: 10.1016/j.atherosclerosis.2018.04.029

101. Lorkowski SW, Brubaker G, Rotroff DM, Kashyap SR, Bhatt DL, Nissen SE, et al. Bariatric surgery improves HDL function examined by apoa1 exchange rate and cholesterol efflux capacity in patients with obesity and type 2 diabetes. *Biomolecules*. (2020) 10:551. doi: 10.3390/biom10040551

102. Thakkar H, Vincent V, Shukla S, Sra M, Kanga U, Aggarwal S, et al. Improvements in cholesterol efflux capacity of HDL and adiponectin contribute to mitigation in cardiovascular disease risk after bariatric surgery in a cohort with morbid obesity. *Diabetol Metab Syndr*. (2021) 13:46. doi: 10.1186/s13098-021-00662-3

103. Osto E, Doytcheva P, Corteville C, Bueter M, Dörig C, Stivala S, et al. Rapid and body weight-independent improvement of endothelial and high-density lipoprotein function after Roux-en-Y gastric bypass: role of glucagon-like peptide-1. *Circulation*. (2015) 131:871–81. doi: 10.1161/CIRCULATIONAHA.114.011791

104. Asztalos BF, Swarbrick MM, Schaefer EJ, Dallal GE, Horvath KV Ai M, et al. Effects of weight loss, induced by gastric bypass surgery, on HDL remodeling in obese women. *J Lipid Res*. (2010) 51:2405–12. doi: 10.1194/jlr.P900015-JLR200

105. van Veldhuisen SL, Gorter TM, van Woerden G, de Boer RA, Rienstra M, Hazebroek EJ, et al. Bariatric surgery and cardiovascular disease: a systematic review and meta-analysis. *Eur Heart J*. (2022) 43:1955–69. doi: 10.1093/eurheartj/ehac071

106. Aly S, Hachey K, Pernar LIM. Gender disparities in weight loss surgery. *Mini-invasive Surgery*. (2020) 4:21. doi: 10.20517/2574-1225.2019.57

107. Aminian A, Zelisko A, Kirwan JP, Brethauer SA, Schauer PR. Exploring the impact of bariatric surgery on high density lipoprotein. *Surg Obes Relat Dis*. (2015) 11:238–47. doi: 10.1016/j.soard.2014.07.017

108. Genua I, Ramos A, Caimari F, Balagué C, Sánchez-Quesada JL, Pérez A, et al. Effects of bariatric surgery on HDL cholesterol. *Obes Surg*. (2020) 30:1793–8. doi: 10.1007/s11695-020-04385-8

109. Arnal JF, Fontaine C, Billon-Galés A, Favre J, Laurell H, Lenfant F, et al. Estrogen receptors and endothelium. *Arterioscler Thromb Vasc Biol*. (2010) 30:1506–12. doi: 10.1161/ATVBAHA.109.191221

110. Celermajer DS, Sorensen KE, Spiegelhalter DJ, Georgakopoulos D, Robinson J, Deanfield JE. Aging is associated with endothelial dysfunction in healthy men years before the age-related decline in women. *J Am Coll Cardiol*. (1994) 24:471–6. doi: 10.1016/0735-1097(94)90305-0

111. Sader MA, Celermajer DS. Endothelial function, vascular reactivity and gender differences in the cardiovascular system. *Cardiovasc Res*. (2002) 53:597–604. doi: 10.1016/S0008-6363(01)00473-4

112. Simoncini T. Mechanisms of action of estrogen receptors in vascular cells: relevance for menopause and aging. *Climacteric*. (2009) 12 (Suppl 1):6–11. doi: 10.1080/13697130902986385

113. Mendelsohn ME, Karas RH. Molecular and cellular basis of cardiovascular gender differences. *Science*. (2005) 308:1583–7. doi: 10.1126/science.1112062

114. Collins P, Rosano GM, Sarrel PM, Ulrich L, Adamopoulos S, Beale CM, et al. 17 beta-Estradiol attenuates acetylcholine-induced coronary arterial constriction in women but not men with coronary heart disease. *Circulation*. (1995) 92:24–30. doi: 10.1161/01.CIR.92.1.24

115. Rossouw JE, Anderson GL, Prentice RL, LaCroix AZ, Kooperberg C, Stefanick ML, et al. Risks and benefits of estrogen plus progestin in healthy postmenopausal women: principal results From the Women's Health Initiative randomized controlled trial. *JAMA*. (2002) 288:321–33. doi: 10.1001/jama.288.3.321

116. Fredette NC, Meyer MR, Prossnitz ER. Role of GPER in estrogen-dependent nitric oxide formation and vasodilation. *J Steroid Biochem Mol Biol*. (2018) 176:65–72. doi: 10.1016/j.jsbmb.2017.05.006

117. Tuma M, Canestrini S, Alwahab Z, Marshall J. Trauma and endothelial glycocalyx: the microcirculation helmet? *Shock*. (2016) 46:352–7. doi: 10.1097/SHK.0000000000000635

118. Hartman RJG, Kapteijn DMC, Haitjema S, Bekker MN, Mokry M, Pasterkamp G, et al. Intrinsic transcriptomic sex differences in human endothelial cells at birth and in adults are associated with coronary artery disease targets. *Sci Rep*. (2020) 10:12367. doi: 10.1038/s41598-020-69451-8

119. Addis R, Campesi I, Fois M, Capobianco G, Dessole S, Fenu G, et al. Human umbilical endothelial cells (HUVECs) have a sex: characterisation of the phenotype of male and female cells. *Biol Sex Differ*. (2014) 5:18. doi: 10.1186/s13293-014-0018-2

120. Cattaneo MG, Banfi C, Brioschi M, Lattuada D, Vicentini LM. Sex-dependent differences in the secretome of human endothelial cells. *Biol Sex Differ*. (2021) 12:7. doi: 10.1186/s13293-020-00350-3

121. Torres-Estay V, Carreño DV, San Francisco IF, Sotomayor P, Godoy AS, Smith GJ. Androgen receptor in human endothelial cells. *J Endocrinol*. (2015) 224:R131–7. doi: 10.1530/JOE-14-0611

122. Liu PY, Death AK, Handelsman DJ. Androgens and cardiovascular disease. *Endocr Rev*. (2003) 24:313–40. doi: 10.1210/er.2003-0005

123. Kim KH, Bender JR. Membrane-initiated actions of estrogen on the endothelium. *Mol Cell Endocrinol*. (2009) 308:3–8. doi: 10.1016/j.mce.2009.03.025

124. Diebel LN, Wheaton M, Liberati DM. The protective role of estrogen on endothelial and glycocalyx barriers after shock conditions: a microfluidic study. *Surgery*. (2021) 169:678–85. doi: 10.1016/j.surg.2020.08.006

125. Rodríguez E, López R, Paez A, Massó F, Montaño LF. 17Beta-estradiol inhibits the adhesion of leukocytes in TNF-alpha stimulated human endothelial cells by blocking IL-8 and MCP-1 secretion, but not its transcription. *Life Sci*. (2002) 71:2181–93. doi: 10.1016/S0024-3205(02)01999-9

126. Caulin-Glaser T, Watson CA, Pardi R, Bender JR. Effects of 17beta-estradiol on cytokine-induced endothelial cell adhesion molecule expression. *J Clin Invest*. (1996) 98:36–42. doi: 10.1172/JCI118774

127. Holm P, Andersen HL, Arrøe G, Stender S. Gender gap in aortic cholesterol accumulation in cholesterol-clamped rabbits: role of the endothelium and mononuclear-endothelial cell interaction. *Circulation*. (1998) 98:2731–7. doi: 10.1161/01.CIR.98.24.2731

128. Usselman CW, Yarovinsky TO, Steele FE, Leone CA, Taylor HS, Bender JR, et al. Androgens drive microvascular endothelial dysfunction in women with polycystic ovary syndrome: role of the endothelin B receptor. *J Physiol*. (2019) 597:2853–65. doi: 10.1113/JP277756

129. Lopes RA, Neves KB, Carneiro FS, Tostes RC. Testosterone and vascular function in aging. *Front Physiol*. (2012) 3:89. doi: 10.3389/fphys.2012.00089

130. Villablanca A, Lubahn D, Shelby L, Lloyd K, Barthold S. Susceptibility to early atherosclerosis in male mice is mediated by estrogen receptor &#x3b1. *Arterioscler Thromb Vasc Biol*. (2004) 24:1055–61. doi: 10.1161/01.ATV.0000130467.65290.d4

131. Villablanca AC, Tetali S, Altman R, Ng KF, Rutledge JC. Testosterone-derived estradiol production by male endothelium is robust and dependent on p450 aromatase via estrogen receptor alpha. *Springerplus*. (2013) 2:214. doi: 10.1186/2193-1801-2-214

132. McCrohon JA, Death AK, Nakhla S, Jessup W, Handelsman DJ, Stanley KK, et al. Androgen receptor expression is greater in macrophages from male than from female donors. A sex difference with implications for atherogenesis. *Circulation*. (2000) 101:224–6. doi: 10.1161/01.CIR.101.3.224

133. Remus EW, Sayeed I, Won S, Lyle AN, Stein DG. Progesterone protects endothelial cells after cerebrovascular occlusion by decreasing MCP-1- and CXCL1-mediated macrophage infiltration. *Exp Neurol*. (2015) 271:401–8. doi: 10.1016/j.expneurol.2015.07.010

134. You Y, Tan W, Guo Y, Luo M, Shang FF, Xia Y, et al. Progesterone promotes endothelial nitric oxide synthase expression through enhancing nuclear progesterone receptor-SP-1 formation. *Am J Physiol Heart Circ Physiol*. (2020) 319:H341–h8. doi: 10.1152/ajpheart.00206.2020

135. Grady D, Herrington D, Bittner V, Blumenthal R, Davidson M, Hlatky M, et al. Cardiovascular disease outcomes during 6.8 years of hormone therapy: heart and estrogen/progestin replacement study follow-up (HERS II). *JAMA*. (2002) 288:49–57. doi: 10.1001/jama.288.1.49

136. Jiang Y, Tian W. The effects of progesterones on blood lipids in hormone replacement therapy. *Lipids Health Dis*. (2017) 16:219. doi: 10.1186/s12944-017-0612-5

137. Kojima K, Abe-Dohmae S, Arakawa R, Murakami I, Suzumori K, Yokoyama S. Progesterone inhibits apolipoprotein-mediated cellular lipid release: a putative mechanism for the decrease of high-density lipoprotein. *Biochim Biophys Acta*. (2001) 1532:173–84. doi: 10.1016/S1388-1981(01)00124-X

138. Wang J, Bingaman S, Huxley VH. Intrinsic sex-specific differences in microvascular endothelial cell phosphodiesterases. *Am J Physiol Heart Circ Physiol*. (2010) 298:H1146–54. doi: 10.1152/ajpheart.00252.2009

139. Du L, Bayir H, Lai Y, Zhang X, Kochanek PM, Watkins SC, et al. Innate gender-based proclivity in response to cytotoxicity and programmed cell death pathway. *J Biol Chem*. (2004) 279:38563–70. doi: 10.1074/jbc.M405461200

140. Du L, Hickey RW, Bayir H, Watkins SC, Tyurin VA, Guo F, et al. Starving neurons show sex difference in autophagy. *J Biol Chem.* (2009) 284:2383–96. doi: 10.1074/jbc.M804396200

141. Lorenz M, Koschate J, Kaufmann K, Kreye C, Mertens M, Kuebler WM, et al. Does cellular sex matter? Dimorphic transcriptional differences between female and male endothelial cells. *Atherosclerosis.* (2015) 240:61–72. doi: 10.1016/j.atherosclerosis.2015.02.018

142. Lorenz M, Blaschke B, Benn A, Hammer E, Witt E, Kirwan J, et al. Sex-specific metabolic and functional differences in human umbilical vein endothelial cells from twin pairs. *Atherosclerosis.* (2019) 291:99–106. doi: 10.1016/j.atherosclerosis.2019.10.007

143. McDonald G, Cabal N, Vannier A, Umiker B, Yin RH, Orjalo AV, et al. Female bias in systemic lupus erythematosus is associated with the differential expression of X-linked toll-like receptor 8. *Front Immunol.* (2015) 6:457. doi: 10.3389/fimmu.2015.00457

144. Khan D, Ansar Ahmed S. The immune system is a natural target for estrogen action: opposing effects of estrogen in two prototypical autoimmune diseases. *Front Immunol.* (2015) 6:635. doi: 10.3389/fimmu.2015.00635

145. Keselman A, Heller N. Estrogen signaling modulates allergic inflammation and contributes to sex differences in asthma. *Front Immunol.* (2015) 6:568. doi: 10.3389/fimmu.2015.00568

146. Messner B, Bernhard D. Smoking and cardiovascular disease: mechanisms of endothelial dysfunction and early atherogenesis. *Arterioscler Thromb Vasc Biol.* (2014) 34:509–15. doi: 10.1161/ATVBAHA.113.300156

147. He BM, Zhao SP, Peng ZY. Effects of cigarette smoking on HDL quantity and function: implications for atherosclerosis. *J Cell Biochem.* (2013) 114:2431–6. doi: 10.1002/jcb.24581

148. Kauss AR, Antunes M, de La Bourdonnaye G, Pouly S, Hankins M, Heremans A, et al. Smoking and apolipoprotein levels: a meta-analysis of published data. *Toxicology Reports.* (2022) 9:1150–71. doi: 10.1016/j.toxrep.2022.05.009

149. Njølstad I, Arnesen E, Lund-Larsen PG. Smoking, serum lipids, blood pressure, and sex differences in myocardial infarction. A 12-year follow-up of the Finnmark Study. *Circulation.* (1996) 93:450–6. doi: 10.1161/01.CIR.93.3.450

150. Bolego C, Poli A, Paoletti R. Smoking and gender. *Cardiovasc Res.* (2002) 53:568–76. doi: 10.1016/S0008-6363(01)00520-X

151. Baron JA, La Vecchia C, Levi F. The antiestrogenic effect of cigarette smoking in women. *Am J Obstet Gynecol.* (1990) 162:502–14. doi: 10.1016/0002-9378(90)90420-C

152. Le-Ha C, Beilin LJ, Burrows S, Huang R-C, Oddy WH, Hands B, et al. Gender difference in the relationship between passive smoking exposure and HDL-cholesterol levels in late adolescence. *J Clin Endocr.* (2013) 98:2126–35. doi: 10.1210/jc.2013-1016

153. Criqui MH, Wallace RB, Heiss G, Mishkel M, Schonfeld G, Jones GT. Cigarette smoking and plasma high-density lipoprotein cholesterol. The lipid research clinics program prevalence study. *Circulation.* (1980) 62:Iv70–6.

154. Forey BA, Fry JS, Lee PN, Thornton AJ, Coombs KJ. The effect of quitting smoking on HDL-cholesterol - a review based on within-subject changes. *Biomark Res.* (2013) 1:26. doi: 10.1186/2050-7771-1-26

155. Nyboe J, Jensen G, Appleyard M, Schnohr P. Smoking and the risk of first acute myocardial infarction. *Am Heart J.* (1991) 122:438–47. doi: 10.1016/0002-8703(91)90997-V

156. Shao Y, Luo L, Ren Z, Guo J, Xiao X, Huang J, et al. Smoking-induced inhibition of number and activity of endothelial progenitor cells and nitric oxide in males were reversed by estradiol in premenopausal females. *Cardiol Res Pract.* (2020) 2020:9352518. doi: 10.1155/2020/9352518

157. Piano MR, Thur LA, Hwang CL, Phillips SA. Effects of alcohol on the cardiovascular system in women. *Alcohol Res.* (2020) 40:12. doi: 10.35946/arcr.v40.2.12

158. Marques-Vidal P, Bochud M, Paccaud F, Waterworth D, Bergmann S, Preisig M, et al. No interaction between alcohol consumption and HDL-related genes on HDL cholesterol levels. *Atherosclerosis.* (2010) 211:551–7. doi: 10.1016/j.atherosclerosis.2010.04.001

159. Fuchs FD, Chambless LE, Folsom AR, Eigenbrodt ML, Duncan BB, Gilbert A, et al. Association between alcoholic beverage consumption and incidence of coronary heart disease in whites and blacks: the Atherosclerosis Risk in Communities Study. *Am J Epidemiol.* (2004) 160:466–74. doi: 10.1093/aje/kwh229

160. Volcik K, Ballantyne CM, Pownall HJ, Sharrett AR, Boerwinkle E. Interaction effects of high-density lipoprotein metabolism gene variation and alcohol consumption on coronary heart disease risk: the atherosclerosis risk in communities study. *J Stud Alcohol Drugs.* (2007) 68:485–92. doi: 10.15288/jsad.2007.68.485

161. Hata Y, Nakajima K. Life-style and serum lipids and lipoproteins. *J Atheroscler Thromb.* (2000) 7:177–97. doi: 10.5551/jat1994.7.177

162. Hannuksela M, Marcel YL, Kesäniemi YA, Savolainen MJ. Reduction in the concentration and activity of plasma cholesteryl ester transfer protein by alcohol. *J Lipid Res.* (1992) 33:737–44. doi: 10.1016/S0022-2275(20)41437-3

163. Williams PT. Quantile-dependent expressivity and gene-lifestyle interactions involving high-density lipoprotein cholesterol. *Lifestyle Genom.* (2021) 14:1–19. doi: 10.1159/000511421

164. Hines LM, Hunter DJ, Stampfer MJ, Spiegelman D, Chu NF, Rifai N, et al. Alcohol consumption and high-density lipoprotein levels: the effect of ADH1C genotype, gender and menopausal status. *Atherosclerosis.* (2005) 182:293–300. doi: 10.1016/j.atherosclerosis.2005.02.005

165. Hines LM, Stampfer MJ, Ma J, Gaziano JM, Ridker PM, Hankinson SE, et al. Genetic variation in alcohol dehydrogenase and the beneficial effect of moderate alcohol consumption on myocardial infarction. *N Engl J Med.* (2001) 344:549–55. doi: 10.1056/NEJM200102223440802

166. Greaves L, Poole N, Brabete AC. Sex, gender, and alcohol use: implications for women and low-risk drinking guidelines. *Int J Environ Res Public Health.* (2022) 19:4523. doi: 10.3390/ijerph19084523

167. Williams GD, Grant BF, Harford TC, Noble J. Population projections using DSM-III criteria: alcohol abuse and dependence, 190-2000. *Alcohol Res Health.* (1989) 1989:366.

168. Zheng YL, Lian F, Shi Q, Zhang C, Chen YW, Zhou YH, et al. Alcohol intake and associated risk of major cardiovascular outcomes in women compared with men: a systematic review and meta-analysis of prospective observational studies. *BMC Public Health.* (2015) 15:773. doi: 10.1186/s12889-015-2081-y

169. Rajendran NK, Liu W, Chu CC, Cahill PA, Redmond EM. Moderate dose alcohol protects against serum amyloid protein A1-induced endothelial dysfunction via both notch-dependent and notch-independent pathways. *Alcohol Clin Exp Res.* (2021) 45:2217–30. doi: 10.1111/acer.14706

170. Badía E, Sacanella E, Fernández-Solá J, Nicolás JM, Antúnez E, Rotilio D, et al. Decreased tumor necrosis factor-induced adhesion of human monocytes to endothelial cells after moderate alcohol consumption. *Am J Clin Nutr.* (2004) 80:225–30. doi: 10.1093/ajcn/80.1.225

171. Tanaka A, Cui R, Kitamura A, Liu K, Imano H, Yamagishi K, et al. Heavy alcohol consumption is associated with impaired endothelial function. *J Atheroscler Thromb.* (2016) 23:1047–54. doi: 10.5551/jat.31641

172. Sacanella E, Estruch R, Badía E, Fernández-Sola J, Nicolás JM, Urbano-Márquez A. Chronic alcohol consumption increases serum levels of circulating endothelial cell/leucocyte adhesion molecules E-selectin and ICAM-1. *Alcohol Alcohol.* (1999) 34:678–84. doi: 10.1093/alcalc/34.5.678

173. Bédard A, Riverin M, Dodin S, Corneau L, Lemieux S. Sex differences in the impact of the Mediterranean diet on cardiovascular risk profile. *Br J Nutr.* (2012) 108:1428–34. doi: 10.1017/S0007114511006969

174. Bédard A, Corneau L, Lamarche B, Dodin S, Lemieux S. Sex differences in the impact of the mediterranean diet on LDL particle size distribution and oxidation. *Nutrients.* (2015) 7:3705–23. doi: 10.3390/nu7053705

175. Turner KM, Keogh JB, Clifton PM. Red meat, dairy, and insulin sensitivity: a randomized crossover intervention study. *Am J Clin Nutr.* (2015) 101:1173–9. doi: 10.3945/ajcn.114.104976

176. Brennan L, Gibbons H. Sex matters: a focus on the impact of biological sex on metabolomic profiles and dietary interventions. *Proc Nutr Soc.* (2020) 79:205–9. doi: 10.1017/S002966511900106X

177. Zhang Y, Klein K, Sugathan A, Nassery N, Dombkowski A, Zanger UM, et al. Transcriptional profiling of human liver identifies sex-biased genes associated with polygenic dyslipidemia and coronary artery disease. *PLoS ONE.* (2011) 6:e23506. doi: 10.1371/journal.pone.0023506

178. Darabi M, Rabbani M, Ani M, Zarean E, Panjehpour M, Movahedian A. Increased leukocyte ABCA1 gene expression in post-menopausal women on hormone replacement therapy. *Gynecol Endocrinol.* (2011) 27:701–5. doi: 10.3109/09513590.2010.507826

179. Shinohata R, Shibakura M, Arao Y, Watanabe S, Hirohata S, Usui S, et al. high-fat/high-cholesterol diet, but not high-cholesterol alone, increases free cholesterol and apoE-rich HDL serum levels in rats and upregulates hepatic ABCA1 expression. *Biochimie.* (2022) 197:49–58. doi: 10.1016/j.biochi.2022.01.011

180. Mauerer R, Ebert S, Langmann T. High glucose, unsaturated and saturated fatty acids differentially regulate expression of ATP-binding cassette transporters ABCA1 and ABCG1 in human macrophages. *Exp Mol Med.* (2009) 41:126–32. doi: 10.3858/emm.2009.41.2.015

181. Singaraja RR, James ER, Crim J, Visscher H, Chatterjee A, Hayden MR. Alternate transcripts expressed in response to diet reflect tissue-specific regulation of ABCA1. *J Lipid Res.* (2005) 46:2061–71. doi: 10.1194/jlr.M500133-JLR200

182. Villarreal-Molina MT, Aguilar-Salinas CA, Rodríguez-Cruz M, Riaño D, Villalobos-Comparan M, Coral-Vazquez R, et al. The ATP-binding cassette transporter A1 R230C variant affects HDL cholesterol levels and BMI in the Mexican population: association with obesity and obesity-related comorbidities. *Diabetes.* (2007) 56:1881–7. doi: 10.2337/db06-0905

183. Jacobo-Albavera L, Posadas-Romero C, Vargas-Alarcón G, Romero-Hidalgo S, Posadas-Sánchez R, González-Salazar Mdel C, et al. Dietary fat and carbohydrate modulate the effect of the ATP-binding cassette A1 (ABCA1) R230C variant on metabolic risk parameters in premenopausal women from the Genetics of Atherosclerotic Disease (GEA) Study. *Nutr Metab (Lond).* (2015) 12:45. doi: 10.1186/s12986-015-0040-3

184. Schoch L, Sutelman P, Suades R, Casani L, Padro T, Badimon L, et al. Hypercholesterolemia-induced HDL dysfunction can be reversed: the impact of diet and statin treatment in a preclinical animal model. *Int J Mol Sci.* (2022) 23:8596. doi: 10.3390/ijms23158596

185. Vogel RA, Corretti MC, Plotnick GD. Effect of a single high-fat meal on endothelial function in healthy subjects. *Am J Cardiol.* (1997) 79:350–4. doi: 10.1016/S0002-9149(96)00760-6

186. Ong PJ, Dean TS, Hayward CS, Della Monica PL, Sanders TA, Collins P. Effect of fat and carbohydrate consumption on endothelial function. *Lancet.* (1999) 354:2134. doi: 10.1016/S0140-6736(99)03374-7

187. Pérez-Jiménez F, Castro P, López-Miranda J, Paz-Rojas E, Blanco A, López-Segura F, et al. Circulating levels of endothelial function are modulated by dietary monounsaturated fat. *Atherosclerosis.* (1999) 145:351–8. doi: 10.1016/S0021-9150(99)00116-1

188. Fuentes F, López-Miranda J, Sánchez E, Sánchez F, Paez J, Paz-Rojas E, et al. Mediterranean and low-fat diets improve endothelial function in hypercholesterolemic men. *Ann Intern Med.* (2001) 134:1115–9. doi: 10.7326/0003-4819-134-12-200106190-00011

189. Cuevas AM, Germain AM. Diet and endothelial function. *Biol Res.* (2004) 37:225–30. doi: 10.4067/S0716-97602004000200008

190. Mata P, Alonso R, Lopez-Farre A, Ordovas JM, Lahoz C, Garces C, et al. Effect of dietary fat saturation on LDL oxidation and monocyte adhesion to human endothelial cells in vitro. *Arterioscler Thromb Vasc Biol.* (1996) 16:1347–55. doi: 10.1161/01.ATV.16.11.1347

191. Shah R, Makarem N, Emin M, Liao M, Jelic S, Aggarwal B. Mediterranean diet components are linked to greater endothelial function and lower inflammation in a pilot study of ethnically diverse women. *Nutr Res.* (2020) 75:77–84. doi: 10.1016/j.nutres.2020.01.004

192. Yusuf S, Joseph P, Rangarajan S, Islam S, Mente A, Hystad P, et al. Modifiable risk factors, cardiovascular disease, and mortality in 155 722 individuals from 21 high-income, middle-income, and low-income countries (PURE): a prospective cohort study. *Lancet.* (2020) 395:795–808. doi: 10.1016/S0140-6736(19)32008-2

193. Gambardella J, Morelli MB, Wang XJ, Santulli G. Pathophysiological mechanisms underlying the beneficial effects of physical activity in hypertension. *J Clin Hypertens (Greenwich).* (2020) 22:291–5. doi: 10.1111/jch.13804

194. Alevizos A, Lentzas J, Kokkoris S, Mariolis A, Korantzopoulos P. Physical activity and stroke risk. *Int J Clin Pract.* (2005) 59:922–30. doi: 10.1111/j.1742-1241.2005.00536.x

195. Madsen TE, Samaei M, Pikula A, Yu AYX, Carcel C, Millsaps E, et al. Sex differences in physical activity and incident stroke: a systematic review. *Clin Ther.* (2022) 44:586–611. doi: 10.1016/j.clinthera.2022.02.006

196. Kiely DK, Wolf PA, Cupples LA, Beiser AS, Kannel WB. Physical activity and stroke risk: the Framingham Study. *Am J Epidemiol.* (1994) 140:608–20. doi: 10.1093/oxfordjournals.aje.a117298

197. Superko HR, Haskell WH. The role of exercise training in the therapy of hyperlipoproteinemia. *Cardiol Clin.* (1987) 5:285–310. doi: 10.1016/S0733-8651(18)30552-6

198. Kokkinos PF, Fernhall B. Physical activity and high density lipoprotein cholesterol levels: what is the relationship? *Sports Med.* (1999) 28:307–14. doi: 10.2165/00007256-199928050-00002

199. Drygas W, Jegler A, Kunski H. Study on threshold dose of physical activity in coronary heart disease prevention. Part I relationship between leisure time physical activity and coronary risk factors. *Int J Sports Med.* (1988) 9:275–8. doi: 10.1055/s-2007-1025021

200. Kokkinos PF, Holland JC, Narayan P, Colleran JA, Dotson CO, Papademetriou V. Miles run per week and high-density lipoprotein cholesterol levels in healthy, middle-aged men. A dose-response relationship. *Arch Intern Med.* (1995) 155:415–20. doi: 10.1001/archinte.1995.00430040091011

201. Durstine JL, Haskell WL. Effects of exercise training on plasma lipids and lipoproteins. *Exerc Sport Sci Rev.* (1994) 22:477–521. doi: 10.1249/00003677-199401000-00017

202. Rohrer L, Ohnsorg Pascale M, Lehner M, Landolt F, Rinninger F, von Eckardstein A. High-density lipoprotein transport through aortic endothelial cells involves scavenger receptor BI and ATP-binding cassette transporter G1. *Circ Res.* (2009) 104:1142–50. doi: 10.1161/CIRCRESAHA.108.190587

203. Hassan HH, Denis M, Krimbou L, Marcil M, Genest J. Cellular cholesterol homeostasis in vascular endothelial cells. *Can J Cardiol.* (2006) 22 (Suppl B):35b—40b. doi: 10.1016/S0828-282X(06)70985-0

204. O'Connell Brian J, Denis M, Genest J. Cellular physiology of cholesterol efflux in vascular endothelial cells. *Circulation.* (2004) 110:2881–8. doi: 10.1161/01.CIR.0000146333.20727.2B

205. Baumer Y, McCurdy S, Weatherby TM, Mehta NN, Halbherr S, Halbherr P, et al. Hyperlipidemia-induced cholesterol crystal production by endothelial cells promotes atherogenesis. *Nat Commun.* (2017) 8:1129. doi: 10.1038/s41467-017-01186-z

206. Catalano G, Duchene E, Julia Z, Le Goff W, Bruckert E, Chapman MJ, et al. Cellular SR-BI and ABCA1-mediated cholesterol efflux are gender-specific in healthy subjects. *J Lipid Res.* (2008) 49:635–43. doi: 10.1194/jlr.M700510-JLR200

207. Villard EF. P EIK, Frisdal E, Bruckert E, Clement K, Bonnefont-Rousselot D, et al. Genetic determination of plasma cholesterol efflux capacity is gender-specific and independent of HDL-cholesterol levels. *Arterioscler Thromb Vasc Biol.* (2013) 33:822–8. doi: 10.1161/ATVBAHA.112.300979

208. Ma F, Darabi M, Lhomme M, Tubeuf E, Canicio A, Brerault J, et al. Phospholipid transfer to high-density lipoprotein (HDL) upon triglyceride lipolysis is directly correlated with HDL-cholesterol levels and is not associated with cardiovascular risk. *Atherosclerosis.* (2021) 324:1–8. doi: 10.1016/j.atherosclerosis.2021.03.002

209. El Khoudary SR, Hutchins PM, Matthews KA, Brooks MM, Orchard TJ, Ronsein GE, et al. Cholesterol efflux capacity and subclasses of HDL particles in healthy women transitioning through menopause. *J Clin Endocrinol Metab.* (2016) 101:3419–28. doi: 10.1210/jc.2016-2144

210. Rubinow KB, Vaisar T, Chao JH, Heinecke JW, Page ST. Sex steroids mediate discrete effects on HDL cholesterol efflux capacity and particle concentration in healthy men. *J Clin Lipidol.* (2018) 12:1072–82. doi: 10.1016/j.jacl.2018.04.013

211. Holmboe SA, Vradi E, Jensen TK, Linneberg A, Husemoen LL, Scheike T, et al. The association of reproductive hormone levels and all-cause, cancer, and cardiovascular disease mortality in men. *J Clin Endocrinol Metab.* (2015) 100:4472–80. doi: 10.1210/jc.2015-2460

212. Bernini G, Versari D, Moretti A, Virdis A, Ghiadoni L, Bardini M, et al. Vascular reactivity in congenital hypogonadal men before and after testosterone replacement therapy. *J Clin Endocrinol Metab.* (2006) 91:1691–7. doi: 10.1210/jc.2005-1398

213. Stanhewicz AE, Wenner MM, Stachenfeld NS. Sex differences in endothelial function important to vascular health and overall cardiovascular disease risk across the lifespan. *Am J Physiol Heart Circ Physiol.* (2018) 315:H1569–h88. doi: 10.1152/ajpheart.00396.2018

214. Bolick David T, Srinivasan S, Kim Kyu W, Hatley Melissa E, Clemens Jeremy J, Whetzel A, et al. Sphingosine-1-phosphate prevents tumor necrosis factor-α-mediated monocyte adhesion to aortic endothelium in mice. *Arterioscler Thromb Vasc Biol.* (2005) 25:976–81. doi: 10.1161/01.ATV.0000162171.30089.f6

215. Ross R. The pathogenesis of atherosclerosis: a perspective for the (1990s). *Nature.* (1993) 362:801–9. doi: 10.1038/362801a0

216. Navab M, Berliner Judith A, Subbanagounder G, Hama S, Lusis Aldons J, Castellani Lawrence W, et al. HDL and the inflammatory response induced by LDL-derived oxidized phospholipids. *Arterioscler Thromb Vasc Biol.* (2001) 21:481–8. doi: 10.1161/01.ATV.21.4.481

217. Mackness B, Durrington P, McElduff P, Yarnell J, Azam N, Watt M, et al. Low paraoxonase activity predicts coronary events in the Caerphilly Prospective Study. *Circulation.* (2003) 107:2775–9. doi: 10.1161/01.CIR.0000070954.00271.13

218. Mackness B, Hine D, Liu Y, Mastorikou M, Mackness M. Paraoxonase-1 inhibits oxidised LDL-induced MCP-1 production by endothelial cells. *Biochem Biophys Res Commun.* (2004) 318:680–3. doi: 10.1016/j.bbrc.2004.04.056

219. Gharavi NM, Gargalovic PS, Chang I, Araujo JA, Clark MJ, Szeto WL. et al. High-density lipoprotein modulates oxidized phospholipid signaling in human endothelial cells from proinflammatory to anti-inflammatory. *Arterioscler Thromb Vasc Biol.* (2007) 27:1346–53. doi: 10.1161/ATVBAHA.107.141283

220. Nicholls SJ, Dusting GJ, Cutri B, Bao S, Drummond GR, Rye K-A, et al. Reconstituted high-density lipoproteins inhibit the acute

pro-oxidant and proinflammatory vascular changes induced by a periarterial collar in normocholesterolemic rabbits. *Circulation*. (2005) 111:1543–50. doi: 10.1161/01.CIR.0000159351.95399.50

221. Vickers KC, Palmisano BT, Shoucri BM, Shamburek RD, Remaley AT. MicroRNAs are transported in plasma and delivered to recipient cells by high-density lipoproteins. *Nat Cell Biol.* (2011) 13:423–33. doi: 10.1038/ncb2210

222. Michell DL, Vickers KC. Lipoprotein carriers of microRNAs. *Biochimica et biophysica acta*. (2016) 1861:2069–74. doi: 10.1016/j.bbalip.2016.01.011

223. Cockerill Gillian W, Rye K-A, Gamble Jennifer R, Vadas Mathew A, Barter Philip J. High-density lipoproteins inhibit cytokine-induced expression of endothelial cell adhesion molecules. *Arterioscler Thromb Vasc Biol.* (1995) 15:1987–94. doi: 10.1161/01.ATV.15.11.1987

224. Tabet F, Vickers KC, Cuesta Torres LF, Wiese CB, Shoucri BM, Lambert G, et al. HDL-transferred microRNA-223 regulates ICAM-1 expression in endothelial cells. *Nat Commun.* (2014) 5:3292. doi: 10.1038/ncomms4292

225. Zhang Q-H, Zu X-Y, Cao R-X, Liu J-H, Mo Z-C, Zeng Y, et al. An involvement of SR-B1 mediated PI3K–Akt–eNOS signaling in HDL-induced cyclooxygenase 2 expression and prostacyclin production in endothelial cells. *Biochem Biophys Res Commun.* (2012) 420:17–23. doi: 10.1016/j.bbrc.2012.02.103

226. Dorris SL, Peebles RS. PGI2 as a regulator of inflammatory diseases. *Mediators Inflamm*. (2012) 2012:926968. doi: 10.1155/2012/926968

227. Madsen CM, Varbo A, Tybjærg-Hansen A, Frikke-Schmidt R, Nordestgaard BG. U-shaped relationship of HDL and risk of infectious disease: two prospective population-based cohort studies. *Eur Heart J.* (2017) 39:1181–90. doi: 10.1093/eurheartj/ehx665

228. Catapano AL, Pirillo A, Bonacina F, Norata GD, HDL. in innate and adaptive immunity. *Cardiovasc Res.* (2014) 103:372–83. doi: 10.1093/cvr/cvu150

229. Shor R, Wainstein J, Oz D, Boaz M, Matas Z, Fux A, et al. Low HDL levels and the risk of death, sepsis and malignancy. *Clin Res Cardiol.* (2008) 97:227–33. doi: 10.1007/s00392-007-0611-z

230. Andersen RV, Wittrup HH, Tybjaerg-Hansen A, Steffensen R, Schnohr P, Nordestgaard BG. Hepatic lipase mutations,elevated high-density lipoprotein cholesterol, and increased risk of ischemic heart disease: the Copenhagen City Heart Study. *J Am Coll Cardiol.* (2003) 41:1972–82. doi: 10.1016/S0735-1097(03)00407-8

231. Feitosa MF, Myers RH, Pankow JS, Province MA, Borecki IB, LIPC. variants in the promoter and intron 1 modify HDL-C levels in a sex-specific fashion. *Atherosclerosis.* (2009) 204:171–7. doi: 10.1016/j.atherosclerosis.2008.09.007

232. Wu DF, Lin D, Lu F, Liao QC, Wu YJ, Wang Z, et al. Sex-specific influence of the SCARB1 Rs5888 SNP on the serum lipid response to atorvastatin in patients with acute coronary syndrome undergoing percutaneous coronary intervention. *Pharmgenomics Pers Med.* (2020) 13:553–61. doi: 10.2147/PGPM.S273346

233. Taleb-Belkadi O, Chaib H, Zemour L, Fatah A, Chafi B, Mekki K. Lipid profile, inflammation, and oxidative status in peri- and postmenopausal women. *Gynecol Endocrinol.* (2016) 32:982–5. doi: 10.1080/09513590.2016.1214257

234. Feingold KR, Grunfeld C. Effect of inflammation on HDL structure and function. *Curr Opin Lipidol.* (2016) 27:521–30. doi: 10.1097/MOL.0000000000000333

235. Chung CP, Oeser A, Raggi P, Sokka T, Pincus T, Solus JF, et al. Lipoprotein subclasses determined by nuclear magnetic resonance spectroscopy and coronary atherosclerosis in patients with rheumatoid arthritis. *J Rheumatol.* (2010) 37:1633–8. doi: 10.3899/jrheum.090639

236. Schulte DM, Paulsen K, Türk K, Brandt B, Freitag-Wolf S, Hagen I, et al. Small dense LDL cholesterol in human subjects with different chronic inflammatory diseases. *Nutr Metab Cardiovasc Dis.* (2018) 28:1100–5. doi: 10.1016/j.numecd.2018.06.022

237. Charakida M, Besler C, Batuca JR, Sangle S, Marques S, Sousa M, et al. Vascular abnormalities, paraoxonase activity, and dysfunctional HDL in primary antiphospholipid syndrome. *JAMA.* (2009) 302:1210–7. doi: 10.1001/jama.2009.1346

238. Mostaza JM, Salinero-Fort MA, Cardenas-Valladolid J, Rodriguez-Artalejo F, Díaz-Almiron M, Vich-Pérez P, et al. Pre-infection HDL-cholesterol levels and mortality among elderly patients infected with SARS-CoV-2. *Atherosclerosis.* (2022) 341:13–9. doi: 10.1016/j.atherosclerosis.2021.12.009

239. Hilser JR, Han Y, Biswas S, Gukasyan J, Cai Z, Zhu R, et al. Association of serum HDL-cholesterol and apolipoprotein A1 levels with risk of severe SARS-CoV-2 infection. *J Lipid Res.* (2021) 62:100061. doi: 10.1016/j.jlr.2021.100061

240. Peng F, Lei S, Zhang Q, Zhong Y, Wu S. Triglyceride/high-density lipoprotein cholesterol ratio is associated with the mortality of COVID-19: a retrospective study in China. *Int J Gen Med.* (2022) 15:985–96. doi: 10.2147/IJGM.S346690

241. Souza Junior DR, Silva ARM, Rosa-Fernandes L, Reis LR, Alexandria G, Bhosale SD, et al. HDL proteome remodeling associates with COVID-19 severity. *J Clin Lipidol.* (2021) 15:796–804. doi: 10.1016/j.jacl.2021.10.005

242. Tanaka S, Begue F, Veeren B, Tran-Dinh A, Robert T, Tashk P, et al. First recombinant high-density lipoprotein particles administration in a severe ICU COVID-19 patient, a multi-omics exploratory investigation. *Biomedicines*. (2022) 10:754. doi: 10.3390/biomedicines10040754

243. Sha J, Qie G, Yao Q, Sun W, Wang C, Zhang Z, et al. Sex differences on clinical characteristics, severity, and mortality in adult patients with COVID-19: a multicentre retrospective study. *Front Med (Lausanne).* (2021) 8:607059. doi: 10.3389/fmed.2021.607059

244. Gebhard C, Regitz-Zagrosek V, Neuhauser HK, Morgan R, Klein SL. Impact of sex and gender on COVID-19 outcomes in Europe. *Biol Sex Differ.* (2020) 11:29. doi: 10.1186/s13293-020-00304-9

245. Kumar N, Zuo Y, Yalavarthi S, Hunker KL, Knight JS, Kanthi Y, et al. SARS-CoV-2 spike protein S1-mediated endothelial injury and pro-inflammatory state is amplified by dihydrotestosterone and prevented by mineralocorticoid antagonism. *Viruses*. (2021) 13:2209. doi: 10.3390/v13112209

246. Gebhard CE, Hamouda N, Gebert P, Regitz-Zagrosek V, Gebhard C, Bengs S, et al. Sex versus gender-related characteristics: which predicts clinical outcomes of acute COVID-19? *Intensive Care Med.* (2022). doi: 10.1007/s00134-022-06836-5

247. Mallat Z, Tedgui A. Apoptosis in the vasculature: mechanisms and functional importance. *Br J Pharmacol.* (2000) 130:947–62. doi: 10.1038/sj.bjp.0703407

248. Winn RK, Harlan JM. The role of endothelial cell apoptosis in inflammatory and immune diseases. *J Thromb Haemost.* (2005) 3:1815–24. doi: 10.1111/j.1538-7836.2005.01378.x

249. Bombeli T, Schwartz BR, Harlan JM. Endothelial cells undergoing apoptosis become proadhesive for nonactivated platelets. *Blood.* (1999) 93:3831–8. doi: 10.1182/blood.V93.11.3831

250. Bombeli T, Karsan A, Tait JF, Harlan JM. Apoptotic vascular endothelial cells become procoagulant. *Blood.* (1997) 89:2429–42. doi: 10.1182/blood.V89.7.2429

251. Escargueil-Blanc I, Salvayre R, Nègre-Salvayre A. Necrosis and apoptosis induced by oxidized low density lipoproteins occur through two calcium-dependent pathways in lymphoblastoid cells. *FASEB J.* (1994) 8:1075–80. doi: 10.1096/fasebj.8.13.7926374

252. Hessler JR, Morel DW, Lewis LJ, Chisolm GM. Lipoprotein oxidation and lipoprotein-induced cytotoxicity. *Arteriosclerosis.* (1983) 3:215–22. doi: 10.1161/01.ATV.3.3.215

253. Escargueil-Blanc I, Meilhac O, Pieraggi MT, Arnal JF, Salvayre R, Nègre-Salvayre A. Oxidized LDLs induce massive apoptosis of cultured human endothelial cells through a calcium-dependent pathway. Prevention by aurintricarboxylic acid. *Arterioscler Thromb Vasc Biol.* (1997) 17:331–9. doi: 10.1161/01.ATV.17.2.331

254. Nofer JR, Levkau B, Wolinska I, Junker R, Fobker M, von Eckardstein A, et al. Suppression of endothelial cell apoptosis by high density lipoproteins (HDL) and HDL-associated lysosphingolipids. *J Biol Chem.* (2001) 276:34480–5. doi: 10.1074/jbc.M103782200

255. Walter M. Interrelationships among HDL metabolism, aging, and atherosclerosis. *Arterioscler Thromb Vasc Biol.* (2009) 29:1244–50. doi: 10.1161/ATVBAHA.108.181438

256. Nofer JR, Kehrel B, Fobker M, Levkau B, Assmann G, von Eckardstein A, et al. and arteriosclerosis: beyond reverse cholesterol transport. *Atherosclerosis.* (2002) 161:1–16. doi: 10.1016/S0021-9150(01)00651-7

257. Riwanto M, Rohrer L, Roschitzki B, Besler C, Mocharla P, Mueller M, et al. Altered activation of endothelial anti- and proapoptotic pathways by high-density lipoprotein from patients with coronary artery disease: role of high-density lipoprotein-proteome remodeling. *Circulation.* (2013) 127:891–904. doi: 10.1161/CIRCULATIONAHA.112.108753

258. Suc I, Escargueil-Blanc I, Troly M, Salvayre R, Nègre-Salvayre A, HDL. and ApoA prevent cell death of endothelial cells induced by oxidized LDL. *Arterioscler Thromb Vasc Biol.* (1997) 17:2158–66. doi: 10.1161/01.ATV.17.10.2158

259. de Souza JA, Vindis C, Nègre-Salvayre A, Rye KA, Couturier M, Therond P, et al. Small, dense HDL 3 particles attenuate apoptosis in endothelial cells: pivotal role of apolipoprotein A-I. *J Cell Mol Med.* (2010) 14:608–20. doi: 10.1111/j.1582-4934.2009.00713.x

260. Op den. Buijs J, Musters M, Verrips T, Post JA, Braam B, van Riel N. Mathematical modeling of vascular endothelial layer maintenance: the role of endothelial cell division, progenitor cell homing, and telomere shortening. *Am J Physiol Heart Circ Physiol.* (2004) 287:H2651–8. doi: 10.1152/ajpheart.00332.2004

261. Tso C, Martinic G, Fan WH, Rogers C, Rye KA, Barter PJ. High-density lipoproteins enhance progenitor-mediated endothelium repair in mice. *Arterioscler Thromb Vasc Biol.* (2006) 26:1144–9. doi: 10.1161/01.ATV.0000216600.37436.cf

262. Pu DR, Liu L, HDL. slowing down endothelial progenitor cells senescence: a novel anti-atherogenic property of HDL. *Med Hypotheses.* (2008) 70:338–42. doi: 10.1016/j.mehy.2007.05.025

263. Cervellati C, Vigna GB, Trentini A, Sanz JM, Zimetti F, Dalla Nora E, et al. Paraoxonase-1 activities in individuals with different HDL circulating levels: Implication in reverse cholesterol transport and early vascular damage. *Atherosclerosis.* (2019) 285:64–70. doi: 10.1016/j.atherosclerosis.2019.04.218

264. Furlong CE, Marsillach J, Jarvik GP, Costa LG. Paraoxonases-1,−2 and−3: what are their functions? *Chem Biol Interact.* (2016) 259:51–62. doi: 10.1016/j.cbi.2016.05.036

265. Rosenblat M, Karry R, Aviram M. Paraoxonase 1 (PON1) is a more potent antioxidant and stimulant of macrophage cholesterol efflux, when present in HDL than in lipoprotein-deficient serum: relevance to diabetes. *Atherosclerosis.* (2006) 187:74–81. doi: 10.1016/j.atherosclerosis.2005.08.026

266. Tavori H, Khatib S, Aviram M, Vaya J. Characterization of the PON1 active site using modeling simulation, in relation to PON1 lactonase activity. *Bioorg Med Chem.* (2008) 16:7504–9. doi: 10.1016/j.bmc.2008.06.008

267. Kelesidis T, Roberts CK, Huynh D, Martínez-Maza O, Currier JS, Reddy ST, et al. A high throughput biochemical fluorometric method for measuring lipid peroxidation in HDL. *PLoS ONE.* (2014) 9:e111716. doi: 10.1371/journal.pone.0111716

268. Farbstein D, Levy AP, HDL. dysfunction in diabetes: causes and possible treatments. *Expert Rev Cardiovasc Ther.* (2012) 10:353–61. doi: 10.1586/erc.11.182

269. Flaherty SM, Wood EK, Ryff CD, Love GD, Kelesidis T, Berkowitz L, et al. Race and sex differences in HDL peroxide content among American adults with and without type 2 diabetes. *Lipids Health Dis.* (2022) 21:18. doi: 10.1186/s12944-021-01608-4

270. Gomez Rosso L, Lhomme M, Meroño T, Dellepiane A, Sorroche P, Hedjazi L, et al. Poor glycemic control in type 2 diabetes enhances functional and compositional alterations of small, dense HDL3c. *Biochim Biophys Acta Mol Cell Biol Lipids.* (2017) 1862:188–95. doi: 10.1016/j.bbalip.2016.10.014

271. Chapman MJ, HDL. functionality in type 1 and type 2 diabetes: new insights. *Curr Opin Endocrinol Diabetes Obes.* (2022) 29:112–23. doi: 10.1097/MED.0000000000000705

272. Rosta V, Trentini A, Passaro A, Zuliani G, Sanz JM, Bosi C, et al. Sex difference impacts on the relationship between paraoxonase-1 (PON1) and type 2 diabetes. *Antioxidants (Basel).* (2020) 9:683. doi: 10.3390/antiox9080683

273. Garrido-Sánchez L, García-Fuentes E, Cardona F, Rojo-Martínez G, Soriguer F, Tinahones FJ. Anti-oxidized LDL antibody levels are reduced in women with hypertension. *Eur J Clin Invest.* (2009) 39:800–6. doi: 10.1111/j.1365-2362.2009.02156.x

274. Lapointe A, Couillard C, Piché ME, Weisnagel SJ, Bergeron J, Nadeau A, et al. Circulating oxidized LDL is associated with parameters of the metabolic syndrome in postmenopausal women. *Atherosclerosis.* (2007) 191:362–8. doi: 10.1016/j.atherosclerosis.2006.03.036

275. Chae JS, Kim OY, Paik JK, Kang R, Seo WJ, Jeong TS, et al. Association of Lp-PLA(2) activity and LDL size with interleukin-6, an inflammatory cytokine and oxidized LDL, a marker of oxidative stress, in women with metabolic syndrome. *Atherosclerosis.* (2011) 218:499–506. doi: 10.1016/j.atherosclerosis.2011.06.036

276. Tikkanen MJ, Vihma V, Jauhiainen M, Höckerstedt A, Helisten H, Kaamanen M. Lipoprotein-associated estrogens. *Cardiovasc Res.* (2002) 56:184–8. doi: 10.1016/S0008-6363(02)00535-7

277. Shwaery GT, Vita JA, Keaney JF. Antioxidant protection of LDL by physiologic concentrations of estrogens is specific for 17-beta-estradiol. *Atherosclerosis.* (1998) 138:255–62. doi: 10.1016/S0021-9150(98)00020-3

278. González F, Nair KS, Daniels JK, Basal E, Schimke JM, Blair HE. Hyperandrogenism sensitizes leukocytes to hyperglycemia to promote oxidative stress in lean reproductive-age women. *J Clin Endocrinol Metab.* (2012) 97:2836–43. doi: 10.1210/jc.2012-1259

279. Sun Y, Li S, Liu H, Bai H, Hu K, Zhang R, et al. Oxidative stress promotes hyperandrogenism by reducing sex hormone-binding globulin in polycystic ovary syndrome. *Fertil Steril.* (2021) 116:1641–50. doi: 10.1016/j.fertnstert.2021.07.1203

280. Li X-A, Titlow WB, Jackson BA, Giltiay N, Nikolova-Karakashian M, Uittenbogaard A, et al. High density lipoprotein binding to scavenger receptor, class b, type I activates endothelial nitric-oxide synthase in a ceramide-dependent manner*. *J Biol Chem.* (2002) 277:11058–63. doi: 10.1074/jbc.M110985200

281. Terasaka N, Westerterp M, Koetsveld J, Fernández-Hernando C, Yvan-Charvet L, Wang N, et al. ATP-binding cassette transporter G1 and high-density lipoprotein promote endothelial NO synthesis through a decrease in the interaction of caveolin-1 and endothelial NO synthase. *Arterioscler Thromb Vasc Biol.* (2010) 30:2219–25. doi: 10.1161/ATVBAHA.110.213215

282. Mineo C, Yuhanna IS, Quon MJ, Shaul PW. High density lipoprotein-induced endothelial nitric-oxide synthase activation is mediated by Akt and MAP Kinases*. *J Biol Chem.* (2003) 278:9142–9. doi: 10.1074/jbc.M211394200

283. Gong M, Wilson M, Kelly T, Su W, Dressman J, Kincer J, et al. HDL-associated estradiol stimulates endothelial NO synthase and vasodilation in an SR-BI-dependent manner. *J Clin Invest.* (2003) 111:1579–87. doi: 10.1172/JCI16777

284. Ruiz M, Okada H, Dahlbäck B. HDL-associated ApoM is anti-apoptotic by delivering sphingosine 1-phosphate to S1P1 & S1P3 receptors on vascular endothelium. *Lipids Health Dis.* (2017) 16:36. doi: 10.1186/s12944-017-0429-2

285. Christoffersen C, Obinata H, Kumaraswamy SB, Galvani S, Ahnström J, Sevvana M, et al. Endothelium-protective sphingosine-1-phosphate provided by HDL-associated apolipoprotein M. *Proc Nat Acad Sci.* (2011) 108:9613. doi: 10.1073/pnas.1103187108

286. Wilkerson BA, Grass GD, Wing SB, Argraves WS, Argraves KM. Sphingosine 1-phosphate (S1P) carrier-dependent regulation of endothelial barrier: high density lipoprotein (HDL)-s1p prolongs endothelial barrier enhancement as compared with albumin-s1p via effects on levels, trafficking, and signaling of S1p1*. *J Biol Chem.* (2012) 287:44645–53. doi: 10.1074/jbc.M112.423426

287. Memon AA, Bennet L, Zöller B, Wang X, Palmér K, Dahlbäck B, et al. The association between apolipoprotein M and insulin resistance varies with country of birth. *Nutr Metab Cardiovasc Dis.* (2014) 24:1174–80. doi: 10.1016/j.numecd.2014.05.007

288. Brinck JW, Thomas A, Lauer E, Jornayvaz FR, Brulhart-Meynet MC, Prost JC, et al. Diabetes mellitus is associated with reduced high-density lipoprotein sphingosine-1-phosphate content and impaired high-density lipoprotein cardiac cell protection. *Arterioscler Thromb Vasc Biol.* (2016) 36:817–24. doi: 10.1161/ATVBAHA.115.307049

289. Frej C, Mendez AJ, Ruiz M, Castillo M, Hughes TA, Dahlbäck B, et al. A Shift in ApoM/S1P between HDL-particles in women with type 1 diabetes mellitus is associated with impaired anti-inflammatory effects of the ApoM/S1P complex. *Arterioscler Thromb Vasc Biol.* (2017) 37:1194–205. doi: 10.1161/ATVBAHA.117.309275

290. Besler C, Heinrich K, Rohrer L, Doerries C, Riwanto M, Shih DM, et al. Mechanisms underlying adverse effects of HDL on eNOS-activating pathways in patients with coronary artery disease. *J Clin Invest.* (2011) 121:2693–708. doi: 10.1172/JCI42946

291. Denimal D, Monier S, Brindisi MC, Petit JM, Bouillet B, Nguyen A, et al. Impairment of the ability of HDL from patients with metabolic syndrome but without diabetes mellitus to activate eNOS: correction by S1P enrichment. *Arterioscler Thromb Vasc Biol.* (2017) 37:804–11. doi: 10.1161/ATVBAHA.117.309287

292. Gomaraschi M, Ossoli A, Favari E, Adorni MP, Sinagra G, Cattin L, et al. Inflammation impairs eNOS activation by HDL in patients with acute coronary syndrome. *Cardiovasc Res.* (2013) 100:36–43. doi: 10.1093/cvr/cvt169

293. Eren E, Yilmaz N, Aydin O. Functionally defective high-density lipoprotein and paraoxonase: a couple for endothelial dysfunction in atherosclerosis. *Cholesterol.* (2013) 2013:792090. doi: 10.1155/2013/792090

294. Shah K, McCormack CE, Bradbury NA. Do you know the sex of your cells? *Am J Physiol Cell Physiol.* (2014) 306:C3–18. doi: 10.1152/ajpcell.00281.2013

295. Taylor KE, Vallejo-Giraldo C, Schaible NS, Zakeri R, Miller VM. Reporting of sex as a variable in cardiovascular studies using cultured cells. *Biol Sex Differ.* (2011) 2:11. doi: 10.1186/2042-6410-2-11

296. Woitowich NC, Woodruff TK. Opinion: Research community needs to better appreciate the value of sex-based research. *Proc Natl Acad Sci U S A.* (2019) 116:7154–6. doi: 10.1073/pnas.1903586116

frontiers | Frontiers in Cardiovascular Medicine

TYPE Original Research
PUBLISHED 17 October 2022
DOI 10.3389/fcvm.2022.934914

OPEN ACCESS

EDITED BY
Chu-Huang Chen,
Texas Heart Institute, United States

REVIEWED BY
Zeneng Wang,
Cleveland Clinic, United States
Maria Giovanna Lupo,
Università degli Studi di Padova, Italy

*CORRESPONDENCE
Daoquan Peng
Pengdq@csu.edu.cn

†These authors have contributed equally to this work and share first authorship

SPECIALTY SECTION
This article was submitted to Lipids in Cardiovascular Disease, a section of the journal Frontiers in Cardiovascular Medicine

RECEIVED 03 May 2022
ACCEPTED 12 September 2022
PUBLISHED 17 October 2022

CITATION
Wang S, Fu D, Liu H and Peng D (2022) Independent association of PCSK9 with platelet reactivity in subjects without statin or antiplatelet agents. *Front. Cardiovasc. Med.* 9:934914. doi: 10.3389/fcvm.2022.934914

Independent association of PCSK9 with platelet reactivity in subjects without statin or antiplatelet agents

Shuai Wang†, Di Fu†, Huixing Liu and Daoquan Peng*

Department of Cardiovascular Medicine, Second Xiangya Hospital of Central South University, Changsha, China

Background and aims: Proprotein convertase subtilisin/kexin type 9 (PCSK9) levels could predict cardiovascular event in patients with well-controlled LDL-C levels, suggesting an LDL-independent mechanism of PCSK9 on the cardiovascular system. Accumulating evidence suggests PCSK9 might be associated with increased platelet reactivity. This study aimed to assess the relationship between PCSK9 levels and platelet reactivity in subjects not taking statins or antiplatelet agents.

Methods: A cross-sectional study was conducted to investigate the independent contribution of PCSK9 to platelet activity by controlling for the potential confounding factors. The study population included 89 subjects from a health examination centre who underwent routine annual health check-ups or had an examination before a selective operation. Subjects taking statins or antiplatelet agents were excluded. Adenosine diphosphate (ADP)-induced platelet aggregation was determined by PL-11 platelet analyzer using impedance aggregometry and plasma PCSK9 levels were determined using an ELISA. Serum Lipid profile was assessed by measuring the concentration of total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), and triglyceride (TG), with low-density lipoprotein cholesterol (LDL-C) being directly measured using enzymatic techniques. The association between PCSK9 and platelet reactivity was investigated.

Results: The study subjects were composed of 53 males and 36 females with an average age of 55 (±11) years old. The univariate correlation analysis showed significant correlation between ADP-induced maximal aggregation rate (MAR) and PCSK9 ($r = 0.55$, $p < 0.001$) as well as TC ($r = 0.23$, $p = 0.028$), LDL-C ($r = 0.27$, $p < 0.001$), and PLT ($r = 0.31$, $p = 0.005$). Being male (41.2% vs. 46.6, $p = 0.04$) and smoking (37.4 vs. 46.2%, $p = 0.016$) were associated with lower ADP-induced MAR than being female and non-smoking. However, there is no correlation between PCSK9 and AA-induced platelet maximal aggregation rate ($r = 0.17$, $p = 0.12$). Multiple regression analysis suggested that PCSK9 contributed independently to ADP-induced maximal aggregation rate ($\beta = 0.08$, $p = 0.004$) after controlling for the effect of TC, LDL-C, PLT, being male, and smoking.

10.3389/fcvm.2022.934914

Conclusions: PCSK9 is positively associated with platelet reactivity, which may partly account for the beneficial effect of PCSK9 inhibition in reducing the risk of major adverse cardiovascular events after acute coronary syndrome (ACS).

KEYWORDS

proprotein convertase subtilisin/kexin type 9, platelet aggregation, ADP, cross-sectional study, impedance aggregometry

Introduction

Atherosclerotic cardiovascular disease (CVD) is a major cause of disease burden. Platelets have an important role in coronary thrombosis pathogenesis and atherogenesis.

Studies have shown that platelet activity varies greatly among individuals. It could explain the variability in the risk for CVD (1–4). Prior clinical studies found an association between platelet activity and incident cardiovascular morbidity and mortality (5, 6). Hypercholesterolemia and its induced reactive oxygen species production can activate platelets (7–9). However, the molecules through which platelets become hyperactive remain not fully understood.

Proprotein Convertase Subtilisin/Kexin Type 9 (PCSK9), mainly synthesized by the liver, kidney, and small intestine, bind and inhibit low density lipoprotein receptor (LDLR) recircularization by promoting its degradation in the lysosomes and consequently increase low density lipoprotein (LDL) particles in the circulation (10). Regulation of cholesterol-rich LDL level is not the only role that PCSK9 has in atherosclerosis pathogenesis. In prospective cohort studies, plasma PCSK9 level was correlated with enhanced atherosclerosis progression and elevated probability of future cardiovascular events independently of LDL plasma levels, suggesting alternative roles for PCSK9 in the pathogenesis of atherosclerosis (11, 12).

A relationship between PCSK9 plasma levels and total number of circulating platelets has been reported in patients with stable coronary artery disease (13). A strong correlation between PCSK9 levels and platelet reactivity was also revealed in patients with recent acute coronary syndromes who underwent coronary intervention and received $P2Y_{12}$ inhibitors (14). However, statin use could increase PCSK9 levels and antiplatelet drugs could affect platelet activity in CAD patients (15, 16). In another study, human recombinant PCSK9 added to healthy human plasma significantly increased platelet aggregation when stimulated with epinephrine (17). But the concentration of human recombinant PCSK9 used in an *in-vitro* study was much higher than the physiological concentration in humans. Therefore, the naive correlation between plasma PCSK9 and platelet reactivity in subjects without lipid lowering or antiplatelet drugs is not known.

In the present study, we revealed a correlation between plasma PCSK9 and platelet reactivity when stimulated by agonist adenosine diphosphate (ADP) *in vitro* in healthy subjects without lipid-lowering or antiplatelet drugs. The ADP-induced maximal aggregation rate of platelets was 15.8% higher in patients with highest tertile PCSK9 value than the patients in the lowest tertile. Additionally, we found that PCSK9 was independently correlated to platelet activity after adjusting for low density lipoprotein cholesterol (LDL-C) and platelet (PLT) count. The results of this study provide another piece of evidence on the correlation between PCSK9 and platelet activity in healthy subjects.

Methods

Study population and design

This study is a cross-sectional, single center clinical study. Eighty-nine subjects who underwent routine annual health check-ups or had an examination before a selective operation were enrolled from a health examination center. Inclusion criteria were: 1) aged between 18 and 80 years and 2) obtained signed informed consent. The exclusion criteria were subjects with atherosclerotic cardiovascular disease (ASCVD), malignant tumor, renal dysfunction, liver dysfunction, thyroid disease, autoimmune disease, or coagulation disorders. Subjects who had lipid lowering drugs or antiplatelet drugs in the past 3 months before screening were excluded. Hypertension and diabetes were defined as being present when an individual self-reported a health professional's diagnosis and was using associated drugs. Written informed consent was obtained from each patient included in the study. The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki and the study protocol has been priorly approved by the ethics committee of Second Xiangya Hospital on research on humans.

Assessment of platelet reactivity

A blood sample was withdrawn after overnight fasting and analyzed for platelet reactivity within 2 h. Whole blood

aggregation was determined using PL-11 platelet analyzer (SINNOWA, Nanjing). The system detects the electrical impedance change due to the adhesion and aggregation of platelet on two independent electrode-set surfaces. Sodium citrate was used as an anticoagulant; adenosine diphosphate and arachidonic acid were used as agonists. A 1:9 dilution of whole blood anticoagulated with sodium citrate and 0.9% NaCl was stirred at 25°C. ADP 5 0μmol/L and arachidonic acid (AA) 2 mg/mL were added.

Assessment of PCSK9 serum levels

Blood samples were collected after overnight fasting and the samples were stored at −80°C until analysis. Plasma PCSK9 concentration was determined by a sandwich enzyme-linked immunosorbent assay (ELISA). Commercial PCSK9 (Quantikine ELISA, R&D systems Inc.) ELISA kits were used to quantify the concentrations of them.

Statistical analysis

For clinical data, continuous data are expressed as mean and standard deviation for normally distributed data or median and interquartile range for non-Gaussian data distribution. For comparison of variables between different groups of tertile PCSK9 values, one-way ANOVA test was used for normally distributed data, with LSD performed for multiple comparisons. Kruskal-Wallis test was used for non-Gaussian distributed data. The distribution of data was examined with the Kolmogorov-Smirnov test. Categorical variables are presented as percentages of subjects and were compared using Pearson X^2 or Fisher's exact tests, as appropriate. The correlation between PCSK9, other lipid parameters, PLT, and platelet activity was evaluated by Spearman's rank test. Continuous PCSK9 levels were categorized into tertiles of equal size to assess the association with ADP-induced maximal aggregation rate (MAR). Platelet reactivity above mean value was classified as higher. Multivariate linear regression was used to assess the association between PCSK9 levels and ADP-induced MAR. Unstandardized *B* and *p*-values were used to present results of the linear regression model. Adjusted R square and *p*-values were used to present for goodness of fit of the multivariate linear regression. All tests were two-sided; a $p < 0.05$ was considered statistically significant. Calculations were performed using SPSS version 26.0 (IBM Corporation, Chicago, USA).

Results

Study population

Between 2020 and 2021, 89 subjects, including 53 male and 36 female with an average age of 55 (±11) years, were recruited at Second Xiangya hospital. All subjects had not taken statins or antiplatelet agents before. Baseline clinical characteristics, comorbidities, and laboratory tests of participants according to tertile of PCSK9 are summarized in Supplementary Table 1.

Factors correlate with platelet reactivity

Correlation analysis including all characteristic parameters revealed a significant correlation of ADP-induced MAR with total cholesterol (TC) ($r = 0.23$, $p = 0.028$), LDL-C ($r = 0.27$, $p < 0.001$), and PLT ($r = 0.312$, $p = 0.005$). No significant correlation was found between ADP-induced MAR with age and other lipid parameters including plasma triglyceride (TG), high-density lipoprotein cholesterol (HDL-C), nonesterified fatty acid (NEFA), apolipoprotein A, [apo(A)], and apolipoprotein B [apo(B)] (Figure 1).

A significantly higher ADP-induced MAR was also observed in females compared to males (46.6 ± 11.1% vs. 41.2 ± 11.7%, $p = 0.039$) and in non-smoking subjects (46.2 ± 11.4% vs. 37.4 ± 11.4%, $p = 0.016$) in comparison to smokers. No significant difference in ADP-induced MAR was found in subjects with or without hypertension and diabetes (Figure 2).

Plasma total cholesterol, low density lipoprotein cholesterol, and platelet reactivity

In accordance with correlation analysis, a significantly higher ADP-induced MAR was observed in subjects with higher TC level (TC≥3.64 mmol/L:46.8 ± 10.3% vs. TC<3.64 mmol/L: 40.7 ± 12.1%, $p = 0.013$; Figure 3A). On the other hand, subjects with ADP-induced MAR higher than the mean value had significantly higher TC level (3.88 ± 0.90 mmol/L vs. 3.44 ± 0.72 mmol/L, $p = 0.013$; Figure 4A).

Although no significant difference of ADP-induced MAR was observed between tertile LDL-C groups or between high vs. low LDL-C groups according to mean value (Figure 3B), those who had higher platelet reactivity had significantly higher LDL-C levels (2.51 ± 0.72 mmol/L vs. 2.13 ± 0.57 mmol/L; Figure 4B).

Platelet count and platelet reactivity

In line with the correlation analysis, ADP-induced MAR was significantly higher in subjects with higher PLT (PLT≥220.6 × 10^{12}/L: 47.6 ± 12.3% vs. PLT<220.6 × 10^{12}/L: 39.4 ± 9.8%; Figure 3C). Vise versa, those who had higher platelet reactivity also had significantly higher PLT [ADP-induced MAR≥mean (43.7%): 248.4 ± 71.9 × 10^{12}/L vs. ADP-induced MAR<43.7%: 201.7 ± 93.0 × 10^{12}/L] (Figure 4C).

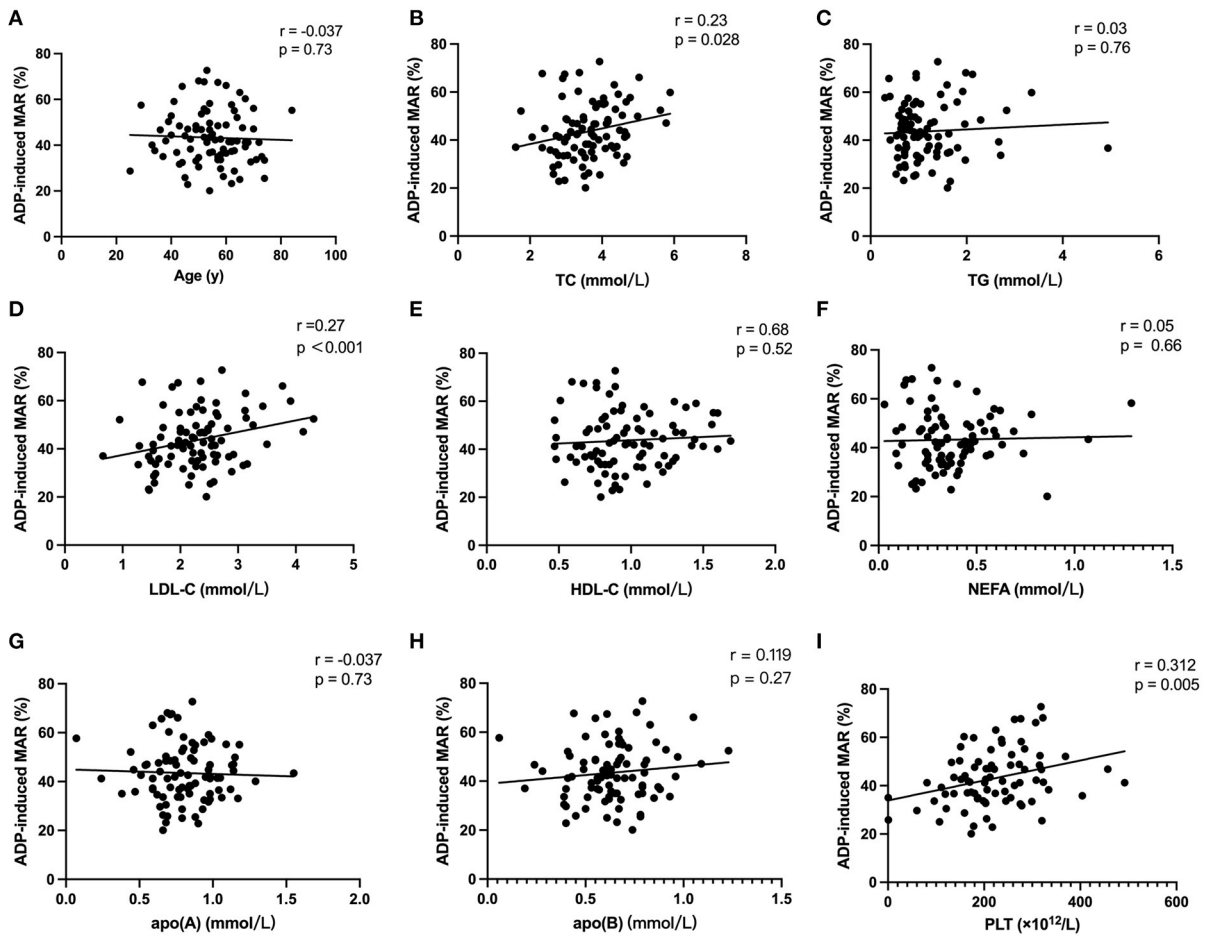


FIGURE 1
Correlation analysis. Univariate linear correlation analysis of subject characteristics including age **(A)**, TC **(B)**, TG **(C)**, LDL-C **(D)**, HDL-C **(E)**, NEFA **(F)**, apoA **(G)**, apoB **(H)**, and PLT **(I)** with ADP-induced platelet maximal aggregation rate (MAR).

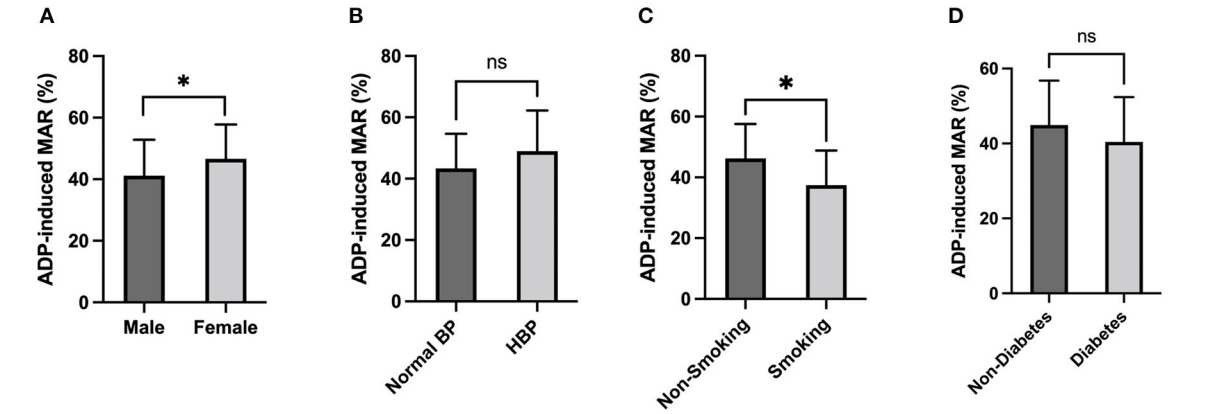


FIGURE 2
ADP-induced platelet aggregation stratified by Sex and Comorbidities. ADP-induced platelet maximal aggregation rate (MAR) in healthy subjects stratified by Sex **(A)**, Hypertension **(B)**, Smoking **(C)**, and Diabetes **(D)**. *: $P < 0.0332$, ns: $P > 0.1234$.

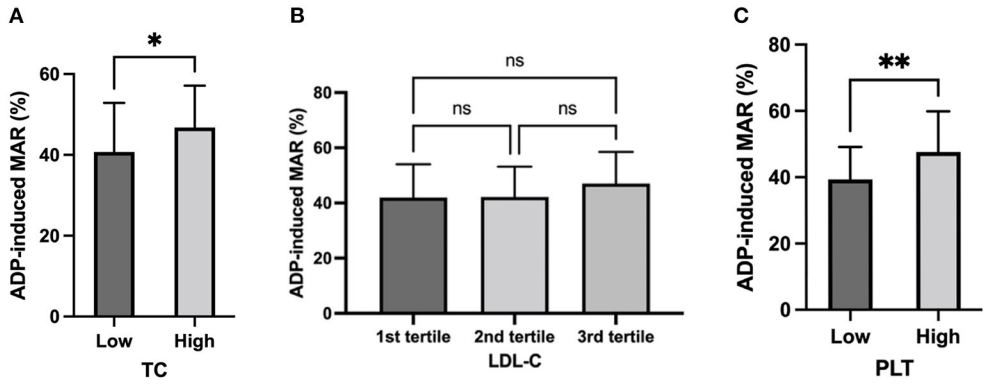


FIGURE 3
ADP-induced platelet aggregation stratified by TC, LDL-C, and PLT. ADP-induced platelet maximal aggregation rate (MAR) in healthy subjects stratified by TC **(A)**, LDL-C **(B)**, and PLT **(C)**. *: $P < 0.033$, **: $P < 0.002$, ns: $P > 0.1234$.

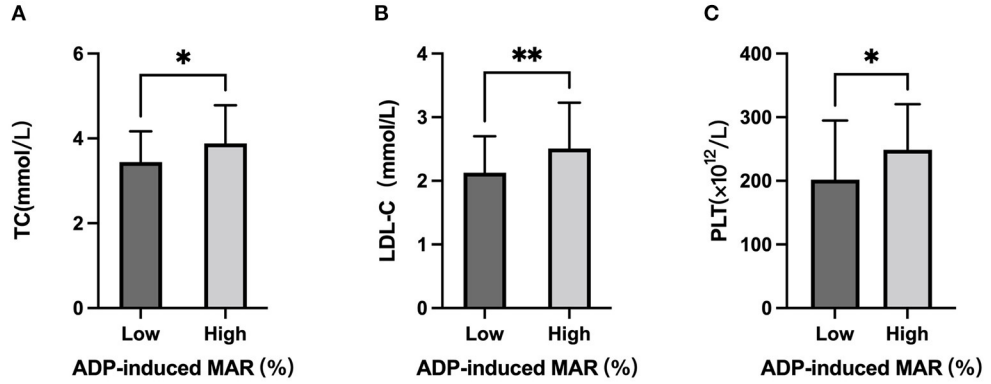


FIGURE 4
TC, LDL-C, and PLT stratified by low and high ADP-induced platelet aggregation rate. Comparison of **(A)** Serum TC level, **(B)** serum LDL-C level, and **(C)** PLT in subjects with ADP-induced maximal platelet aggregation rate (MAR) lower and higher than mean value. *: $P < 0.033$, **: $P < 0.002$, ns: $P > 0.1234$.

Plasma PCSK9 concentration and platelet reactivity

Analysis of the correlation between plasma PCSK9 concentration and baseline characteristics revealed that PCSK9 concentration was not correlated with age. Except for LDL-C ($r = 0.23$, $p = 0.03$), PCSK9 concentration was not significantly correlated with other lipid parameters. A higher plasma PCSK9 was observed in subjects with hypertension (246.1 ± 53.8 vs. 207.6 ± 57.0, $p = 0.03$) and in non-smoking subjects (221.8 ± 58.6 vs. 185.8 ± 43.3, $p = 0.043$). No significant difference in PCSK9 level was observed between females and males, nor in subjects with and without diabetes (Supplementary Figure 1).

A direct linear correlation was found between increased plasma PCSK9 levels and adenosine diphosphate (ADP)-induced maximal aggregation rate (MAR) ($r = 0.555$, $p<0.001$; Figure 5A). This correlation was also observed in both female and male subgroups despite difference in ADP-induced MAR between sex (Figures 5B,C).

The distribution of ADP-induced MAR between subgroups of subjects with different PCSK9 value exhibited a trend of increment when PCSK9 increase (Supplementary Figure 2A). Vice versa, the distribution of PCSK9 in subjects with higher ADP-induced MAR above the mean value compared to subjects with low ADP-induced MAR showed a correlation with high PCSK9 level (Supplementary Figure 2B).

When assessed according to tertile values of PCSK9, there was a significant increase in ADP-induced MAR in the 2nd-tertile compared to 1st-tertile (43.5 ± 11.7% vs. 35.0 ± 7.6%; $p<0.001$). In addition, an increase of ADP-induced MAR was observed in the 3rd-tertile compared to 2nd-tertile (48.7 ± 9.3% vs. 43.5 ± 11.7%; $p = 0.035$; Figure 5D). On the other hand, subjects with high ADP-induced MAR had significantly higher plasma level of PCSK9 (Figure 5E).

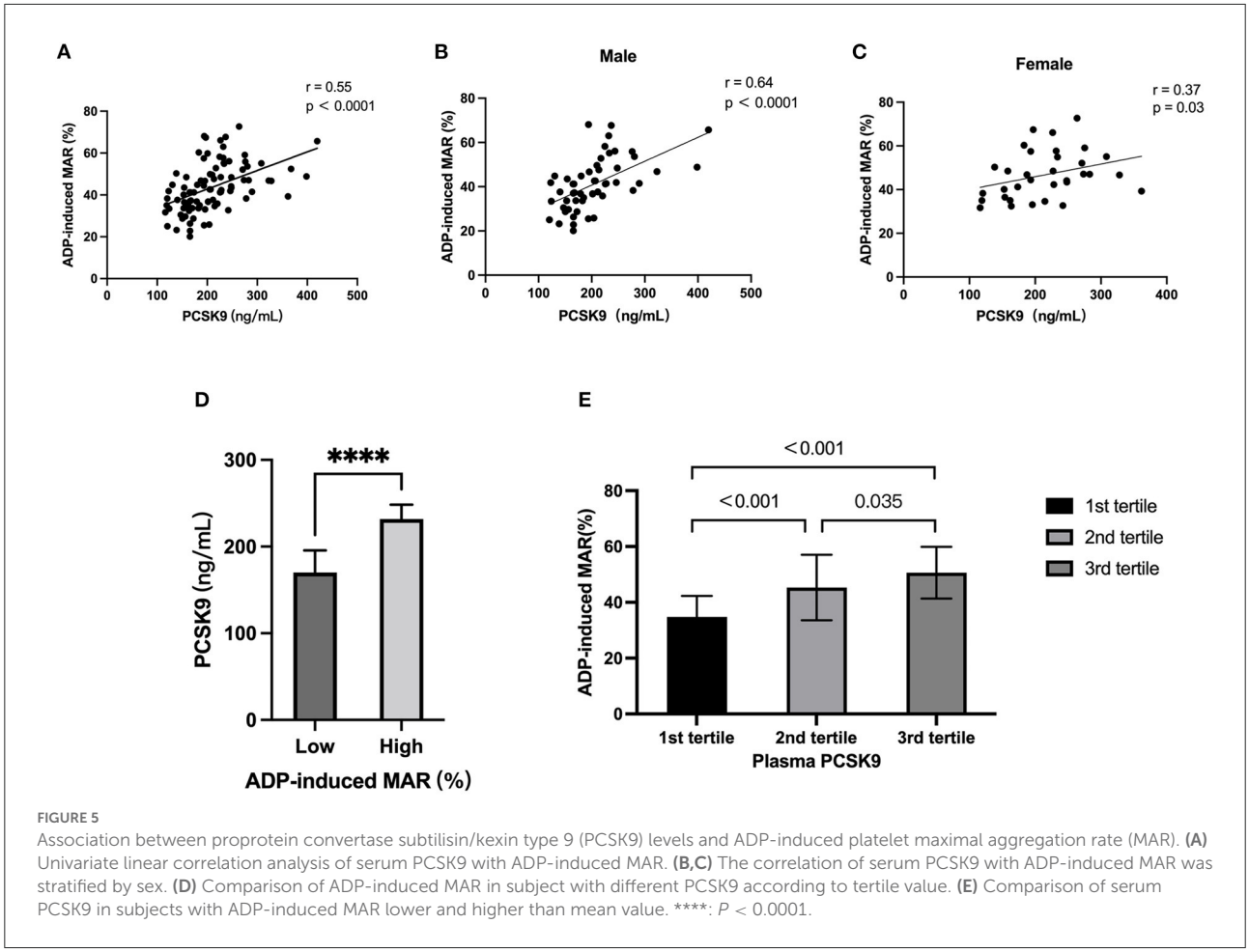


FIGURE 5
Association between proprotein convertase subtilisin/kexin type 9 (PCSK9) levels and ADP-induced platelet maximal aggregation rate (MAR). **(A)** Univariate linear correlation analysis of serum PCSK9 with ADP-induced MAR. **(B,C)** The correlation of serum PCSK9 with ADP-induced MAR was stratified by sex. **(D)** Comparison of ADP-induced MAR in subject with different PCSK9 according to tertile value. **(E)** Comparison of serum PCSK9 in subjects with ADP-induced MAR lower and higher than mean value. ****: $P < 0.0001$.

TABLE 1 Univariate linear regression analysis regarding the association of ADP-induced MAR and characteristics.

Variate	Univariate	
	β	*p*-value
PCSK9	0.089	<0.001
TC	3.254	0.028
LDL-C	4.779	0.009
PLT	0.041	0.005
Sex	−4.026	0.096
Smoking	−8.832	0.016

ADP, Adenosine diphosphate; PCSK9, Proprotein Convertase Subtilisin/Kexin Type 9; MAR, maximal aggregation rate; TC, total cholesterol; LDL-C, low density lipoprotein-cholesterol; PLT, platelet.

Plasma PCSK9 level was not associated with AA-induced platelet aggregation in the correlation analysis. In addition, AA-induced platelet MAR was not different according to tertile value of PCSK9. Plasma PCSK9 concentration in subjects with high ADP-induced MAR was similar compared to those with low ADP-induced MAR (Supplementary Figure 3).

Univariate and multivariate linear regression analysis

In univariate linear regression analysis, PCSK9 ($\beta = 0.089$, p<0,001), TC ($\beta = 3.254$, p =0.028), LDL-C ($\beta = 4.779$, $p = 0.009$), PLT ($\beta = 0.041$, $p = 0.005$), and smoking ($\beta = -8.832$, $p = 0.016$) were factors that could significantly predict the value of ADP-induced MAR (Table 1). In multivariate regression analysis, only parameters of covariates that were retained in the model during stepwise elimination procedure are included in model 2. PCSK9 ($\beta = 0.09$, $p = 0.001$), LDL-C ($\beta = 4.81$, $p = 0.046$), and PLT ($\beta = 0.05$, $p = 0.005$) were found to predict ADP-induced MAR. The adjusted R^2 of the multivariate model was 0.284, p<0.001 (Table 2).

Discussion

The role of PCSK9 in altering plasma LDL-C *via* PCSK9-LDLR axis has been well established; recent studies have suggested a possible role of PCSK9 in regulating platelet function. Our study is the first to confirm an independent and

TABLE 2 Multivariate linear regression analysis of the association of ADP-induced MAR and variables.

Variables	Model 1 (adjusted R^2 0.27, $p = 0.002$)		Model 2 (adjusted R^2 0.284, $p = 0.001$)	
	β	*p*-value	β	*p*-value
PCSK9	0.08	0.004	0.09	0.001
TC	−2.92	0.591	/	/
LDL-C	7.58	0.236	4.81	0.046
PLT	0.04	0.073	0.05	0.005
Sex	−0.63	0.83	/	/
Smoking	−4.73	0.262	/	/

ADP, Adenosine diphosphate; PSCK9, Proprotein Convertase Subtilisin/kexin Type 9; MAR, maximal aggregation rate; TC, total cholesterol; LDL-C, low density lipoprotein-cholesterol; PLT, platelet.

positive correlation between PCSK9 level and platelet reactivity in populations without established CVD, who did not take statin or antiplatelet agents.

Platelet reactivity refers to the degree of the response of blood platelets to an external stimulus. Agonists such as ADP and collagen activate platelets by binding to specific receptors that are presented on the platelet surface membrane. Platelet activation leads to an increase of intra-cytoplasmatic concentration of calcium and platelet shape change, enabling platelets to interact with each other and aggregate (18). On the other hand, platelet activation induces conformational changes in GPIIb/IIIa that transform it into its fibrinogen binding form through activating phospholipase Cβ (PLCβ) or phospholipase Cγ (PLCγ) (19). The receptor-bound fibrinogen connects two GPIIb/IIIa molecules on nearby platelets. This process is the final common pathway of agonist-induced platelet aggregation (20).

Platelet aggregation is modified by activating and inactivating biomolecules and conditions. Some components circulating in the blood can potentiate the activation process in the presence of a strong agonist. For example, adrenaline lowers cytosolic cAMP levels and augments platelet activation; insulin-like growth factor I and thrombopoietin enhance platelet activation *via* phosphatidylinositol 3-kinase (PI3K) signaling pathway (21). Diabetes mellitus and states of increased vascular stress might increase the responsiveness of platelets to agonists (22). On the other hand, bioactive mediators released from endothelial cells, such as prostaglandin I_2 (PGI_2), prostaglandin E_2 (PGE2), and nitric oxide (NO), were negative platelet-priming substances (23). A study indicated that polyunsaturated fatty acid products of 12-lipoxygenase can also hamper platelet activation (24).

Measurement of platelet aggregation in platelet-rich plasma using light transmission aggregometry (LTA) is considered the "gold standard" for measurement but is complex and technically demanding. Newer approaches to measuring platelet aggregation uses impedance aggregometry, which is based on the measurement of the electrical resistance between two electrodes immersed in stirred whole blood. As platelets aggregate and bind to the electrodes, there is a change in electrical impedance that corresponds to the degree of aggregation that has occurred. Therefore, it is deemed to be more physiological than studies performed in platelet-rich plasma (25).

ADP is one of the major components released from activated platelets and it acts as an agonist at two platelet purinergic G-protein coupled receptors—the Gq-coupled $P2Y_1$ and Gi-coupled $P2Y_{12}$ receptor. While $P2Y_1$ activation is responsible for intracellular calcium mobilization, shape change, and initiation of aggregation, the $P2Y_{12}$ receptor is responsible for the completion of the aggregation to ADP (26). In the present study we found that ADP-induced platelet aggregation was associated with PLT, PCSK9, and LDL-C in multivariate regression model.

In multivariate regression analysis, ADP-induced platelet aggregation was associated with PLT, which has been described previously in patients after recent coronary stent-implantation and on dual-antiplatelet therapy (27).

Previous studies have found a correlation between plasma PCSK9 and platelet reactivity in patients with coronary artery disease and hypercholesterolemia. In a cohort of stable coronary artery disease patients, plasma PCSK9 levels were positively correlated with the platelet count and plateletcrit (13). The PCSK9-REACT study found that, in patients with a recent acute coronary syndrome (ACS) undergoing percutaneous coronary intervention and receiving P2Y12 inhibitor, there was a direct association between PCSK9 plasma level and high-on-treatment platelet reactivity (14). In patients with hypercholesterolemia who received background statin and acetyl salicylic acid therapy, platelet function parameters were significantly reduced after 12 months of treatment with the monoclonal antibody (mAb) anti-PCSK9 alirocumab or evolocumab (28). However, in these studies, use of statin and aspirin will affect both the plasma PCSK9 level and platelet activity (15, 16). In our study, a significant association of PCSK9 with increased ADP-induced MAR was detected in healthy subjects without statin and antiplatelets. Importantly, this association was significant even after adjusting for other covariates.

Similar to a previous study in CAD, platelet reactivity correlated with plasma LDL-C level in the present study (29, 30). Hypercholesterolemia may influence platelet reactivity through several mechanisms. 1) Formation of ox-LDL, which was induced by high LDL-C, could activate platelets by binding with CD36 and LOX-1 receptors (31–33). 2)

Cholesterol incorporation in plasma membranes induces platelet hypersensitivity to stimuli, whereas its depletion strikingly reduces platelet reactivity (34–36). However, PCSK9 correlated with platelet reactivity after adjusting LDL-C level in multivariate linear regression in our study, suggesting PCSK9 may affect platelet activity through a lipid-lowering independent mechanism. A recent study found PCSK9 can directly enhance agonist-induced platelet activation by binding to platelet CD36 and thus activating Src kinase and MAPK-extracellular signal-regulated kinase 5 and c-Jun N terminal kinase, increasing the generation of reactive oxygen species and activating the p38MAPK/cytosolic phospholipase A2/cyclooxygenase-1/thromboxane A2 signaling pathway (10).

Another finding of this study is AA-induced platelet aggregation was not correlated to plasma PCSK9. Arachidonic acid is derived from membrane phospholipids through phospholipase A2 (PLA2). AA is transformed into prostaglandin G2 and prostaglandin H2 by cyclooxygenase-1(COX-1), then transformed into TXA2, which is a strong activator of platelets. Our finding suggests PCSK9 does not affect PLA2/Cox-1/TXA2 pathway.

The limitation of the present study is its cross-sectional nature. The findings of our study could only indicate associations, not causality. Another limitation is the relatively small sample size of the population.

Conclusions

In summary, PCSK9 levels are associated with platelet activation in subjects not taking statins or antiplatelet agents. Subjects with increased concentration of PCSK9 have significantly higher platelet activation. The finding of this study provides additional evidence of the correlation between PCSK9 level and platelet activation beyond CVD patients. Future studies are warranted to further elucidate the role of PCSK9 as a risk factor for ASCVD.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by the Ethics Committee of Second Xiangya Hospital on research on humans. The patients/participants provided their written informed consent to participate in this study.

Author contributions

SW analyzed the result and wrote the manuscript. DF and HL collected the datasets. DP conceived the study and revised the manuscript. All authors contributed to the article and approved the submitted version.

Funding

This work was supported by grants from the National Nature Science Foundation Youth Project (81600359) and the National Nature Science Foundation General Project (81870336).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcvm.2022.934914/full#supplementary-material

References

1. Berger JS, Becker RC, Kuhn C, Helms MJ, Ortel TL, Williams R. Hyperreactive platelet phenotypes: relationship to altered serotonin transporter number, transport kinetics and intrinsic response to adrenergic co-stimulation. *Thromb Haemost.* (2013) 109:85–92. doi: 10.1160/TH12-03-0202

2. Kondkar AA, Bray MS, Leal SM, Nagalla S, Liu DJ, Jin Y, et al. VAMP8/endobrevin is overexpressed in hyperreactive human platelets: suggested role for platelet microRNA. *J Thromb Haemost.* (2010) 8:369–78. doi: 10.1111/j.1538-7836.2009.03700.x

3. Montenont E, Echagarruga C, Allen N, Araldi E, Suarez Y, Berger JS. Platelet WDR1 suppresses platelet activity and is associated with cardiovascular disease. *Blood.* (2016) 128:2033–42. doi: 10.1182/blood-2016-03-703157

4. Sharma G, Berger JS. Platelet activity and cardiovascular risk in apparently healthy individuals: a review of the data. *J Thromb Thrombolysis.* (2011) 32:201–8. doi: 10.1007/s11239-011-0590-9

5. Trip MD, Cats VM, van Capelle FJ, Vreeken J. Platelet hyperreactivity and prognosis in survivors of myocardial infarction. *N Engl J Med.* (1990) 322:1549–54. doi: 10.1056/NEJM199005313222201

6. Tofler GH, Brezinski D, Schafer AI, Czeisler CA, Rutherford JD, Willich SN, et al. Concurrent morning increase in platelet aggregability and the risk of myocardial infarction and sudden cardiac death. *N Engl J Med.* (1987) 316:1514–8. doi: 10.1056/NEJM198706113162405

7. Fuentes F, Palomo I, Fuentes E. Platelet oxidative stress as a novel target of cardiovascular risk in frail older people. *Vascul Pharmacol.* (2017) 93-95:14–9. doi: 10.1016/j.vph.2017.07.003

8. El Haouari M. Platelet oxidative stress and its relationship with cardiovascular diseases in type 2 diabetes mellitus patients. *Curr Med Chem.* (2019) 26:4145–65. doi: 10.2174/0929867324666171005114456

9. Wang N, Tall AR. Cholesterol in platelet biogenesis and activation. *Blood.* (2016) 127:1949–53. doi: 10.1182/blood-2016-01-631259

10. Qi Z, Hu L, Zhang J, Yang W, Liu X, Jia D, et al. PCSK9 (proprotein convertase subtilisin/kexin 9) enhances platelet activation, thrombosis, and myocardial infarct expansion by binding to platelet CD36. *Circulation.* (2021) 143:45–61. doi: 10.1161/CIRCULATIONAHA.120.046290

11. Leander K, Malarstig A, Van't Hooft FM, Hyde C, Hellenius ML, Troutt JS, et al. Circulating proprotein convertase subtilisin/kexin type 9 (PCSK9) predicts future risk of cardiovascular events independently of established risk factors. *Circulation.* (2016) 133:1230–9. doi: 10.1161/CIRCULATIONAHA.115.018531

12. Xie W, Liu J, Wang W, Wang M, Qi Y, Zhao F, et al. Association between plasma PCSK9 levels and 10-year progression of carotid atherosclerosis beyond LDL-C: a cohort study. *Int J Cardiol.* (2016) 215:293–8. doi: 10.1016/j.ijcard.2016.04.103

13. Li S, Zhu CG, Guo YL, Xu RX, Zhang Y, Sun J, et al. The relationship between the plasma PCSK9 levels and platelet indices in patients with stable coronary artery disease. *J Atheroscler Thromb.* (2015) 22:76–84. doi: 10.5551/jat.25841

14. Navarese EP, Kolodziejczak M, Winter MP, Alimohammadi A, Lang IM, Buffon A, et al. Association of PCSK9 with platelet reactivity in patients with acute coronary syndrome treated with prasugrel or ticagrelor: the PCSK9-REACT study. *Int J Cardiol.* (2017) 227:644–9. doi: 10.1016/j.ijcard.2016.10.084

15. Sahebkar A, Simental-Mendia LE, Guerrero-Romero F, Golledge J, Watts GF. Effect of statin therapy on plasma proprotein convertase subtilisin kexin 9 (PCSK9) concentrations: a systematic review and meta-analysis of clinical trials. *Diabetes Obes Metab.* (2015) 17:1042–55. doi: 10.1111/dom.12536

16. Nenna A, Nappi F, Lusini M, Satriano UM, Schiliro D, Spadaccio C, et al. Effect of statins on platelet activation and function: from molecular pathways to clinical effects. *Biomed Res Int.* (2021) 2021:6661847. doi: 10.1155/2021/6661847

17. Camera M, Rossetti L, Barbieri SS, Zanotti I, Canciani B, Trabattoni D, et al. PCSK9 as a positive modulator of platelet activation. *J Am Coll Cardiol.* (2018) 71:952–4. doi: 10.1016/j.jacc.2017.11.069

18. Varga-Szabo D, Braun A, Nieswandt B. Calcium signaling in platelets. *J Thromb Haemost.* (2009) 7:1057–66. doi: 10.1111/j.1538-7836.2009.03455.x

19. Varga-Szabo D, Pleines I, Nieswandt B. Cell adhesion mechanisms in platelets. *Arterioscler Thromb Vasc Biol.* (2008) 28:403–12. doi: 10.1161/ATVBAHA.107.150474

20. Koltai K, Kesmarky G, Feher G, Tibold A, Toth K. Platelet aggregometry testing: molecular mechanisms, techniques and clinical implications. *Int J Mol Sci.* (2017) 18:1803. doi: 10.3390/ijms18081803

21. van der Meijden PEJ, Heemskerk JWM. Platelet biology and functions: new concepts and clinical perspectives. *Nat Rev Cardiol.* (2019) 16:166–79. doi: 10.1038/s41569-018-0110-0

22. Kraakman MJ, Lee MK, Al-Sharea A, Dragoljevic D, Barrett TJ, Montenont E, et al. Neutrophil-derived S100 calcium-binding proteins A8/A9 promote reticulated thrombocytosis and atherogenesis in diabetes. *J Clin Invest.* (2017) 127:2133–47. doi: 10.1172/JCI92450

23. Naseem KM, Roberts W. Nitric oxide at a glance. *Platelets.* (2011) 22:148–52. doi: 10.3109/09537104.2010.522629

24. Tourdot BE, Adili R, Isingizwe ZR, Ebrahem M, Freedman JC, Holman TR, et al. 12-HETrE inhibits platelet reactivity and thrombosis in part through the prostacyclin receptor. *Blood Adv.* (2017) 1:1124–31. doi: 10.1182/bloodadvances.2017006155

25. Algahtani M, Heptinstall S. Novel strategies for assessing platelet reactivity. *Future Cardiol.* (2017) 13:33–47. doi: 10.2217/fca-2016-0054

26. Gremmel T, Frelinger III AL, Michelson AD. Platelet physiology. *Semin Thromb Hemost.* (2016) 42:191–204. doi: 10.1055/s-0035-1564835

27. Olivier CB, Meyer M, Bauer H, Schnabel K, Weik P, Zhou Q, et al. The ratio of ADP- to TRAP-induced platelet aggregation quantifies P2Y12-dependent platelet inhibition independently of the platelet count. *PLoS ONE.* (2016) 11:e0149053. doi: 10.1371/journal.pone.0149053

28. Barale C, Bonomo K, Frascaroli C, Morotti A, Guerrasio A, Cavalot F, et al. Platelet function and activation markers in primary hypercholesterolemia treated with anti-PCSK9 monoclonal antibody: a 12-month follow-up. *Nutr Metab Cardiovasc Dis.* (2020) 30:282–91. doi: 10.1016/j.numecd.2019.09.012

29. Petersen-Uribe A, Kremser M, Rohlfing AK, Castor T, Kolb K, Dicenta V, et al. Platelet-derived PCSK9 is associated with LDL metabolism and modulates atherothrombotic mechanisms in coronary artery disease. *Int J Mol Sci.* (2021) 22:1179. doi: 10.3390/ijms222011179

30. DiMinno G, Silver MJ, Cerbone AM, Rainone A, Postiglione A, Mancini M. Increased fibrinogen binding to platelets from patients with familial hypercholesterolemia. *Arteriosclerosis.* (1986) 6:203–11. doi: 10.1161/01.ATV.6.2.203

31. Magwenzi S, Woodward C, Wraith KS, Aburima A, Raslan Z, Jones H, et al. Oxidized LDL activates blood platelets through CD36/NOX2-mediated inhibition of the cGMP/protein kinase G signaling cascade. *Blood.* (2015) 125:2693–703. doi: 10.1182/blood-2014-05-574491

32. Chen K, Febbraio M, Li W, Silverstein RL. A specific CD36-dependent signaling pathway is required for platelet activation by oxidized low-density lipoprotein. *Circ Res.* (2008) 102:1512–9. doi: 10.1161/CIRCRESAHA.108.172064

33. Hofmann A, Brunssen C, Morawietz H. Contribution of lectin-like oxidized low-density lipoprotein receptor-1 and LOX-1 modulating compounds to vascular diseases. *Vascul Pharmacol.* (2017) 19:S1537-1891(17)30171-4. doi: 10.1016/j.vph.2017.10.002

34. Shattil SJ, Anaya-Galindo R, Bennett J, Colman RW, Cooper RA. Platelet hypersensitivity induced by cholesterol incorporation. *J Clin Invest.* (1975) 55:636–43. doi: 10.1172/JCI107971

35. Tomizuka T, Yamamoto K, Hirai A, Tamura Y, Yoshida S. Hypersensitivity to thromboxane A2 in cholesterol-rich human platelets. *Thromb Haemost.* (1990) 64:594–9. doi: 10.1055/s-0038-1647364

36. Korporaal SJ, Meurs I, Hauer AD, Hildebrand RB, Hoekstra M, Cate HT, et al. Deletion of the high-density lipoprotein receptor scavenger receptor BI in mice modulates thrombosis susceptibility and indirectly affects platelet function by elevation of plasma free cholesterol. *Arterioscler Thromb Vasc Biol.* (2011) 31:34–42. doi: 10.1161/ATVBAHA.110.210252

frontiers | Frontiers in Cardiovascular Medicine

TYPE Original Research
PUBLISHED 28 November 2022
DOI 10.3389/fcvm.2022.1026243

OPEN ACCESS

EDITED BY
Alexander Akhmedov,
University of Zurich, Switzerland

REVIEWED BY
Federica Fogacci,
University of Bologna, Italy
Željko Reiner,
University Hospital Centre Zagreb, Croatia

*CORRESPONDENCE
Jinchun He
hejch@lzu.edu.cn

SPECIALTY SECTION
This article was submitted to Lipids in Cardiovascular Disease, a section of the journal Frontiers in Cardiovascular Medicine

RECEIVED 23 August 2022
ACCEPTED 07 November 2022
PUBLISHED 28 November 2022

CITATION
Wang Y and He J (2022) Correlation of cardiovascular risk predictors with overweight and obesity in patients with familial hypercholesterolemia. *Front. Cardiovasc. Med.* 9:1026243. doi: 10.3389/fcvm.2022.1026243

Correlation of cardiovascular risk predictors with overweight and obesity in patients with familial hypercholesterolemia

Yaodong Wang[1,2] and Jinchun He[3]*

[1]Laboratory Medicine Center, Lanzhou University Second Hospital, Lanzhou University, Lanzhou, Gansu, China, [2]The First School of Clinical Medicine, Lanzhou University, Lanzhou, Gansu, China, [3]Department of Laboratory Medicine, The First Hospital of Lanzhou University, The First School of Clinical Medicine, Lanzhou University, Lanzhou, Gansu, China

Purpose: We aimed to analyze the correlation between overweight and obesity-related indicators and cardiovascular risk predictors in patients with familial hypercholesterolemia (FH) and to evaluate their mutual predictive properties.

Methods: A total of 103 patients with FH included from 2004 to 2017 were retrospectively analyzed. Pearson correlation analysis and multiple linear regression analysis were used to assess the correlation between overweight and obesity-related indicators and cardiovascular risk predictors in FH patients. Subject operating characteristic (ROC) curve was used to analyze their reciprocal predictive performance.

Results: (1) Atherogenic index of plasma (AIP) (β = 0.020) and ApoB/ApoA1 Ratio (BAR) (β = 0.015) were independently correlated with body mass index (BMI) ($P < 0.05$); AIP (β = 1.176) was independently correlated with waist-to-hip ratio (WHR) ($P < 0.01$); AIP (β = 1.575), BAR (β = 0.661) and atherogenic coefficient (AC) (β = 0.427) were independently correlated with waist-to-height ratio (WHtR) ($P < 0.05$). (2) The area under the ROC (AUC) for overweight corresponding to AIP, BAR, and AC were 0.695 (95% *CI* = 0.593–0.797, $P < 0.01$), 0.660 (95% *CI* = 0.555–0.766, $P < 0.01$), and 0.632 (95% *CI* = 0.525–0.740, $P < 0.05$), respectively; and AUCs for central obesity corresponding to AIP, BAR and AC were 0.757 (95% *CI* = 0.656–0.857, $P < 0.001$), 0.654 (95% *CI* = 0.536–0.771, $P < 0.05$) and 0.651 (95% *CI* = 0.538–0.764, $P < 0.05$), respectively. The AUCs for moderate risk of AIP corresponding to BMI, WHR, and WHtR were 0.709 (95% *CI* = 0.608–0.811, $P < 0.001$), 0.773 (95% *CI* = 0.678–0.867, $P < 0.001$), 0.739 (95% *CI* = 0.641–0.836, $P < 0.001$), respectively, and BMI, WHR and WHtR corresponded to an AUC of 0.691 (95% *CI* = 0.585–0.797, $P < 0.01$), 0.734 (95% *CI* = 0.632–0.835, $P < 0.001$), and 0.706 (95% *CI* = 0.603–0.810, $P < 0.01$) for high risk of AIP, respectively.

Conclusion: AIP has independent positive linear correlation with indicators related to overweight and obesity in FH patients; AIP has good predictive performance for overweight and obesity in FH patients, and WHR has good performance for identifying moderate and high risk of AIP in FH patients.

KEYWORDS

hypercholesterolemia, cardiovascular, obesity, atherogenic, correlation

Introduction

Familial hypercholesterolemia (FH) is an autosomal dominant disorder with an estimated prevalence of 1/300 to 1/500 in heterozygous populations and at least 20 million people worldwide with FH (1–5). It is well known that hypercholesterolemia predisposes to the formation of atherosclerotic plaques in the vascular wall and has a high risk of cardiovascular disease (CVD) events. The prevalence of FH is higher in patients who have experienced a CVD event, and control of other CVD risk factors appears to be less optimal than in other patients (6). The results of a cross-sectional survey of FH in China showed that the prevalence of FH in the Chinese population is similar to that in other countries; however, FH in China is mainly found in patients with early-onset coronary heart disease and their lipid levels are poorly controlled and at higher risk of CVD (7). Together with the fact that cholesterol is involved in the formation of cellular barriers for many basic physiological processes and acts as an important component of signal transduction (1), many studies have emphasized that patients with FH should be identified early and given early intervention (8–11).

The detection of traditional lipid profiles and their associated calculated indices are the main methods currently used to assess the risk of CVD. However, in the absence of an abnormal lipid profile, the possibility of coronary artery disease (CAD) cannot be excluded. Therefore, it has been suggested that different combinations of these lipid profile parameters could be used to identify such high-risk individuals. The atherogenic index of plasma (AIP), ApoB/ApoA1 Ratio (BAR) and the atherogenic coefficient (AC) have been considered as high-quality predictors of cardiovascular risk (12, 13). In recent years, AIP has gained widespread interest as a screening tool for dyslipidemia and is considered a major cardiometabolic risk factor and an emerging indicator to predict CVD risk (14), reflecting the balance between atherogenic and antiatherogenic factors in an integrated manner (15, 16). BAR has also been proposed to be better than LDL-C and superior to non-high-dense lipoprotein cholesterol (non-HDL-C) as a marker of CVD risk (17–20). This ratio has also been considered as a potential marker of cardiovascular risk because it can often be abnormal in the presence of normal conventional lipid levels (21). A study by Lu et al. indicated that BAR is a valid predictor of coronary heart disease risk in overweight and obese people (22). Another ratio index that is HDL-C dependent and has significance in predicting CAD risk is AC, calculated as [(TC-HDL-C)/HDL-C] (23). It has been demonstrated that AC reflects the atherogenic potential of the entire lipoprotein fraction profile and can be used for therapeutic management (12).

It is well known that overweight and obesity-related indicators, including body mass index (BMI), waist-to-height ratio (WHtR) and waist-to-hip ratio (WHR), are among the good criteria to reflect the degree of body fatness and healthiness, and are widely used to screen overweight and centrally obese people. A large epidemiological survey showed that more than two-thirds of deaths associated with high BMI were due to CVD (24). Abdominal obesity (also central obesity) involves the accumulation of abdominal fat and is considered an independent risk factor for obesity-related diseases and death (25). It has been reported that when AIP values of 0.12–0.21 and $>$ 0.21 indicate the likelihood of critical abdominal obesity and abdominal obesity, respectively, while the combination of waist circumference and AIP may increase the specificity and sensitivity of abdominal obesity detection in clinical practice, thus suggesting that AIP may be used as a reference for estimating abdominal obesity (26).

In this study, to determine whether atherogenic lipid indicators such as AIP are independently associated with overweight and obesity-related indicators such as BMI, we analyzed the correlation between lipid parameters (i.e., lipid calculation indicators) such as AIP, BAR, and AC with overweight and obesity-related indicators, and finally evaluated the predictive performance of cardiovascular risk predictors for overweight and overweight and obesity-related indicators for AIP in risk and high risk identification performance. It is also hoped that these findings will highlight the threat of overweight and even obesity in FH patients and promote the benefits of weight control in FH patients, thus reducing the risk of atherogenic disease in this population.

Patients and methods

Inclusion of study subjects

The original data of the FH study samples were obtained from the subproject "Collection and clinical epidemiology of hereditary hyperlipidemia blood specimens in family lines" under the "Collection, preservation and utilization of genetic resources of major diseases" of the "Tenth Five-Year Plan" of China. The original data was initially screened from the population who attended the outpatient clinic of the First Hospital of Lanzhou University from 2004 to 2017 based on the initial fasting lipid levels, and then invited them and their first-degree relatives to undergo a physical examination again on a specified date, which included biochemical tests such as lipid panel, physical examination, electrocardiogram and face-to-face questionnaires, and all study subjects signed an informed consent form to voluntarily participate in this study. In addition, two of the following three criteria were met and included in the project: (1) At least two family members in each family line with dyslipidemia, as determined by the National Cholesterol Education Program (NCEP) (27), with TC $\geq$ 6.20 mmol/L and/or LDL-C $\geq$ 4.10 mmol/L without secondary causes; (2) at least 2 generations of involvement per family line; and (3) at least 1 member of each family line with hypercholesterolemia with an age of onset no older than 50 years.

In order to make the above information meet the needs of the current study, we initially screened out cases in which the above information might bias the results, including non-Han, non-first-degree relatives, age < 18 years, and TG > 5.6 mmol/L. Finally, we followed the Dutch Lipid Clinic Network (DLCN) (28) for clinical lipid monitoring guidelines and included Patients with scores of 6 and above (i.e., definite FH and probable FH) were included in the current study the screening process for the study sample is shown in **Figure 1**.

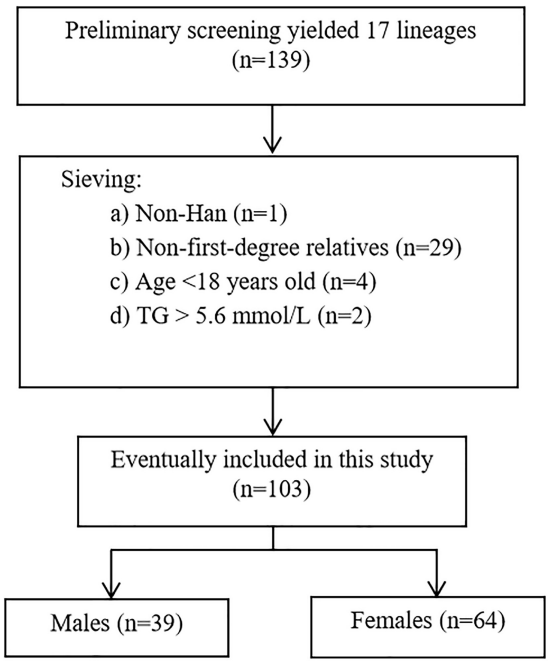


FIGURE 1
Flowchart of the study sample. FH, familial hypercholesterolemia; TG, triglycerides.

Questionnaire survey and clinical evaluation

Questionnaire

The questionnaire for the hyperlipidemia family blood specimen collection project was pre-designed by the researchers to obtain general demographic information, life and dietary habits, disease history and medication history of the participants. The survey involved in this study mainly included the following aspects: (1) general demographic information: such as ethnicity, gender, age, etc.; (2) lifestyle habits: such as smoking, alcohol consumption, physical exercise, etc.; (3) dietary habits: dietary preferences; (4) disease history: past history, current disease history and family history, etc.; (5) medication history: type of medication, name of medication, start date of medication, dose of medication, etc.

Clinical assessment

Clinical assessments were performed by uniformly trained clinicians. BMI was calculated by dividing weight (kg) by the square of height (m^2), using an overweight cut-off point of 24 kg/m^2 suitable for BMI in Asian populations (29), and the study population was divided into overweight and non-overweight groups. WHR was obtained by dividing waist circumference (cm) by hip circumference (cm), and WHtR calculated as waist circumference (cm)/height (cm.) WHtR $\geq$ 0.5 and WHR $\geq$ 0.9 in men and $\geq$ 0.85 in women were considered centrally obese (30). Xanthoma included tendinous xanthoma (which could be located the back of the fingers, elbows, knees, or elsewhere and also included the thickening of the Achilles tendon) and tuberous xanthoma, as well as rash and flat xanthoma. Fasting blood glucose (FBG) $\geq$ 7.0 mmol/L or those who had been treated with hypoglycemic therapy were identified as having diabetes. With regard to family history of disease, it was defined as a family history of appropriate disease in first-degree relatives (i.e., children, parents, and siblings) and grandparents in second-degree relatives of study subjects, including diabetes, hypertension, hypercholesterolemia, and CVD (coronary heart disease, stroke). Systolic blood pressure (SBP) $\geq$ 140 *mmHg* or diastolic blood pressure (DBP) $\geq$ 90 *mmHg* measured three times on non-same day or taking antihypertensive medication was defined as hypertension. Mean arterial pressure (MAP) was calculated as (SBP + 2DBP)/3. Those who smoked within the

past 6 months and reached 1 cigarette/day were classified as smokers, those who never smoked or smoked occasionally but did not meet the smoking criteria or quit smoking for more than 1 year were classified as non-smokers. Those who drank alcohol continuously for more than 6 months and drank alcohol at least once a week on average were classified as alcohol drinkers, and those who never drank alcohol or drank occasionally but did not meet the criteria for drinking alcohol were classified as non-drinkers. The AIP risk was divided into three groups: (1) low risk, AIP < 0.11; (2) moderate risk, AIP ≥ 0.11 and ≤ 0.21; and (3) high risk, AIP > 0.21 (31, 32).

Laboratory tests

The results of the laboratory analyses were obtained from the subjects' data profiles. The analysis of biochemical items such as the full lipid panel was performed at the Laboratory Department of the First Hospital of Lanzhou University, and all blood sampling was performed on the following morning after 8–12 h of fasting, with appropriate quality control, using the same fully automated biochemical analyzer. Serum TC, HDL-C, LDL-C, and TG concentrations were measured by applying enzyme colorimetric method, serum ApoA1, ApoB and Lipoprotein (a) [Lp(a)] levels were measured by applying immunoturbidimetric method, and serum FBG levels were measured by applying hexokinase method. The above biochemical items were performed in an automated biochemical analyzer (Beckman Coulter, Brea, CA, USA). Based on independent lipid parameters, the following clinical indicators were calculated: non-HDL-C, AIP, BAR, LDL-C/ApoB ratio, HDL-C/ApoA1 ratio, LDL-C/HDL-C ratio, and AC. non-HDL-C values were obtained by subtracting HDL-C values from TC values. AIP was calculated as Log_{10} (TG/HDL-C) (15).

Statistical analysis

Data were analyzed using SPSS version 20.0 (SPSS, Inc., Chicago, IL, USA). For continuous variables, normal distribution was expressed as mean ± standard deviation ($\bar{x} \pm s$) and non-normal distribution was expressed as median and quartiles [M (P25, P75)]; for categorical variables, it was expressed as number and percentage (N/%). Normality of continuous variables was tested using Shapiro-Wilk test and Q-Q plot test. In clinical characteristics and biochemical parameters between groups, for some physical and blood indicators such as MAP, BMI, WHR, which are normally distributed continuous variables, the Student's *t*-test was applied to analyze the differences between two independent variables, and the chi-square test was performed before the *t*-test; for TG and Lp(a), which are two continuous variables that do not obey normal distribution, the Mann-Whitney *U*-test was applied to analyze the differences between two analysis of variance between independent variables. For the analysis of variance of categorical variables such as males, smokers, and alcohol drinkers, we applied the chi-square test. Pearson correlation analysis was used to determine the relationship between BMI, WHR, and WHtR and the levels of AIP, BAR, and AC. Multiple stepwise linear regression analysis was used to determine the independent correlations between BMI, WHR, and WHtR and the levels of AIP, BAR, and AC. Receiver operating characteristic (ROC) analysis was used to explore the performance of cardiovascular risk predictors in identifying overweight and central obesity. $P < 0.05$ was considered statistically significant, and all tests were two-sided.

Results

Basic information of familial hypercholesterolemia patients

After excluding samples with missing key data such as basic information, overweight and obesity-related indicators and lipid indicators, a total of 103 patients with FH from 17 family lines were finally included in this study. As shown in **Table 1**, the study subjects were all Han Chinese, including 39 (37.9%) males and 64 (62.1%) females, with an age range of 18–85 years, an overall mean age of (46.12 ± 14.29) years, and an overall mean BMI of (23.63 ± 3.39) kg/m^2. The study subjects were classified according to BMI into overweight group (53, 51.4%) and non-overweight group (50, 48.6%), and the results were compared between the two groups for basic conditions showing age ($P = 0.019$), xanthoma ($P = 0.027$), hypertension ($P < 0.001$), MAP ($P < 0.001$), BMI ($P < 0.001$), WHR ($P < 0.001$), WHtR ($P < 0.001$), FBG ($P = 0.012$), TG ($P = 0.002$), HDL-C ($P = 0.016$), ApoA1 ($P = 0.007$), AIP ($P = 0.001$), BAR ($P = 0.003$), AC ($P = 0.029$), and LDL-C/HDL-C ratio ($P = 0.035$) were statistically significant between the two groups, while gender, smoking, alcohol consumption, dietary oiliness, dietary saltiness, history of coronary heart disease, TC, LDL-C, ApoB, Lp(a), non-HDL-C, LDL-C/ApoB, and HDL-C/ApoA1 were not statistically significant between the two groups ($P > 0.05$). The study subjects were divided into central obesity group (59 cases, 59%) and non-central obesity group (41 cases, 41%) according to WHR and WHtR, and the basic conditions were compared between the two groups, which showed that age ($P = 0.001$), male ($P = 0.023$), smoking ($P = 0.024$), xanthoma ($P = 0.014$), hypertension ($P = 0.003$), MAP ($P < 0.001$), BMI ($P < 0.001$), WHR ($P < 0.001$), WHtR ($P < 0.001$), FBG ($P = 0.007$), TG ($P < 0.001$), HDL-C ($P = 0.010$), ApoA1 ($P = 0.007$), and AIP ($P < 0.001$) were statistically significant between the two groups. statistically significant, whereas the differences in alcohol consumption, dietary oiliness, dietary salinity, history

TABLE 1 Basic Clinical features of patients with familial hypercholesterolemia.

Variables	All $n = 103$	BMI ≥ 24 kg/m^2		P	Central obesity		P
		No ($n = 53$)	Yes ($n = 50$)		No ($n = 59$)	Yes ($n = 41$)	
Age (years)	46.12 ± 14.29	42.94 ± 16.27	49.48 ± 11.04	**0.019**	42.19 ± 15.80	51.54 ± 10.24	**0.001**
Male (*N*/%)	39 (37.9%)	17 (32.1%)	22 (44%)	0.212	17 (28.8%)	21 (51.2%)	**0.023**
Smokers (*N*/%)	28 (27.2%)	12 (22.6%)	16 (32%)	0.286	11 (18.6%)	16 (39%)	**0.024**
Alcohol drinkers (*N*/%)	45 (43.7%)	23 (43.4%)	22 (44%)	0.286	26 (44.1%)	16 (39%)	0.615
Dietary oiliness (*N*/%)	21 (20.4%)	12 (22.6%)	9 (18%)	0.559	12 (20.3%)	9 (22%)	0.846
Salty diet (*N*/%)	27 (26.2%)	10 (18.9%)	17 (34%)	0.081	11 (18.6%)	15 (36.6%)	0.063
CHD history (*N*/%)	5 (4.8%)	2 (3.8%)	3 (6%)	0.599	3 (5.1%)	2 (4.9%)	0.925
Xathoma (*N*/%)	18 (17.5%)	5 (9.4%)	13 (26%)	**0.027**	6 (10.2%)	12 (29.3%)	**0.014**
Hypertension (*N*/%)	34 (33%)	8 (15.1%)	26 (52%)	**<0.001**	12 (20.3%)	20 (48.8%)	**0.003**
MAP (mmHg)	91.24 ± 14.11	85.77 ± 12.40	97.03 ± 13.58	**<0.001**	86.47 ± 12.49	97.19 ± 13.83	**<0.001**
BMI (kg/m^2)	23.63 ± 3.39	20.94 ± 1.91	26.48 ± 1.99	**<0.001**	21.85 ± 2.72	25.97 ± 2.73	**<0.001**
WHR	0.87 ± 0.08	0.83 ± 0.07	0.91 ± 0.08	**<0.001**	0.82 ± 0.06	0.94 ± 0.06	**<0.001**
WHtR	0.51 ± 0.06	0.47 ± 0.04	0.55 ± 0.04	**<0.001**	0.47 ± 0.04	0.56 ± 0.04	**<0.001**
FBG (mmol/L)	4.96 ± 0.79	4.77 ± 0.68	5.16 ± 0.85	**0.012**	4.78 ± 0.62	5.21 ± 0.93	**0.007**
TC (mmol/L)	5.86 ± 1.41	5.82 ± 1.21	5.92 ± 1.59	0.724	5.95 ± 1.27	5.74 ± 1.61	0.483
TG (mmol/L)	1.48 (0.90–2.34)	1.10 (0.76–1.88)	1.78 (1.10–2.61)	**0.002**	1.1 (0.77–1.72)	1.92 (1.38–2.78)	**<0.001**
HDL-C (mmol/L)	1.33 ± 0.27	1.39 ± 0.28	1.26 ± 0.25	**0.016**	1.39 ± 0.26	1.25 ± 0.27	**0.010**
LDL-C (mmol/L)	3.95 ± 1.30	3.82 ± 1.12	4.08 ± 1.46	0.313	3.95 ± 1.20	3.90 ± 1.46	0.876
ApoA1 (g/L)	1.43 ± 0.27	1.50 ± 0.29	1.36 ± 0.24	**0.007**	1.49 ± 0.28	1.34 ± 0.25	**0.007**
ApoB (g/L)	0.93 ± 0.50	0.86 ± 0.22	0.91 ± 0.25	0.262	0.89 ± 0.25	0.87 ± 0.22	0.658
Lp(a) (mg/L)	249.5 (168–309)	227.5 (175–324)	256.5 (156–304)	0.905	238 (168–329)	256.5 (147–286)	0.762
AIP	0.05 ± 0.30	-0.05 ± 0.29	0.15 ± 0.28	**0.001**	−0.06 ± 0.27	0.21 ± 0.29	**<0.001**
BAR	0.63 ± 0.17	0.58 ± 0.15	0.68 ± 0.18	**0.003**	0.61 ± 0.16	0.66 ± 0.19	0.103
AC	3.50 ± 1.17	3.26 ± 0.90	3.76 ± 1.35	**0.029**	3.35 ± 0.94	3.68 ± 1.34	0.168
LDL-C/HDL-C	3.05 ± 1.14	2.82 ± 0.92	3.28 ± 1.29	**0.035**	2.91 ± 0.96	3.19 ± 0.16	0.227
LDL-C/ApoB	4.49 ± 0.95	4.53 ± 1.04	4.44 ± 0.86	0.649	4.50 ± 1.03	4.42 ± 0.86	0.693
HDL-C/ApoA1	0.93 ± 0.12	0.93 ± 0.12	0.93 ± 0.13	0.911	0.94 ± 0.12	0.93 ± 0.13	0.845
Non-HDL-C (mmol/L)	4.54 ± 1.32	4.42 ± 1.11	4.65 ± 1.51	0.385	4.55 ± 1.19	4.49 ± 1.52	0.822

Data are presented as mean ± standard deviation, median (25th–75th percentile) or *n* (%). Bold values indicate statistical significance.

of coronary heart disease, TC, LDL-C, ApoB, Lp(a), non-HDL-C, LDL-C/ApoB, BAR, AC, LDL-C/HDL-C, LDL-C/ApoB, and HDL-C/ApoA1 were not statistically significant between the two groups ($P > 0.05$).

Relationship between overweight and obesity-related indicators and cardiovascular risk predictors in patients with familial hypercholesterolemia

Simple correlation analysis

Further analysis of linear relationships between overweight and obesity-related indicators and conventional lipid profiles and lipid-related calculated parameters in patients with FH, as shown in Table 2, revealed that BMI was significantly negatively correlated ($P < 0.01$) with HDL-C ($r = -0.284$) and ApoA1 ($r = -0.269$), and with AIP ($r = 0.385$), BAR ($r = 0.348$) and AC ($r = 0.256$) were significantly positively correlated ($P < 0.01$); WHR was significantly negatively correlated with HDL-C ($r = -0.303$) and ApoA1 ($r = -0.361$) ($P < 0.01$) and positively correlated with TG ($r = 0.329$), AIP ($r = 0.501$) and BAR ($r = 0.287$) ($P < 0.01$). WHtR showed significant negative correlations ($P < 0.05$) with HDL-C ($r = -0.196$) and ApoA1 ($r = -0.203$), and significant positive correlations ($P < 0.01$). The overall significant correlations of overweight and obesity-related indicators with AIP, BAR and AC among lipid parameters in FH patients were shown, and the scatter plots of correlations between BMI, WHR and WHtR and AIP, BAR, and AC were plotted in Figure 2.

Independent correlation analysis

Given the above correlations, independent correlation analyses between BMI, WHR and WHtR and AIP, BAR and AC

TABLE 2 Correlation analysis of overweight and obesity indicators and lipid parameters in patients with familial hypercholesterolemia.

	BMI		WHR		WHtR	
	r	*P*	*r*	*P*	*r*	*P*
TC (mmol/L)	0.048	0.634	−0.112	0.272	0.136	0.173
TG (mmol/L)	0.233	0.018	0.329	**0.001**	0.310	**0.002**
HDL-C (mmol/L)	−0.284	**0.004**	−0.303	**0.002**	−0.196	**0.048**
LDL-C (mmol/L)	0.126	0.207	−0.017	0.865	0.216	**0.029**
ApoA1 (g/L)	−0.269	**0.006**	−0.361	**<0.001**	−0.203	**0.041**
ApoB (g/L)	0.161	0.105	0.031	0.760	0.186	0.061
Lp(a) (mg/L)	−0.058	0.689	0.035	0.810	−0.069	0.633
AIP	0.385	**<0.001**	0.501	**<0.001**	0.465	**<0.001**
BAR	0.348	**<0.001**	0.287	**0.004**	0.327	**0.001**
AC	0.256	**0.009**	0.121	0.233	0.275	**0.005**
LDL-C/HDL-C	0.247	0.012	0.123	0.224	0.282	0.004
LDL-C/ApoB	−0.059	0.554	−0.128	0.206	0.022	0.823
HDL-C/ApoA1	−0.050	0.615	0.056	0.584	−0.009	0.929
Non-HDL-C (mmol/L)	0.109	0.274	−0.057	0.577	0.185	0.062

Pearson correlation analyses were used. Bold values indicate statistical significance.

were performed by applying multiple stepwise linear regression, as shown in Tables 3–5. After adjusting for age, sex, smoking, xanthoma, MAP and FBG, the results showed that independent correlations with BMI were AIP (β = 0.020, P = 0.013) and BAR (β = 0.015, P = 0.003), AIP (β = 1.176, P = 0.001) independently associated with WHR, and AIP (β = 1.575, P = 0.001), BAR (β = 0.661, P = 0.024) and AC (β = 0.427, P = 0.035) independently associated with WHtR. It can be seen that overweight and obesity-related indicators BMI, WHR, and WHtR all had independent positive linear correlations with AIP.

Predictive performance analysis of cardiovascular risk predictors for overweight and obesity in patients with familial hypercholesterolemia

To further assess the role of AIP, BAR and AC in identifying overweight as well as central obesity conditions in FH patients, we plotted ROC curves, which showed that the area under the ROC (AUC) for overweight when AIP, BAR, AC and combined triple indicators were 0.695 (95% CI = 0.593–0.797, P = 0.001), 0.660 (95% CI = 0.555–0.766, P = 0.005), 0.632 (95% CI = 0.525–0.740, P = 0.021) and 0.710 (95% CI = 0.611–0.810, $P < 0.001$), respectively, as shown in Figure 3A; the AUCs for central obesity with AIP, BAR and AC and the combination of all three were 0.757 (95% CI = 0.656–0.857, $P < 0.001$), 0.654 (95% CI = 0.536–0.771, P = 0.012), 0.651 (95% CI = 0.538–0.764, P = 0.013) and 0.762 (95% CI = 0.666–0.858, $P < 0.001$), Figure 3B. It can be seen that AIP has the best predictive performance for overweight and obesity among cardiovascular risk predictors, while the area under the curve suggests the possibility that AIP its predictive performance for obesity is better than that for overweight; in addition, the combined AIP, BAR, and AC three indicators have a moderate predictive performance for overweight in FH patients.

Analysis of the identification performance of overweight and obesity-related indicators in familial hypercholesterolemia patients for moderate and high risk of atherogenic index of plasma

AIP is known to have the best predictive performance for overweight and obesity based on BMI, WHR and WHtR judgments, however, to explore which indicator is more accurate for identifying the risk level of AIP, the AUC was further used to compare the three overweight and obesity related indicators for identifying moderate risk of AIP and high risk of AIP, respectively, and the results showed that the AUC for BMI, WHR, and WHtR for moderate risk were 0.709 (95% CI = 0.608–0.811, $P < 0.001$), 0.773 (95% CI = 0.678–0.867, $P < 0.001$), and 0.739 (95% CI = 0.641–0.836, $P < 0.001$), respectively, as shown in Figure 4A; the AUCs of BMI, WHR, and WHtR for AUC for high risk of AIP were 0.691 (95% CI = 0.585–0.797, P = 0.002), 0.734 (95% CI = 0.632–0.835, $P < 0.001$), and 0.706 (95% CI = 0.603–0.810, P = 0.001), respectively, as shown in Figure 4B. It can be seen that the three overweight and obesity-related indicators BMI, WHR, and WHtR have good identification performance for both moderate and high risk of AIP, with WHR having the largest AUC, followed by WHtR, and BMI having the smallest. It is suggested that WHR has better and more robust performance in identifying moderate and high risk of AIP. As for the combined diagnostic effectiveness, the AUC of combining BMI, WHR, and WHtR was 0.782 (95% CI = 0.689–0.874, $P < 0.001$) for moderate risk of AIP and 0.749 (95% CI = 0.648–0.850, $P < 0.001$) for high risk of AIP. It showed that the combination of BMI, WHR, and WHtR had a moderate level of discrimination ability for moderate and high risk of AIP although the discrimination performance was not significantly improved compared to the individual indicators.

Discussion

In this study, by analyzing the correlation between cardiovascular risk predictors and overweight and obesity-related indicators in patients with FH, the results showed

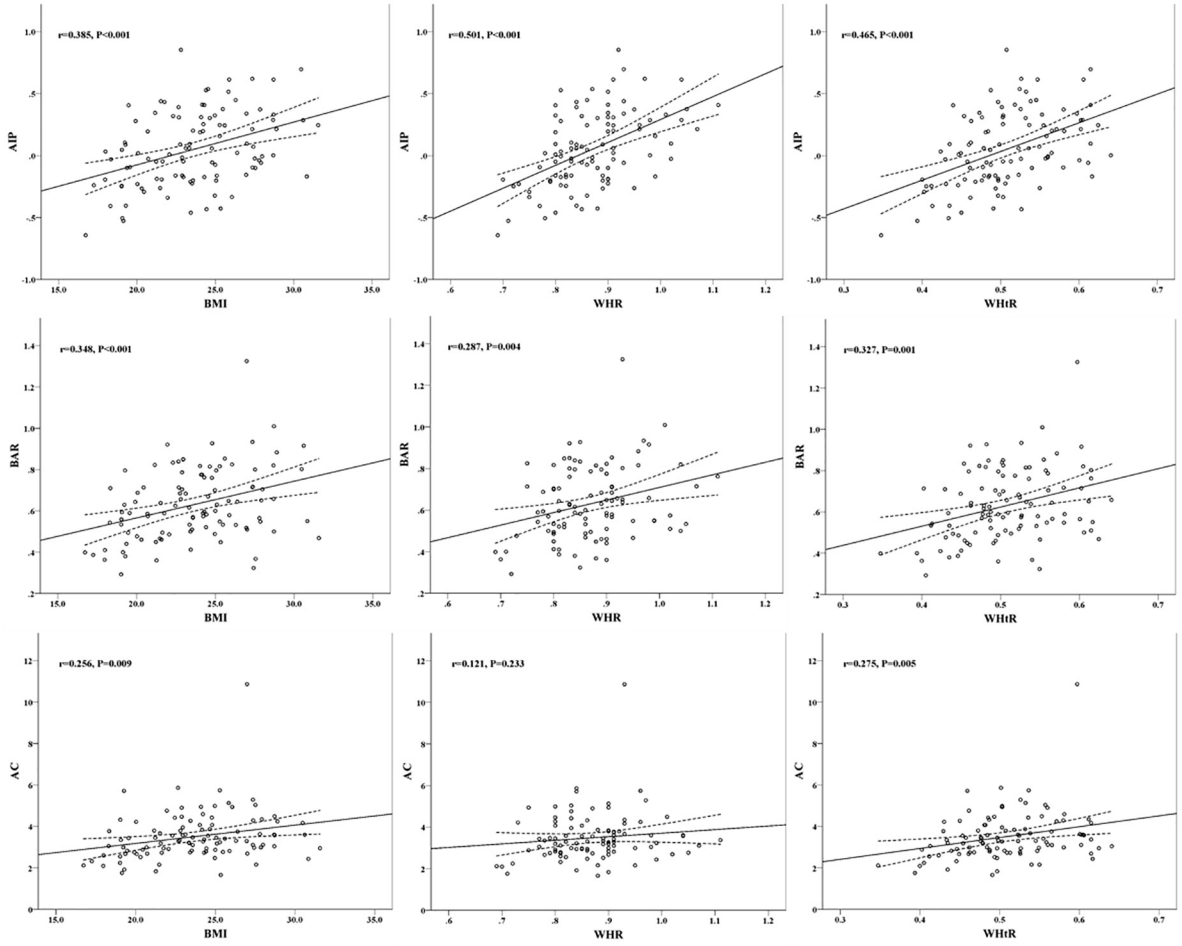


FIGURE 2
Scatter plots of correlation between overweight and obesity indicators and cardiovascular risk predictors.

TABLE 3 Independent correlation analysis of cardiovascular risk predictors with BMI.

	BMI (un-adjusted)			BMI (adjusted)		
	Constant	β	*P*	Constant	β	*P*
AIP	−0.765	0.035	<**0.001**	−1.169	0.020	**0.013**
BAR	0.210	0.018	<**0.001**	0.138	0.015	**0.003**
AC	1.412	0.088	**0.009**	/[a]	/	/

Multivariable stepwise linear regression models are shown. Adjusted confounders included age, sex, smoking, xanthoma, MAP and FBG. Bold values indicate statistical significance. [a]"/"denotes no independent correlation.

TABLE 4 Independent correlation analysis of cardiovascular risk predictors with WHR.

	WHR (un-adjusted)			WHR (adjusted)		
	Constant	β	*P*	Constant	β	*P*
AIP	−1.557	1.848	<**0.001**	−1.692	1.176	**0.001**
BAR	0.105	0.605	**0.004**	/	/	/
AC	1.991	1.716	0.233	/	/	/

Multivariable stepwise linear regression models are shown. Adjusted confounders included age, sex, smoking, xanthoma, MAP and FBG. Bold values indicate statistical significance.

that AIP was independently associated with BMI, WHR and WHtR, BAR was independently associated with BMI and WHtR, and AC was independently associated with WHtR. Although independent correlations between AIP and BMI have been reported (33) and between BAR and waist circumference (34), up to now, in patients with FH, the present study is the first to report correlations regarding the group of cardiovascular risk predictors in patients with FH with the group of overweight and obesity-related indicators. The significance of this study is that (1) by analyzing the correlation between cardiovascular risk predictors and overweight and obesity-related indicators in patients with

TABLE 5 Independent correlation analysis of cardiovascular risk predictors with WHtR.

	WHtR (un-adjusted)			WHtR (adjusted)		
	Constant	β	*P*	Constant	β	*P*
AIP	−1.124	2.314	**<0.001**	−0.967	1.575	**0.001**
BAR	0.160	0.927	**0.001**	0.046	0.661	**0.024**
AC	0.841	5.244	**0.005**	1.288	0.427	**0.035**

Multivariable stepwise linear regression models are shown. Adjusted confounders included age, sex, smoking, xanthoma, MAP and FBG. Bold values indicate statistical significance.

FH families, it provides a theoretical basis for actively controlling overweight and obesity-related indicators in FH patients and thus reducing CVD risk, which has important public health implications; (2) among cardiovascular risk predictors, AIP was found to have the strongest predictive effect on overweight, especially central obesity, which provides a basis for identifying overweight, especially obesity, through cardiovascular risk predictors.

AIP is a more comprehensive indicator of the balanced relationship between atheroprotective and atherogenic factors than a simple lipid index. The results of this study also showed that AIP among cardiovascular risk predictors has a better performance than BAR and AC both in terms of independent correlation with overweight and obesity-related indicators and in terms of identification of overweight and obesity. In fact, Shen et al. have reported that AIP can be a valid indicator for the assessment of abdominal obesity (26), and a recent cross-sectional study from a Chinese population also concluded that AIP was a novel and good biomarker associated with abdominal obesity (35). This is consistent with the results of the ROC curve analysis in the present study. Given the superiority of AIP over lipid indices alone, coupled with the fact that it can be easily calculated from conventional lipid profiles, AIP has gradually been increasingly favored and used in clinical practice to guide the assessment of CVD risk and disease prognosis in recent years (16, 13, 36–38). Therefore, it is reasonable to assume that CVD risk is further elevated in those obese populations in FH patients by the analysis of this study. As for the study on AIP in FH, Tomáš Freiberger's team compared the levels of lipid-related parameters between FH patients with and without a history of CVD and found that AIP and TG were significantly higher in those with CVD events in FH, concluding that AIP is associated with a history of CVD in FH patients, which reflects the atherosclerotic small LDL and small HDL particles presence, which may be associated with the risk of CVD in FH patients (34). The results of the present study showed significantly higher TG and AIP as well as significantly lower HDL-C in overweight individuals with FH, suggesting an imbalance between atherogenic and anti-atherogenic factors. Moreover, in further multifactorial

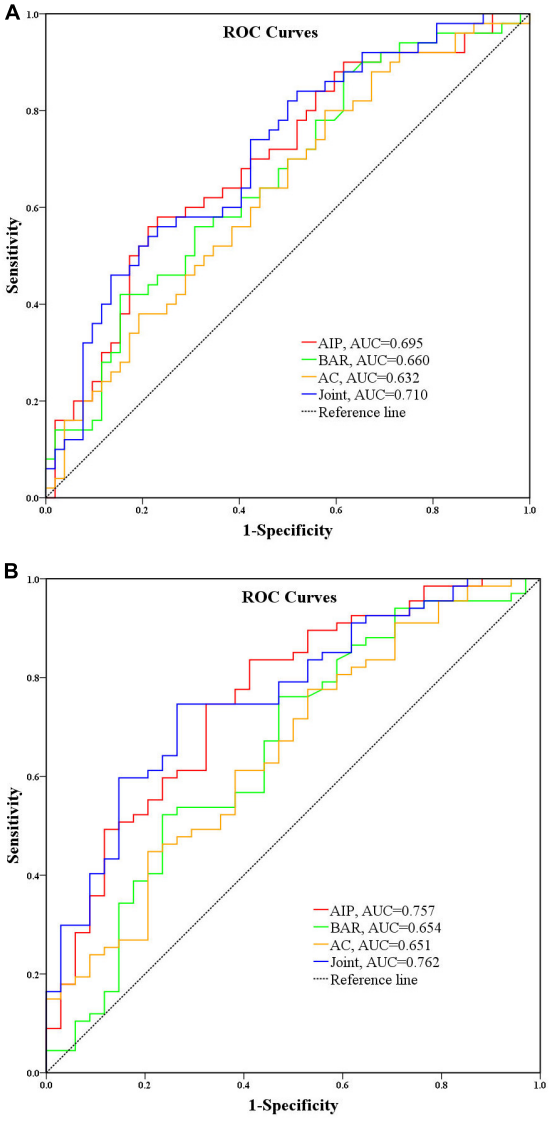


FIGURE 3
(A) Predictive performance analysis of AIP, BAR, and AC for overweight. **(B)** Predictive performance analysis of AIP, BAR, and AC for central obesity.

regression analysis in this study, BMI was independently associated with AIP, and BMI was an independent risk factor for increased risk level of AIP, suggesting that overweight patients with FH are at higher risk of CVD (33). It is well known that the main clinical manifestations of FH patients are significantly elevated atherogenic lipid indicators LDL-C and TC, and CVD events are the main cause of death in FH patients, and if BMI, an indicator associated with overweight and obesity, is not effectively controlled in these patients, it will add to their high CVD risk.

BAR represents the balance between ApoB-rich atherogenic particles and ApoA1-rich anti-atherogenic particles (17, 39) and

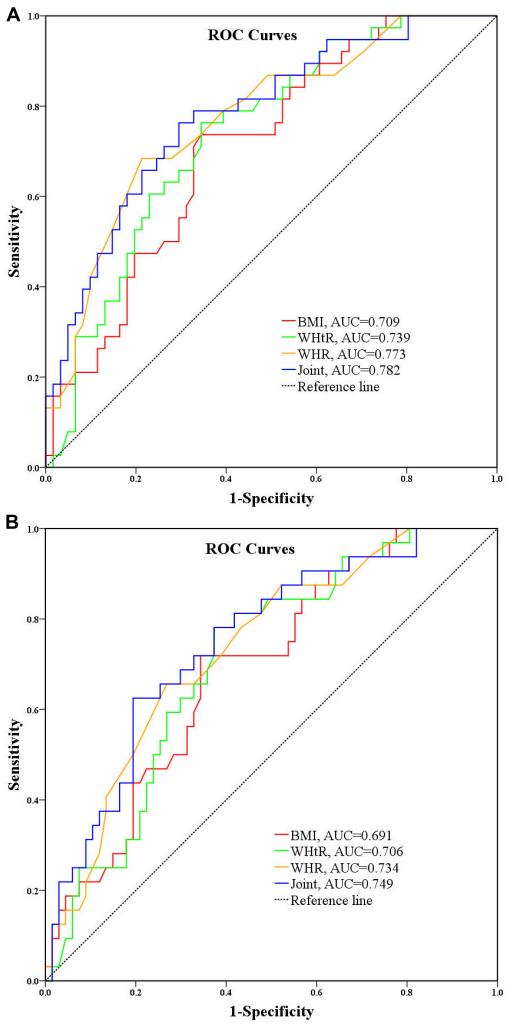


FIGURE 4
(A) Performance analysis of BMI, WHR, and WHtR for identifying moderate risk of AIP. **(B)** Performance analysis of BMI, WHR, and WHtR for identifying high risk of AIP.

is also considered as a potential marker of cardiovascular risk due to the fact that this ratio can often be abnormal in the presence of normal conventional lipid levels (21). It is generally accepted that a BAR above 0.9 is associated with a high risk of CVD (40), along with higher TG levels, AIP values and lower HDL-C levels (17). Showing that BAR was significantly and positively correlated with AIP, which is also consistent with what we observed in our results, some studies have also pointed out that AIP reflects the qualitative composition of lipoproteins, while BAR shows their quantity. Since they have different but complementary emphases, we suspect that they are intrinsically linked and hypothesize that there should be consistency in the manifestation of some diseases, and that combining these two indices to predict certain diseases may be promising. Currently, although the relationship between BMI and BAR is unclear, the results of some studies suggest that there may be an intrinsic association between BMI and BAR. A cohort study from China showed that both BMI and BAR were significantly elevated in patients with lactinoma (41), suggesting that there may be some intrinsic association between BMI and BAR in the disease state. Consistent with this, the results of the present study showed that BMI was independently associated with BAR in FH patients, suggesting that BMI is an independent influence on the elevated BAR in FH patients, and the present study also found that BAR indicators were significantly higher in overweight individuals than in non-overweight individuals, suggesting that overweight factors further increase the risk of CVD events in FH patients.

Several other lipid-related parameters, including non-HDL-C (42), AC (12), LDL-C/HDL-C (43), LDL-C/ApoB ratio (44, 45), and HDL-C/ApoA1 ratio (46, 47), have also been reported to be associated with CVD risk. However, the results of the current study did not show an independent correlation between BMI and these indicators. Although the results of the present study showed a significant correlation between BMI and LDL-C/HDL-C and AC in patients with FH, the correlation between them was found to disappear after adjusting for confounding factors.

It is generally accepted that age, male and blood pressure are important risk factors for cardiovascular events, and this is also true in patients with FH. Consistently, the results of the current study also showed that AIP was significantly correlated with age, male and MAP in addition to BMI independently, suggesting a higher risk of CVD in men with FH than in women, and the possibility that blood pressure is also a risk factor for CVD in patients with FH (27, 36, 48).

The results of this study showed that both AIP and BAR had significant independent correlations for BMI. However, by plotting ROC curves, it was shown that AIP was slightly better than BAR in predicting overweight. Our results also showed that cardiovascular risk predictors AIP, BAR, and AC were all independently correlated with WHtR among overweight and obesity-related indicators, but comparative analysis of ROC curves revealed that AIP was the strongest identifier of central obesity among the three cardiovascular risk predictors. Consistent with this result, one study found that AIP was significantly associated with BMI but not BAR by analyzing changes in cardiometabolic markers in overweight/obese children before and after lifestyle interventions; their results also showed that AIP was strongly associated with obesity, whereas BAR was not significantly associated with obesity (49). Although AIP is a calculated value, it is a sensitive indicator of dyslipidemia and may indirectly reflect the diameter of LDL-C particles (50). Therefore, we hypothesized that the combination of BMI and AIP could increase the specificity and sensitivity of overweight and even obesity detection in clinical practice. From Shen et al. showed that an AIP of 0.11–0.21 or > 0.21 suggested the possibility of borderline abdominal obesity or

abdominal obesity, respectively, by examining the relationship between waist circumference and AIP, suggesting that AIP can be used as a reference for estimating abdominal obesity (26). Similarly, our results show a linear correlation between BMI and AIP, according to our derived mathematical expression for the relationship between AIP and BMI, an increase in BMI of 1.0 kg/m^2 causes an increase in AIP of 0.035, an AIP value of 0.110 when BMI is 25 kg/m^2, and an AIP $\geq$ 0.215 when BMI $\geq$ 28 kg/m^2, which is essentially consistent with BMI $\geq$ 25 and $\geq$ 28 kg/m^2 correspond to moderate risk ($\geq$ 0.11) and high risk ($\geq$ 0.21) for AIP, respectively, which also indicates that moderate risk AIP indicates overweight, while high risk AIP indicates the presence of obesity. WHtR $\geq$ 0.5 and/or WHR $\geq$ 0.9 in men and $\geq$ 0.85 in women are known to be considered centrally obese (30). According to the mathematical expression of the present study results AIP and WHR, AIP = 1.848WHR-1.557, bringing the AIP values of 0.11 and 0.24 into the formula, the resulting WHR values are 0.90 and 0.97, respectively, indicating that central obesity judged based on WHR corresponds to a moderate risk of AIP, and when WHR exceeds 0.97, patients with FH are at high risk of AIP. According to the mathematical expression of AIP and WHtR of the results of this study, AIP = 2.314WHtR-1.124, bringing the AIP values of 0.11 and 0.24 into the formula, the resulting WHtR values are 0.53 and 0.59, respectively, and it can be basically concluded that central obesity judged based on WHtR corresponds to moderate risk of AIP, and when WHtR exceeds 0.59 FH patients would have a high risk of AIP. Thus, it can be seen that if obesity judged based on BMI has a high risk of AIP, while central obesity judged based on WHtR and WHR has a moderate risk of AIP. On the other hand, the assessment of AIP risk based on BMI may be more sensitive than the assessment of AIP risk based on WHtR and WHR. However, the AIP risk level corresponding to obesity judged based on BMI and the AIP risk level corresponding to central obesity judged based on WHR combined with WHtR derived from this study contradict each other. In view of this, which of BMI, WHtR and WHR identifies the more reliable AIP risk, the present study again compared the identification of these three overweight and obesity-related indicators for intermediate AIP risk and high AIP risk, respectively, using AUC, and the results showed that WHR had the largest AUC, WHtR the second largest, and BMI the smallest for both intermediate AIP risk and high AIP risk. It is suggested that WHR may be a better and more robust identifier of overweight and obesity-related indicators for moderate and high risk of AIP.

However, there are still some shortcomings in this study: (1) Although our conclusions were obtained based on retrospective data analysis, the causal relationship between BMI and cardiovascular risk predictors such as AIP has not been clearly answered in this study, and deeper mechanisms based on genetic diagnosis need to be further explored. (2) With the accelerated urbanization in China, the increase of "small family" has made the collection of FH family cases more difficult. Although the sample size of this study is eligible for this small incidence genetic disease, a larger sample size study is still necessary to improve the robustness of the results and the reliability of the conclusions.

Conclusion

(1) Overweight and obesity-related indicators BMI, WHR and WHtR in FH patients all had independent positive linear correlations with AIP; (2) among cardiovascular risk predictors, AIP has better performance for predicting overweight and obesity; (3) overweight and obesity-related indicators had better performance in identifying both medium and high risk for AIP, among which WHR had the best performance in identifying medium and high risk for AIP in patients with FH.

Data availability statement

The data analyzed in this study is subject to the following licenses/restrictions: The data presented in this study are available on reasonable request from the corresponding author. Requests to access these datasets should be directed to JH, hejch@lzu.edu.cn.

Ethics statement

Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

JH: conceptualization, data curation, funding acquisition, project administration, resources, and supervision. YW: formal analysis, investigation, methodology, software, and writing—original draft. JH and YW: validation and writing—review and editing. Both authors contributed to the article and approved the submitted version.

Funding

This work was supported by the National Science and Technology Major Project of the ministry of Science and Technology of China during the "10th Five-Year Plan" (Grant no. 2002DA711A028-17) and the Natural Science Foundation of Gansu Province (Grant no. 1308RJZA218), China.

Acknowledgments

We would appreciate the families with FH for their positive contribution and willingness to participate.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

References

1. Benito-Vicente A, Uribe KB, Jebari S, Galicia-Garcia U, Ostolaza H, Martin C. Familial hypercholesterolemia: the most frequent cholesterol metabolism disorder caused disease. *Int J Mol Sci.* (2018) 19:3426. doi: 10.3390/ijms19113426

2. Hu P, Dharmayat KI, Stevens CAT, Sharabiani MTA, Jones RS, Watts GF, et al. Prevalence of familial hypercholesterolemia among the general population and patients with atherosclerotic cardiovascular disease: a systematic review and meta-analysis. *Circulation.* (2020) 141:1742–59. doi: 10.1161/circulationaha.119.044795

3. Kitahara H, Mori N, Saito Y, Nakayama T, Fujimoto Y, Kobayashi Y. Prevalence of achilles tendon xanthoma and familial hypercholesterolemia in patients with coronary artery disease undergoing percutaneous coronary intervention. *Heart Vessels.* (2019) 34:1595–9. doi: 10.1007/s00380-019-01400-6

4. Bell DA, Hooper AJ, Watts GF, Burnett JR. Mipomersen and other therapies for the treatment of severe familial hypercholesterolemia. *Vasc Health Risk Manag.* (2012) 8:651–9. doi: 10.2147/vhrm.S28581

5. Watts GF, Gidding S, Wierzbicki AS, Toth PP, Alonso R, Brown WV, et al. Integrated guidance on the care of familial hypercholesterolaemia from the international FH foundation. *Int J Cardiol.* (2014) 171:309–25. doi: 10.1016/j.ijcard.2013.11.025

6. De Backer G, Besseling J, Chapman J, Hovingh GK, Kastelein JJ, Kotseva K, et al. Prevalence and management of familial hypercholesterolaemia in coronary patients: an analysis of EUROASPIRE IV, a study of the European society of cardiology. *Atherosclerosis.* (2015) 241:169–75. doi: 10.1016/j.atherosclerosis.2015.04.809

7. Tomlinson, B, Hu M, Chow E. Current status of familial hypercholesterolemia in Chinese populations. *Curr Opin Lipidol.* (2019) 30:94–100. doi: 10.1097/MOL.0000000000000580

8. Daniels SR. How to identify children with familial hypercholesterolemia. *J Pediatr.* (2017) 183:2.

9. Alonso R, Mata P, Zambón D, Mata N, Fuentes-Jiménez F. Early diagnosis and treatment of familial hypercholesterolemia: improving patient outcomes. *Expert Rev Cardiovasc Ther.* (2013) 11:327–42.

10. Plana N, Rodríguez-borjabad C, Ibarretxe D, Ferré R, Feliu A, Caselles A, et al. Lipid and lipoprotein parameters for detection of familial hypercholesterolemia in childhood. The DECOPIN project. *Clin Investig Arterioscler.* (2018) 30:170–8. doi: 10.1016/j.arteri.2017.12.003

11. Cicero AFG, Fogacci F, Giovannini M, Bove M, Debellis G, Borghi C. Effect of quantitative and qualitative diet prescription on children behavior after diagnosis of heterozygous familial hypercholesterolemia. *Int J Cardiol.* (2019) 293:193–6. doi: 10.1016/j.ijcard.2019.05.069

12. Kalelioglu T, Genc A, Karamustafalioglu N, Emul M. Assessment of cardiovascular risk via atherogenic indices in patients with bipolar disorder manic episode and alterations with treatment. *Diabetes Metab Syndr.* (2017) 11(Suppl. 1):S473–5. doi: 10.1016/j.dsx.2017.03.038

13. Cao B, Fan Z, Zhang Y, Li T. Independent association of severity of obstructive sleep apnea with lipid metabolism of atherogenic index of plasma (AIP) and apoB/apoAI ratio. *Sleep Breath.* (2020) 24:1507–13. doi: 10.1007/s11325-020-02016-1

14. Niroumand S, Khajedaluee M, Khadem-Rezaiyan M, Abrishami M, Juya M, Khodaee G, et al. Atherogenic index of plasma (AIP): a marker of cardiovascular disease. *Med J Islam Repub Iran.* (2015) 29:240.

15. Dobiásová M, Frohlich J. The plasma parameter log (TG/HDL-C) as an atherogenic index: correlation with lipoprotein particle size and esterification rate in apoB-lipoprotein-depleted plasma (FER(HDL)). *Clin Biochem.* (2001) 34:583–8. doi: 10.1016/s0009-9120(01)00263-6

16. Dobiásová M, Frohlich J, Sedová M, Cheung MC, Brown BG. Cholesterol esterification and atherogenic index of plasma correlate with lipoprotein size and findings on coronary angiography. *J Lipid Res.* (2011) 52:566–71. doi: 10.1194/jlr.P011668

17. Kaneva AM, Potolitsyna NN, Bojko ER, Odland J. The apolipoprotein B/apolipoprotein A-I ratio as a potential marker of plasma atherogenicity. *Dis Markers.* (2015) 2015:591454. doi: 10.1155/2015/591454

18. Walldius G, Jungner I, Holme I, Aastveit AH, Kolar W, Steiner E. High apolipoprotein B, low apolipoprotein A-I, and improvement in the prediction of fatal myocardial infarction (AMORIS study): a prospective study. *Lancet.* (2001) 358:2026–33. doi: 10.1016/s0140-6736(01)07098-2

19. Lamarche B, Moorjani S, Lupien PJ, Cantin B, Bernard PM, Dagenais GR, et al. Apolipoprotein A-I and B levels and the risk of ischemic heart disease during a five-year follow-up of men in the Québec cardiovascular study. *Circulation.* (1996) 94:273–8. doi: 10.1161/01.cir.94.3.273

20. Long-Term Intervention with Pravastatin in Ischaemic Disease (Lipid) Study Group. Prevention of cardiovascular events and death with pravastatin in patients with coronary heart disease and a broad range of initial cholesterol levels. *N Engl J Med.* (1998) 339:1349–57. doi: 10.1056/nejm199811053391902

21. Walldius G, Jungner I. The apoB/apoA-I ratio: a strong, new risk factor for cardiovascular disease and a target for lipid-lowering therapy–a review of the evidence. *J Intern Med.* (2006) 259:493–519. doi: 10.1111/j.1365-2796.2006.01643.x

22. Lu M, Lu Q, Zhang Y, Tian G. ApoB/apoA1 is an effective predictor of coronary heart disease risk in overweight and obesity. *J Biomed Res.* (2011) 25:266–73. doi: 10.1016/s1674-8301(11)60036-5

23. Igharo OG, Akinfenwa Y, Isara AR, Idomeh FA, Nwobi NL, Anetor JI, et al. Lipid profile and atherogenic indices in nigerians occupationally exposed to e-waste: a cardiovascular risk assessment study. *Maedica.* (2020) 15:196–205. doi: 10.26574/maedica.2020.15.2.196

24. Afshin A, Forouzanfar MH, Reitsma MB, Sur P, Estep K, Lee A, et al. Health effects of overweight and obesity in 195 countries over 25 years. *N Engl J Med.* (2017) 377:13–27. doi: 10.1056/NEJMoa1614362

25. Matsuzawa Y. Establishment of a concept of visceral fat syndrome and discovery of adiponectin. *Proc Jpn Acad Ser B Phys Biol Sci.* (2010) 86:131–41. doi: 10.2183/pjab.86.131

26. Shen SW, Lu Y, Li F, Yang CJ, Feng YB, Li HW, et al. Atherogenic index of plasma is an effective index for estimating abdominal obesity. *Lipids Health Dis.* (2018) 17:11. doi: 10.1186/s12944-018-0656-1

27. National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). Third report of the national cholesterol education program (NCEP) expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (adult treatment panel III) final report. *Circulation.* (2002) 106:3143–421.

28. Nordestgaard BG, Chapman MJ, Humphries SE, Ginsberg HN, Masana L, Descamps OS, et al. Familial hypercholesterolaemia is underdiagnosed and undertreated in the general population: guidance for clinicians to prevent coronary heart disease: consensus statement of the European Atherosclerosis Society. *Eur Heart J.* (2013) 34:3478–90a. doi: 10.1093/eurheartj/eht273

29. WHO Expert Consultation. Appropriate body-mass index for Asian populations and its implications for policy and intervention strategies. *Lancet.* (2004) 363:157–63. doi: 10.1016/s0140-6736(03)15268-3

30. Browning LM, Hsieh SD, Ashwell M. A systematic review of waist-to-height ratio as a screening tool for the prediction of cardiovascular disease and diabetes: 0·5 could be a suitable global boundary value. *Nutr Res Rev.* (2010) 23:247–69. doi: 10.1017/s0954422410000144

31. Dobiásová M. Atherogenic index of plasma [log(triglycerides/HDL-cholesterol)]: theoretical and practical implications. *Clin Chem.* (2004) 50:1113–5. doi: 10.1373/clinchem.2004.033175

32. Sapunar J, Aguilar-Farías N, Navarro J, Araneda G, Chandía-Poblete D, Manríquez V, et al. [High prevalence of dyslipidemia and high atherogenic index of plasma in children and adolescents]. *Rev Med Chil.* (2018) 146:1112–22. doi: 10.4067/s0034-98872018001001112

33. Rašlová K, Dobiášová M, Hubáèek JA, Bencová D, Siváková D, Danková Z, et al. Association of metabolic and genetic factors with cholesterol esterification rate in HDL plasma and atherogenic index of plasma in a 40 years old Slovak population. *Physiol Res.* (2011) 60:785–95. doi: 10.33549/physiolres.932069

34. Soška V, Jarkovskı J, Ravèuková B, Tichı L, Fajkusová L, Freiberger T. The logarithm of the triglyceride/HDL-cholesterol ratio is related to the history of cardiovascular disease in patients with familial hypercholesterolemia. *Clin Biochem.* (2012) 45:96–100. doi: 10.1016/j.clinbiochem.2011.11.001

35. Zhu X, Yu L, Zhou H, Ma Q, Zhou X, Lei T, et al. Atherogenic index of plasma is a novel and better biomarker associated with obesity: a population-based cross-sectional study in China. *Lipids Health Dis.* (2018) 17:37. doi: 10.1186/s12944-018-0686-8

36. Tautu OF, Darabont R, Onciul S, Deaconu A, Comanescu I, Andrei RD, et al. New cardiovascular risk factors and their use for an accurate cardiovascular risk assessment in hypertensive patients. *Maedica.* (2014) 9:127–34.

37. Zhou K, Qin Z, Tian J, Cui K, Yan Y, Lyu S. The atherogenic index of plasma: a powerful and reliable predictor for coronary artery disease in patients with type 2 diabetes. *Angiology.* (2021) 72:934–41. doi: 10.1177/0003319721101 2129

38. Garg R, Knox N, Prasad S, Zinzuwadia S, Rech MA. The atherogenic index of plasma is independently associated with symptomatic carotid artery stenosis. *J Stroke Cerebrovasc Dis.* (2020) 29:105351. doi: 10.1016/j.jstrokecerebrovasdis.2020.105351

39. Thompson A, Danesh J. Associations between apolipoprotein B, apolipoprotein AI, the apolipoprotein B/AI ratio and coronary heart disease: a literature-based meta-analysis of prospective studies. *J Intern Med.* (2006) 259:481–92. doi: 10.1111/j.1365-2796.2006.01644.x

40. Walldius G, Jungner I. Apolipoprotein B and apolipoprotein A-I: risk indicators of coronary heart disease and targets for lipid-modifying therapy. *J Intern Med.* (2004) 255:188–205. doi: 10.1046/j.1365-2796.2003.01276.x

41. Jiang XB, He DS, Mao ZG, Fan X, Lei N, Hu B, et al. BMI, apolipoprotein B/apolipoprotein A-I ratio, and insulin resistance in patients with prolactinomas: a pilot study in a Chinese cohort. *Tumour Biol.* (2013) 34:1171–6. doi: 10.1007/s13277-013-0660-z

42. Brunner FJ, Waldeyer C, Ojeda F, Salomaa V, Kee F, Sans S, et al. Application of non-HDL cholesterol for population-based cardiovascular risk stratification: results from the multinational cardiovascular risk consortium. *Lancet.* (2019) 394:2173–83. doi: 10.1016/s0140-6736(19)32519-x

43. Nair D, Carrigan TP, Curtin RJ, Popovic ZB, Kuzmiak S, Schoenhagen P, et al. Association of total cholesterol/ high-density lipoprotein cholesterol ratio with proximal coronary atherosclerosis detected by multislice computed tomography. *Prev Cardiol.* (2009) 12:19–26. doi: 10.1111/j.1751-7141.2008.00011.x

44. Kaneva AM, Potolitsyna NN, Bojko ER. Usefulness of the LDL-C/apoB ratio in the overall evaluation of atherogenicity of lipid profile. *Arch Physiol Biochem.* (2017) 123:16–22. doi: 10.1080/13813455.2016.1195411

45. Chang TY, Chen JD. Low-density lipoprotein cholesterol/apolipoprotein B ratio is superior to apolipoprotein B alone in the diagnosis of coronary artery calcification. *Coron Artery Dis.* (2021) 32:561–6. doi: 10.1097/mca.0000000000001004

46. Mazer NA, Giulianini F, Paynter NP, Jordan P, Mora S. A comparison of the theoretical relationship between HDL size and the ratio of HDL cholesterol to apolipoprotein A-I with experimental results from the Women's health study. *Clin Chem.* (2013) 59:949–58. doi: 10.1373/clinchem.2012.196949

47. Jieni L, Yazhi X, Xiaorong Z, Dan L, Yusheng M, Jiahuan R, et al. Effect of renal function on high-density lipoprotein particles in patients with coronary heart disease. *BMC Cardiovasc Disord.* (2021) 21:534. doi: 10.1186/s12872-021-02354-2

48. Paquette M, Dufour R, Baass A. The montreal-FH-SCORE: a new score to predict cardiovascular events in familial hypercholesterolemia. *J Clin Lipidol.* (2017) 11:80–6. doi: 10.1016/j.jacl.2016.10.004

49. Vrablík M, Dobiášová M, Zlatohlávek L, Urbanová Z, Èeška R. Biomarkers of cardiometabolic risk in obese/overweight children: effect of lifestyle intervention. *Physiol Res.* (2014) 63:743–52. doi: 10.33549/physiolres.932895

50. Dobiásová M. [AIP–atherogenic index of plasma as a significant predictor of cardiovascular risk: from research to practice]. *Vnitr Lek.* (2006) 52:64–71.

frontiers | Frontiers in Cardiovascular Medicine

TYPE Original Research
PUBLISHED 04 January 2023
DOI 10.3389/fcvm.2022.960419

OPEN ACCESS

EDITED BY
Chu-Huang Chen,
Texas Heart Institute, United States

REVIEWED BY
Nardos Abebe,
Eötvös Loránd Research Network (ELKH) ELKH-DE Public Health Research Group, Hungary
Robert Hegele,
Western University, Canada

*CORRESPONDENCE
Szilard Voros
szilard.voros@globalgenomicsgroup.com

SPECIALTY SECTION
This article was submitted to Lipids in Cardiovascular Disease, a section of the journal Frontiers in Cardiovascular Medicine

RECEIVED 02 June 2022
ACCEPTED 23 November 2022
PUBLISHED 04 January 2023

CITATION
Voros S, Bansal AT, Barnes MR, Narula J, Maurovich-Horvat P, Vazquez G, Marvasty IB, Brown BO, Voros ID, Harris W, Voros V, Dayspring T, Neff D, Greenfield A, Furchtgott L, Church B, Runge K, Khalil I, Hayete B, Lucero D, Remaley AT and Newton RS (2023) Bayesian network analysis of panomic biological big data identifies the importance of triglyceride-rich LDL in atherosclerosis development. *Front. Cardiovasc. Med.* 9:960419. doi: 10.3389/fcvm.2022.960419

Bayesian network analysis of panomic biological big data identifies the importance of triglyceride-rich LDL in atherosclerosis development

Szilard Voros[1]*, Aruna T. Bansal[2], Michael R. Barnes[2], Jagat Narula[3], Pal Maurovich-Horvat[4], Gustavo Vazquez[5], Idean B. Marvasty[1], Bradley O. Brown[1], Isaac D. Voros[1], William Harris[6], Viktor Voros[1,7], Thomas Dayspring[5], David Neff[1], Alex Greenfield[8], Leon Furchtgott[8], Bruce Church[8], Karl Runge[8], Iya Khalil[8], Boris Hayete[8], Diego Lucero[9], Alan T. Remaley[9] and Roger S. Newton[1]

[1]Global Genomics Group, Atlanta, GA, United States, [2]Acclarogen, Ltd, Cambridge, United Kingdom, [3]Mount Sinai School of Medicine, New York, NY, United States, [4]MTA-SE Cardiovascular Imaging Research Group, Heart and Vascular Center, Semmelweis University, Budapest, Hungary, [5]Global Institute for Research, LLC, Richmond, VA, United States, [6]OmegaQuant, Sioux Falls, SD, United States, [7]Department of Psychiatry, Medical School, University of Pécs, Pécs, Hungary, [8]GNS Healthcare, Somerville, MA, United States, [9]Lipoprotein Metabolism Laboratory, National Heart, Lung and Blood Institute, National Institutes of Health, Bethesda, MD, United States

Introduction: We sought to explore biomarkers of coronary atherosclerosis in an unbiased fashion.

Methods: We analyzed 665 patients (mean ± SD age, 56 ± 11 years; 47% male) from the GLOBAL clinical study (NCT01738828). Cases were defined by the presence of any discernable atherosclerotic plaque based on comprehensive cardiac computed tomography (CT). De novo Bayesian networks built out of 37,000 molecular measurements and 99 conventional biomarkers per patient examined the potential causality of specific biomarkers.

Results: Most highly ranked biomarkers by gradient boosting were interleukin-6, symmetric dimethylarginine, LDL-triglycerides [LDL-TG], apolipoprotein B48, palmitoleic acid, small dense LDL, alkaline phosphatase, and asymmetric dimethylarginine. In Bayesian analysis, LDL-TG was directly linked to atherosclerosis in over 95% of the ensembles. Genetic variants in the genomic region encoding hepatic lipase (LIPC) were associated with LIPC gene expression, LDL-TG levels and with atherosclerosis.

Discussion: Triglyceride-rich LDL particles, which can now be routinely measured with a direct homogenous assay, may play an important role in atherosclerosis development.

Clinical trial registration: GLOBAL clinical study (Genetic Loci and the Burden of Atherosclerotic Lesions); [https://clinicaltrials.gov/ct2/show/NCT01738828?term=NCT01738828&rank=1], identifier [NCT01738828].

KEYWORDS

triglyceride-rich LDL, LDL-triglycerides, cardiovascular risk, Bayesian network analysis, omics, hepatic lipase

Highlights

- In our Bayesian analysis, LDL-TG was directly upstream from atherosclerosis.
- LDL-TG was associated with atherosclerosis independently of well-known factors.
- Hepatic lipase's genetic variants correlated with LDL-TG levels and atherosclerosis
- LDL-TG was positively linked to triglycerides, sd-LDL, and inflammatory markers.

Introduction

Cardiovascular disease remains the leading cause of mortality and morbidity worldwide (1). Acute manifestations of coronary artery disease (CAD) are caused by at least three relevant biological processes: underlying coronary arterial atherosclerosis that develops over decades (2), acute plaque rupture/erosion (3) followed by coronary arterial thrombosis (4). Many long-term studies of cardiovascular outcomes have identified low-density lipoprotein cholesterol (LDL-C) and apolipoprotein-B (Apo-B) as key causal risk factors for cardiovascular events (5, 6).

Such long-term cardiovascular outcomes studies are very helpful in establishing clinically relevant risk factors that can be monitored and modified in clinical practice, such as LDL-C and Apo-B. A limitation of the current cardiovascular biomarker studies is that they primarily rely on clinical events, which is a combination of the three underlying biological processes with different time scales, namely atherogenesis, plaque rupture/erosion and thrombosis. As a consequence, the current cardiovascular biomarker studies do not efficiently identify and discriminate which of these three specific biological processes is associated with and causally linked to a risk factor.

Non-invasive coronary arterial imaging with cardiac CT presents a unique opportunity to isolate causal factors of atherosclerosis *per se*. Accordingly, we designed a nested case-control analysis within the Genetic Loci and the Burden of Atherosclerotic Lesions (GLOBAL) clinical study (ClinicalTrials.gov number NCT01738828) (7) to identify additional causal factors. We used *de novo* Bayesian network analysis, a hypothesis-free approach (8), to enrich for associations with risk of CT for causal relevance to the development of atherosclerosis. In order to examine causal relevance of relationships among the multi-modal covariates of CAD (genetics, gene expression, proteomics, etc.), we used a specific technique successfully employed to infer biological pathways from steady-state cross-sectional data, namely Bayesian belief networks (8, 9). In addition, our network analysis incorporated whole genome sequencing data and other data modalities to avoid latent confounding and to study potential causal biomarkers revealed by Bayesian network analysis.

Materials and methods

Patients

The analyses for the present study were performed in a subgroup from the Genetic Loci and the Burden of Atherosclerotic Lesions (GLOBAL) multicentric clinical study (ClinicalTrials.gov number NCT01738828). The present nested Case-Control study was performed in the pre-specified Pilot Discovery (340 patients) and Pilot Validation (340 patients) cohorts, which, in combination, included 680 patients. Entirely complete clinical, imaging, multiomic and genetic data with zero missingness that is required to build the integrated data frame for Bayesian analysis was available in 665 patients. Of these, 317 subjects had no discernable atherosclerosis on comprehensive CT and were therefore designated as "Controls" and 348 subjects had discernable plaque on CT and were designated as "Cases." The GLOBAL study included subjects of 18–90 years of age and self-referred as Caucasian, with the indication of or undergoing coronary computerized tomography (CT). Subjects under immunosuppressive or immunomodulatory therapy or chemotherapy were excluded from the study. Those with major surgery and blood transfusion within the last two months, contraindicated CT, or preexisting cardiac affections were also excluded from the study. Blood draw for all blood-based biomarker analysis, "omics" testing and genetic testing was performed at the time of the CT imaging procedure. For further details about the GLOBAL study design, please go to Voros et al. (7). The study was conducted according to the criteria

set by the declaration of Helsinki and all included subjects signed informed consent for the use of genetic material for research purposes. The study was approved by institutional review boards and ethics committee as appropriate. Cardiac CT was evaluated as previously described (7). Subjects with any evidence of atherosclerotic plaque in coronary CT were considered cases, and those without, controls. Peripheral blood samples were obtained from enrolled subjects, and plasma, serum, whole blood, and buffy coat were adequately stored for further analysis.

Data analysis approach

In order to examine causal relevance of relationships among the multi-modal covariates of CAD (genetics, gene expression, proteomics, etc.), we used Bayesian belief networks (8, 9). Although Bayesian Belief networks may not always be able to establish a unique relationship between covariates, we relied on Markov equivalence to take advantage of additional information in order to break synonymous probabilistic relationships. This approach can take the form of intentional perturbations (8) or of genetic constraints, the use of which in Bayesian networks permits analysis that is statistically related to mendelian randomization (10). Furthermore, we investigated our networks of causally enriched probabilistic relationships by means of per-patient counterfactual simulations (11), where the genetic constraints played a role similar to that of instruments in instrumental variable analysis. In addition, our networks incorporated our whole genome sequencing data and other data modalities to avoid latent confounding and to study potential causal biomarkers revealed by Bayesian network analysis.

Detailed sample size calculations for Bayesian network analysis

Tanner and Donoho have pioneered a compressed sensing approach which bounds inferable complexity given available data and assumed sparseness (12). In their simulations, for example, if number of samples, n = 300, number of useful predictors, k, is 3 on average, and number of variables, p, is 100,000 (as in our case), the x-axis – delta – in **Figure 1** is n/p = 300/100000 ~ 0, the worst possible case, but the y-axis – rho – is k/n = 3/300 ~ 0 < < 0.15. While the specific numbers do not map to our problem domain, they illustrate that statistical inference depends on k, n, and p, and in our case is expected to be very hard. In order to ensure that we do not suffer from overfitting, we have applied a number of priors, as documented in the manuscript. In particular, these include the probability of the local model (modeled by BIC, or penalized likelihood) multiplied by the prior probability of the model of a given complexity and has been described by us in the supplement to a prior work (13). In the case of a single class (e.g., only gene expression), the total overall penalty simplifies to E-BIC with gamma = 1/2, or simply BIC + log(| S|), where | S| is the number of all possible models (network fragments) of the same size as S. When multiple data types are present, our incremental penalty for adding a term of class C to a model is defined as deltaBIC + log(| C|) + log(| S_c|), where deltaBIC is the change in BIC due to this addition, | C| is the number of classes, and | S_c| is the number of elements in class C. Effectively, this formula computes E-BIC subject to the Bayesian belief that all classes are equally informative *a priori*, before any data is seen, thus penalizing large classes, e.g., genetics, more than small ones, e.g., clinical data. Subject to this regularization strategy, the network's default state is to be fully disconnected, and it can only become connected through the preponderance of evidence that overcomes these two penalties. Further, the use of large model ensembles makes it virtually impossible that the network overtrains systematically; in a way that would be repeatable in simulations. Any overtraining would be diluted by the entropy of the ensemble. Performing simulations on a per-network basis and averaging their predictions allows us to shrink the overall standard error of the estimate by the aforementioned dilution of errors. This property of ensemble methods is well-studied and has been reflected in a number of popular approaches to classification and regression, as described in the manuscripts cited above.

Conventional biomarker analysis

A panel of conventional biomarkers were evaluated using commercially available kits and reagents, as listed in **Supplementary Table 2**.

Isolation of genomic deoxyribonucleic acid

Isolation of genomic DNA was performed using the QIAamp DNA Blood Midi Kit (Qiagen part no. 51185). Starting with 0.3–1 ml of whole blood, a lysis buffer and protease were added to each sample for cell lysis. After lysis, the lysate was loaded onto a QIAamp spin column. DNA remained bound to the QIAamp membrane, while impurities were washed away in 2 vacuum steps. Upon drying the membrane, DNA was eluted in 200 µl of elution buffer. The yield of genomic DNA was subsequently determined by PicoGreen quantitation or by using the Qubit fluorometer.

Whole genome sequencing (Illumina Service Laboratory)

Whole-genome sequencing was performed by the Illumina Service Laboratory.

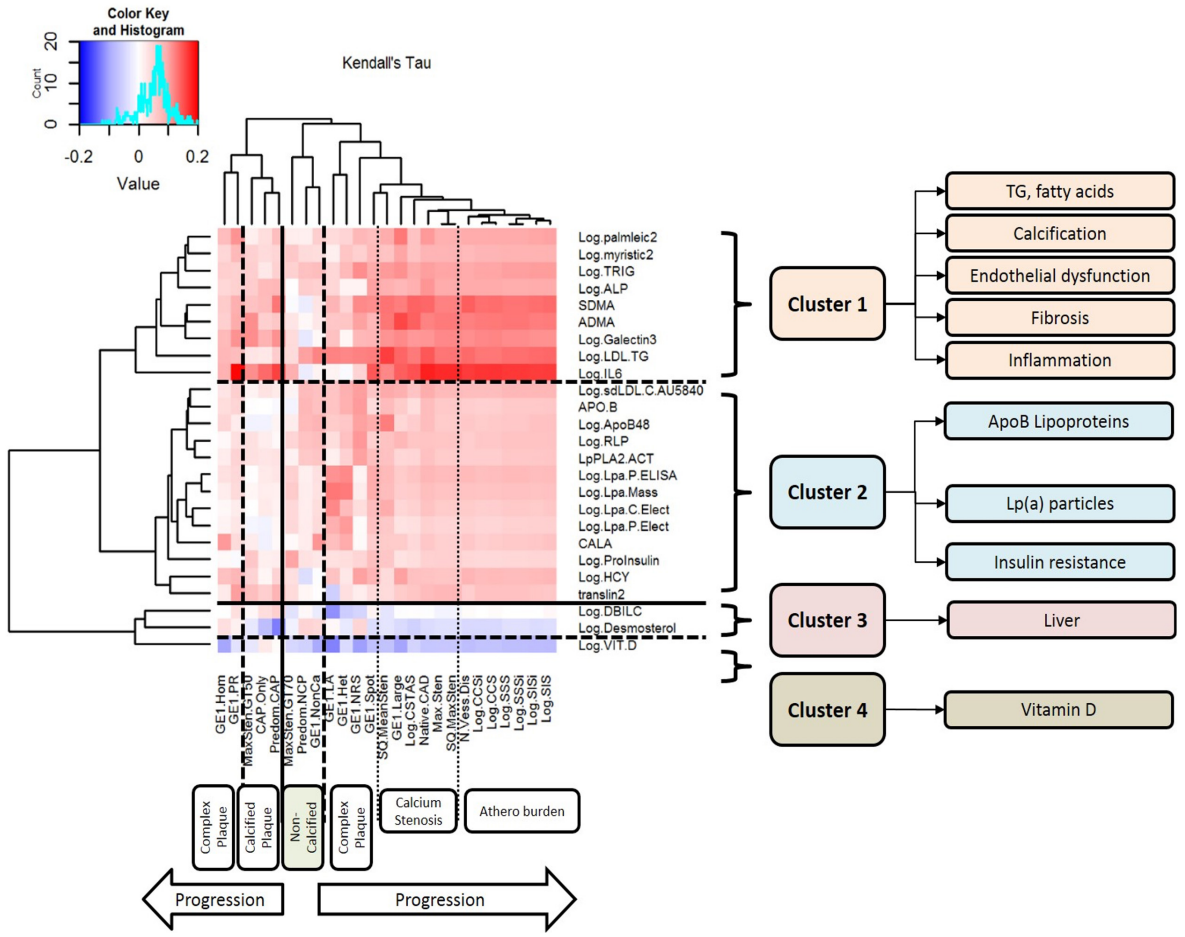


FIGURE 1
Clusters of circulating biomarkers against CT measures of atherosclerotic plaque, stenosis, and disease burden. This figure displays the results of unsupervised hierarchical clustering of circulating biomarkers against CT measures of atherosclerotic plaque, stenosis, and disease burden. Each row represents a biomarker that was nominally associated with atherosclerosis, and each column represents a CT measurement of atherosclerosis. The dendrograms on both axes show the results of hierarchical cluster analysis. Inside the heat map, positive correlations are shown in red and negative correlations are shown in blue; the intensity of the color represents the strength of association, as quantified by Kendall's tau. There are six major clusters of CT measurements of atherosclerosis: complex plaque, calcified plaque, non-calcified plaque, calcification/stenosis, disease burden, and a second cluster of complex plaque. There are four clusters of circulating biomarkers with different patterns of association with different measures of atherosclerosis: Cluster 1 includes triglycerides, fatty acids, calcification, endothelial dysfunction, fibrosis, and inflammation. LDL-TG is strongly associated with early-stage, non-calcified plaque and complex plaque, and IL-6 is strongly associated with later-stage, calcified plaque and atherosclerosis burden. Cluster 2 includes ApoB-containing lipoproteins, lipoprotein (a), and biomarkers of insulin resistance; Cluster 3 includes hepatic biomarkers and markers of hepatic cholesterol synthesis; and Cluster 4 contains vitamin D alone.

Genomic deoxyribonucleic acid quantitation

Genomic DNA was quantified prior to library construction using PicoGreen (Quant-iT^{TM} PicoGreen® dsDNA Reagent, Invitrogen, Catalog #: P11496). Quants were read with Spectromax Gemini XPS (Molecular Devices).

Library construction – Polymerase chain reaction-free

Paired-end libraries were manually generated from 500 ng to 1 μg of genomic DNA using the Illumina TruSeq DNA Sample Preparation Kit (Catalog #: FC-121-2001), based on the protocol in the TruSeq DNA PCR-free Sample Preparation Guide. Prefragmentation genomic DNA cleanup was performed using paramagnetic sample purification beads (Agencourt® AMPure® XP reagents, Beckman Coulter). Samples were fragmented and libraries were size-selected following fragmentation and end-repair using paramagnetic sample purification beads, targeting 300 bp inserts. Final libraries were quality controlled for size using a gel electrophoretic separation system and were quantified.

Clustering and sequencing – v3 chemistry

Following library quantitation, DNA libraries were denatured, diluted, and clustered onto v3 flow cells using the

Illumina cBot™ system. cBot runs were performed based on the cBot User Guide, using the reagents provided in Illumina TruSeq Cluster Kit v3. Clustered v3 flow cells were loaded onto HiSeq 2000 instruments and sequenced on 100 bp paired-end, non-indexed runs. All samples were sequenced on independent lanes. Sequencing runs were performed based on the HiSeq 2000 User Guide, using Illumina TruSeq SBS v3 Reagents. Illumina HiSeq Control Software and Real-time Analysis were used on HiSeq 2000 sequencing runs for real-time image analysis and base calling.

Genotyping

Samples were processed using Infinium chemistry, based on the Infinium LCG Assay Guide, and run on the HumanOmni2.5-8 array. Resulting intensity.idat files were loaded into GenomeStudio® software to export genotyping calls.

Ribonucleic acid isolation from PAXGene tubes

RNA isolation was completed using the PAXgene Blood miRNA Kit (Qiagen, Venlo, The Netherlands). PAXgene Blood RNA Tubes were first centrifuged to pellet the samples, then washed with water and resuspended. After digestion with proteinase K, the samples were homogenized by centrifugation through PAXgene Shredder spin columns. Isopropanol was added to the samples to optimize binding conditions, and the samples were then centrifuged through PAXgene RNA spin columns, where total RNA >18 nucleotides (including miRNA) was bound to the silica membrane. The bound RNA was treated with DNase to remove genomic DNA contamination and washed. Pure RNA was then eluted.

Small ribonucleic acid sequencing methods and materials

Libraries were prepared for small RNA sequencing using the TruSeq Small RNA Sample Prep Kit (Illumina). Prior to library preparation, RNA samples were quantitated by spectrophotometry using a Nanodrop ND-8000 spectrophotometer and assessed for RNA integrity using an Agilent 2100 BioAnalyzer or Caliper LabChip GX. RNA samples with A260/A280 ratios ranging from 1.6 to 2.2, with RNA integrity number values ≥7.0, and for which at least 1,000 ng of total RNA was available proceeded to library preparation. Total RNA samples must have been prepared using extraction chemistry that does not exclude small RNA species (e.g., the QIAGEN miRNeasy Kit).

Library preparation began with 1,000 ng of total RNA in 5 μl of nuclease-free water, to which an adapter oligonucleotide was added that was then ligated to the 3′ hydroxyl present on miRNA species using T4 RNA ligase (New England Biolabs). Similarly, a different adapter sequence was ligated to the 5′ end of RNAs that possessed a 5′ phosphate, in order to create a single-stranded molecule with defined sequences at both the 5′ and 3′ ends. This molecule was reverse-transcribed and amplified using 14 cycles of PCR with primers that include sequences complementary to the 5′ and 3′ adapter sequences, a specific index sequence, and Illumina sequencing adapter sequences. The resulting product was analyzed using an Agilent 2100 BioAnalyzer, and the molar amount of mature miRNA present in the library was estimated by integrating the area under the curve in the 145–160 bp range. Individual libraries were mixed to create multiplexed pools, and the mixture was purified by gel electrophoresis, wherein the 145–160 bp range was excised from the gel, crushed using a Gel Breaker tube (IST Engineering), eluted into nuclease-free water, and concentrated by precipitation with ethanol. The concentration of the final library pool was determined using PicoGreen (Invitrogen), and the size distribution of the pool was determined using an Agilent 2100 BioAnalyzer. Library pools were normalized to 2 nM in preparation for sequencing.

mRNA sequencing

Prior to library preparation, alpha and beta globin mRNA was reduced using the GLOBINclear™-Human Kit (Life Technologies, Carlsbad, CA), following the manufacturers protocol. Total RNA samples were converted into cDNA libraries using the TruSeq Stranded mRNA Sample Prep Kit (Illumina, #RS-122-2103). Starting with 100 ng of total RNA, polyadenylated RNA (primarily mRNA) was selected and purified using oligo-dT conjugated magnetic beads. This mRNA was chemically fragmented and converted into single-stranded cDNA using reverse transcriptase and random hexamer primers, with the addition of Actinomycin D to suppress DNA-dependent synthesis of the second strand. Double-stranded cDNA was created by removing the RNA template and synthesizing the second strand in the presence of dUTP instead of dTTP. A single A base was added to the 3′ end to facilitate ligation of sequencing adapters, which contained a single T base overhang. Adapter-ligated cDNA was amplified by polymerase chain reaction to increase the amount of sequence-ready library. During this amplification, the polymerase stalls when it encounters a U base, rendering the second strand a poor template. Accordingly, amplified material used the first strand as a template, thereby preserving the strand information. Final cDNA libraries were analyzed for size distribution using an Agilent BioAnalyzer (DNA 1000 Kit, Agilent #5067-1504), quantitated by qPCR (KAPA Library Quant Kit, KAPA Biosystems #KK4824), and then normalized to 2 nM in preparation for sequencing.

Mass-spectrometry–based proteomics methods

We performed proteomics discovery experiments in 2 stages; the first stage was performed using non-targeted mass spectrometry, followed by the second stage of targeted mass spectrometry using multiple reaction monitoring.

Discovery experiments using non-targeted mass spectrometry

Samples were processed essentially as described previously (14). Briefly, each 30 μl sample was depleted of high abundance proteins using an affinity resin (IgY14/Supermix, Sigma). All columns were prepared with the same manufacturing batch of affinity resin and tested for consistent performance prior to use. Control samples, consisting of aliquots of a pooled human plasma sample, were inserted at the start, middle, and end of each set of 20 paired study samples, resulting in a batch size of 23. After depletion, samples were frozen, freeze-dried, digested with trypsin (1:10, w:w, Promega), and desalted on Empore C18 plates (3M Bioanalytical Technologies). Resulting peptides were separated by strong cation exchange (SCX, Waters) chromatography into 6 fractions with a linear salt gradient and desalted on Oasis HLB plates (Waters). Samples were distributed into two 96-well plates (one test plate and one backup plate). Samples were then dried and resuspended in 96.25/3.75 (v/v) water/acetonitrile and 0.1% formic acid, containing 19 internal standard peptides. Mass spectrometry analysis was performed by nanoflow reversed phase liquid chromatography (NanoAcquity UPLC, Waters), coupled by electrospray (Michrom ADVANCE CaptiveSpray MS Source) to a high-resolution mass spectrometer (Q Exactive, ThermoScientific) in liquid chromatography mass spectrometry (LC-MS) and liquid chromatography/tandem mass spectrometry (LC-MS/MS) mode. The LC column was used at a flow rate of 1.8 μl/min (Waters nanoAcquity UPLC column BEH130 C18, 150 μm × 100 mm, 1.7 μm). Each of the 6 fractions was run as a separate set of 338 samples plus control samples.

Intensity data files for each LC-MS run within a SCX fraction were aligned using Elucidator (Rosetta Biosoftware). Peak intensities for each peptide ion were then extracted across all files. LC-MS/MS files were analyzed by Mascot (Matrix Sciences) and the Uniprot human protein database (version 2013_08) to assign high confidence peptide sequences to the observed peptide ions. All sequenced peptides were then clustered by their parent proteins. Potential intensity bias introduced by sample processing and/or loss of sensitivity of the mass spectrometer over the time of the experiment was corrected by normalization. The normalization procedure was based on a regression model, which predicted log-intensity level on a per-peptide basis. First, the mean raw log-intensity for each peptide was calculated. Then the regression model (linear regression or natural cubic spline smoothing) for sample processing variables was fit to the data. Finally, the normalized log-intensity was computed as the raw log-intensity minus the regression-predicted log-intensity plus the mean raw log-intensity.

The statistical significance of the intensity differences between the various clinical groups was assessed using a paired *t* test, which was performed independently on each peptide and each protein, for the matched case and control samples. An analysis of variance model was also used to compare the same two groups to account for dyslipidemia, hypertension, and diabetes status covariates, which were not matched between sample pairs. All statistical test *P* values were adjusted for multiple testing by conversion to *Q* values using Storey's method.

Metabolomics and lipidomics methods by mass spectrometry

Sample preparation for global metabolomics

Samples were stored at −70°C until processed. Sample preparation was carried out as described previously (15) at Metabolon, Inc. Briefly, recovery standards were added prior to the first step in the extraction process for quality control purposes. To remove protein, dissociate small molecules bound to protein or trapped in the precipitated protein matrix, and to recover chemically diverse metabolites, proteins were precipitated with methanol under vigorous shaking for 2 min (Glen Mills Genogrinder 2000), followed by centrifugation. The resulting extract was divided into 4 fractions: 1 for analysis by ultra-high performance liquid chromatography tandem mass spectrometry (UPLC-MS/MS; positive mode), 1 for analysis by UPLC-MS/MS (negative mode), 1 for analysis by gas chromatography–mass spectrometry (GC-MS), and 1 sample was reserved for backup.

Three types of controls were analyzed in concert with the experimental samples: samples generated from a pool of human plasma (extensively characterized by Metabolon, Inc.) served as technical replicates throughout the data set; extracted water samples served as process blanks; and a cocktail of standards spiked into every analyzed sample allowed for instrument performance monitoring. Instrument variability was determined by calculating the median relative standard deviation (RSD) for the standards that were added to each sample prior to injection into the mass spectrometers (median RSD = 5%; n = 30 standards). Overall process variability was determined by calculating the median RSD for all endogenous metabolites (i.e., non-instrument standards) present in 100% of the pooled human plasma samples (median RSD = 11%; n = 610 metabolites). Experimental samples and controls were randomized across the platform run.

Mass spectrometry analysis

Non-targeted MS analysis was performed at Metabolon, Inc. Extracts were subjected to either GC-MS (16) or UPLC-MS/MS (15). The chromatography was standardized and, once the method was validated, no further changes were made. As part of Metabolon's general practice, all columns were purchased from a single manufacturer's lot at the outset of the experiments. All solvents were similarly purchased in bulk from a single manufacturer's lot in sufficient quantity to complete all related experiments. For each sample, vacuum-dried samples were dissolved in injection solvent containing 8 or more injection standards at fixed concentrations, depending on the platform. The internal standards were used to assure both injection and chromatographic consistency. Instruments were tuned and calibrated for mass resolution and mass accuracy daily.

The UPLC-MS/MS platform utilized a Waters Acquity UPLC with Waters UPLC BEH C18-2.1 × 100 mm, 1.7 μm columns and a Thermo Scientific Q-Exactive high resolution/accurate mass spectrometer interfaced with a heated electrospray ionization source and Orbitrap mass analyzer operated at 35,000 mass resolution. The sample extract was dried and then reconstituted in acidic or basic LC-compatible solvents, each of which contained 8 or more injection standards at fixed concentrations to ensure injection and chromatographic consistency. One aliquot was analyzed using acidic, positive ion–optimized conditions, and the other using basic, negative ion–optimized conditions in 2 independent injections using separate dedicated columns. Extracts reconstituted in acidic conditions were gradient eluted using water and methanol containing 0.1% formic acid, while the basic extracts, which also used water/methanol, contained 6.5 mM ammonium bicarbonate. The MS analysis alternated between MS and data-dependent MS^2 scans using dynamic exclusion, and the scan range was from 80 to 1,000 *m/z*.

The samples destined for analysis by GC-MS were dried under vacuum desiccation for a minimum of 18 h prior to being derivatized under dried nitrogen using bistrimethyl-silyltrifluoroacetamide. Derivatized samples were separated on a 5% phenyldimethyl silicone column with helium as carrier gas and a temperature ramp from 60 to 340°C within a 17-min period. All samples were analyzed on a Thermo-Finnigan Trace DSQ MS operated at unit mass resolving power with electron impact ionization and a 50–750 atomic mass unit scan range.

Compound identification, quantification, and data curation

Metabolites were identified by automated comparison of the ion features in the experimental samples to a reference library of chemical standard entries that included retention time, molecular weight (*m/z*), preferred adducts, and in-source fragments as well as associated MS spectra and curated by visual inspection for quality control using software developed at Metabolon. Identification of known chemical entities was based on comparison to metabolomic library entries of purified standards. Over 2,500 commercially available purified standard compounds have been acquired and registered into the Laboratory Information Management System for distribution to both the LC-MS and GC-MS platforms for determination of their detectable characteristics. An additional 250 mass spectral entries have been created for structurally unnamed biochemicals, which have been identified by virtue of their recurrent nature (both chromatographic and mass spectral). These compounds have the potential to be identified by future acquisition of a matching purified standard or by classical structural analysis. Peaks were quantified using area-under-the-curve. Raw area counts for each metabolite in each sample were normalized to correct for variation resulting from instrument inter-day tuning differences by the median value for each run-day; therefore, the medians were set to 1.0 for each run. This preserved variation between samples but allowed metabolites of widely different raw peak areas to be compared on a similar graphical scale. Missing values were imputed with the observed minimum after normalization.

TrueMass® lipomic panel

Lipids were extracted in the presence of authentic internal standards by the method of Folch et al. (17) using chloroform:methanol (2:1 v/v). For the separation of neutral lipid classes [FFA, TAG, DAG, CE], a solvent system consisting of petroleum ether/diethyl ether/acetic acid (80:20:1) was employed. Individual phospholipid classes within each extract [PC, PE] were separated using the Agilent Technologies 1100 Series LC. Each lipid class was transesterified in 1% sulfuric acid in methanol in a sealed vial under a nitrogen atmosphere at 100°C for 45 min. The resulting fatty acid methyl esters were extracted from the mixture with hexane containing 0.05% butylated hydroxytoluene and prepared for GC by sealing the hexane extracts under nitrogen. Fatty acid methyl esters were separated and quantified by capillary GC (Agilent Technologies 6890 Series GC) equipped with a 30 m DB 88 capillary column (Agilent Technologies) and a flame ionization detector.

Evaluation of associations between low density lipoprotein triglycerides and plasma lipoproteins

For a confirmatory study, eight hundred and six subjects were included from the National Institutes of Health CT study. The cohort included both males and females that were at least 18 years of age and with clinical indication for a coronary CT angiography. There were no additional inclusion criteria. Exclusion criteria were current pregnancy and severely decreased renal function (estimated glomerular filtration rate < 30 mL/min/1.73m2 body surface area). The study protocol was approved by

the National Heart, Lung, and Blood Institute's Institutional Review Board and all subjects provided informed consent at enrolment. ClinicalTrials.gov identifier: NCT01621594. Plasma LDL-TG was determined by homogeneous assay (Denka Seiken Co., Ltd., Tokyo, Japan), and subjects were divided according to LDL-TG terciles. Fasting lipid panel was determined by standard enzymatic methods on a Cobas 6000 analyzer (Roche Diagnostics, Indianapolis, IN, USA). LDL cholesterol and very low-density lipoprotein (VLDL) cholesterol were calculated using Sampson's formula (18). Small dense LDL cholesterol was measured by a homogeneous assay (Denka Seiken Co, Ltd., Tokyo, Japan). Lipoprotein subclass profile was determined in Vantera Clinical NMR Analyzer (Labcorp, Burlington, NC, USA). The LipoProfile-3 or 4 algorithm was used to determine the particle number of lipoprotein subclasses: Number of triglyceride-rich lipoprotein particles (TRL-P) and the following subclasses: very small-, small-, medium- and large-TRL-P; LDL particle number (LDL-P), and its subclasses: small-, medium-, large-LDL-P; HDL particle number (HDL-P), as well as HDL subclasses: small-, medium-, and large-HDL-P. GlycA levels were determined in a Vantera Clinical NMR Analyzer (Labcorp, Burlington, NC, USA). Plasma high sensitivity (hs-CRP) was measured on the Cobas 6000 analyzer (Roche Diagnostics, Indianapolis, IN, USA).

Results

Demographic features and atherosclerosis in the patient population

A total of 665 patients were included in our analysis; general demographic features are shown in **Supplementary Table 1**. Typical angina (62 vs. 64%) and atypical angina (36.5 vs. 36%) were similar in cases and controls. In general, the mean ± SD age in the overall study population was 56 ± 11 years, 47% of patients were male, and the mean Diamond-Forrester score was 26% (range, 0–94%). LDL-C, high-density lipoprotein cholesterol (HDL-C), and triglycerides in cases and controls are also shown in **Supplementary Table 1**. The prevalence of atherosclerosis (i.e., cases) was 52% in the overall cohort. Seven percent of patients had a coronary calcium score of zero but had a non-calcified plaque. Predominantly non-calcified, partially calcified, and calcified plaques were present in 7, 36, and 57% of cases, respectively. Napkin ring sign, a high-risk feature by CT, was observed in 10% of patients. Moderate stenosis (50–69%) was the highest degree of stenosis in 7% of patients, and 16% of patients had moderate-to-severe stenosis (≥50% luminal stenosis). Mean ± SD segment involvement score and segment involvement score index were 2.2 ± 3.1 and 2.4 ± 3.3%, respectively.

Biomarker associations with atherosclerosis

In a preliminary coarse filter of the biomarkers, nominal univariate associations (raw $P < 0.05$) with atherosclerosis were identified for 30 of the 99 conventional biomarkers; these are illustrated in a heatmap in **Figure 1**. The dendrogram on the left of the plot was generated by unsupervised hierarchical clustering and indicates four (4) clusters. Cluster 1 included total plasma triglycerides and LDL-TG, as well as fatty acids and measures of endothelial dysfunction, inflammation, and fibrosis. Cluster 2 included ApoB-containing lipoprotein measurements, lipoprotein(a), and measures of insulin resistance. Cluster 3 included hepatic measurements of bilirubin metabolism and a marker of cholesterol biosynthesis. Cluster 4 contained vitamin D alone.

The thirty biomarkers identified by univariate analysis were further subjected to gradient boosting analysis to identify the strongest predictors of atherosclerosis **Figure 2A** indicates the relative influence of the eight biomarkers ranked most highly. Interleukin-6 [IL-6], symmetric dimethylarginine, and LDL-TG emerged as the top 3 predictors of case-control status, with a relative influence of over ∼30% for IL-6 and symmetric dimethylarginine and ∼15% for LDL-TG. As described below, of these eight (8) biomarkers strongly associated with atherosclerosis, only LDL-TG was directly connected to atherosclerosis in the Bayesian network analysis.

Bayesian network analysis using reverse engineering with forward simulation

The primary result and output from the hypothesis-free Bayesian network analysis is shown in **Figure 2B**. The ensemble of Bayesian networks identified consisted of 24,929 nodes and 110,350 edges, which occurred in >5% of the models in the ensemble. LDL-TG was the only biomarker directly upstream from the presence of atherosclerotic CAD (ASCAD), which occurred in 95% of networks in the ensemble. This suggests a potential causal role of triglyceride-rich LDL particles, as measured by LDL-TG levels, in the development and progression of atherosclerosis. Given the central role of LDL-TG in the Bayesian networks, we further explored the potential contribution of LDL-TG to atherosclerosis. It is important to point out that clinical features, such as age and gender, were also included in the Bayesian analysis and therefore, the Bayesian findings do normalize our findings for age and gender.

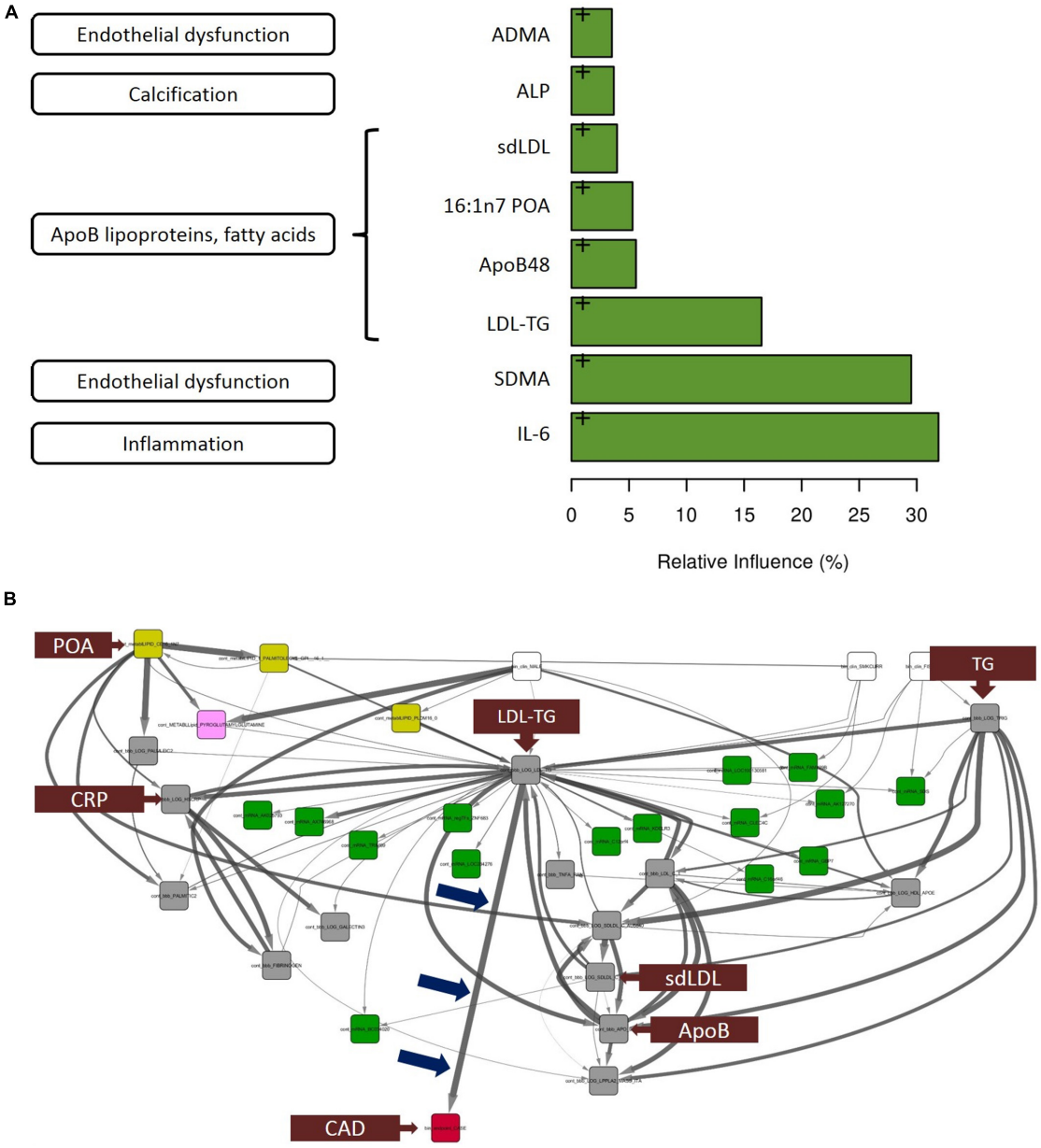


FIGURE 2
Gradient boosting analysis identified the strongest predictors of atherosclerotic coronary artery disease. Hypothesis-free Bayesian network analysis using reverse engineering with forward simulation (REFS^{TM}) Suggests a potential causal role of triglyceride-rich LDL particles, as measured by LDL-TG levels, in the development and progression of atherosclerosis. **(A)** Out of the 30 conventional biomarkers included in the multivariate analysis, the top 8 analytes are shown in the bar graph. The length of the bar corresponds to the relative influence the biomarker in predicting atherosclerotic coronary artery disease. The biomarkers include IL-6 (inflammation), symmetric dimethylarginine (endothelial dysfunction), and LDL-TG (ApoB-containing lipoprotein cluster). **(B)** This figure is not an illustration; it is an actual output from the hypothesis-free Bayesian network analysis. A total of 24,929 nodes and 110,350 edges were discovered in more than 5% of the networks in the ensemble; shown is the subnetwork of measurements with 1 degree of separation from LDL-TG. Arrow thickness indicates the fraction of networks in which the causal edge appears; different colored boxes represent different types of measurements (yellow: mass-spectrometry–based lipidomics; pink: mass-spectrometry–based metabolomics; green: gene expression [mRNA]; gray: conventional biomarker measurements). Notably, among all of the biomarkers that were measured and included in the model, LDL-TG was the only biomarker with a direct connection to ASCAD (see blue arrows pointing to the edge connecting LDL-TG to ASCAD). This suggests that triglyceride-rich lipoprotein particles, as measured by LDL-TG levels, may have a causal role in atherosclerosis. Interestingly, in this causal model, sd-LDL, ApoB, and C-reactive protein are downstream from LDL-TG, while palmitoleic acid and total triglyceride levels appear upstream from LDL-TG. Fibrinogen and galectin-3 are downstream from C-reactive protein. ADMA, asymmetric dimethylarginine; ALP, alkaline phosphatase; ApoB, apolipoprotein B; IL-6, interleukin-6; LDL-TG, low-density lipoprotein-triglycerides; SDMA, symmetric dimethylarginine, ASCAD, atherosclerotic coronary artery disease; CAD, coronary artery disease; CRP, C-reactive protein; POA, palmitoleic acid; sd-LDL, small, dense low-density lipoprotein; TG, triglycerides.

Low density lipoprotein triglycerides and atherosclerosis

As an independent biomarker, LDL-TG levels were significantly higher in cases versus controls (mean ± SE, 20.19 ± 0.93 vs 17.21 ± 0.40 mg/dL; $P < 0.001$). The four quartiles of LDL-TG measurements were examined; odds ratios in the second, third, and fourth quartiles were 1.38 (95% CI, 0.86–2.24), 1.43 (95% CI, 0.89–2.31), and 2.84 (95% CI, 1.75–4.64), respectively, compared to the first (i.e., reference) quartile (**Table 1**). Adjusting the model for age, sex, LDL-C and ApoB levels demonstrated that the association of LDL-TG with atherosclerosis was independent of these well-known factors.

Cumulative incidence curves

To examine the relative contribution of each key biomarker to atherosclerosis, we constructed cumulative incidence curves for ApoB, LDL-C, and LDL-TG levels against the cumulative incidence of atherosclerosis in all patients and in patients who were not on statins (**Figure 3**). Our data confirmed the well-described relationship between LDL-C levels and the incidence of atherosclerosis, which was most apparent in patients who were not on statin therapy (**Figure 3E**). A similar pattern was also observed for ApoB (**Figure 3D**). It is acknowledged that the left tails of the cumulative incidence curves are likely to be highly influenced by patients with known ASCAD whose LDL-C and ApoB levels were likely lowered by recent statin therapy. Importantly, statin therapy appears to have little influence on the cumulative incidence of ASCAD as a function of LDL-TG measurements (**Figures 3C,F**).

Hepatic lipase (LIPC), low density lipoprotein triglycerides and atherosclerosis

Since previous publications (19–21) have *demonstrated an association* between hepatic lipase (encoded by the LIPC gene), LDL-TG and atherosclerosis, we performed a genomic screen of the LIPC gene region. The SNP rs261336 was associated with both higher levels of LDL-TG and higher odds of atherosclerosis, while rs12898984, rs12900448, rs4774301, rs4775064 and rs4775065 were associated with lower circulating levels of LDL-TG and lower odds of atherosclerosis (**Table 2** and **Figure 4**).

In addition, LIPC gene expression in circulating mononuclear cells was significantly lower in Cases than in Controls (mean expression: 1.20 (0.08) vs. 1.49 (0.07); $p = 0.015$).

Associations of low density lipoprotein triglycerides with other known risk markers

We analyzed lipid panel test results and lipoprotein subclass profile, determined by proton nuclear magnetic resonance (^{1}H-NMR) spectroscopy, in 800 patients from the National Institutes of Health CT cohort, subdivided by LDL-TG terciles (**Supplementary Table 3**). Total Cholesterol and LDL-C increased from low to high LDL-TG terciles ($p < 0.0001$ for trend). The same trend was observed for triglycerides and calculated VLDL-C (18). Small dense LDL (sd-LDL) cholesterol as measured by Denka assay increased along the LDL-TG tertials. Lipoprotein subclass analysis by ^{1}H-NMR spectroscopy revealed that total, large, medium, and very small triglyceride-rich lipoprotein (TRL) particle number increased from low to high LDL-TG terciles ($p < 0.0001$ for trend). Furthermore, the number of LDL particles was higher in high LDL-TG terciles at the expense of smaller LDL particles ($p < 0.0001$), probably due to the poorer sdLDL recognition by LDL receptor (22), leading to its accumulation in the plasma.

Finally, LDL-TG was positively associated with GlycA ($p < 0.0001$ for trend), a recently identified systemic inflammation marker derived from the ^{1}H-NMR signal of N-acetyl groups on the glycan portion of acute-phase proteins in plasma (23). Overall, these results suggest that increased LDL-TG is linked to a more pro-inflammatory and pro-atherogenic phenotype and are, therefore, aligned with the findings from the Bayesian Network Analysis.

Discussion

Our results suggest that, while several serum biomarkers are associated with human ASCAD, triglyceride-rich LDL particles, as measured by LDL-TG levels, may have *an important central* role, potentially as a result of abnormal hepatic lipase function. Our study design provided a unique opportunity to assess biomarker associations in the context of the impact of genetic predisposition on atherosclerosis. We completed precise and detailed quantitative phenotyping measurements of human coronary arterial atherosclerosis in a prospective study using comprehensive cardiac CT, analyzed in a central core laboratory. This precision phenotyping was coupled with measuring and ranking 99 circulating biomarkers and 37,000 "omics" measurements. We built hypothesis-free, causal Bayesian networks of biological pathways to examine the *potential role* of serum biomarkers in a comprehensive manner.

Our initial analysis identified four main biomarker clusters, thus providing unique high-level insights into the pathogenesis of ASCAD (**Figure 1**). The content of these clusters is consistent with prevailing hypotheses of the development of atherosclerosis as a result of atherogenic lipoproteins,

TABLE 1 Odds ratios (95% CI) for atherosclerosis against the lowest quartile of LDL-TG.

	Quartile 1 [8.5–14.1 mg/dl]	Quartile 2 [14.1–16.9 mg/dl]	Quartile 3 [16.9–22.1 mg/dl]	Quartile 4 [22.1–45.7 mg/dl]	*P*-value versus fourth quartile	*P*-value for trend
LDL-TG Unadjusted	Reference	1.38 (0.86–2.24)	1.43 (0.89–2.31)	2.84 (1.75–4.64)	2.67e-05	5.35e-05
LDL-TG Model 1[a]	Reference	1.31 (0.80–2.13)	1.39 (0.86–2.27)	3.00 (1.84–4.95)	1.38e-05	2.50e-05
LDL-TG Model 2[b]	Reference	1.43 (0.88–2.33)	1.56 (0.95–2.56)	3.36 (1.95–5.85)	1.50e-05	3.88e-05
LDL-TG Model 3[c]	Reference	1.34 (0.82–2.20)	1.48 (0.89–2.46)	3.37 (1.94–5.91)	1.86e-05	4.49e-05
LDL-TG Model 4[d]	Reference	1.44 (0.89–2.35)	1.56 (0.94–2.60)	3.42 (1.88–6.29)	6.31e-05	1.99e-04
LDL-TG Model 5[e]	Reference	1.34 (0.82–2.20)	1.46 (0.87–2.47)	3.32 (1.81–6.16)	1.19e-04	3.55e-04

CI, confidence interval; LDL-TG, low-density lipoprotein–triglycerides.
[a]Adjusted for age and gender.
[b]Adjusted for LDL-C.
[c]Adjusted for age, gender, and LDL-C.
[d]Adjusted for APOB.
[e]Adjusted for age, gender, and APOB.

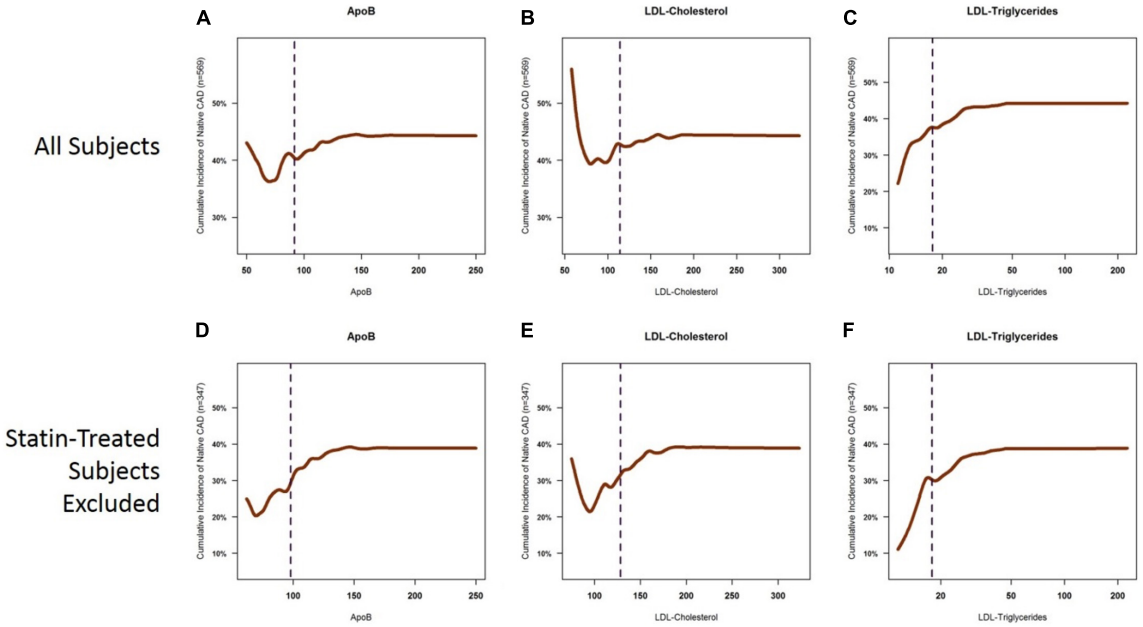


FIGURE 3
Cumulative incidence curves for the presence of coronary atherosclerosis as a function of ApoB, LDL-C, and LDL-TG. Cumulative incidence curves demonstrate the well-described relationship between ApoB and LDL-C levels and the incidence of atherosclerosis, which is primarily apparent in the ApoB range of 50–150 mg/dl and in the LDL-C range of 60–200 mg/dl. The left tails of the curves are distorted by statin-treated patients **(A,B)**, in which you see a high cumulative incidence of atherosclerosis despite very low levels of ApoB (panel A) and LDL-C **(B)**. This likely represents patients with known ASCAD whose LDL-C and ApoB levels have been lowered by aggressive statin therapy. The sigmoid relationship for ApoB and LDL-C is more apparent when statin-treated patients are excluded **(D,E)**. On the other hand, a clear, near-exponential relationship is seen for the incidence of atherosclerosis as a function of serum LDL-TG levels, with no apparent effect of statin therapy **(C,F)**. ApoB, apolipoprotein B; LDL, low density lipoprotein.

inflammation, and endothelial dysfunction, in some instances in the context of insulin resistance and diabetes (24–26).

Furthermore, in univariate (**Figure 1**) and multivariable analyses (**Figure 2A**), we also found that ApoB-containing lipoproteins, insulin resistance, endothelial dysfunction, inflammation, and fibrosis are all strongly associated with human coronary atherosclerosis, consistent with decades of hypothesis-driven data.

TABLE 2 Genetic association between LIPC gene variants, LDL-TG and atherosclerosis.

Variant (SNP)	LDL-TG				ASCAD ("Case")			
	Beta	SE	CI	*P*-value	OR	Ln(SE)	CI	*P*-value
rs261336	0.07	0.0297	0.01_0.13	0.0223	1.5	0.2011	1.01_2.23	0.0434
rs12898984	−0.06	0.0285	−0.11 to 0.00	0.0424	0.67	0.1899	0.46_0.97	0.0342
rs12900448	−0.06	0.0285	−0.11_−0.00	0.0424	0.67	0.1899	0.46_0.97	0.0342
rs4774301	−0.06	0.0285	−0.11_−0.00	0.0424	0.67	0.1899	0.46_0.97	0.0342
rs4775064	−0.06	0.0285	−0.12_−0.00	0.0386	0.67	0.1899	0.46_0.98	0.0374

SNP, single nucleotide polymorphism. LDL-TG, low density lipoprotein triglycerides; ASCAD, atherosclerotic coronary artery disease; SE, standard error; CI, confidence interval; Ln, natural log.

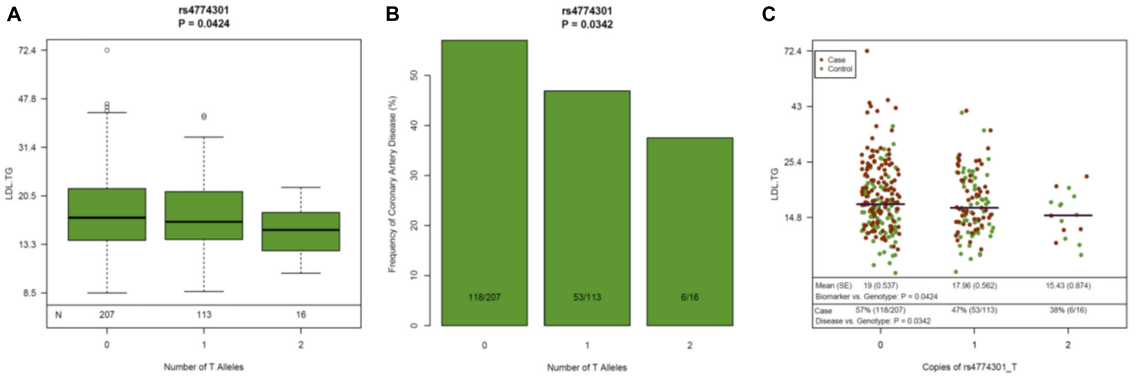


FIGURE 4
Genetic variants in the LIPC Gene, Circulating Levels of LDL-TG and Atherosclerosis. The genetic variant rs4774301 in the LIPC genomic region is associated with significantly lower circulating levels of LDL-TG **(A)** and simultaneously, with significantly lower prevalence of atherosclerosis **(B)**. Jitter plot **(C)** demonstrates the same concept in a single graph, demonstrating that the number of the "T" alleles at rs4774301 is associated with lower circulating levels of LDL-TG and with lower prevalence of ASCAD. LIPC: hepatic lipase gene; LDL-TG: low density lipoprotein triglycerides.

A key finding was that, out of tens of thousands of blood-based molecules and biomarkers, LDL-TG emerged with a potential central role in human coronary atherosclerosis, potentially as a function of abnormal hepatic lipase activity. This was seen in our hypothesis-free, causal, Bayesian network analysis, which included 24,929 variables and 110,350 significant edges in the models. LDL-TG was directly connected to human coronary atherosclerosis in 95% of the models in the ensemble. The output of the Bayesian network analysis shown in Figure 2B (not an illustration) indicates the potential central role of triglyceride-rich LDL particles, as measured by LDL-TG levels. In this model, triglyceride levels and palmitoleic acid were upstream from LDL-TG, while small, dense LDL (sd-LDL) inflammatory markers (e.g., C-reactive protein, fibrinogen, and lipoprotein-associated phospholipase A2), and fibrosis markers (e.g., galectin-3) were downstream. In addition to these potentially "positive" controls, the absence of HDL-C, ApoAI, CETP and vitamin-D may serve as relevant "negative controls" in the Bayesian networks.

The hierarchal organization of the lipid/lipoprotein-related biomarkers, inflammatory biomarkers and fibrosis-related biomarkers in the Bayesian networks are consistent with a mechanistic hypothesis in which triglyceride-rich LDL particles drive downstream inflammation and a fibrotic response, directly contributing to the initiation and progression of human coronary atherosclerotic plaques (Figure 2B). It is important to emphasize, however, that our findings do not suggest that triglyceride-rich LDL particles themselves physically localize in the coronary vessel wall to initiate the atherosclerosis process. The overall lipoprotein milieu in patients with elevated LDL-TG may lead to the increased formation of sd-LDL particles, which then may physically localize to the arterial wall.

Our panomic dataset offers a unique and unprecedented opportunity to assess causality of certain biomarkers, as we can assess *simultaneous* associations between genotypes, gene expression levels, circulating biomarkers and the atherosclerotic phenotype via comprehensive cardiovascular CT. Overall, our data is consistent with the potential *central* hypothesis that loss-of-function variants in the hepatic lipase gene may be associated with lower hepatic lipase activity, higher LDL-TG levels resulting in atherosclerosis. In our data, LIPC gene expression levels were significantly lower and LDL-TG levels were significantly higher in patients with atherosclerosis. Furthermore, single nucleotide polymorphisms (SNP's) in the LIPC gene that were associated with elevated LDL-TG levels were simultaneously associated with increased prevalence of

atherosclerosis, suggesting the *potential role* of LDL-TG based on the principles of natural randomization (**Figure 4**) (27). Our data is consistent with historical findings that polymorphisms in the LIPC gene are associated with circulating levels of LDL-TG (28–30).

From a mechanistic point of view, our results are consistent with a potential hypothesis whereby lower hepatic lipase activity may result in decreased lipolysis, decreased remodeling, and decreased initial clearance of TRL's, such as very low-density lipoprotein (VLDL) particles. The increased residence time of TRL's may lead to prolonged exposure of TRL's to CETP activity, resulting in more TG-rich IDL and LDL particles, as reflected in elevated LDL-TG levels. The presence of TG-rich IDL and LDL particles favor the generation of sd-LDL particles, which physically may localize to the arterial wall, resulting in the retention of these atherogenic lipoprotein particles, triggering an inflammatory reaction and endothelial dysfunction, culminating in the initiation and propagation of atherosclerosis.

In general, our findings are consistent with the literature but also add to those findings by demonstrating a possible *central* role of LDL-TG, potentially as a function of abnormal hepatic lipase activity, as revealed by our unique causal Bayesian network analysis and through genetic validation. A large epidemiologic study has examined the association of LDL-TG with angiographic ASCAD (31). In that study, LDL-TG levels were measured by the ultracentrifugation-precipitation method ("beta-quantification"), a cumbersome reference procedure not used for routine diagnostic testing. The main finding was that LDL-TG was a stronger predictor of ASCAD compared to LDL-C and was independent of LDL-C, with an overall odds ratio of 1.3 (95% CI, 1.19–1.43; $P < 0.001$). Although consistent with our findings, the odds ratio in our study was much higher at 3.41 (95% CI, 1.94–6.01), likely due to the use of precision phenotyping in our approach. Also consistent with our findings, they also identified significant correlations between LDL-TG and IL-6 and between LDL-TG and C-reactive protein. In a smaller more mechanistic sub-study of 114 patients, it has also been reported that in patients with high LDL-TG levels, LDL particles are enriched in triglycerides and depleted in cholesterol esters. VLDL particles showed the opposite trend; they were enriched in cholesterol esters and depleted in triglycerides. These observations are in line with the association of LDL-TG with very small TRL particle number that we observed. It is also consistent with our mechanistic hypothesis on the central role of the remodeling of apoB-containing lipoprotein particles in the development of atherosclerosis.

Several other large clinical trials have also provided important information related to the association of LDL-TG with atherosclerosis. Albers et al. examined the potential role of LDL-TG, sd-LDL and HDL subclasses in 3,094 subjects in the AIM-HIGH clinical trial (19), which was evaluating the effect of extended-release niacin in a secondary prevention population on statin background. The primary endpoint was the composite of death from coronary artery disease, non-fatal myocardial infarction, ischemic stroke, hospitalization for acute coronary syndrome or symptom-driven coronary or cerebrovascular revascularization. In their study, sd-LDL and LDL-TG were not event related. The advantage of our study is a very clear phenotype of coronary atherosclerosis based on comprehensive cardiovascular CT. In addition, the AIM-HIGH study was a secondary prevention population on the background of statin therapy, different from our patient population.

Saeed et al. also examined the potential role of LDL-TG in 9,334 subjects without prevalent CAD the ARIC study (20), using a direct homogenous assay that can be routinely applied in clinical laboratories. They found that LDL-TG were significantly associated with cardiovascular disease, even after adjusting for traditional risk factors, including lipids. This is consistent with our own findings in a similar patient population. Similarly, the authors also found that variants in the promoter region of the LIPC gene were associated with lower hepatic lipase activity, consistent with our own findings.

Finally, Silbernagel et al. (21) demonstrated that LDL-TG was associated with cardiovascular mortality in 3,140 subjects. Genome-wide association study in this cohort demonstrated that variants in the LIPC gene were significantly associated with circulating LDL-TG levels, consistent with our own findings. Furthermore, in a two-sample Mendelian randomization analysis, the authors found that low hepatic lipase activity may be the causal factor behind elevated LDL-TG levels, driving atherosclerotic cardiovascular risk. The authors suggested that LDL-TG may be on the causal pathway related to cardiovascular disease. Our combined unbiased, causal Bayesian network analysis and genomic analysis *is consistent with* these findings and *propose* a more detailed biological network explaining the hepatic lipase/LDL-TG axis of atherosclerosis (**Figure 5**).

In summary, we performed an unbiased, causal Bayesian network analysis to identify potential novel causal factors in human coronary atherosclerosis, revealing the potential key role of TG-rich lipoprotein particles. We then used our panomic data, including genetic validation, to further explore the potential *central* role of LDL-TG, demonstrating that the hepatic lipase/LDL-TG axis may be *an important* pathway in ASCAD.

Limitations

Although this was a prospective, multicenter study with central core laboratory analysis of all imaging and biochemical measurements, it has some limitations. First, we only included Caucasian subjects in our study, as it was powered for genome-wide association analyses based on a single ethnic background,

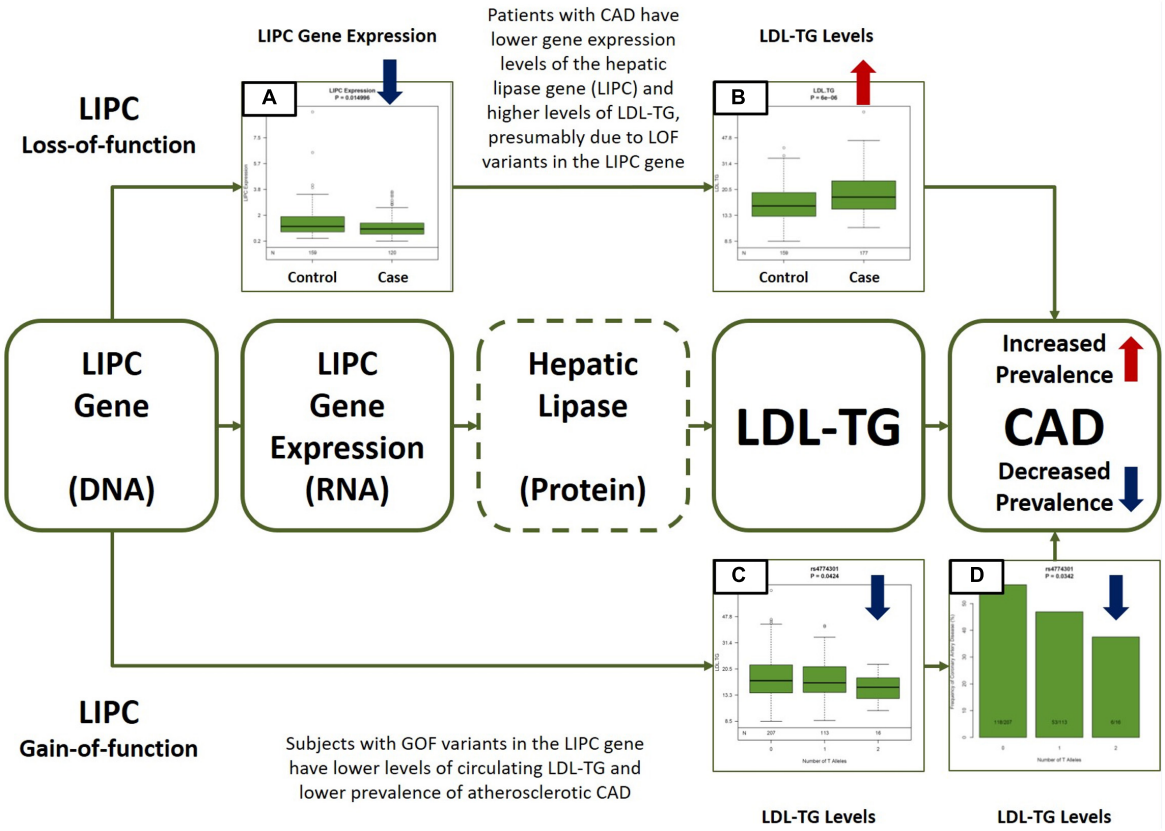


FIGURE 5
Central Illustration. Multiomic Validation of the Hepatic Lipase (LIPC)/LDL-TG Axis in Atherosclerotic CAD along the Central Dogma of Biology. Gene expression levels of hepatic lipase (LIPC) are significantly lower **(A)** and circulating LDL-TG levels are significantly higher **(B)** in patients with atherosclerotic CAD, presumably due to LOF variants in the LIPC gene (upper part of the Figure). On the other hand, GOF variants in the LIPC gene are simultaneously associated with lower circulating LDL-TG **(C)** levels AND with lower prevalence of atherosclerotic CAD **(D)**. Except for hepatic lipase activity measurements (indicated by dotted line around hepatic lipase in the Figure), the GLOBAL study database contains all other multiomic measurements along the hepatic lipase/LDL-TG/atherosclerosis axis. The overall data presented here is consistent with our hypothesis that circulating triglyceride-rich LDL particles may have a potential causal role in atherosclerosis, due to abnormal hepatic lipase activity. LIPC, hepatic lipase; LDL-TG, low density lipoprotein triglycerides; CAD, coronary artery disease; LOF, loss-of-function; GOF, gain-of-function.

requiring at least 6,700 subjects (7). Second, we have limited longitudinal follow-up of the patients. Nevertheless, a key feature of Bayesian network analysis with the implementation of REFS is its ability to generate causal biological models, even in the absence of longitudinal outcomes. In addition, since we had whole genome sequence data, we were also able to demonstrate causality through genetic methods. Third, although the genetic analysis is consistent with a potential central role of the hepatic lipase/LDL-TG axis, we did not have functional measurements of hepatic lipase. Finally, although we had in *a priori* Discovery and Validation dataset in our own GLOBAL clinical study, we did not validate our findings in external datasets, technically limiting our findings to the GLOBAL clinical study population.

Summary and conclusion

While ApoB-containing lipoproteins, inflammatory biomarkers, and markers of endothelial dysfunction and fibrosis were all associated with human coronary atherosclerosis, triglyceride-rich LDL particles, as measured by LDL-TG levels, emerged as a potentially key factor, within a sub-network that includes apoB and LDL-C. Furthermore, genetic analysis revealed the potential *central* role of the hepatic lipase/LDL-TG axis in atherosclerosis. With the recent introduction of a simple and fully automated method for the quantification of LDL-TG levels (32), this biomarker may become an important tool in the clinical assessment of patients at risk for, or with, atherosclerosis. Furthermore, the results from this study have

confirmed a possible role of hepatic lipase in human coronary atherosclerosis, which in the future can be explored as a target for drug development. It is also already known that several approved lipid-lowering drugs, such as fibrates, and statins, have a differential effect on LDL-TG versus LDL-C (33), which will be useful to further investigate to better understand their overall impact in cardiovascular event reduction.

Data availability statement

The Global Genomics Group, LLC company has spent in excess of $30M USD to collect the raw data that was used in the present analysis. Value creation in the company occurs by commercializing insights from the raw data, and that is how invested capital is returned to investors. Public disclosure of the raw data would compromise the full potential of value creation by the company at the present stage. Once the company returns invested capital to its investors, the raw data may be deposited in public data sources. In addition, under appropriate non-disclosure agreements, the company may disclose raw data to interested parties. Enquirers regarding raw data access are to be directed to BB, bbrown@g3therapeutics.com.

Ethics statement

The studies involving human participants were reviewed and approved by Western Institutional Review Board, Inc. The patients/participants provided their written informed consent to participate in this study.

Author contributions

SV, AB, DL, AR, and RN: study design, data analysis and manuscript preparation. MB: study design and statistical analysis. IM and BB: study execution. JN, PM-H, GV, IV, WH, VV, TD, DN, and BH: data analysis and manuscript preparation. AG, LF, BC, KR, and IK: data analysis. All authors contributed to the article and approved the submitted version.

Funding

Global Genomics Group sponsored and funded the study and has filed patents on biomarker diagnosis of coronary artery disease. Denka Seiken provided financial support for manuscript preparation. Research by DL and AR was supported by intramural DIR funds from the National Heart, Lung and Blood Institute, NIH, Bethesda, MD.

Conflict of interest

SV was Founder, CEO, and a full-time employee of Global Genomics Group. PM-H and LF report grants from Global Genomics Group during the conduct of the study. IM was a founder of Global Genomics Group. BB was a co-founder, VP of Clinical Affairs, and a full-time employee of Global Genomics Group. IV was the son of the Founder and CEO of Global Genomics Group. BC, KR, and BH were salaried employees of GNS Healthcare. WH was a shareholder of Global Genomics Group. AB and MB were employed by Acclarogen, Ltd, Cambridge, United Kingdom.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcvm.2022.960419/full#supplementary-material

References

1. Benjamin E, Muntner P, Alonso A, Bittencourt M, Callaway C, Carson A, et al. Heart disease and stroke statistics-2019 update: a report from the American heart association. *Circulation.* (2019) 139: e56–528.

2. Tabas I, Williams K, Boren J. Subendothelial lipoprotein retention as the initiating process in atherosclerosis: update and therapeutic implications. *Circulation.* (2007) 116:1832–44.

3. Bentzon J, Otsuka F, Virmani R, Falk E. Mechanisms of plaque formation and rupture. *Circ Res.* (2014) 114:1852–66.

4. Libby P, Theroux P. Pathophysiology of coronary artery disease. *Circulation.* (2005) 111:3481–8.

5. Ference B, Ginsberg H, Graham I, Ray K, Packard C, Bruckert E, et al. Low-density lipoproteins cause atherosclerotic cardiovascular disease. 1. Evidence

from genetic, epidemiologic, and clinical studies. A consensus statement from the European atherosclerosis society consensus panel. *Eur Heart J.* (2017) 38:2459–72. doi: 10.1093/eurheartj/ehx144

6. Imes C, Austin M. Low-density lipoprotein cholesterol, apolipoprotein B, and risk of coronary heart disease: from familial hyperlipidemia to genomics. *Biol Res Nurs.* (2013) 15:292–308. doi: 10.1177/10998004124 36967

7. Voros S, Maurovich-Horvat P, Marvasty I, Bansal A, Barnes M, Vazquez G, et al. Precision phenotyping, panomics, and system-level bioinformatics to delineate complex biologies of atherosclerosis: rationale and design of the "Genetic loci and the burden of atherosclerotic lesions" study. *J Cardiovasc Comput Tomogr.* (2014) 8:442–51.doi: 10.1016/j.jcct.2014.08.006

8. Sachs K, Perez O, Pe'er D, Lauffenburger D, Nolan G. Causal protein-signaling networks derived from multiparameter single-cell data. *Science.* (2005) 308:523–9. doi: 10.1126/science.1105809

9. Zhu J, Zhang B, Smith E, Drees B, Brem R, Kruglyak L, et al. Integrating large-scale functional genomic data to dissect the complexity of yeast regulatory networks. *Nat Genet.* (2008) 40:854–61. doi: 10.1038/ng.167

10. Howey R, Shin S, Relton C, Davey Smith G, Cordell H. Bayesian network analysis incorporating genetic anchors complements conventional Mendelian randomization approaches for exploratory analysis of causal relationships in complex data. *PLoS Genet.* (2020) 16:e1008198. doi: 10.1371/journal.pgen.1008198

11. Pearl J. *Causality : Models, Reasoning, and Inference*. Cambridge: Cambridge University Press (2000). 384 p.

12. Donoho D, Tanner J. Observed universality of phase transitions in high-dimensional geometry, with implications for modern data analysis and signal processing. *Philos Trans A Math Phys Eng Sci.* (2009) 367:4273–93. doi: 10.1098/rsta.2009.0152

13. Latourelle J, Beste M, Hadzi T, Miller R, Oppenheim J, Valko M, et al. Large-scale identification of clinical and genetic predictors of motor progression in patients with newly diagnosed Parkinson's disease: a longitudinal cohort study and validation. *Lancet Neurol.* (2017) 16:908–16. doi: 10.1016/S1474-4422(17)30328-9

14. Gendelman R, Xing H, Mirzoeva O, Sarde P, Curtis C, Feiler H, et al. Bayesian network inference modeling identifies TRIB1 as a novel regulator of cell-cycle progression and survival in cancer cells. *Cancer Res.* (2017) 77:1575–85. doi: 10.1158/0008-5472.CAN-16-0512

15. Xing H, McDonagh P, Bienkowska J, Cashorali T, Runge K, Miller R, et al. Causal modeling using network ensemble simulations of genetic and gene expression data predicts genes involved in rheumatoid arthritis. *PLoS Comput Biol.* (2011) 7:e1001105. doi: 10.1371/journal.pcbi.1001105

16. Hayete B, Wuest D, Laramie J, McDonagh P, Church B, Eberly S, et al. A Bayesian mathematical model of motor and cognitive outcomes in Parkinson's disease. *PLoS One.* (2017) 12:e0178982. doi: 10.1371/journal.pone.0178982

17. Folch J, Lees M, Sloane Stanley G. A simple method for the isolation and purification of total lipides from animal tissues. *J Biol Chem.* (1957) 226:497–509.

18. Sampson M, Ling C, Sun Q, Harb R, Ashmaig M, Warnick R, et al. A new equation for calculation of low-density lipoprotein cholesterol in patients with normolipidemia and/or hypertriglyceridemia. *JAMA Cardiol.* (2020) 5:540–8.doi: 10.1001/jamacardio.2020.0013

19. Albers J, Slee A, Fleg J, O'Brien K, Marcovina S. Relationship of baseline HDL subclasses, small dense LDL and LDL triglyceride to cardiovascular events in the AIM-HIGH clinical trial. *Atherosclerosis.* (2016) 251:454–9. doi: 10.1016/j.atherosclerosis.2016.06.019

20. Saeed A, Feofanova E, Yu B, Sun W, Virani S, Nambi V, et al. Remnant-like particle cholesterol, low-density lipoprotein triglycerides, and incident cardiovascular disease. *J Am Coll Cardiol.* (2018) 72:156–69.doi: 10.1016/j.jacc.2018.04.050

21. Silbernagel G, Scharnagl H, Kleber M, Delgado G, Stojakovic T, Laaksonen R, et al. LDL triglycerides, hepatic lipase activity, and coronary artery disease: an epidemiologic and Mendelian randomization study. *Atherosclerosis.* (2019) 282:37–44. doi: 10.1016/j.atherosclerosis.2018.12.024

22. Diffenderfer M, Schaefer E. The composition and metabolism of large and small LDL. *Curr Opin Lipidol.* (2014) 25:221–6.doi: 10.1097/MOL.0000000000000067

23. Ballout R, Remaley A. GlycA: a new biomarker for systemic inflammation and cardiovascular disease (CVD) risk assessment. *J Lab Precis Med.* (2020) 5:17.doi: 10.21037/jlpm.2020.03.03

24. Hopkins P. Molecular biology of atherosclerosis. *Physiol Rev.* (2013) 93:1317–542.

25. Libby P, Buring J, Badimon L, Hansson G, Deanfield J, Bittencourt M, et al. Atherosclerosis. *Nat Rev Dis Primers.* (2019) 5:56.

26. Petersen M, Shulman G. Mechanisms of insulin action and insulin resistance. *Physiol Rev.* (2018) 98:2133–223.doi: 10.1152/physrev.00063.2017

27. Ference B, Robinson J, Brook R, Catapano A, Chapman M, Neff D, et al. Variation in PCSK9 and HMGCR and risk of cardiovascular disease and diabetes. *N Engl J Med.* (2016) 375:2144–53. doi: 10.1056/NEJMoa1604304

28. Hegele R, Little J, Vezina C, Maguire G, Tu L, Wolever T, et al. Hepatic lipase deficiency. Clinical, biochemical, and molecular genetic characteristics. *Arterioscler Thromb.* (1993) 13:720–8. doi: 10.1161/01.atv.13.5.720

29. Ruel I, Couture P, Gagne C, Deshaies Y, Simard J, Hegele R, et al. Characterization of a novel mutation causing hepatic lipase deficiency among French Canadians. *J Lipid Res.* (2003) 44:1508–14.doi: 10.1194/jlr.M200479-JLR200

30. Hegele R, Breckenridge W, Cox D, Maguire G, Little J, Connelly P. Elevated LDL triglyceride concentrations in subjects heterozygous for the hepatic lipase S267F variant. *Arterioscler Thromb Vasc Biol.* (1998) 18:1212–6. doi: 10.1161/01.atv.18.8.1212

31. Marz W, Scharnagl H, Winkler K, Tiran A, Nauck M, Boehm B, et al. Low-density lipoprotein triglycerides associated with low-grade systemic inflammation, adhesion molecules, and angiographic coronary artery disease: the ludwigshafen risk and cardiovascular health study. *Circulation.* (2004) 110:3068–74. doi: 10.1161/01.CIR.0000146898.06923.80

32. Ito YO, Ikezaki H, Hirao Y, Machida A, Schaefer E, Furusyo N. Development and population results of a fully automated homogeneous assay for LDL triglyceride. *J Appl Lab Med.* (2018) 2:11. doi: 10.1373/jalm.2017.024554

33. Hirano, T, Kodera R, Hirashima T, Suzuki N, Aoki E, Hosoya M, et al. Metabolic properties of lowdensity lipoprotein (LDL) triglycerides in patients with type 2 diabetes, comparison with small dense LDL-cholesterol. *J Atheroscler Thromb.* (2021) 29:762–74. doi: 10.5551/jat.62789

frontiers | Frontiers in Cardiovascular Medicine

TYPE Systematic Review
PUBLISHED 16 January 2023
DOI 10.3389/fcvm.2022.1023651

OPEN ACCESS

EDITED BY
Chu-Huang Chen,
Texas Heart Institute, United States

REVIEWED BY
Federica Fogacci,
University of Bologna, Italy
Xianwei Wang,
Xinxiang Medical University, China

*CORRESPONDENCE
Alexander V. Sorokin
✉ alexander.sorokin2@nih.gov

SPECIALTY SECTION
This article was submitted to Lipids in Cardiovascular Disease, a section of the journal Frontiers in Cardiovascular Medicine

RECEIVED 20 August 2022
ACCEPTED 30 December 2022
PUBLISHED 16 January 2023

CITATION
Hong CG, Florida E, Li H, Parel PM, Mehta NN and Sorokin AV (2023) Oxidized low-density lipoprotein associates with cardiovascular disease by a vicious cycle of atherosclerosis and inflammation: A systematic review and meta-analysis.
Front. Cardiovasc. Med. 9:1023651.
doi: 10.3389/fcvm.2022.1023651

Oxidized low-density lipoprotein associates with cardiovascular disease by a vicious cycle of atherosclerosis and inflammation: A systematic review and meta-analysis

Christin G. Hong, Elizabeth Florida, Haiou Li, Philip M. Parel, Nehal N. Mehta and Alexander V. Sorokin*

Section of Inflammation and Cardiometabolic Diseases, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, MD, United States

Background: Low-density lipoprotein cholesterol (LDL-C) is an established marker for cardiovascular disease (CVD) and a therapeutic target. Oxidized LDL (oxLDL) is known to be associated with excessive inflammation and abnormal lipoprotein metabolism. Chronic inflammatory diseases confer an elevated risk of premature atherosclerosis and adverse cardiovascular events. Whether oxLDL may serve as a potential biomarker for CVD stratification in populations with chronic inflammatory conditions remains understudied.

Objective: To perform a systematic review and meta-analysis evaluating the relationship between oxLDL and CVD (defined by incident CVD events, carotid intima-media thickness, presence of coronary plaque) in patients with chronic inflammatory diseases.

Methods: A systematic literature search was performed using studies published between 2000 and 2022 from PubMed, Cochrane Library, Embase (Elsevier), CINHAL (EBSCOhost), Scopus (Elsevier), and Web of Science: Core Collection (Clarivate Analytics) databases on the relationship between oxLDL and cardiovascular risk on inflamed population. The pooled effect size was combined using the random effect model and publication bias was assessed if $P < 0.05$ for the Egger or Begg test along with the funnel plot test.

Results: A total of three observational studies with 1,060 participants were ultimately included in the final meta-analysis. The results demonstrated that oxLDL is significantly increased in participants with CVD in the setting of chronic inflammatory conditions. This meta-analysis suggests that oxLDL may be a useful biomarker in risk stratifying cardiovascular disease in chronically inflamed patients.

KEYWORDS

cardiovascular disease, atherosclerosis, inflammation, oxidized low-density lipoprotein, lipids

Introduction

Atherosclerosis is a complex pathophysiological process driven by metabolic derangements, lipid accumulation, and inflammation (1–3). While it may be clinically silent at early stages, atherosclerotic lesions often transform into vulnerable plaques prone to rupture and incite subsequent adverse events, including myocardial infarction, stroke and death (4, 5). Coronary artery disease (CAD) is an atherosclerotic cardiovascular disorder and continues to be the leading cause of mortality worldwide, despite advancements in treatments (6, 7). While traditional cardiovascular (CV) risk factors contribute to the pathogenesis of CAD, other novel risk factors may be involved. In particular, systemic inflammation has thought to play a role in the development and progression of CAD (1, 8). Growing body of evidence has shown that chronic inflammatory diseases, such as psoriasis (PSO), rheumatoid arthritis (RA), human immunodeficiency virus (HIV), and systemic lupus erythematosus (SLE) are associated with accelerated atherosclerosis and premature adverse CV events (9–14). In fact, such conditions are now considered independent risk factors for cardiovascular disease (CVD) (15). However, traditional CV risk stratification using Framingham risk score and age is suboptimal in assessing CVD risk in patients with chronic inflammatory conditions (16–18). For example, severe psoriasis has been shown to confer an additional 6.2% increase in long-term risk of CVD based on Framingham Risk score (19). Given the elevated CVD risk and current challenges in evaluating CVD in these inflamed populations, it is necessary to identify prognostic tools that will adequately capture and assess CVD risk.

Low-density lipoprotein cholesterol (LDL-C) is a known biomarker of cardiovascular disease (CVD) (20, 21). Pharmacological reduction of LDL-C is considered a main tool in the primary prevention of atherosclerotic cardiovascular disease (ASCVD); however, the issue of residual atherosclerotic risk that remains in patients with decreased LDL-C and elevated high density lipoprotein-cholesterol (HDL-C) is of additional clinical concern (22). Alternativly, other LDL-related lipoprotein species, such as small-dense LDL (sdLDL), lipoprotein (a) [Lp (a)], and oxLDL, have been shown to be reliable markers of CVD risk prognosis as well (23–25). Oxidative stress contributes to atherosclerotic plaque formation by stimulating activation of macrophages and vascular smooth muscle cells, increasing extracellular cholesterol accumulation within vessel walls, and transforming macrophages into pro-inflammatory and pro-thrombotic phenotypes (26). The observed critical step in atherosclerotic plaque build-up, the foam cell formation, is triggered by the uptake of oxLDL by macrophages through scavenger receptors, such as CD36, as well as lectin-like oxLDL receptor (LOX-1) (27–29). Previous studies have found that circulating oxLDL associates with every stage of atherosclerosis, from subclinical atherosclerosis to overt cardiovascular disease, including hypertension, coronary and peripheral arterial disease, acute coronary syndromes, and ischemic cerebral infarction, and has prognostic value in estimating CVD risk (25, 30, 31). Indeed, elevated levels of oxLDL were shown to predict myocardial infarction in the Health ABC cohort, even after adjusting for age, gender, race, smoking, and metabolic syndrome (30). OxLDL may even be associated with arterial aging, as a recent study found that oxLDL demonstrated predictive value of arterial stiffness, as measured by pulse-wave velocity, in patients with normal to mildly reduced renal function (32). Further, oxLDL is linked with metabolically dysfunctional pathologies frequently associated with CVD, including obesity, metabolic syndrome, and diabetes mellitus (25). Thus, oxLDL has recently become an important therapeutic target for CVD and has been recognized as a biomarker for CAD and other age-related atherosclerotic processes (24, 31, 33, 34). However, to what extent oxLDL contributes to CVD within systemic inflammation and whether it has any clinical utility in CVD risk stratification for such populations remain understudied. Therefore, we conducted a systematic review to examine the available evidence and aimed to investigate the association between oxLDL levels and CVD in the setting of chronic inflammation by meta-analysis.

Methods

Search strategy

The systematic review and meta-analysis were conducted according with the Preferred Reporting Items for Systematic reviews and Meta-Analyses guidelines (35) and the protocol was registered with the PROSPERO International Prospective Register of Systematic Reviews (PROSPERO 2022 CRD42022354525). This meta-analysis was not based on the individual participant data, thus ethical approval was not applicable.

A systematic search of studies published between 2000 and 2022 was conducted through PubMed, Cochrane Library, Embase (Elsevier), CINHAL (EBSCOhost), Scopus (Elsevier), and Web of Science: Core Collection (Clarivate Analytics) databases. The initial search strategies were performed: "oxidized phospholipid" OR "oxPLs" OR "oxidized LDL-C" OR "oxidized low-density lipoprotein" OR "oxLDL" OR "low-density lipoprotein receptor-1" OR "LOX-1" OR "sLOX-1" OR "apoA-I" OR "Apolipoprotein A-I" OR "Apolipoproteins E" OR "apolipoprotein E" OR "ApoE" OR "ApoC2" OR "ApoC3" OR "oxHDL" OR "Lipoproteins, LDL" OR "Lipoproteins, HDL" OR "modified lipoprotein" and ("Myocardial Infarction" OR "Stroke" OR "Cerebral" OR "Angina Pectoris" OR "Arteriosclerosis" OR "atherogenes" OR "atherosclerotic" OR "coronary artery disease" OR "Psoriasis") and ("Patient Outcome Assessment" OR "Risk Assessment" OR "Treatment Outcome"). While we initially planned to include all oxidized lipids in our systematic review, the results of the search strategy were ultimately focused on oxidized low-density lipoprotein as this search term yielded the greatest number of relevant studies. We also considered reference lists and review articles for other potentially relevant citations. The references of retrieved articles were also reviewed to identify any relevant study. Language restriction of English was applied. We used Endnote software (Clarivate Analytics, Philadelphia, PA) for management of the studies.

Study selection criteria

A 2-step selection process was conducted using Covidence (Covidence, Melbourne, Victoria, Australia) screening software. In the first step, titles and abstracts generated from the search strategy were reviewed by two independent researchers. Studies that did not examine the association between oxidized low-density lipoprotein, chronic inflammatory conditions, and cardiovascular disease measures were excluded. In the second step, studies

successfully screened in after the first step were reviewed in full text to confirm if they reported the mean with standard deviation, or median with interquartile range for observational studies.

Inclusion and exclusion criteria

Inclusion criteria were: observational studies investigating the relationship between oxLDL and CVD in patient populations with chronic inflammatory diseases, including psoriasis, systemic lupus erythematosus, rheumatoid arthritis, and human immunodeficiency virus.

Exclusion criteria were: incorrect study design; literature reviews, discussions, editorials, opinion pieces, and abstracts-only texts; wrong comparators; incorrect setting; wrong patient population; studies that did not report mean and standard deviation or median with interquartile range for observational studies; unavailable full text articles.

Data extraction and quality assessment

After the 47 available full-text articles were selected, 3 full-text sources were examined for representative data containing effect size (ES) of oxLDL measured by mean and standard deviation or median and interquartile range. For these studies, Covidence software was used to extract the data. The following data were extracted from each included study: first author's name, publication year, number of subjects, participant population, type of publication, patient characteristics (mean or median age in years, percentage of men, baseline body mass index), effect size of oxLDL (represented by mean with standard deviation, median with interquartile range), and study outcomes [defined as incident CVD event, carotid intima-media thickness, or coronary plaque presence as measured by coronary computed tomographic angiography (CCTA)]. To standardize the different measurements and units of oxLDL reported in the studies used in our analysis, we utilized the standardized mean difference with 95% confidence interval to consistently compare oxLDL across studies. Any studies representing results through median with interquartile range (IQR) were converted to mean with standardized mean difference based on methods from Wan et al. (36). All data extractions were completed by two reviewers (EF, HL) and checked by another reviewer (CGH).

Statistical analyses

The pooled standardized mean difference with its 95% confidence interval (CI) was calculated for oxLDL to account for the different units of oxLDL measurement across all studies. Statistical heterogeneity was identified if the P value for Cochran Q was <0.05 or the I^2 statistics was $>50\%$ (37). The Hedges random effects model was chosen if heterogeneity was detected (38). Otherwise, an inverse variance fixed effect model was used. Publication bias was considered if $P<0.05$ for the Egger or Begg test along with the funnel plot method (Supplementary Figure 1). All statistical analyses were performed using R Statistical Software (version 4.2.0, R Foundation for Statistical Computing, Vienna, Austria).

Results

Study selection

The screening and selection process is demonstrated using a flowchart diagram in Figure 1. Initially, a total of 7,309 relevant studies were imported into Covidence with 846 duplicates immediately removed. Of the 6,463 remaining references, 6,416 were excluded in the first step of the selection strategy based on title and abstract screening. Review of the remaining 47 studies in full text form during the second step of the selection strategy yielded 3 final studies (39–41) with 1,060 participants that were included in the meta-analysis. While excluded from the meta-analysis, 4 additional studies from the 47 full-text sources were included in our discussion for their findings on other promising biomarkers of LDL oxidation, including LDL-conjugated dienes, soluble lectin-like oxidized LDL receptor-1 (sLOX-1), oxidized phospholipids (Ox-PLs), and other oxidation-modified lipoproteins (OMLs) (27, 33, 42, 43).

Study characteristics

The studies included in our meta-analysis are shown in Table 1. The sample size of individual study ranged from 105 to 755 participants. Of the three studies, one was cross-sectional (40) and the rest were cohort studies (39, 41). All the studies were published between 2010 and 2021 and only included participants without known CVD history. The enrolled participants had a mean age range from 38.96 to 51 years. The sex by percent male in the studies ranged from 31.5 to 77%. The baseline body mass index (BMI) ranged from 23.3 to 28.0. All three studies measured oxLDL concentration using an enzyme-linked immunosorbent assay (ELISA) (Mercodia, Uppsala, Sweden). One study reported oxLDL as per the change in oxLDL levels (ΔoxLDL) (39), one study reported oxLDL levels in U/L (40), and one study reported oxLDL levels in mU/L (41). Thus, to account for the different measurements of oxLDL reported in these studies, the pooled standardized mean difference with its 95% confidence interval (CI) was calculated. In order to assess the effect size (ES) of oxLDL and CVD, two studies utilized the odds ratio (OR) (40, 41) and one study used the hazard ratio (HR) (39) (Table 1). All quality scores of the included studies were calculated as >5 according to the Newcastle-Ottawa Scale (NOS) (44). NOS scores of the studies included in the meta-analysis are presented in Table 2.

Elevated oxLDL significantly associates with CVD in inflamed populations

The individual studies and pooled meta-analysis results are demonstrated in Figure 2. Of the three studies, two assessed oxLDL levels in 468 participants with HIV disease and associated CVD (defined by carotid intima-media thickness or coronary plaque presence on coronary CTA) vs. 487 participants with HIV disease without CVD and found that increased oxLDL levels were significantly associated with CVD compared to those without CVD (ES for Parra: 0.75 (0.46, 1.03]; ES for Hoffman: 0.34 [0.20, 0.48]). In one study of participants with rheumatoid arthritis, there was no significant association between oxLDL ES and CVD. As shown

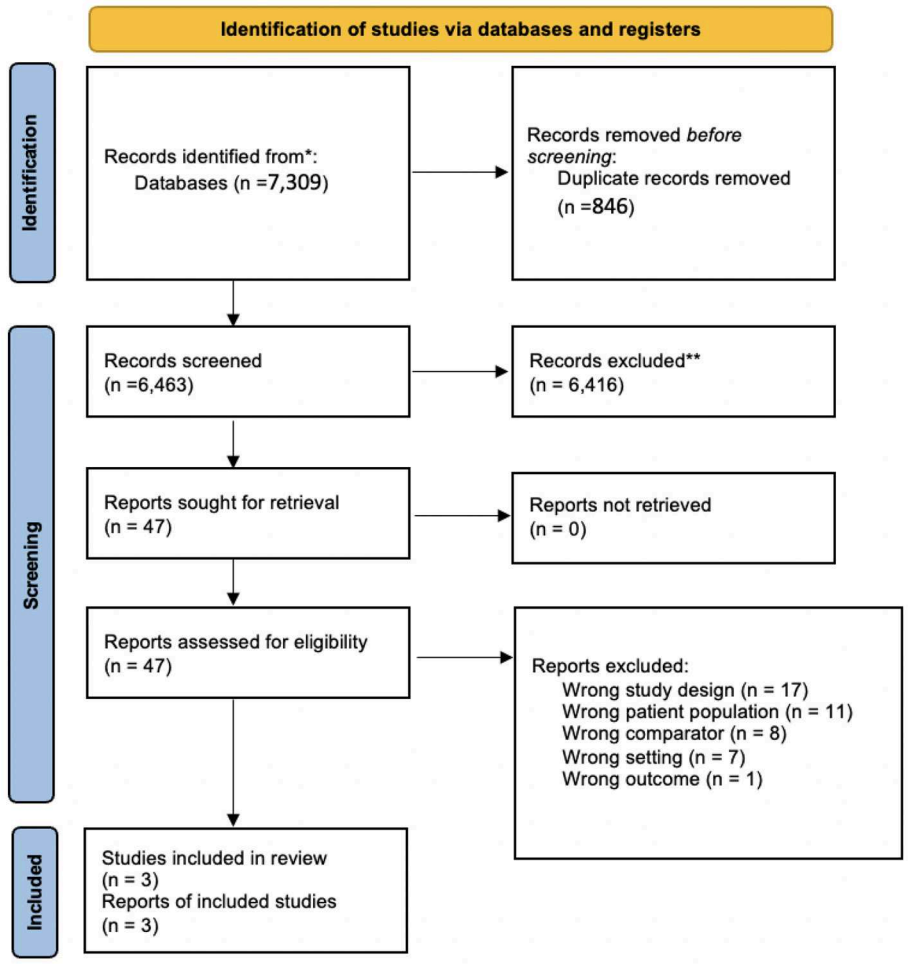


FIGURE 1
Flow chart of the study selection generated by PRISMA.

in Figure 2, the pooled total effect size of elevated oxLDL indicated that participants with chronic inflammatory diseases with associated CVD had a significant increase in oxLDL compared to those without CVD (0.44 [95% CI: 0.11, 0.77]). Cochran Q and I^2 index indicated that there was heterogeneity observed for the marginal analysis. The heterogeneity may be secondary to different methods of measurements and study participants. *P*-value of the Egger's test for funnel plot asymmetry was 0.86, which does not suggest the presence of publication bias.

Discussion

Chronic inflammatory conditions, such as psoriasis, RA, HIV, and SLE, have increased oxLDL levels, accelerated atherosclerosis, and premature adverse cardiovascular outcomes (9–13, 45). Thus, these pathologies provide suitable human models to study the mechanisms of inflammatory atherosclerosis and associated CVD in humans. In our systematic review with meta-analysis, we aimed to use these diseases to better understand oxLDL as a CV risk biomarker and its relationship with atherosclerotic CVD within the context of chronic inflammation. We found that compared to chronically inflamed subjects without CVD, elevated oxLDL levels were significantly associated with higher CVD presence in patients with chronic inflammatory conditions (ES total: 0.44 [95% CI: 0.11, 0.77]). These results extend the current understanding of the clinical utility of oxLDL as a potential biomarker for CV risk assessment in chronically inflamed populations.

Oxidized LDL is known to have pro-inflammatory and pro-atherogenic properties (46) and can predict increased risk of myocardial infarction (MI) (47–49). Additionally, many studies have demonstrated elevated levels of oxLDL in chronic inflammatory populations. Autoantibodies against oxLDL (auAb-oxLDL) were shown to be elevated in patients with psoriasis compared to matched controls, with 42% of psoriasis patients and 3.3% of control subjects having higher auAb-oxLDL levels than the cut-off point (352 mU/mL) (50). The autoantibody levels were also found to significantly correlate with the Psoriasis Area Severity Index score, a tool used to assess the severity and extent of psoriasis (50). OxLDL also significantly associated with noncalcified coronary burden, a marker of subclinical atherosclerosis, in patients with psoriasis (33). A recent study comparing female lupus patients with and without CVD found that oxLDL was significantly higher in those with CVD (14). However, to our knowledge, this is the first meta-analysis

TABLE 1 Baseline characteristics of studies included in the meta-analysis.

Reference	Design	Study Population	Age	Sex, male%	BMI	oxLDL mean (SD)	oxLDL assay	Adjusted variables		CVD outcome	Effect size (95% CI)
								oxLDL exposure	Other variables		
Ajeganova et al. (38)	Cohort (hospital-based)	114 RA patients from the BARFOT trial	50.6 ±11.2	31.5	24.93 ± 4	Case: 56.4 (12.69); Control: 54.2 (15.5)	Mercodia	ΔoxLDL	Age	Occurrence of MI, Angina pectoris, congestive HF, ischemic cerebrovascular event	HR 1.03 [1.0–1.06] 0.035
Parra et al. (39)	Cross-sectional	187 HIV patients at Hospital Universitari de Sant Joan	38.96 ± 0.61	68.8	23.31 ± 0.27	Case: 97.8 (29.22); Control: 76.22 (28.43)	Mercodia	U/L	Age, gender, smoking status, SBP, DBP, glucose, LDL-C, HDL-C, TG, BMI, HIV-1 basal viral load, basal CD4 cell count, lipodystrophy, exposure time to NRTI, NNRTI and (PI) treatments, inflammatory markers, and oxidative markers	Atherosclerosis evaluated by carotid intima-media thickness (CIMT). CVD risk estimated using FRS, low risk (<10%), moderate (10–20%) and high risk (>20%)	OR 1.026 [1.001–1.05]
Hoffmann et al. (40)	Cohort (community-based)	755 HIV-positive participants from the REPRIEVE study	51 ± 6	77	28.0 ± 6.0	Case: 382.27 (138.09) Control: 340.03 (116.48)	Mercodia	mU/L	ASCVD risk, HIV Parameters (ART duration, CD4, nadir CD4), age, sex, and race, LDL-C level, HTN, and current smoking	Prevalence and composition of CAD measured as coronary plaque on coronary CTA	OR 1.01 [0.90–1.15]

BARFOOT, Better Anti-Rheumatic Pharmaco-Therapy; REPRIEVE, Randomized Trial to Prevent Vascular Events in HIV; SBP, systolic blood pressure; DBP, diastolic blood pressure; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; TG, triglycerides; BMI, body mass index; HIV-1, human immunodeficiency virus-1; NRTI, nucleoside reverse transcriptase inhibitors; NNRTI, Non-nucleoside reverse transcriptase inhibitors; ASCVD, atherosclerotic cardiovascular disease; ART, anti-retroviral therapy; HTN, hypertension; MI, myocardial infarct; HF, heart failure; FRS, Framingham risk score; CAD, coronary artery disease; CTA, computed tomography angiography.

10.3389/fcvm.2022.1023651

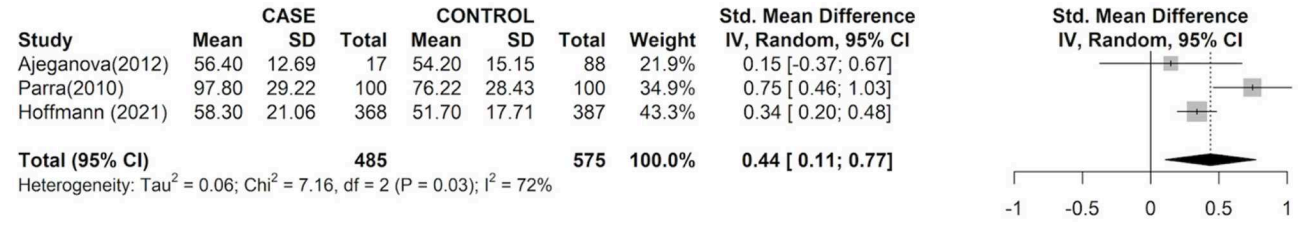


FIGURE 2
Effect size between circulating oxidized low-density lipoprotein and atherosclerotic cardiovascular disease risk. Forest plots describing effect size (ES), also known as the standard mean difference, with 95% confidence interval (CI) for all included observational studies. Squares represent study-specific estimates; box sizes represent individual study weight; horizontal line represent 95% CIs; diamonds represent the total mean difference with its 95% CI.

to observe the association between oxLDL and CVD only within chronic inflammatory disease populations. Thus, our aim for this systematic review and meta-analysis was to: (1) to summarize current literature on the relationship between oxLDL and CVD in chronic inflammatory populations, and (2) to provide a standardized representation of measured oxLDL levels across various studies. Currently, no standardized units or reference levels exist for reporting oxLDL measured by different biochemical assays (51–54). Thus, our findings utilized the standardized mean difference to unify the reporting of oxLDL across different studies.

Pharmacological perspective on oxLDL as a potential therapeutic target

Low-density lipoprotein cholesterol is a prognostic circulating biomarker for stratifying general cardiovascular risk (20, 21). Consequently, lipid-lowering treatments, such as statins and fibrates, are the mainstay treatments of lowering LDL-C levels as well as decreasing triglycerides, increasing HDL-C levels, and reducing hepatic cholesterol biosynthesis (55, 56). However, there is still a need for more specific biomarkers with pathological relevance, especially in chronically inflamed populations, to improve the risk stratification of cardiovascular events (57). OxLDL may be a promising candidate, as increased oxLDL levels are central to atherosclerotic plaque formation and thus may be more causally associated with CVD outcomes than LDL-C (31, 58). Several studies have illustrated the association between elevated circulating oxLDL and adverse CVD outcomes (31, 58, 59). More importantly, Tsimikas et al. demonstrated that high dose atorvastatin reduced total plasma oxidized phospholipids complexed with apolipoprotein B-100 (ApoB-100), the primary protein of the LDL particle, suggesting that statins may partly exert protective cardiovascular effects through mobilization of pro-inflammatory oxidation species from atherosclerotic lesions (60). Further, the "Standard vs. high-dose therApy with Rosuvastatin for lipiD lowering" (SARD) randomized clinical trial found that high dose rosuvastatin significantly reduced levels of oxLDL when compared to low dose rosuvastatin (61). In the setting of HIV, several studies showed that statin therapy reduced noncalcified coronary plaque volume, total plaque volume, and positively remodeled plaque in patients with HIV (62, 63) (Table 1). Thus, using current statin therapy to treat elevated oxLDL levels in addition to LDL-C may provide increased benefits in potentially reducing the risk of adverse CVD events in such populations.

While statins are the mainstay lipid-lowering therapy, many people are statin intolerant or cannot achieve goal LDL-C levels on statin therapy alone and thus require alternative therapy. Injectable lipid-lowering therapy currently used for inherited hypercholesterolemia and high-risk CV patients have demonstrated great benefit for such patients (64). Proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitors are a type of injectable lipid-lowering therapy targeting PCSK9, a protease enzyme produced in hepatocytes involved in LDL binding, internalization, and degradation (65, 66). Interestingly, PCSK9 is also implicated in the oxLDL-induced inflammatory pathway. In adult rat ventricular cardiomyocytes, oxLDL significantly impaired contractile function *via* induction of PCSK9 (67). Tang et al. found that PCSK9 small interfering RNA suppressed oxLDL-induced inflammatory response in THP-1-derived macrophages (68). Thus, PCSK9 inhibitors offer a novel therapeutic opportunity in targeting oxLDL-related atherosclerotic outcomes (67). Evolocumab is a PCSK9 inhibitor that when added to maximally tolerated statin therapy was found to reduce the risk of cardiovascular outcomes in patients with atherosclerotic CVD, while data from the "ODYSSEY OUTCOMES" trial demonstrated decreased risk of recurrent ischemic cardiovascular events in patients with previous acute coronary syndrome treated with alirocumab in addition to high-intensity statins (65, 69, 70). These findings illustrate the importance of discovering additional treatment modalities for patients at high risk for CVD complications.

In addition to oxLDL as a candidate biomarker for CVD, there is a growing body of literature showing the potential role of anti-oxLDL antibodies and other oxLDL-related moieties for CVD risk stratification and promising therapeutic targets (71). Autoantibodies to oxidation-specific epitopes on LDL, such as MDA-modified LDL (MDA-LDL), are found in atherosclerotic lesions of humans and animals (72, 73) and there is significant research on the clinical correlates of these antibodies (74–76). Karvonen et al. demonstrated that IgM autoantibodies to MDA-LDL epitope had an inverse association with carotid atherosclerosis in a population cohort study of 1,022 middle-aged men and women (77). Soluble lectin-like oxLDL receptor 1 (sLOX-1) is an inflammation-induced receptor for oxLDL that has been shown to induce myocardial ischemia through unstable atherosclerotic

10.3389/fcvm.2022.1023651

plaque formation, suggesting an important role of LOX-1 in the pathogenesis of oxLDL-related CVD (78, 79). Interestingly, increased sLOX-1 levels have been associated with systemic inflammatory diseases. sLOX-1 levels were higher in patients with RA with positive rheumatoid factor and anti-citrullinated protein antibody serology than those without, and continued to remain at high levels in non-remission patients compared to those in remission irrespective of treatment, highlighting the potential utility of sLOX-1 as a biomarker for disease activity and remission in RA (80). SLE patients had two-fold higher levels of sLOX-1, which positively associated with high-sensitivity CRP levels, oxLDL, proinflammatory HDL, and impaired HDL efflux, instead of traditional risk factors and SLE disease activity (79). Further, the authors found that SLE patients with higher sLOX-1 levels were younger than those with low levels, which is concerning given that SLE patients are at greater risk of CVD and is particularly evident in the younger female SLE population, as 54% of cardiac events that occur in female SLE patients are under the age of 44 (81). Thus, elevated sLOX-1 levels may serve as an useful biomarker of increased CVD risk, and sLOX-1 inhibition may be a therapeutic opportunity for decreasing atherosclerosis in these patients. Additionally, genetic modulation has become a promising therapeutic approach for oxLDL treatment. For instance, overexpression of the long non-coding RNA LINC00452 has been shown to reverse oxLDL injury in human umbilical vein endothelial cells (HUVECS) by regulating the miR-194-5p/IGF1R axis (82). Additionally, miR-214-3p in HUVECS regulates oxLDL-initiated macrophage autophagy, thus suggesting a potential therapeutic role for miRNAs in atherosclerosis (83). Further studies are necessary to elucidate more therapeutic targets aimed at the function and quantity of oxLDL in the pathogenesis of cardiovascular disease.

Several articles excluded based on our exclusion criteria for the meta-analysis were deemed important to include here for their discussion of other oxLDL-related potential biomarkers for optimization of CVD risk stratification in inflamed populations. In a multicenter observational study, Nyyssönen et al. found that increased LDL-conjugated diene concentrations, identified as one of the first stages of LDL oxidation and subclinical atherosclerosis, exhibited a positive relationship with increased CIMT in high-risk subjects presenting with at least three vascular risk factors (VRF) (27). Oxidation-specific biomarkers primarily oxidized phospholipids (Ox-PLs) on apolipoprotein B-100-containing lipoproteins (oxPL/ApoB-100), have been demonstrated as essential in identifying the risk of peripheral artery disease (43). Other studies have focused on the uptake pathway of oxLDL through soluble lectin-like oxidized LDL receptor-1 (sLOX-1) to better understand atherosclerosis. Dey et al. showed that in patients with psoriasis, sLOX-1 associated with imaging markers of subclinical atherosclerosis and increased psoriasis severity (42). Moreover, patients with psoriasis had decrease in plasma levels of oxidation-modified lipoproteins, including oxLDL under specific biologic treatment (33, 42).

Other potential oxLDL-related biomarkers of CVD and atherosclerosis

Other LDL-related lipoproteins, including sdLDL and Lp (a), are prone to oxidation and associated with elevated cardiovascular risk

TABLE 2 Quality assessment of studies included in meta-analysis.

Reference	Is the case definition adequate?	Representativeness of the cases	Selection of controls	Definition of controls	Comparability of groups of basis of design or analysis	Ascertainment of exposure	Ascertainment of both groups with same method	Overall NOS scores
Ajeganova et al. (38)	★	★	-	★	★	★	★	6
Parra et al. (39)	★	★	-	★	★★	★	★	7
Hoffmann et al. (40)	★	★	★	★	★★	★	★	8

NOS, Newcastle-Ottawa Scale.

(84–86). Because of their physical and compositional characteristics, they have higher affinity for extracellular matrix, reduced binding to LDL receptor, and increased residence time in the circulation compared to large (buoyant) LDL particles (23, 87, 88). In patients with psoriatic arthritis, sdLDL concentration was increased independently of the presence of metabolic syndrome, suggesting a potential mediation by sdLDL of atherosclerosis development in psoriatic arthritis (89). Furthermore, a study comparing HIV-positive with HIV-negative participants found increased sdLDL levels in those with HIV (63). Both HIV infection and combination antiretroviral therapy are thought to induce endothelial dysfunction through endothelial cell activation, oxidative stress, and inflammation that leads to increased cardiovascular disease in these patients (90). Indeed, in a study by Post et al., the authors found that suboptimal HIV RNA suppression and combined antiretroviral therapy adherence were the main determinants of coronary artery stenosis progression during a median follow-up of 4.5 years (91). Lp (a) is also a candidate biomarker and has been demonstrated to predict CIMT in HIV-positive females (92). While these findings are promising, meta-analyses investigating the role of oxidized lipids within systemically inflamed populations are lacking and thus future studies will continuously be necessary to further elucidate these relationships (93, 94).

Our meta-analysis had several limitations. Firstly, the causal association between oxLDL and CVD outcomes in our populations of interest could not be defined because of the cohort or cross-sectional nature of the included studies. Another limitation is that studies using other techniques to estimate CVD outcomes were not included in this meta-analysis. As observational studies show more heterogeneity than randomized control trials and several of the included studies were observational studies, this factor must also be considered given that heterogeneity interferes with the detection of publication bias (95, 96). The heterogeneity sources may correlate with study design, participant ages, and whether patients have atherosclerotic risk factors. While oxLDL is a promising biomarker for CVD risk stratification, oxLDL is not yet used in the clinic as a diagnostic tool for CVD. Finally, we were unable to determine the effects of populational characteristics or pharmacologic therapy on the progression of CVD outcomes in relation to oxLDL in patients with chronic inflammatory diseases.

Conclusion

Our systematic review and meta-analysis demonstrate that patients with chronic inflammatory diseases, particularly RA and HIV, have significantly higher levels of circulating oxLDL as measured by effect size in relation to increased cardiovascular risk. Thus, oxLDL may offer insight into optimizing CVD risk stratification in chronically inflamed populations. We also discussed additional atherogenic lipoprotein parameters associated with oxLDL that offer a more nuanced understanding of lipoprotein modifications linked with CVD in the setting of inflammation. Larger meta-analysis and future mechanistic studies are necessary to further elucidate the relationship between oxidized lipoproteins and cardiovascular disease in patients with long-standing inflammatory conditions.

Data availability statement

The original contributions presented in the study are publicly available. This data can be found here: https://www.crd.york.ac.uk/prospero/#searchadvanced, accession number: CRD42022354525.

Author contributions

CH, EF, HL, and AS were involved in designing the concept of the review and oversight and in the literature search, summation of the literature, and revisions of the manuscript. CH, EF, HL, PP, NM, and AS drafted the manuscript. All authors read, reviewed, and approved the final manuscript.

Funding

This study was supported by the National Heart, Lung, and Blood Institute Intramural Research Program (HL006193-07). This research was made possible through the NIH Medical Research Scholars Program, a public-private partnership supported jointly by the NIH and contributions to the Foundation for the NIH from the Doris Duke Charitable Foundation, Genentech, the American Association for Dental Research, the Colgate-Palmolive Company, and other private donors. The funding sources had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication.

Acknowledgments

We would like to thank the National Institutes of Health Library of Medicine for the resources to make this systematic review possible. We would especially like to acknowledge Gisela Butera, MEd, MLIS, Biomedical and Informationist Librarian, for her valuable expertise and help throughout this entire process.

Conflict of interest

NM has served as a consultant for Amgen, Eli Lilly, and Leo Pharma receiving grants/other payments, as a principal investigator and/or investigator for AbbVie, Celgene, AstraZeneca, Janssen Pharmaceuticals, Inc., Novartis, and Abcentra receiving grants and/or research funding, and as a principal investigator for the NIH receiving grants and/or research funding.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of

their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcvm.2022.1023651/full#supplementary-material

References

1. Libby P, Ridker PM, Hansson GK. Inflammation in atherosclerosis: from pathophysiology to practice. *J Am Coll Cardiol.* (2009) 54:2129–38. doi: 10.1016/j.jacc.2009.09.009

2. Harrington RA. Targeting inflammation in coronary artery disease. *N Engl J Med.* (2017) 377:1197–8. doi: 10.1056/NEJMe1709904

3. Lusis AJ. Atherosclerosis. *Nature.* (2000) 407:233–41. doi: 10.1038/35025203

4. Arbab-Zadeh A, Fuster V. The myth of the "vulnerable plaque": transitioning from a focus on individual lesions to atherosclerotic disease burden for coronary artery disease risk assessment. *J Am Coll Cardiol.* (2015) 65:846–55. doi: 10.1016/j.jacc.2014.11.041

5. Chen YC, Huang AL, Kyaw TS, Bobik A, Peter K. Atherosclerotic plaque rupture: identifying the straw that breaks the camel's back. *Arterioscler Thromb Vasc Biol.* (2016) 36:e63–72. doi: 10.1161/ATVBAHA.116.307993

6. Virani SS, Alonso A, Aparicio HJ, Benjamin EJ, Bittencourt MS, Callaway CW, et al. Heart disease and stroke statistics-2021 update: a report from the American heart association. *Circulation.* (2021) 143:e254–743. doi: 10.1161/CIR.0000000000000950

7. Ahmad FB, Anderson RN. The leading causes of death in the Us for 2020. *Jama.* (2021) 325:1829–30. doi: 10.1001/jama.2021.5469

8. Ellulu MS, Patimah I, Khaza'ai H, Rahmat A, Abed Y, Ali F. Atherosclerotic cardiovascular disease: a review of initiators and protective factors. *Inflammopharmacology.* (2016) 24:1–10. doi: 10.1007/s10787-015-0255-y

9. Mason JC, Libby P. Cardiovascular disease in patients with chronic inflammation: mechanisms underlying premature cardiovascular events in rheumatologic conditions. *Eur Heart J.* (2015) 36:482–9c. doi: 10.1093/eurheartj/ehu403

10. Funderburg NT, Mehta NN. Lipid abnormalities and inflammation in HIV inflection. *Curr HIV/AIDS Rep.* (2016) 13:218–25. doi: 10.1007/s11904-016-0321-0

11. Aksentijevich M, Lateef SS, Anzenberg P, Dey AK, Mehta NN. Chronic inflammation, cardiometabolic diseases and effects of treatment: psoriasis as a human model. *Trends Cardiovasc Med.* (2020) 30:472–8. doi: 10.1016/j.tcm.2019.11.001

12. Harrington CL, Dey AK, Yunus R, Joshi AA, Mehta NN. Psoriasis as a human model of disease to study inflammatory atherogenesis. *Am J Physiol Heart Circ Physiol.* (2017) 312:H867–h73. doi: 10.1152/ajpheart.00774.2016

13. McMahon M, Grossman J, Skaggs B, Fitzgerald J, Sahakian L, Ragavendra N, et al. Dysfunctional proinflammatory high-density lipoproteins confer increased risk of atherosclerosis in women with systemic lupus erythematosus. *Arthritis Rheum.* (2009) 60:2428–37. doi: 10.1002/art.24677

14. Khairy N, Ezzat Y, Naeem N, Taha R, Wesam R. Atherosclerosis biomarkers in female systemic lupus erythematosus patients with and without cardiovascular diseases. *Egypt Rheumatol.* (2017) 39:7–12. doi: 10.1016/j.ejr.2016.03.003

15. Grundy SM, Stone NJ, Bailey AL, Beam C, Birtcher KK, Blumenthal RS, et al. 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. *J Am Coll Cardiol.* (2019) 73:e285–350. doi: 10.1016/j.jacc.2018.11.003

16. Finckh A, Courvoisier DS, Pagano S, Bas S, Chevallier-Ruggeri P, Hochstrasser D, et al. Evaluation of cardiovascular risk in patients with rheumatoid arthritis: do cardiovascular biomarkers offer added predictive ability over established clinical risk scores? *Arthritis Care Res (Hoboken).* (2012) 64:817–25. doi: 10.1002/acr.21631

17. Lloyd-Jones DM, Wilson PW, Larson MG, Beiser A, Leip EP, D'Agostino RB, et al. Framingham risk score and prediction of lifetime risk for coronary heart disease. *Am J Cardiol.* (2004) 94:20–4. doi: 10.1016/j.amjcard.2004.03.023

18. Chung CP, Oeser A, Avalos I, Gebretsadik T, Shintani A, Raggi P, et al. Utility of the framingham risk score to predict the presence of coronary atherosclerosis in patients with rheumatoid arthritis. *Arthritis Res Ther.* (2006) 8:R186. doi: 10.1186/ar2098

19. Torres T, Sales R, Vasconcelos C, Martins da Silva B, Selores M. Framingham risk score underestimates cardiovascular disease risk in severe psoriatic patients: implications in cardiovascular risk factors management and primary prevention of cardiovascular disease. *J Dermatol.* (2013) 40:923–6. doi: 10.1111/1346-8138.12267

20. Packard C, Chapman MJ, Sibartie M, Laufs U, Masana L. Intensive low-density lipoprotein cholesterol lowering in cardiovascular disease prevention: opportunities and challenges. *Heart.* (2021) 107:1369–75. doi: 10.1136/heartjnl-2020-318760

21. Borén J, 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) 41:2313–30. doi: 10.1093/eurheartj/ehz962

22. Ridker PM, Genest J, Boekholdt SM, Libby P, Gotto AM, Nordestgaard BG, et al. Hdl cholesterol and residual risk of first cardiovascular events after treatment with potent statin therapy: an analysis from the jupiter trial. *Lancet.* (2010) 376:333–9. doi: 10.1016/S0140-6736(10)60713-1

23. Packard CJ. Small dense low-density lipoprotein and its role as an independent predictor of cardiovascular disease. *Curr Opin Lipidol.* (2006) 17:412–7. doi: 10.1097/01.mol.0000236367.42755.c1

24. Tsimikas S, A. Test in context: lipoprotein(a): diagnosis, prognosis, controversies, and emerging therapies. *J Am Coll Cardiol.* (2017) 69:692–711. doi: 10.1016/j.jacc.2016.11.042

25. Trpkovic A, Resanovic I, Stanimirovic J, Radak D, Mousa SA, Cenic-Milosevic D, et al. Oxidized low-density lipoprotein as a biomarker of cardiovascular diseases. *Crit Rev Clin Lab Sci.* (2015) 52:70–85. doi: 10.3109/10408363.2014.992063

26. Di Pietro N, Formoso G, Pandolfi A. Physiology and pathophysiology of oxldl uptake by vascular wall cells in atherosclerosis. *Vascul Pharmacol.* (2016) 84:1–7. doi: 10.1016/j.vph.2016.05.013

27. Nyyssönen K, Kurl S, Karppi J, Nurmi T, Baldassarre D, Veglia F, et al. Ldl oxidative modification and carotid atherosclerosis: results of a multicenter study. *Atherosclerosis.* (2012) 225:231–6. doi: 10.1016/j.atherosclerosis.2012.08.030

28. Febbraio M, Silverstein RL. Cd36: Implications in cardiovascular disease. *Int J Biochem Cell Biol.* (2007) 39:2012–30. doi: 10.1016/j.biocel.2007.03.012

29. Barreto J, Karathanasis SK, Remaley A, Sposito AC. Role of Lox-1 (lectin-like oxidized low-density lipoprotein receptor 1) as a cardiovascular risk predictor: mechanistic insight and potential clinical use. *Arterioscler Thromb Vasc Biol.* (2021) 41:153–66. doi: 10.1161/ATVBAHA.120.315421

30. Holvoet P. Oxidized Ldl and coronary heart disease. *Acta Cardiol.* (2004) 59:479–84. doi: 10.2143/AC.59.5.2005219

31. Holvoet P, Mertens A, Verhamme P, Bogaerts K, Beyens G, Verhaeghe R, et al. Circulating oxidized ldl is a useful marker for identifying patients with coronary artery disease. *Arterioscler Thromb Vasc Biol.* (2001) 21:844–8. doi: 10.1161/01.ATV.21.5.844

32. Cicero AFG, Kuwabara M, Johnson R, Bove M, Fogacci F, Rosticci M, et al. Ldl-oxidation, serum uric acid, kidney function and pulse-wave velocity: data from the brisighella heart study cohort. *Int J Cardiol.* (2018) 261:204–8. doi: 10.1016/j.ijcard.2018.03.077

33. Sorokin AV, Kotani K, Elnabawi YA, Dey AK, Sajja AP, Yamada S, et al. Association between oxidation-modified lipoproteins and coronary plaque in psoriasis. *Circ Res.* (2018) 123:1244–54. doi: 10.1161/CIRCRESAHA.118.313608

34. Meisinger C, Baumert J, Khuseyinova N, Loewel H, Koenig W. Plasma oxidized low-density lipoprotein, a strong predictor for acute coronary heart disease events in apparently healthy, middle-aged men from the general population. *Circulation.* (2005) 112:651–7. doi: 10.1161/CIRCULATIONAHA.104.529297

35. Page MJ, Moher D, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. Prisma 2020 explanation and elaboration: updated guidance and exemplars for reporting systematic reviews. *BMJ.* (2021) 372:n160. doi: 10.1136/bmj.n160

36. Wan X, Wang W, Liu J, Tong T. Estimating the sample mean and standard deviation from the sample size, median, range and/or interquartile range. *BMC Med Res Methodol.* (2014) 14:135. doi: 10.1186/1471-2288-14-135

37. Higgins JP, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-analyses. *BMJ.* (2003) 327:557–60. doi: 10.1136/bmj.327.7414.557

38. Borenstein M, Hedges LV, Higgins JPT, Rothstein HR. *Introduction to Meta-Analysis*. Hoboken, NJ: Wiley (2021). doi: 10.1002/9781119558378

39. Ajeganova S, Huizinga T. Sustained remission in rheumatoid arthritis: latest evidence and clinical considerations. *Ther Adv Musculoskelet Dis.* (2017) 9:249–62. doi: 10.1177/1759720X17720366

40. Parra S, Coll B, Aragonés G, Marsillach J, Beltrán R, Rull A, et al. Nonconcordance between subclinical atherosclerosis and the calculated framingham risk score in HIV-infected patients: relationships with serum markers of oxidation and inflammation. *HIV Med.* (2010) 11:225–31. doi: 10.1111/j.1468-1293.2009.00766.x

41. Hoffmann U, Lu MT, Foldyna B, Zanni MV, Karady J, Taron J, et al. Assessment of coronary artery disease with computed tomography angiography and inflammatory and immune activation biomarkers among adults with HIV eligible for primary cardiovascular prevention. *JAMA Netw Open.* (2021) 4:e2114923. doi: 10.1001/jamanetworkopen.2021.14923

42. Dey AK, Gaddipati R, Elnabawi YA, Ongstad E, Goyal A, Chung JH, et al. Association between soluble lectinlike oxidized low-density lipoprotein receptor-1 and coronary artery disease in psoriasis. *JAMA Dermatol.* (2020) 156:151–7. doi: 10.1001/jamadermatol.2019.3595

43. Bertoia ML, Pai JK, Lee JH, Taleb A, Joosten MM, Mittleman MA, et al. Oxidation-specific biomarkers and risk of peripheral artery disease. *J Am Coll Cardiol.* (2013) 61:2169–79. doi: 10.1016/j.jacc.2013.02.047

44. Wells GA, Wells G, Shea B, Shea B, O'Connell D, Peterson J, et al. *The Newcastle-Ottawa Scale (Nos) for Assessing the Quality of Nonrandomised Studies in Meta-Analyses.* (2014).

45. Coll B, Parra S, Alonso-Villaverde C, Aragonés G, Montero M, Camps J, et al. The role of immunity and inflammation in the progression of atherosclerosis in patients with HIV infection. *Stroke.* (2007) 38:2477–84. doi: 10.1161/STROKEAHA.106.479030

46. Navab M, Berliner JA, Watson AD, Hama SY, Territo MC, Lusis AJ, et al. The Yin and Yang of oxidation in the development of the fatty streak: a review based on the 1994 george lyman duff memorial lecture. *Arterioscler Thromb Vasc Biol.* (1996) 16:831–42. doi: 10.1161/01.ATV.16.7.831

47. Vaarala O, Mänttäri M, Manninen V, Tenkanen L, Puurunen M, Aho K, et al. Anti-cardiolipin antibodies and risk of myocardial infarction in a prospective cohort of middle-aged men. *Circulation.* (1995) 91:23–7. doi: 10.1161/01.CIR.91.1.23

48. Hamsten A, Björkholm M, Norberg R, De Faire U, Holm G. Antibodies to cardiolipin in young survivors of myocardial infarction: an association with recurrent cardiovascular events. *Lancet.* (1986) 327:113–6. doi: 10.1016/S0140-6736(86)92258-0

49. Palinski W, Ylä-Herttuala S, Rosenfeld ME, Butler SW, Socher SA, Parthasarathy S, et al. Antisera and monoclonal antibodies specific for epitopes generated during oxidative modification of low density lipoprotein. *Arteriosclerosis.* (1990) 10:325–35. doi: 10.1161/01.ATV.10.3.325

50. Orem A, Cimşit G, Deger O, Orem C, Vanizor B. The significance of autoantibodies against oxidatively modified low-density lipoprotein (Ldl) in patients with psoriasis. *Clin Chim Acta.* (1999) 284:81–8. doi: 10.1016/S0009-8981(99)00062-5

51. Elkan AC, Sjöberg B, Kolsrud B, Ringertz B, Hafström I, Frostegård J. Gluten-free vegan diet induces decreased ldl and oxidized ldl levels and raised atheroprotective natural antibodies against phosphorylcholine in patients with rheumatoid arthritis: a randomized study. *Arthritis Res Ther.* (2008) 10:R34. doi: 10.1186/ar2388

52. Tsimikas S, Witztum JL. Measuring circulating oxidized low-density lipoprotein to evaluate coronary risk. *Circulation.* (2001) 103:1930–2. doi: 10.1161/01.CIR.103.15.1930

53. Holvoet P, Collen D, Van de Werf F. Malondialdehyde-modified ldl as a marker of acute coronary syndromes. *JAMA.* (1999) 281:1718–21. doi: 10.1001/jama.281.18.1718

54. Itabe H, Yamamoto H, Imanaka T, Shimamura K, Uchiyama H, Kimura J, et al. Sensitive detection of oxidatively modified low density lipoprotein using a monoclonal antibody. *J Lipid Res.* (1996) 37:45–53. doi: 10.1016/S0022-2275(20)37634-3

55. Pahan K. Lipid-lowering drugs. *Cell Mol Life Sci.* (2006) 63:1165–78. doi: 10.1007/s00018-005-5406-7

56. Stone NJ, Robinson JG, Lichtenstein AH, Bairey Merz CN, Blum CB, Eckel RH, et al. 2013 Acc/Aha guideline on the treatment of blood cholesterol to reduce atherosclerotic cardiovascular risk in adults: a report of the american college of cardiology/American Heart Association task force on practice guidelines. *Circulation.* (2014) 129:S1–45. doi: 10.1161/01.cir.0000437738.63853.7a

57. Sandhu PK, Musaad SM, Remaley AT, Buehler SS, Strider S, Derzon JH, et al. Lipoprotein biomarkers and risk of cardiovascular disease: a laboratory medicine best practices (Lmbp) systematic review. *J Appl Lab Med.* (2016) 1:214–29. doi: 10.1373/jalm.2016.021006

58. Choi SH, Chae A, Miller E, Messig M, Ntanios F, DeMaria AN, et al. 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) 52:24–32. doi: 10.1016/j.jacc.2008.02.066

59. Gao S, Zhao D, Wang M, Zhao F, Han X, Qi Y, et al. Association between circulating oxidized Ldl and atherosclerotic cardiovascular disease: a meta-analysis of observational studies. *Can J Cardiol.* (2017) 33:1624–32. doi: 10.1016/j.cjca.2017.07.015

60. Tsimikas S, Witztum JL, Miller ER, Sasiela WJ, Szarek M, Olsson AG, 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) 110:1406–12. doi: 10.1161/01.CIR.0000141728.23033.B5

61. Nishikido T, Oyama J, Keida T, Ohira H, Node K. High-dose statin therapy with rosuvastatin reduces small dense ldl and mda-ldl: the standard vs. high-dose therapy with rosuvastatin for lipid lowering (sard) trial. *J Cardiol.* (2016) 67:340–6. doi: 10.1016/j.jjcc.2015.05.017

62. Nou E, Lo J, Grinspoon SK. Inflammation, immune activation, and cardiovascular disease in HIV. *Aids.* (2016) 30:1495–509. doi: 10.1097/QAD.0000000000001109

63. Duong M, Petit JM, Martha B, Galland F, Piroth L, Walldner A, et al. Concentration of circulating oxidized Ldl in HIV-infected patients treated with antiretroviral agents: relation to hiv-related lipodystrophy. *HIV Clin Trials.* (2006) 7:41–7. doi: 10.1310/7381-M1YD-RTV5-4RYT

64. Strilchuk L, Fogacci F, Cicero AF. Safety and tolerability of injectable lipid-lowering drugs: an update of clinical data. *Expert Opin Drug Saf.* (2019) 18:611–21. doi: 10.1080/14740338.2019.1620730

65. Steffens D, Bramlage P, Scheeff C, Kasner M, Hassanein A, Friebel J, et al. Pcsk9 inhibitors and cardiovascular outcomes. *Expert Opin Biol Ther.* (2020) 20:35–47. doi: 10.1080/14712598.2020.1677604

66. Rosenson RS, Hegele RA, Fazio S, Cannon CP. The evolving future of pcsk9 inhibitors. *J Am Coll Cardiol.* (2018) 72:314–29. doi: 10.1016/j.jacc.2018.04.054

67. Schlüter KD, Wolf A, Weber M, Schreckenberg R, Schulz R. Oxidized low-density lipoprotein (Oxldl) affects load-free cell shortening of cardiomyocytes in a proprotein convertase subtilisin/kexin 9 (Pcsk9)-dependent way. *Basic Res Cardiol.* (2017) 112:63. doi: 10.1007/s00395-017-0650-1

68. Tang Z, Jiang L, Peng J, Ren Z, Wei D, Wu C, et al. Pcsk9 sirna suppresses the inflammatory response induced by oxldl through inhibition of Nf-?b activation in Thp-1-derived macrophages. *Int J Mol Med.* (2012) 30:931–8. doi: 10.3892/ijmm.2012.1072

69. Schwartz GG, Steg PG, Szarek M, Bhatt DL, Bittner VA, Diaz R, et al. Alirocumab and cardiovascular outcomes after acute coronary syndrome. *N Engl J Med.* (2018) 379:2097–107. doi: 10.1056/NEJMoa1801174

70. Sabatine MS, Giugliano RP, Keech AC, Honarpour N, Wiviott SD, Murphy SA, et al. Evolocumab and clinical outcomes in patients with cardiovascular disease. *N Engl J Med.* (2017) 376:1713–22. doi: 10.1056/NEJMoa1615664

71. Hartley A, Haskard D, Khamis R. Oxidized Ldl and anti-oxidized Ldl antibodies in atherosclerosis - novel insights and future directions in diagnosis and therapy. *Trends Cardiovasc Med.* (2019) 29:22–6. doi: 10.1016/j.tcm.2018.05.010

72. Hammer A, Kager G, Dohr G, Rabl H, Ghassempur I, Jürgens G. Generation, characterization, and histochemical application of monoclonal antibodies selectively recognizing oxidatively modified apob-containing serum lipoproteins. *Arterioscler Thromb Vasc Biol.* (1995) 15:704–13. doi: 10.1161/01.ATV.15.5.704

73. Palinski W, Ord VA, Plump AS, Breslow JL, Steinberg D, Witztum JL. Apoe-deficient mice are a model of lipoprotein oxidation in atherogenesis. Demonstration of oxidation-specific epitopes in lesions and high titers of autoantibodies to malondialdehyde-lysine in serum. *Arterioscler Thromb.* (1994) 14:605–16. doi: 10.1161/01.ATV.14.4.605

74. Tsimikas S, Brilakis ES, Lennon RJ, Miller ER, Witztum JL, McConnell JP, et al. Relationship of Igg and Igm autoantibodies to oxidized low density lipoprotein with coronary artery disease and cardiovascular events. *J Lipid Res.* (2007) 48:425–33. doi: 10.1194/jlr.M600361-JLR200

75. Grönlund H, Hallmans G, Jansson JH, Boman K, Wikström M, de Faire U, et al. Low levels of igm antibodies against phosphorylcholine predict development of acute myocardial infarction in a population-based cohort from Northern Sweden. *Eur J Cardiovasc Prev Rehabil.* (2009) 16:382–6. doi: 10.1097/HJR.0b013e32832a05df

76. Ravandi A, Boekholdt SM, Mallat Z, Talmud PJ, Kastelein JJP, Wareham NJ, et al. Relationship of Igg and Igm autoantibodies and immune complexes to oxidized Ldl with markers of oxidation and inflammation and cardiovascular events: results from the epic-norfolk study. *J Lipid Res.* (2011) 52:1829–36. doi: 10.1194/jlr.M015776

77. Karvonen J, Päivänsalo M, Kesäniemi YA, Hörkkö S. Immunoglobulin M type of autoantibodies to oxidized low-density lipoprotein has an inverse relation to carotid artery atherosclerosis. *Circulation.* (2003) 108:2107–12. doi: 10.1161/01.CIR.0000092891.55157.A7

78. Pothineni NVK, Karathanasis SK, Ding Z, Arulandu A, Varughese KI, Mehta JL. Lox-1 in atherosclerosis and myocardial ischemia: biology, genetics, and modulation. *J Am Coll Cardiol.* (2017) 69:2759–68. doi: 10.1016/j.jacc.2017.04.010

79. Sagar D, Gaddipati R, Ongstad EL, Bhagroo N, An LL, Wang J, et al. Lox-1: a potential driver of cardiovascular risk in sle patients. *PLoS ONE.* (2020) 15:e0229184. doi: 10.1371/journal.pone.0229184

80. Ishikawa M, Ito H, Furu M, Hashimoto M, Fujii T, Okahata A, et al. Plasma slox-1 is a potent biomarker of clinical remission and disease activity in patients with seropositive Ra. *Mod Rheumatol.* (2016) 26:696–701. doi: 10.3109/14397595.2015.1128871

81. Manzi S, Meilahn EN, Rairie JE, Conte CG, Medsger TA, Jansen-McWilliams L, et al. Age-specific incidence rates of myocardial infarction and angina in women with systemic lupus erythematosus: comparison with the framingham study. *Am J Epidemiol.* (1997) 145:408–15. doi: 10.1093/oxfordjournals.aje.a009122

82. Yuan L, Wang D, Zhou Z. Linc00452 overexpression reverses oxldl-induced injury of human umbilical vein endothelial cells (Huvecs) *via* regulating Mir-194-5p/Igf1r axis. *Front Cardiovasc Med.* (2022) 9:975640. doi: 10.3389/fcvm.2022.975640

83. Wang J, Wang WN, Xu SB, Wu H, Dai B, Jian DD, et al. Microrna-214-3p: a link between autophagy and endothelial cell dysfunction in atherosclerosis. *Acta Physiol (Oxf).* (2018) 222:3. doi: 10.1111/apha.12973

84. Gentile M, Iannuzzo G, Mattiello A, Marotta G, Iannuzzi A, Panico S, et al. Association between Lp (a) and atherosclerosis in menopausal women without metabolic syndrome. *Biomark Med.* (2016) 10:397–402. doi: 10.2217/bmm.16.4

85. St-Pierre AC, Cantin B, Dagenais GR, Mauriège P, Bernard PM, Després JP, et al. Low-density lipoprotein subfractions and the long-term risk of ischemic heart disease in men: 13-year follow-up data from the Québec cardiovascular study. *Arterioscler Thromb Vasc Biol.* (2005) 25:553–9. doi: 10.1161/01.ATV.0000154144.73236.f4

86. Lamarche B, Tchernof A, Moorjani S, Cantin B, Dagenais GR, Lupien PJ, et al. Small, dense low-density lipoprotein particles as a predictor of the risk of ischemic heart disease in men. Prospective results from the québec cardiovascular study. *Circulation.* (1997) 95:69–75. doi: 10.1161/01.CIR.95.1.69

87. Gardner CD, Fortmann SP, Krauss RM. Association of small low-density lipoprotein particles with the incidence of coronary artery disease in men and women. *Jama.* (1996) 276:875–81. doi: 10.1001/jama.1996.03540110029028

88. Stampfer MJ, Krauss RM, Ma J, Blanche PJ, Holl LG, Sacks FM, et al. A prospective study of triglyceride level, low-density lipoprotein particle diameter, and risk of myocardial infarction. *JAMA*. (1996) 276:882–8. doi: 10.1001/jama.1996.03540110036029

89. Gentile M, Peluso R, Di Minno MND, Costa L, Caso F, de Simone B, et al. Association between small dense ldl and sub-clinical atherosclerosis in patients with psoriatic arthritis. *Clin Rheumatol.* (2016) 35:2023–9. doi: 10.1007/s10067-016-3344-4

90. Kovacs L, Kress TC, Belin de. Chantemèle EJ. HIV, combination antiretroviral therapy, and vascular diseases in men and women. *JACC.* (2022) 7:410–21. doi: 10.1016/j.jacbts.2021.10.017

91. Fogacci F, Borghi C, Grassi D, Cicero AFG. People living with human immunodeficiency virus: cardiovascular risk screening for an early and effective risk management. *Atherosclerosis.* (2022) 353:28–9. doi: 10.1016/j.atherosclerosis.2022.06.001

92. Enkhmaa B, Anuurad E, Zhang W, Li CS, Kaplan R, Lazar J, et al. Lipoprotein(a) and HIV: allele-specific apolipoprotein(a) levels predict carotid intima-media thickness in HIV-infected young women in the women's interagency HIV study. *Arterioscler Thromb Vasc Biol.* (2017) 37:997–1004. doi: 10.1161/ATVBAHA.117.309137

93. Peluso R, Caso F, Tasso M, Sabbatino V, Lupoli R, Dario Di Minno MN, et al. Biomarkers of subclinical atherosclerosis in patients with psoriatic arthritis. *Open Access Rheumatol.* (2019) 11:143–56. doi: 10.2147/OARRR.S206931

94. Colaco K, Ocampo V, Ayala AP, Harvey P, Gladman DD, Piguet V, et al. Predictive utility of cardiovascular risk prediction algorithms in inflammatory rheumatic diseases: a systematic review. *J Rheumatol.* (2020) 47:928–38. doi: 10.3899/jrheum.190261

95. Egger M, Schneider M, Davey Smith G. Spurious precision? Meta-analysis of observational studies. *BMJ.* (1998) 316:140–4. doi: 10.1136/bmj.316.7125.140

96. Delgado-Rodríguez M. Systematic reviews of meta-analyses: applications and limitations. *J Epidemiol Community Health.* (2006) 60:90–2. doi: 10.1136/jech.2005.035253

frontiers | Frontiers in Cardiovascular Medicine

TYPE Systematic Review
PUBLISHED 19 January 2023
DOI 10.3389/fcvm.2022.951523

OPEN ACCESS

EDITED BY
Chu-Huang Chen,
The Texas Heart Institute, United States

REVIEWED BY
Rizanda Machmud,
Andalas University, Indonesia
Chueh-Lung Hwang,
The University of Texas at Arlington,
United States

*CORRESPONDENCE
Han Wang
✉ wanghan@swjtu.edu.cn
Zhen Zhang
✉ zhangzhen@swjtu.edu.cn

SPECIALTY SECTION
This article was submitted to
Lipids in Cardiovascular Disease,
a section of the journal
Frontiers in Cardiovascular Medicine

RECEIVED 24 May 2022
ACCEPTED 30 December 2022
PUBLISHED 19 January 2023

CITATION
Tian Y, Wu W, Qin L, Yu X, Cai L, Wang H and Zhang Z (2023) Prognostic value of remnant cholesterol in patients with coronary heart disease: A systematic review and meta-analysis of cohort studies.
Front. Cardiovasc. Med. 9:951523.
doi: 10.3389/fcvm.2022.951523

Prognostic value of remnant cholesterol in patients with coronary heart disease: A systematic review and meta-analysis of cohort studies

Yun Tian, Wenli Wu, Li Qin, Xiuqiong Yu, Lin Cai, Han Wang* and Zhen Zhang*

Department of Cardiology, The Affiliated Hospital of Southwest Jiaotong University, The Third People's Hospital of Chengdu, Chengdu, Sichuan, China

Background: The relationship between abnormal lipid levels and atherosclerotic cardiovascular diseases is well established, but the association between remnant cholesterol (RC) and coronary heart disease (CHD) remains uncertain. The aim of this meta-analysis is to systematically evaluate the prognostic value of RC concentration in patients with CHD.

Methods: PubMed, EMBASE, Cochrane, and Web of Science databases were reviewed to identify relevant observational cohort studies published in English up to December 2021. Random-effects meta-analysis compared the highest and lowest RC concentration. The primary outcome was a composite of major adverse cardiovascular events (MACEs) and all-cause mortality in patients with CHD.

Results: A total of 10 studies recruiting 30,605 patients with CHD were selected to be included in this meta-analysis. Patients with CHD with elevated RC concentration had an increased risk of the composite endpoint events (RR = 1.54, 95% CI: 1.26–1.87) and MACEs (RR = 1.70, 95% CI: 1.54–1.88), but the risk of all-cause mortality was not statistically significant (RR = 1.16, 95% CI: 0.79–1.69, P = 0.44). Subgroup analysis showed consistent results.

Conclusion: Our results suggest that elevated concentration RC may independently predict MACEs in patients with CHD. Determination of RC concentration may improve risk stratification of prognosis in patients with CHD. However, more high-quality studies are necessary to confirm this association.

KEYWORDS

remnant cholesterol, coronary heart disease, prognosis, dyslipidemia, meta-analysis

Introduction

Coronary heart disease (CHD) is the most common type of organ disease caused by atherosclerosis, which is seriously threatening people's life and health (1, 2). The prevalence of CHD in the world is approximately 4.6–9.2%, and in 2019, the disease caused 9.14 million deaths worldwide (1). Dyslipidemia is one of the important risk factors for CHD. Currently,

lowering low-density lipoprotein cholesterol (LDL-C) is one of the main intervention targets in the treatment of CHD. However, previous studies found that even after reducing LDL-C to an appropriate level and controlling other risk factors, there may be significant differences in the prognosis of patients with CHD (3). Therefore, it is important to find more reliable prognostic indicators to evaluate the long-term prognosis of CHD and formulate the best treatment plan.

In recent years, remnant cholesterol (RC) has been reported to have a critical role in atherosclerosis and CHD, which might indicate it may also be a critical component of the residual risk of patients with CHD (3, 4). RC is the cholesterol content of all non-low-density lipoprotein (LDL) and non-high-density lipoprotein (HDL). Compared with LDL-C, RC had a stronger atherogenic ability because it possesses a larger quantity and volume, carries more cholesterol, and does not need oxidative modification (5, 6). Some observational cohort studies have linked high RC concentrations with an increased risk of CHD (7, 8). Furthermore, RC was found to be causally associated with CHD development in previously healthy individuals (9). However, the prognostic value of plasma RC levels in secondary prevention settings is still undefined because previous studies showed inconsistent and controversial results (10–12). Meanwhile, it is yet to be established whether the prognostic value of RC varies among populations, ages, or the classification of CHDs. In particular, there is still a lack of a standardized method for RC measurement, with strikingly different RC concentrations across studies (10, 12, 13), which may also have contributed to the discrepancy in the outcomes (14–16).

Therefore, our study aims to systematically review and compile meta-analyses of the evidence on the relationship between RC concentration and CHD outcome, by identifying the potential confounders and investigating the prognostic value, which may provide a novel perspective for risk assessment and treatment in patients with CHD.

Materials and methods

Our meta-analysis was performed according to the recommendation of the meta-analysis of Observational Studies in Epidemiology (MOOSE) (17) and the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement (18).

Search strategy

We comprehensively searched four medical databases, including PubMed, EMBASE, Cochrane, and Web of Science, to identify cohort studies assessing the relationship between RC concentration and cardiovascular outcomes published in English from inception up to 25 December 2021. The query syntax was set using Medical Subject Headings (MeSH) and thesaurus search terms, including ("remnant cholesterol" OR "remnant-like particle cholesterol" OR "triglyceride-rich lipoprotein cholesterol") AND ("coronary heart disease" OR "coronary disease" OR "coronary artery disease"). The detailed search strategy was presented in the **Supplementary material**. References retrieved from the studies, as well as relevant reports, were also hand-searched to reduce the likelihood of missing any publications.

Inclusion and exclusion criteria

The inclusion criteria for this study were presented as follows: (1) cohort studies; (2) an inception cohort involving adults with CHD; CHD included stable or unstable angina, and myocardial infarction (MI); (3) the exposure factor was RC concentration; (4) the endpoint was major adverse cardiovascular events (MACEs) and all-cause mortality. MACEs included cardiac death, MI, ischemic stroke, myocardial ischemia, heart failure, unstable angina requiring readmission, and coronary revascularization; and (5) the highest and lowest RC concentration groups of multivariate-adjusted relative risks (RRs), odds ratios (ORs), or hazard ratios (HRs) and their 95% confidence intervals (95% CI) or the above indicators could be calculated with the complete data (19).

Exclusion criteria included (1) case reports, commentary, and conference abstracts; (2) animal, cross-sectional studies, or randomized clinical trials; (3) studies carried out among pregnant women or children; and (4) examined non-relevant outcomes.

YT did the screening of the titles and abstracts of the identified articles, and pertinent articles were independently reviewed in full text by two investigators (YT and WW). Thus, disagreement was resolved through consensus.

Data collection and quality assessment

Data extraction was in a standardized style. Two investigators (YT and LQ) independently extracted the following data: the first author, publication year, population, study design, type of CHD, sample size, percentage of women, age, exposure assessment method, fasting status, follow-up duration, outcome assessment, categorical or continuous, adjusted risk estimates, and adjustment for variables.

The quality of observational cohort studies was assessed using the Newcastle–Ottawa Scale (NOS) (20), which was ranked as poor (score 1–3), fair (score 4–6), or good (score 7–9) according to the quality of study participant selection, comparability, and outcome. Studies with NOS $\geq$7 points were considered high quality. Any disagreements were discussed and resolved by a chief investigator (HW), and a consensus was reached in all cases.

Statistical analysis

Review Manager 5.4 software (The Cochrane Collaboration, Oxford, UK) and Stata 16.0 (Stata Corporation, College Station, TX, USA) were employed for statistical analysis. The I^2 statistics and chi-square Cochran's Q-test were used to assess the heterogeneity across studies. If $P \geq 0.05$ and $I^2 < 50\%$, suggesting that no significant heterogeneity could be found, a fixed-effect model was also applied. In addition, if $P < 0.05$ and $I^2 \geq 50\%$, a random-effect model was cautiously applied, and then subgroup analysis was used to explore the source of heterogeneity (21). The elimination of individual studies one by one was also performed for sensitivity analysis in order to explore the heterogeneity and assess the stability of the meta-analysis. A funnel plot combined with Egger's test was employed to investigate the potential publication bias of the involved studies. Finally, the RR with 95% CI was employed for the effect estimation metric, and HR and OR were converted into RR (22–24). The p-value of < 0.05 meant the difference was statistically significant.

Results

Literature search results

A total of 2,308 articles were retrieved, and after 381 duplicates were removed, 1,927 unique records remained. After screening the titles and abstracts of the articles, 38 records were considered for a detailed full-text screening. Of these studies, 28 articles were excluded: studies with a non-CHD population (n = 11) (16, 25–34), those with no interest outcome (n = 5) (35–39), those with potential patients' duplication with other articles (n = 5) (8, 9, 40–42), and studies with ineligible study design (n = 7) (43–49). Finally, 10 articles (4, 10–13, 50–54) covering 12 cohorts enrolling 30,605 subjects met the selection criteria. **Figure 1** depicts the literature screening process and results in detail.

Study characteristics and quality assessment

The main characteristics of the included studies are summarized in **Table 1**. Of the 10 included studies, three were performed in China (11, 50, 54), three in Japan (4, 13, 51), two in Denmark (52, 53), one in the United States (12), and one collaborative study involving multiple countries (10). The studies were published from 1999 (13) to 2021 (11), of which nine were prospective cohort designs (4, 11–13, 50–54) and one was retrospective cohort design (10). In addition, two studies included two cohorts (50, 52). The sample size ranged from 120 (51) to 6723 (11). The follow-up time ranged from 1.7 (51) to 7.0 (52) years, and participants' age varied from 57.7 (11) to 68.0 (53) years. The average NOS scores for these studies included were 7.3, demonstrating that the quality of the cohort study was good.

Meta-analysis results

The results demonstrated that elevated RC concentration was related to an increased risk of composite endpoint events (MACEs and all-cause death) (RR = 1.54, 95% CI: 1.26–1.87, $P < 0.0001$) in a random-effect model (**Figure 2**). Significant heterogeneity between studies was observed (I^2 = 85%, $P < 0.0001$), and sensitivity analysis indicated that the total combined effect size did not change significantly in each step, demonstrating that the meta-analysis results were relatively stable. However, if the study conducted by

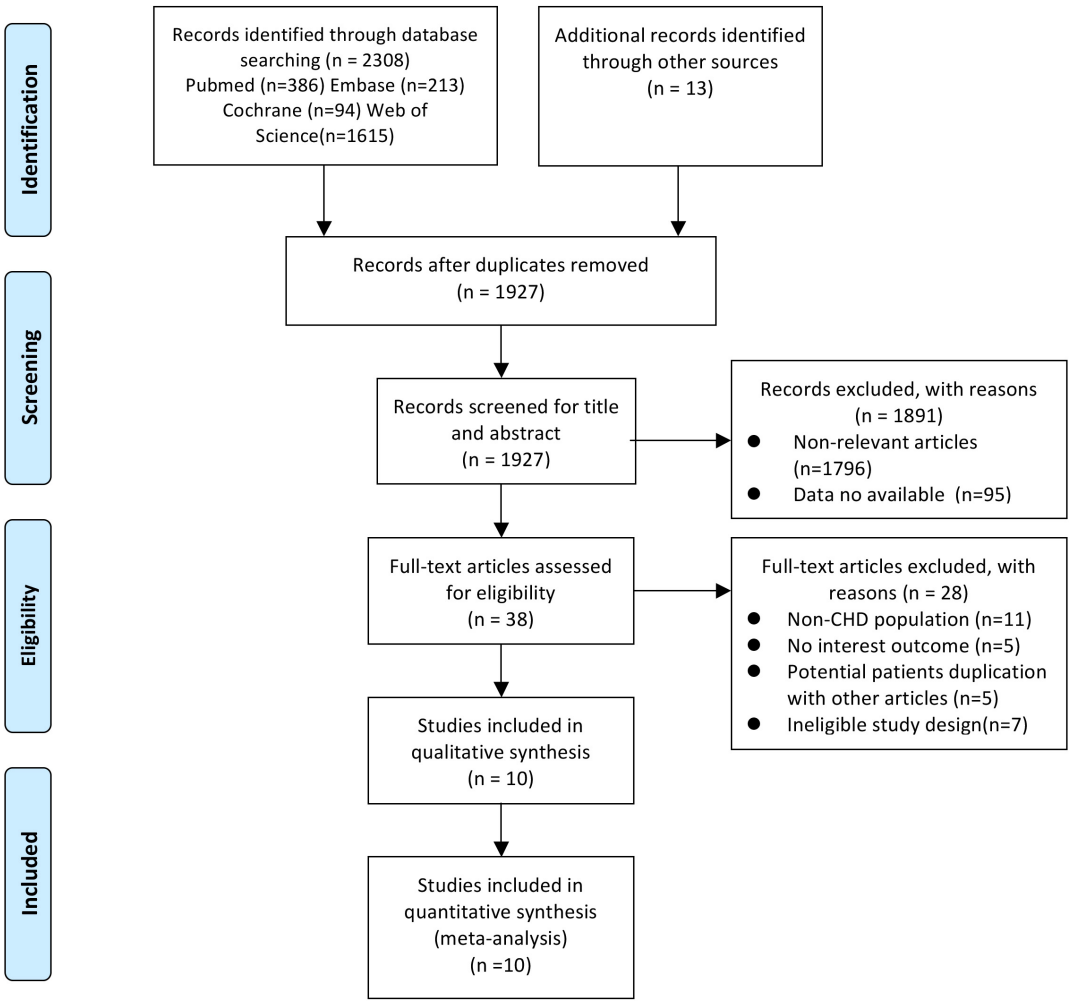


FIGURE 1
Flow diagram of the study search and selection process. From Moher et al. (65).

TABLE 1 Main characteristics of the included studies.

First author, year	Population	Type of study	Partici-pation	Sample size (number)	% females	Age (year)	RC concen-tration (mg/dl)	Exposure assessment	Fasting status	Follow-up duration (year)	Outcome assessment	OR, RR, or HR (95%CI)	Categorical or continuous	Variables adjusted[1]	NOS score
Cao et al. (50)	Chinese	prospective cohort study	CAD	4355	28.9	58.2 ± 9.7	5.0 (2.7–9.7)	Automated assay	Fasting	5.1	MACEs	HR: 1.53 (1.16–2.02)	Q5 vs. Q1	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12	9
Cao et al. (50)	Chinese	prospective cohort study	CAD	4355	28.9	58.2 ± 9.7	9.0 (6.5–12.4)	Immunosepa-ration	Fasting	5.1	MACEs	HR: 1.49 (1.12–2.09)	Q5 vs. Q1	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12	9
Elshazly et al. (10)	North and South American, etc.	retrospective cohort study	CAD	5754	28.0	58.1 ± 9.2	23.8 (19.1–30.8)	Calculation	Fasting	2.0	MACEs	HR 1.62 (1.27–2.07)	Q4 vs. Q1	–	6
Fujihara et al. (4)	Japanese	prospective cohort study	CAD	247	9.0	67 (60–74)	3.6 (2.5–5.5)	Immunosepa-ration	Fasting	3.2	MACEs	HR 1.62 (1.26–2.07)	≥ 3.9 mg/dl vs. < 3.9 mg/dl	4, 11, 13, 14, 15	8
Fukushima et al. (51)	Japanese	prospective cohort study	CAD + DM	120	37.5	65.6 ± 8.4	5.8 (3.1–6.2)	Immunosepa-ration	Fasting	1.7	MACEs	OR 2.2 (1.2–6.4)	> 4.7 mg/dl vs. ≤ 4.7 mg/dl	1, 4, 7, 8, 9, 10, 11, 13, 16, 17, 18	6
Jepsen et al. (52)	Danish	prospective cohort study	IHD	5414	30.3	64.4	14.4 (9.0–21.6)	Calculation	non-fasting	7.0	mortality	HR: 1.5 (1.2–2.0)	Q4 vs. Q1	1, 2, 4, 6, 16	8
Jepsen et al. (52)	Danish	prospective cohort study	IHD	5414	30.3	64.4	1.4 (0.7–3.4)	Automated assay	non-fasting	7.0	mortality	HR: 1.2 (1.0–1.5)	Q4 vs. Q1	1, 2, 4, 6, 16	8
Kugiyama et al. (13)	Japanese	prospective cohort study	CAD	135	34.0	65.0 ± 9.7	3.4	Immunosepa-ration	Fasting	2.2	MACEs	OR 6.38 (2.3–17.6)	highest vs. lowest tertile	1, 2, 4, 5, 8, 10, 11, 16, 19, 20	7
Langsted et al. (53)	Danish	prospective cohort study	MI/IS	2973	32.0	68 (61–74)	NP	Calculation	non-fasting	NR	MACEs	HR 1.71 (1.24–2.36)	Q4 vs. Q1	4, 9, 15, 16, 22	7
Liu et al. (11)	Chinese	prospective cohort study	CAD	6723	26.2	57.7 ± 10.8	9.2 ± 5.0	NP	NP	4.9	MACEs	HR 1.79 (1.18–2.71)	Q4 vs. Q1	1, 2, 3, 4, 5, 6, 7, 9, 10, 11, 12, 16, 18, 21, 33	8
Martin et al. (12)	American	prospective cohort study	AMI	2465	32.0	58 ± 12	20 (14–27)	VLDL3-C + IDL-C	NP	2.0	mortality	HR 0.76 (0.64–0.91)	T3 vs. T1	1, 2, 3, 4, 5, 6, 9, 10, 16, 20, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36	7
Zhao et al. (54)	Chinese	prospective cohort study	NSTE-ACS	2419	28.2	60.08 ± 8.97	12.4 ± 7.6	Calculation	Fasting	3.0	MACEs and mortality	MACEs: HR 1.960 (1.558–2.465); mortality: HR 2.207 (0.612–7.959);	highest vs. lowest	–	7

NP, not provided; HR, hazard ratio; OR, odds ratio; RR, risk ratio; CI, confidence intervals; CAD, coronary artery disease; DM, diabetes mellitus; IHD, ischemic heart disease; MI, myocardial infarction; IS, ischemic stroke; AMI, acute myocardial infarction; NSTE-ACS, non-ST segment elevation acute coronary syndrome; MACEs, major adverse cardiovascular events; T, tertile; Q4, quartile; Q5, quintile; NOS, Newcastle–Ottawa Scale.

[1] Adjustments: age (1), sex (2), body mass index (3), smoking (4), diabetes (5), statin use (6), family history of CAD (7), TC (8), LDL-C (9), HDL-C (10), triglyceride (11), hsCRP (12), HbA1c (13), ApoB (14), lipoprotein (a) (15), hypertension (16), three-vessel disease (17), left ventricular ejection fraction (18), stenosis of left main coronary artery (19), number of diseased coronary arteries (20), creatinine (21), lipid-lowering therapy (22), the GRACE 1.0 score (23), site (24), race (25), insurance (26), education (27), alcohol use (28), physical activity (29), kidney disease (30), heart failure (31), prior MI (32), ezetimibe (33), niacin (34), fibrate (35), and fish oil (36).

10.3389/fcvm.2022.951523

Martin et al. (12) was eliminated, the heterogeneity decreased significantly (I^2 = 44%, RR = 1.61, 95% CI: 1.43–1.80).

In addition, as shown in **Figure 3**, eight of 10 studies reported the MACEs as an outcome, and the other two studies addressed the all-cause mortality outcome, in which one study reported both MACEs risk and all-cause mortality risk. Furthermore, patients with CHD with elevated RC concentration had an increased risk of MACEs (RR = 1.70, 95% CI: 1.54–1.88, $P < 0.0001$) without significant heterogeneity (I^2 = 0%, P = 0.56) in a fixed-effect model. However, the risk of all-cause mortality was not statistically significant (RR = 1.16, 95% CI: 0.79–1.69, P = 0.44), with significant heterogeneity (I^2 = 89%, $P < 0.0001$) in a random effect model. In addition, sensitivity analysis showed that after removing the study of Martin et al. (12), the heterogeneity decreased significantly, and the total combined effect size changed as well (I^2 = 32%, RR = 1.34, 95% CI: 1.10–1.63, P = 0.003).

Subgroup analysis

In the subgroup analysis, the association remained constant, suggesting a positive association between RC concentration and CHD risks in studies conducted in the Asian region (RR = 1.72, 95% CI: 1.53–1.95, $P < 0.0001$) for those with the diagnosis of MI (RR = 1.64, 95% CI: 1.46–1.85, $P < 0.0001$) and with older age (≥65 years old) (RR = 1.78, 95% CI: 1.46–2.15, $P < 0.0001$). In addition, this constant

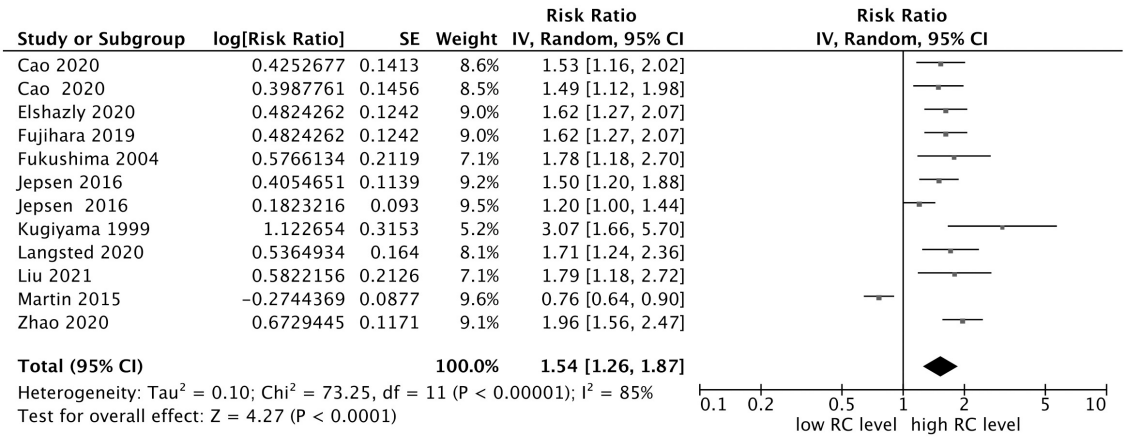


FIGURE 2
Forest plots showing the pooled RR with 95% CI of composite endpoint events for the highest versus lowest remnant cholesterol concentration.

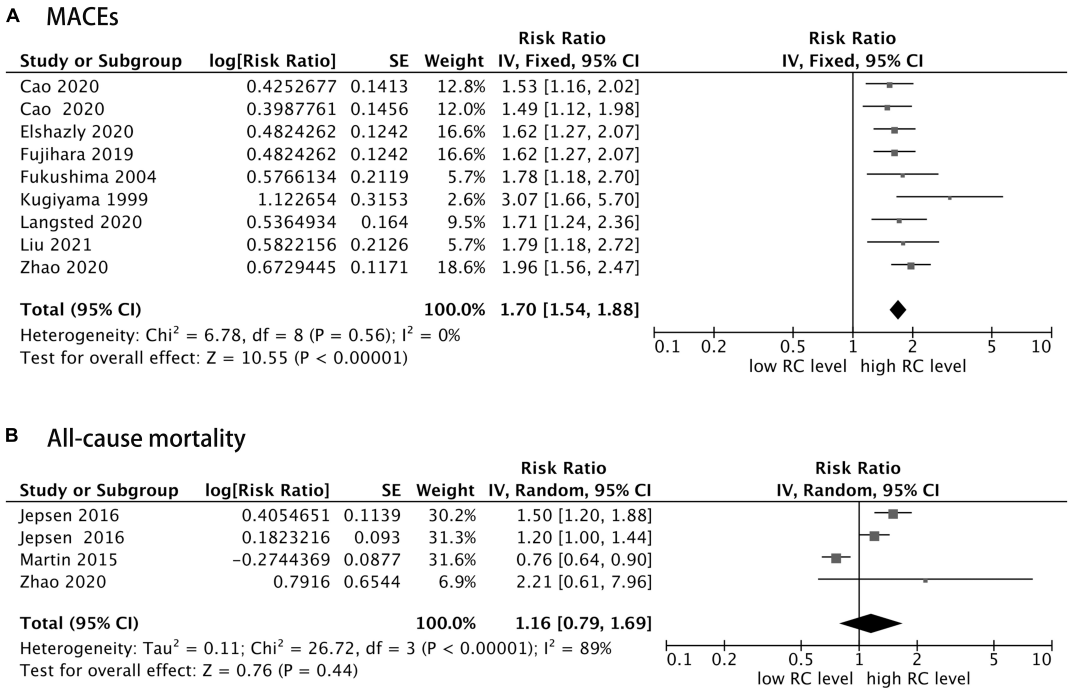


FIGURE 3
Forest plots showing the pooled RR with 95% CI of major adverse cardiovascular events (MACEs) **(A)** and all-cause mortality **(B)** for the highest versus lowest remnant cholesterol concentration.

association continued in selected studies for those publications with sample size <1,000 cases (RR = 1.88, 95% CI: 1.38–2.55, $P < 0.0001$), those that used the immunoseparation method (RR = 1.72, 95% CI: 1.39–2.12, $P < 0.0001$) and fasting status test (RR = 1.70, 95% CI: 1.51–1.91, $P < 0.0001$) for RC assessment, and studies with a long follow-up time (≥3 years) (RR = 1.54, 95% CI: 1.35–1.76, $P < 0.0001$) (Table 2).

Publication bias test

As shown in Figure 4, the funnel plot was asymmetrical, and for further quantitative analysis using Egger's test, a publication bias was suggested ($P < 0.05$).

Discussion

In the present study, the relationship between RC concentration and the prognosis of the patients with CHD was evaluated by meta-analysis for the first time. The results illustrated that elevated RC concentration was significantly correlated with an increased risk of the composite endpoint events and MACEs in patients with CHD, but the risk of all-cause mortality was not statistically significant. In addition, the prognostic significance of higher RC concentration on CHD risks was also confirmed in the subgroup analysis. This meta-analysis contributes to the increasing evidence that higher RC concentration may be an independent predictor of poor cardiovascular outcomes in patients with CHD.

Remnant cholesterol, also known as triglyceride-rich lipoprotein cholesterol, is the cholesterol content of all non-LDL and non-HDL. In the fasting state, RC is composed of liver-derived very low-density lipoprotein (VLDL) and intermediate-density lipoprotein (IDL) in the fasting state, as well as intestinal-derived chylomicron remnants (CM) (27). Recently, an increasing number of studies have demonstrated that RC concentration had a relationship to the occurrence and development of atherosclerosis (27, 53). Particularly, when LDL-C was controlled at an appropriate level, RC was assumed to be the main reason for mediating residual risks in the patients with CHD and even a better predictor of risk than LDL-C (52). Unlike LDL-C, RC could easily penetrate the vessel wall and is directly taken up by the scavenging receptors on macrophages without oxidative modification, leading to forming foam cells and promoting atherosclerotic plaque formation (55, 56). In addition, it could also increase the production of reactive oxygen species free radicals, cause endothelial cell dysfunction (57), and induce the expression of pro-inflammatory mediators, as well as the production of cytokines, interleukin, and atherosclerotic adhesion molecules (58). All of the earlier mechanisms can lead to plaque formation and progressive rupture and promote the occurrence of MACEs, which in turn influences the prognosis of the patients.

TABLE 2 Subgroup analysis on composite endpoint events.

Subgroup	No. of studies	Pooled risk ration	95% confidence interval	*P*-value	Heterogeneity between studies
Region					
Asian	6	1.72	1.53–1.95	<0.0001	$I^2 = 8.9\%$, $P = 0.361$
No-Asian	4	1.55	1.25–1.93	<0.0001	$I^2 = 63\%$, $P = 0.030$
Participation					
CAD	6	1.64	1.46–1.85	<0.0001	$I^2 = 0\%$, $P = 0.55$
MI	3	1.36	0.70–2.64	0.37	$I^2 = 96\%$, $P < 0.001$
Sample size					
≥ 1000	7	1.44	1.15–1.81	0.0020	$I^2 = 87\%$, $P < 0.001$
< 1000	3	1.88	1.38–2.55	<0.0001	$I^2 = 44\%$, $P = 0.17$
Age					
≥ 65 years old	4	1.78	1.46–2.15	0.005	$I^2 = 88\%$, $P < 0.001$
< 65 years old	6	1.42	1.11–1.81	0.04	$I^2 = 91\%$, $P < 0.001$
RC assessment					
Calculation	4	1.69	1.49–1.91	<0.0001	$I^2 = 0\%$, $P = 0.42$
Immunosepa-ration	4	1.72	1.39–2.12	<0.0001	$I^2 = 33\%$, $P = 0.21$
Automated assay	2	1.32	1.05–1.67	0.0200	$I^2 = 52\%$, $P = 0.15$
Fasting status					
Fasting	6	1.70	1.51–1.91	<0.0001	$I^2 = 11\%$, $P = 0.35$
Non-fasting	2	1.41	1.15–1.73	0.0009	$I^2 = 55\%$, $P = 0.11$
Follow-up duration					
≥ 3 year	5	1.54	1.35–1.76	<0.0001	$I^2 = 50\%$, $P = 0.06$
< 3 year	4	1.54	0.87–2.74	0.14	$I^2 = 93\%$, $P < 0.001$

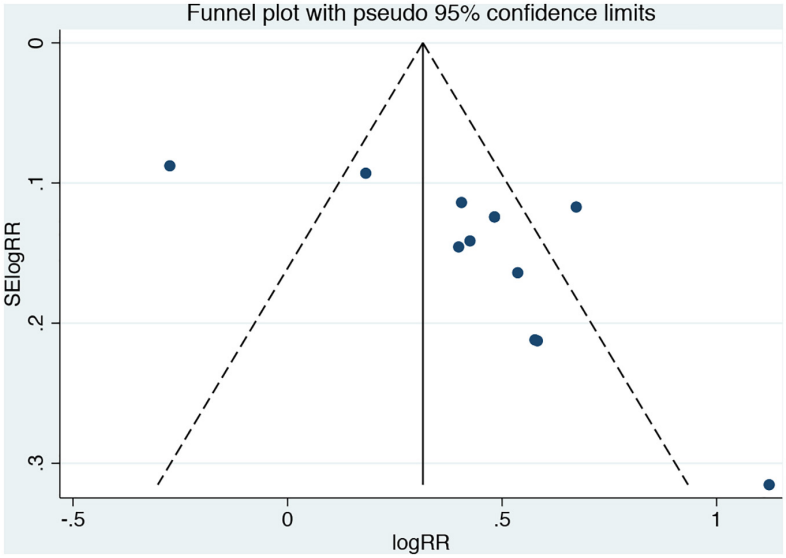


FIGURE 4
Funnel plots for the analysis of remnant cholesterol concentration and composite endpoint events. Results compare participants in the highest versus lowest remnant cholesterol concentration.

The previous clinical studies demonstrated similar conclusions between RC concentration and prognosis in the general population but not in patients with CHD. The latest study by Wadstrom et al. (8) revealed that in the Copenhagen General Population Study, during the 15-year follow-up of 106,937 people, elevated RC concentration was relevant to an increased risk of MI up to multivariable-adjusted HR of 4.2, as well as corresponding HRs were 1.8 for ischemic stroke, and 4.8 for peripheral artery disease (PAD). In addition, in the Copenhagen City Heart Study, corresponding HRs were 2.6 for MI, 2.1 for ischemic stroke, and 4.9 for PAD (8). Castañer et al. (27) also reported that in the PREDIMED cohort study of high cardiovascular-risk groups, every 10 mg/dl increase in RC concentration would increase the risk of cardiovascular events by 21%. After multivariate-adjusted analysis, it was concluded that the levels of triglyceride and RC rather than LDL-C were related to the occurrence of MACEs in the population who were overweight or obese and had a high risk of cardiovascular diseases, which were independent of lifestyle and other risk factors. A few other studies have also reached a similar conclusion (32, 33, 59). However, in our study, RC concentration had no effect on the risk of all-cause mortality, possibly due to the available small sample size and high heterogeneity. Furthermore, it seems the results were consistent across the populations and ages in our subgroup analysis. Wang et al. (31) also indicated the importance of preventive efforts across the adult life course. Obviously, these results need further confirmation in more stratified cases.

In addition, genetic evidence has also been found that RC was the risk factor for atherosclerosis. Varbo et al. (9) performed the Mendelian randomization method by detecting the genes of 73,513 people from the Copenhagen study and selected 15 genotypes to observe the incidence of ischemic heart disease (IHD) for each type of gene. The results indicated that for every 1 mmol/l increase of non-fasting RC concentration, the risk of IHD increased by 2.8 times. In another Mendelian randomized trial, Varbo et al. (60) found that elevated RC concentrations in non-fasting status were causally related to inflammation and IHD, whereas increased LDL-C was only related causally to IHD without inflammation. Jørgensen et al. (61) also indicated that genetic variation in ApoA5 related to stepwise increases of the RC concentration and with comparable increases in the risk of MI. Thus, these results illustrated that exposure to elevated RC concentrations caused by genetic abnormalities could bring a greater risk of cardiovascular diseases.

In our study, sensitivity analysis found that the source of heterogeneity might be the research conducted by Martin et al. (12). In this study, the RC evaluation method was significantly different from others, in which the sum of $VLDL_3$-C and IDL-C was used to calculate RC and fasting state was unknown. At present, no uniform method to measure RC concentration has been provided, and accurate measurement is still challenging, which might be the main reason for conflict in the findings (14–16). This was mainly because RC was composed of different lipids and lipoproteins. Then, its rapid and continuous catabolism, the size, quantity, density, and composition of lipoprotein residues were highly dynamic, which was difficult to distinguish from its precursors (non-remnant lipoproteins) (47). Currently, the simplest way to estimate RC concentration is through calculation method (62); that is, RC was calculated as total cholesterol (TC) minus LDL-C minus HDL-C [i.e., RC = (TC)–(LDL-C)–(HDL-C)]. Although it was not as accurate as the method to direct the detection of RC, it has been widely applied at present due to its convenience and simplicity (10, 52–54). Apart from the calculation, there were also several direct methods to identify and quantify RC depending on their specific ingredients, such as immunoseparation (4, 13, 50, 51), direct homogenous assays (50, 52), preparative ultrafiltration (63), and nuclear magnetic resonance (64). In our study, subgroup analysis indicated that elevated RC concentration measured by the immunoseparation method had higher cardiovascular risk. Furthermore, whether in a fasting state during the detection also had an impact on the RC measurement. The subgroup analysis of our study indicated that the CHD patients with elevated RC concentrations in the fasting state had a higher risk of poor prognosis. Apparently, no optimal way of accurately quantifying RC measurement currently exists, so there was a lack of uniform RC cut-off levels to define high RC

concentration. However, as increasing importance has been attached to RC, a consensus definition of RC with accurate and reproducible quantitative measurement approaches is eagerly required.

Limitations

This study also had potential limitations. First, the studies included in this study were all published in English, which might have a language bias. Second, the differences in the types of CHD and RC measurement approaches in each study may lead to clinical heterogeneity. Third, the included studies adjusted some confounders, but other unadjusted risk factors may exist. Some traditional CHD factors cannot be extracted adequately from the included studies, which might also lead to bias. Thus, further studies of stratified analysis for the risk factors of CHD outcome are necessary. Fourth, there were insufficient relevant data to compare the prognostic effect between LDL-C and RC from the included studies. Then, it is worth answering this valuable question in future studies. Finally, there was a significant publication bias in our study, suggesting the possible presence of negative results that were not published. Therefore, future studies are needed before a firm conclusion can be drawn concerning the association between RC concentration and CHD outcome.

Conclusion

This meta-analysis of 10 cohort studies showed that CHD patients with elevated RC concentrations had a higher risk of adverse cardiovascular outcomes. Measurement of RC concentration has the potential to improve risk classification in patients with CHD. However, future larger sample sizes and higher quality studies are still required to confirm the findings.

Data availability statement

The original contributions presented in this study are included in this article/**Supplementary material**, further inquiries can be directed to the corresponding authors.

Author contributions

YT, WW, and LQ performed the literature search, data extraction, and quality assessment. YT performed data analysis and drafted the manuscript. XY and LC critically revised the study. HW and ZZ designed the study, interpreted the data, and revised the manuscript. All authors contributed intellectually to this manuscript and have approved this final version.

Funding

This study was supported by the Chengdu High-level Key Clinical Specialty Construction Project, Sichuan Province, China, No. 202112001.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcvm.2022.951523/full#supplementary-material

References

1. Roth G, Mensah G, Johnson C, Addolorato G, Ammirati E, Baddour L, et al. Global burden of cardiovascular diseases and risk factors, 1990-2019: update from the GBD 2019 Study. *J Am Coll Cardiol.* (2020) 76:2982–3021. doi: 10.1016/j.jacc.2020.11.010

2. Fuster V, Kelly B, Vedanthan R. Promoting global cardiovascular health: moving forward. *Circulation.* (2011) 123:1671–8. doi: 10.1161/CIRCULATIONAHA.110.009522

3. Salinas C, Chapman M. Remnant lipoproteins: are they equal to or more atherogenic than LDL? *Curr Opin Lipidol.* (2020) 31:132–9. doi: 10.1097/mol.0000000000000682

4. Fujihara Y, Nakamura T, Horikoshi T, Obata J, Fujioka D, Watanabe Y, et al. Remnant lipoproteins are residual risk factor for future cardiovascular events in patients with stable coronary artery disease and on-statin low-density lipoprotein cholesterol levels < 70 mg/dL. *Circ J.* (2019) 83:1302–8. doi: 10.1253/circj.CJ-19-0047

5. Miller Y, Choi S, Fang L, Tsimikas S. Lipoprotein modification and macrophage uptake: role of pathologic cholesterol transport in atherogenesis. *Subcell Biochem.* (2010) 51:229–51. doi: 10.1007/978-90-481-8622-8_8

6. Rosenson R, Davidson M, Hirsh B, Kathiresan S, Gaudet D. Genetics and causality of triglyceride-rich lipoproteins in atherosclerotic cardiovascular disease. *J Am Coll Cardiol.* (2014) 64:2525–40. doi: 10.1016/j.jacc.2014.09.042

7. Holmes M, Millwood I, Kartsonaki C, Hill M, Bennett D, Boxall R, et al. Lipids, lipoproteins, and metabolites and risk of myocardial infarction and stroke. *J Am Coll Cardiol.* (2018) 71:620–32. doi: 10.1016/j.jacc.2017.12.006

8. Wadström B, Wulff A, Pedersen K, Jensen G, Nordestgaard B. Elevated remnant cholesterol increases the risk of peripheral artery disease, myocardial infarction, and ischaemic stroke: a cohort-based study. *Eur Heart J.* (2021) 43:3258–69. doi: 10.1093/eurheartj/ehab705

9. Varbo A, Benn M, Tybjærg-Hansen A, Jørgensen A, Frikke-Schmidt R, Nordestgaard B. Remnant cholesterol as a causal risk factor for ischemic heart disease. *J Am Coll Cardiol.* (2013) 61:427–36. doi: 10.1016/j.jacc.2012.08.1026

10. Elshazly M, Mani P, Nissen S, Brennan D, Clark D, Martin S, et al. Remnant cholesterol, coronary atheroma progression and clinical events in statin-treated patients

with coronary artery disease. *Eur J Prev Cardiol.* (2020) 27:1091–100. doi: 10.1177/2047487319887578

11. Liu H, Li S, Cao Y, Guo Y, Zhu C, Wu N, et al. Association of triglyceride-rich lipoprotein-cholesterol with recurrent cardiovascular events in statin-treated patients according to different inflammatory status. *Atherosclerosis.* (2021) 330:29–35. doi: 10.1016/j.atherosclerosis.2021.06.907

12. Martin S, Faridi K, Joshi P, Blaha M, Kulkarni K, Khokhar A, et al. Remnant lipoprotein cholesterol and mortality after acute myocardial infarction: further evidence for a hypercholesterolemia paradox from the TRIUMPH Registry. *Clin Eardiol.* (2015) 38:660–7. doi: 10.1002/clc.22470

13. Kugiyama K, Doi H, Takazoe K, Kawano H, Soejima H, Mizuno Y, et al. Remnant lipoprotein levels in fasting serum predict coronary events in patients with coronary artery disease. *Circulation.* (1999) 99:2858–60. doi: 10.1161/01.cir.99.22.2858

14. Wilson P, Jacobson T, Martin S, Jackson E, Le N, Davidson M, et al. Lipid measurements in the management of cardiovascular diseases: practical recommendations a scientific statement from the national lipid association writing group. *J Clin Lipidol.* (2021) 15:629–48. doi: 10.1016/j.jacl.2021.09.046

15. Toth P, Bays H, Brown W, Catapano A, Davidson M, Farnier M, et al. Comparing remnant lipoprotein cholesterol measurement methods to evaluate efficacy of ezetimibe/statin vs statin therapy. *J Clin Lipidol.* (2019) 13:997–1007.e8. doi: 10.1016/j.jacl.2019.09.001

16. Joshi P, Khokhar A, Massaro J, Lirette S, Griswold M, Martin S, et al. Remnant lipoprotein cholesterol and incident coronary heart disease: the jackson heart and framingham offspring cohort studies. *J Am Heart Assoc.* (2016) 5:e002765. doi: 10.1161/jaha.115.002765

17. Stroup D, Berlin J, Morton S, Olkin I, Williamson G, Rennie D, et al. Meta-analysis of observational studies in epidemiology: a proposal for reporting. meta-analysis of observational studies in epidemiology (MOOSE) group. *JAMA.* (2000) 283:2008–12. doi: 10.1001/jama.283.15.2008

18. Moher D, Liberati A, Tetzlaff J, Altman D, PRISMA Group. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *PLoS Med.* (2009) 6:e1000097.

19. Tierney J, Stewart L, Ghersi D, Burdett S, Sydes M. Practical methods for incorporating summary time-to-event data into meta-analysis. *Trials.* (2007) 8:16. doi: 10.1186/1745-6215-8-16

20. Stang A. Critical evaluation of the Newcastle-Ottawa scale for the assessment of the quality of nonrandomized studies in meta-analyses. *Eur J Epidemiol.* (2010) 25:603–5. doi: 10.1007/s10654-010-9491-z

21. Higgins J, Thompson S, Deeks J, Altman D. Measuring inconsistency in meta-analyses. *BMJ.* (2003) 327:557–60. doi: 10.1136/bmj.327.7414.557

22. Zhang J, Yu K. What's the relative risk? A method of correcting the odds ratio in cohort studies of common outcomes. *JAMA.* (1998) 280:1690–1. doi: 10.1001/jama.280.19.1690

23. McNutt L, Wu C, Xue X, Hafner J. Estimating the relative risk in cohort studies and clinical trials of common outcomes. *Am J Epidemiol.* (2003) 157:940–3. doi: 10.1093/aje/kwg074

24. Ronksley P, Brien S, Turner B, Mukamal K, Ghali W. Association of alcohol consumption with selected cardiovascular disease outcomes: a systematic review and meta-analysis. *BMJ.* (2011) 342:d671. doi: 10.1136/bmj.d671

25. Quispe R, Martin S, Michos E, Lamba I, Blumenthal R, Saeed A, et al. Remnant cholesterol predicts cardiovascular disease beyond LDL and ApoB: a primary prevention study. *Eur Heart J.* (2021) 19:4324–32. doi: 10.1093/eurheartj/ehab432

26. Chen Y, Li G, Guo X, Ouyang N, Li Z, Ye N, et al. The effects of calculated remnant-like particle cholesterol on incident cardiovascular disease: insights from a general Chinese population. *J Clin Med.* (2021) 10:3388. doi: 10.3390/jcm10153388

27. Castañer O, Pintó X, Subirana I, Amor A, Ros E, Hernáez Á, et al. Remnant cholesterol, not LDL cholesterol, is associated with incident cardiovascular disease. *J Am Coll Cardiol.* (2020) 76:2712–24. doi: 10.1016/j.jacc.2020.10.008

28. Bonfiglio C, Leone C, Silveira L, Guerra R, Misciagna G, Caruso M, et al. Remnant cholesterol as a risk factor for cardiovascular, cancer or other causes mortality: a competing risks analysis. *Nutr Metab Cardiov Dis.* (2020) 30:2093–102. doi: 10.1016/j.numecd.2020.07.002

29. Saeed A, Feofanova E, Yu B, Sun W, Virani S, Nambi V, et al. Remnant-like particle cholesterol, low-density lipoprotein triglycerides, and incident cardiovascular disease. *J Am Coll Cardiol.* (2018) 72:156–69. doi: 10.1016/j.jacc.2018.04.050

30. Varbo A, Nordestgaard B. Remnant cholesterol and risk of ischemic stroke in 112,512 individuals from the general population. *Ann Neurol.* (2019) 85:550–9. doi: 10.1002/ana.25432

31. Wang A, Tian X, Zuo Y, Chen S, Meng X, Chen P, et al. Age dependent association between remnant cholesterol and cardiovascular disease. *Atheroscler Plus.* (2021) 45:18–24. doi: 10.1016/j.athplu.2021.09.004

32. Ikezaki H, Lim E, Cupples L, Liu C, Asztalos B, Schaefer E. Small dense low-density lipoprotein cholesterol is the most atherogenic lipoprotein parameter in the prospective framingham offspring study. *J Am Heart Assoc.* (2021) 10:e019140. doi: 10.1161/jaha.120.019140

33. Hussain A, Sun C, Selvin E, Nambi V, Coresh J, Jia X, et al. Triglyceride-rich lipoproteins, apolipoprotein C-III, angiopoietin-like protein 3, and cardiovascular events in older adults: atherosclerosis risk in communities (ARIC) study. *Eur J Prev Cardiol.* (2021) 14:e53–64. doi: 10.1093/eurjpc/zwaa152

34. Varbo A, Nordestgaard B. Directly measured vs. calculated remnant cholesterol identifies additional overlooked individuals in the general population at higher risk of myocardial infarction. *Eur Heart J.* (2021) 42:4833–43. doi: 10.1093/eurheartj/ehab293

35. Imke C, Rodriguez B, Grove J, McNamara J, Waslien C, Katz A, et al. Are remnant-like particles independent predictors of coronary heart disease incidence? The Honolulu Heart Study. *Arterioscler Thromb Vasc Biol.* (2005) 25:1718–22. doi: 10.1161/01.ATV.0000173310.85845.7b

36. Cao Y, Zhang H, Jin J, Liu H, Zhang Y, Gao Y, et al. The longitudinal association of remnant cholesterol with cardiovascular outcomes in patients with diabetes and pre-diabetes. *Cardiovasc Diabetol.* (2020) 19:104. doi: 10.1186/s12933-020-01076-7

37. Hong L, Yan X, Lu Z, Fan Y, Ye F, Wu Q, et al. Predictive value of non-fasting remnant cholesterol for short-term outcome of diabetics with new-onset stable coronary artery disease. *Lipids Health Dis.* (2017) 16:7. doi: 10.1186/s12944-017-0410-0

38. Winter M, Wiesbauer F, Blessberger H, Pavo N, Sulzgruber P, Huber K, et al. Lipid profile and long-term outcome in premature myocardial infarction. *Eur J Clin Invest.* (2018) 48:e13008. doi: 10.1111/eci.13008

39. Sakai K, Koba S, Nakamura Y, Yokota Y, Tsunoda F, Shoji M, et al. Small dense low-density lipoprotein cholesterol is a promising biomarker for secondary prevention in older men with stable coronary artery disease. *Geriatr Gerontol Int.* (2018) 18:965–72. doi: 10.1111/ggi.13287

40. Varbo A, Freiberg J, Nordestgaard B. Extreme nonfasting remnant cholesterol vs extreme LDL cholesterol as contributors to cardiovascular disease and all-cause mortality in 90000 individuals from the general population. *Clin Chem.* (2015) 61:533–43. doi: 10.1373/clinchem.2014.234146

41. Varbo A, Freiberg J, Nordestgaard B. Remnant cholesterol and myocardial infarction in normal weight, overweight, and obese individuals from the copenhagen general population study. *Clin Chem.* (2018) 64:219–30. doi: 10.1373/clinchem.2017.279463

42. Jorgensen A, Frikke-Schmidt R, West A, Grande P, Nordestgaard B, Tybjaerg-Hansen A. Genetically elevated non-fasting triglycerides and calculated remnant cholesterol as causal risk factors for myocardial infarction. *Eur Heart J.* (2013) 34:1826–33.

43. Wang K, Ding Y, Gao W, Wang R, Yang J, Liu X, et al. Association of increased remnant cholesterol and the risk of coronary artery disease: a retrospective study. *Front Cardiovasc Med.* (2021) 8:740596. doi: 10.3389/fcvm.2021.740596

44. Kim J, Park J, Jeong S, Schellingerhout D, Park J, Lee D, et al. High levels of remnant lipoprotein cholesterol is a risk factor for large artery atherosclerotic stroke. *J Clin Neurol.* (2011) 7:203–9. doi: 10.3988/jcn.2011.7.4.203

45. McNamara J, Shah P, Nakajima K, Cupples L, Wilson P, Ordovas J, et al. Remnant-like particle (RLP) cholesterol is an independent cardiovascular disease risk factor in women: results from the Framingham Heart Study. *Atherosclerosis.* (2001) 154:229–36. doi: 10.1016/s0021-9150(00)00484-6

46. Goliasch G, Wiesbauer F, Blessberger H, Demyanets S, Wojta J, Huber K, et al. Premature myocardial infarction is strongly associated with increased levels of remnant cholesterol. *J Clin Lipidol.* (2015) 9:801–6. doi: 10.1016/j.jacl.2015.08.009

47. Duran E, Pradhan A. Triglyceride-rich lipoprotein remnants and cardiovascular disease. *Clin Chem.* (2021) 67:183–96. doi: 10.1093/clinchem/hvaa296

48. Wulff A, Nordestgaard B, Tybjaerg-Hansen A. APOC3 loss-of-function mutations, remnant cholesterol, low-density lipoprotein cholesterol, and cardiovascular risk: mediation- and meta-analyses of 137895 individuals. *Arterioscler Thromb Vasc Biol.* (2018) 38:660–8. doi: 10.1161/atvbaha.117.310473

49. Feng X, Guo Q, Zhou S, Sun T, Liu Y, Zhou Z, et al. Could remnant-like particle cholesterol become a risk factor in diabetic menopausal women with coronary artery disease? A cross-sectional study of single academic center in China. *Lipids Health Dis.* (2020) 19:44. doi: 10.1186/s12944-020-01224-8

50. Cao Y, Zhang H, Jin J, Liu H, Zhang Y, Xu R, et al. Prognostic utility of triglyceride-rich lipoprotein-related markers in patients with coronary artery disease. *J Lipid Res.* (2020) 61:1254–62. doi: 10.1194/jlr.RA120000746

51. Fukushima H, Sugiyama S, Honda O, Koide S, Nakamura S, Sakamoto T, et al. Prognostic value of remnant-like lipoprotein particle levels in patients with coronary artery disease and type II diabetes mellitus. *J Am Coll Cardiol.* (2004) 43:2219–24. doi: 10.1016/j.jacc.2003.09.074

52. Jepsen A, Langsted A, Varbo A, Bang L, Kamstrup P, Nordestgaard B. Increased remnant cholesterol explains part of residual risk of all-cause mortality in 5414 patients with ischemic heart disease. *Clin Chem.* (2016) 62:593–604. doi: 10.1373/clinchem.2015.253757

53. Langsted A, Madsen C, Nordestgaard B. Contribution of remnant cholesterol to cardiovascular risk. *J Intern Med.* (2020) 288:116–27. doi: 10.1111/joim.13059

54. Zhao Q, Zhang T, Cheng Y, Ma Y, Xu Y, Yang J, et al. Prognostic impact of estimated remnant-like particle cholesterol in patients with differing glycometabolic status: an observational cohort study from China. *Lipids Health Dis.* (2020) 19:179. doi: 10.1186/s12944-020-01355-y

55. Sandesara P, Virani S, Fazio S, Shapiro M. The forgotten lipids: triglycerides, remnant cholesterol, and atherosclerotic cardiovascular disease risk. *Endocr Rev.* (2019) 40:537–57. doi: 10.1210/er.2018-00184

56. Boren J, Olin K, Lee I, Chait A, Wight T, Innerarity T. Identification of the principal proteoglycan-binding site in LDL. A single-point mutation in apo-B100 severely affects proteoglycan interaction without affecting LDL receptor binding. *J Clin Invest.* (1998) 101:2658–64. doi: 10.1172/JCI2265

57. Wang L, Gill R, Pedersen T, Higgins L, Newman J, Rutledge J. Triglyceride-rich lipoprotein lipolysis releases neutral and oxidized FFAs that induce endothelial cell inflammation. *J Lipid Res.* (2009) 50:204–13. doi: 10.1194/jlr.M700505-JLR200

58. Shin H, Kim Y, Kim K, Lee J, Hong K. Remnant lipoprotein particles induce apoptosis in endothelial cells by NAD(P)H oxidase-mediated production of superoxide and cytokines via lectin-like oxidized low-density lipoprotein receptor-1 activation: prevention by cilostazol. *Circulation.* (2004) 109:1022–8. doi: 10.1161/01.CIR.0000117403.64398.53

59. Duran E, Aday A, Cook N, Buring J, Ridker P, Pradhan A. Triglyceride-rich lipoprotein cholesterol, small dense LDL cholesterol, and incident cardiovascular disease. *J Am Coll Cardiol.* (2020) 75:2122–35. doi: 10.1016/j.jacc.2020.02.059

60. Varbo A, Benn M, Tybjaerg-Hansen A, Nordestgaard B. Elevated remnant cholesterol causes both low-grade inflammation and ischemic heart disease, whereas elevated low-density lipoprotein cholesterol causes ischemic heart disease without inflammation. *Circulation.* (2013) 128:1298–309. doi: 10.1161/CIRCULATIONAHA.113.003008

61. Jørgensen A, Frikke-Schmidt R, West A, Grande P, Nordestgaard B, Tybjærg-Hansen A. Genetically elevated non-fasting triglycerides and calculated remnant cholesterol as causal risk factors for myocardial infarction. *Eur Heart J.* (2013) 34:1826–33.

62. Jacobson T, Ito M, Maki K, Orringer C, Bays H, Jones P, et al. National lipid association recommendations for patient-centered management of dyslipidemia: part 1–full report. *J Clin Lipidol.* (2015) 9:129–69. doi: 10.1016/j.jacl.2015.02.003

63. Kulkarni K. Cholesterol profile measurement by vertical auto profile method. *Clin Lab Med.* (2006) 26:787–802. doi: 10.1016/j.cll.2006.07.004

64. Otvos J. Measurement of triglyceride-rich lipoproteins by nuclear magnetic resonance spectroscopy. *Clin Cardiol.* (1999) 22:II21–7. doi: 10.1002/clc.4960221405

65. Moher D, Liberati A, Tetzlaf J, Altman DG, The PRISMA Group. Preferred reporting items for systematic reviews and meta-analyses: The PRISMA statement. *PLoS Med.* 6:e1000097 doi: 10.1371/journal.pmed.1000097

frontiers | Frontiers in Cardiovascular Medicine

TYPE Review
PUBLISHED 16 February 2023
DOI 10.3389/fcvm.2023.1065967

OPEN ACCESS

EDITED BY
Tatsuya Sawamura,
Shinshu University, Japan

REVIEWED BY
Matti Sakari Jauhiainen,
Minerva Foundation Institute for Medical Research, Finland
Jerzy Beltowski,
Medical University of Lublin, Poland
Corina Rosales,
Houston Methodist Research Institute, United States

*CORRESPONDENCE
Paul N. Durrington
✉ pdurrington@manchester.ac.uk

SPECIALTY SECTION
This article was submitted to Lipids in Cardiovascular Disease, a section of the journal Frontiers in Cardiovascular Medicine

RECEIVED 10 October 2022
ACCEPTED 30 January 2023
PUBLISHED 16 February 2023

CITATION
Durrington PN, Bashir B and Soran H (2023) Paraoxonase 1 and atherosclerosis. *Front. Cardiovasc. Med.* 10:1065967. doi: 10.3389/fcvm.2023.1065967

Paraoxonase 1 and atherosclerosis

Paul N. Durrington[1]*, Bilal Bashir[1,2] and Handrean Soran[1,2]

[1]Cardiovascular Research Group, Faculty of Biology, Medicine and Health, The University of Manchester, Manchester, United Kingdom, [2]Department of Diabetes, Endocrinology and Metabolism, Peter Mount Building, Manchester University NHS Foundation Trust, Manchester, United Kingdom

Paraoxonase 1 (PON1), residing almost exclusively on HDL, was discovered because of its hydrolytic activity towards organophosphates. Subsequently, it was also found to hydrolyse a wide range of substrates, including lactones and lipid hydroperoxides. PON1 is critical for the capacity of HDL to protect LDL and outer cell membranes against harmful oxidative modification, but this activity depends on its location within the hydrophobic lipid domains of HDL. It does not prevent conjugated diene formation, but directs lipid peroxidation products derived from these to become harmless carboxylic acids rather than aldehydes which might adduct to apolipoprotein B. Serum PON1 is inversely related to the incidence of new atherosclerotic cardiovascular disease (ASCVD) events, particularly in diabetes and established ASCVD. Its serum activity is frequently discordant with that of HDL cholesterol. PON1 activity is diminished in dyslipidaemia, diabetes, and inflammatory disease. Polymorphisms, most notably Q192R, can affect activity towards some substrates, but not towards phenyl acetate. Gene ablation or over-expression of human *PON1* in rodent models is associated with increased and decreased atherosclerosis susceptibility respectively. PON1 antioxidant activity is enhanced by apolipoprotein AI and lecithin:cholesterol acyl transferase and diminished by apolipoprotein AII, serum amyloid A, and myeloperoxidase. PON1 loses this activity when separated from its lipid environment. Information about its structure has been obtained from water soluble mutants created by directed evolution. Such recombinant PON1 may, however, lose the capacity to hydrolyse non-polar substrates. Whilst nutrition and pre-existing lipid modifying drugs can influence PON1 activity there is a cogent need for more specific PON1-raising medication to be developed.

KEYWORDS

paraoxonase 1, paraoxonase 1 activity, cardiovascular disease, high density lipoprotein, lipid peroxidation, PON1 polymorphism

Introduction

It is 20 years since our last review of the role of paraoxonase in atherogenesis (1). In that time much has been learnt regarding the strength of the relationship of serum paraoxonase activity with atherosclerotic cardiovascular disease (ASCVD). Despite this, all too frequently the involvement of paraoxonase in atherogenesis is still regarded as controversial. However, whilst some aspects of the role of paraoxonase may be as yet poorly understood, a great deal has been clearly established. That will be the subject of this review.

The development of the concept that PON1 is anti-atherosclerotic

Paraoxonase was identified by Aldridge in 1953 as an enzyme present in serum with the capacity to hydrolyse diethyl *para*-nitrophenyl phosphate (2). It was originally termed "A" esterase to distinguish it from "B" esterases, such as acetylcholinesterase and butyrylcholinesterase, which are inhibited by diethyl *para*-nitrophenyl phosphate, an organophosphate. Diethyl *para*-nitrophenyl phosphate, which is now more commonly referred to as paraoxon, is the potent neurotoxin produced by the metabolism of the organophosphate pesticide, parathion. The "A" enzymes hydrolysing paraoxon (EC.3.1.8.1, aryldialkylphosphatase) have thus come to be known as paraoxonases. Paraoxonase, circulating in blood and tissue fluid now designated as paraoxonase 1 (PON1), was found to constitute the first line of defence against a panoply of organophosphate toxins, including insecticides and military nerve gasses. Because of its importance in toxicology, it was intensively studied most notably by the research groups headed by Furlong (3) in Seattle and Draganov and La Du (4) in Ann Arbor. However, it was not until the mid-1980's that Mackness, working in the toxicology department at the University of Reading, demonstrated that the location of paraoxonase was almost exclusively on HDL (5). Because of the known association of low HDL with ASCVD, it was but a short step to discovering that the serum activity was diminished in myocardial infarction (MI) survivors (6). Towards the end of that decade Mackness joined the lipoprotein group in Manchester and we began to study PON1 in diseases associated with accelerated atherosclerosis. We soon discovered that its activity was diminished both in diabetes (7, 8) and familial hypercholesterolaemia (8).

The prevailing dogma to explain the epidemiologically observed inverse relationship between HDL and ASCVD was that HDL was critical for reverse cholesterol transport. The evidence that HDL is rate-limiting for this process in typical human atherosclerosis was and remains scant (9). However, there were reports that HDL might protect LDL against potentially atherogenic modifications to its structure. As early as 1979 it had been found that the cytotoxicity of LDL to human vascular smooth muscle and endothelial cells in tissue culture could be abolished, if HDL was also present (10). Later it was found that:

(a) HDL decreased lipid peroxidation products measured as thiobarbituric acid reacting substances accumulating on LDL during Cu^{2+}- or endothelial cell—induced oxidation (11).
(b) HDL diminished the increase in electrophoretic mobility of LDL following Cu^{2+}-induced oxidation (12).
(c) HDL decreased the accumulation of malondiadehyde in LDL during oxidation induced by Fe^{2+} or by prolonged incubation (13). Fogelman's group in Los Angeles then found that HDL prevented the minimally oxidised LDL-induced migration of human blood monocytes through a layer of cultured endothelial cells (14).

It was uncertain whether these effects (11–14) were due to transfer of lipid peroxides from LDL to HDL (probably for subsequent disposal by the liver) or was due to their breakdown by an enzyme present on HDL. Lecithin: cholesterol acyl transferase (LCAT) was considered for the latter role, but none of the groups was aware of the presence of PON1 on HDL. We began to speculate that PON1, which had no known physiological role, might be involved (15). We studied Cu^{2+}-induced *in vitro* oxidation of LDL in the presence and absence of HDL, using the method of El-Saadani et al. to measure lipid peroxides (16) on LDL and HDL. We discovered in 1991 that Cu^{2+}-induced LDL lipid peroxidation was not only less when co-incubated with HDL, but that it was also unaccompanied by any increase in lipid peroxides on the HDL and that the total lipid peroxides in the system were less when HDL was present (17; **Figure 1**). It was thus likely that HDL did not simply receive lipid peroxidation products from LDL, but that it also catalysed their conversion to products not detected as lipid peroxides in our assay. To explain this phenomenon, PON1 free of LCAT activity was isolated from HDL in lipid micelles and found to be a potent inhibitor of the accumulation of lipid peroxides on LDL when incubated with Cu^{2+} (17). A series of publications from our group followed supporting the hypothesis that PON1 was critical for the protection afforded to LDL against oxidative modification and extending this to include the concept that HDL, which is the predominant lipoprotein in tissue fluid, protected cell membranes against oxidative and other damaging processes (18–20). This seemed to provide an attractive anti-atherosclerotic role for PON1 on HDL. Interest in the oxidative theory of atherogenesis was, however, waning because of the failure of fat-soluble antioxidant vitamins to suppress atherosclerosis in clinical trials (see later) despite evidence that they prevent the formation of conjugated dienes in the initial stage of LDL lipid peroxidation by being more susceptible to oxidation than unsaturated fatty acyl groups. However, once they themselves are oxidised they are pro-oxidant and furthermore they increase cholesteryl ester transfer protein (CETP) activity (21, 22). PON1 on the other hand decreases the accumulation of the lipid peroxides generated after conjugate diene formation and does so over a longer time period.

In 1995 independent confirmation that prevention of oxidative modification of LDL by HDL was due to PON1 was provided

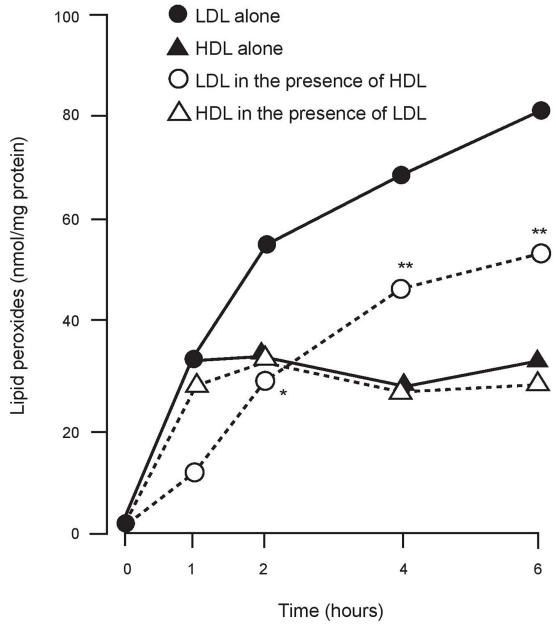


FIGURE 1
The accumulation of lipid peroxides on LDL and HDL when incubated alone and together in the presence of Cu^{2+}. LDL + HDL significantly different from LDL alone. $^{*}P < 0.05$; $^{**}P < 0.001$.

by Fogelman's group in Los Angeles (23). It was reported that the minimally oxidised LDL-induced migration of human blood monocytes through a layer of cultured endothelial cells was diminished in the presence of PON1 purified from HDL. Furthermore, by a mass spectrometry method, when purified PON1 was incubated with minimally oxidised LDL, it was found that oxidation products of phosphatidyl choline were decreased. Later, using electrospray ionisation mass spectrometry, we confirmed the marked decrease in histidine residues modified by 4-hydroxy-2-non-enal (HNE) (a break-down product of peroxidised linoleic acid typically present at the Sn2 position of phosphatidyl choline) in the tryptic fragments of LDL, which had been subject to Cu^{2+}-induced oxidation when co-incubated with HDL possessing PON1 (24).

The oxidation products observed by the Los Angeles group to be present on minimally oxidised LDL in the absence of PON1 could themselves stimulate monocyte migration. The effect of PON1 in decreasing monocyte migration and phosphatidyl choline peroxidation products was enhanced by platelet activating factor acetyl hydrolase (PAFAH) (23). However, as we discuss later much of the PAFAH activity on HDL is probably due to PON1. Other factors proposed to explain the protective effect of HDL against lipid peroxide accumulation were LCAT and apo A1 (11, 25, 26). Experiments reported by our group showed that neither of these acting alone were effective in protecting LDL against lipid peroxidation in comparison to PON1 (27). However, enhancement of the effect of PON1 was observed from the addition of either LCAT or apo A1 to purified PON1 (27). This was greater after 4–8 h of co-incubation with LDL and PON1 than in the first 4 h (28). These observations were made under experimental conditions designed to determine whether PON1 has the capacity to prevent oxidative modification of LDL and, as we discuss later, the intensely hydrophobic environment created on HDL produced by the presence of apoA1 and the action of LCAT in converting pre-beta HDL to more mature HDL (29) is likely to be more critical *in vivo* for PON1 to exert its anti-oxidative, anti-atherogenic role. These and other HDL components which may contribute to anti-oxidant activity have recently been reviewed by us (30). However, in support of an important role for PON1, genetic deficiency of neither apoA1 nor LCAT in humans is, unlike PON1 deficiency, conspicuously associated with premature atherosclerosis (31, 32). Susceptibility to experimental atherosclerosis in *APOA1* or *LCAT* ablated mouse models requires an additional mutation, such as apoE deficiency or LDL receptor deficiency (33, 34) and even then may involve decreased PON1, whereas *PON1* knock-out mice are prone to atherosclerosis induced simply with atherogenic chow even without cross-breeding with apolipoprotein E (apoE) ablated mice (35).

Under oxidising conditions, regardless of the presence or absence of LDL, lipid peroxides begin to form on HDL at an early stage, but their accumulation then ceases remaining at a low level relative to LDL (**Figure 1**). In 1998 a consortium in Ann Arbor and Haifa (36) showed in experiments, involving enrichment of HDL with purified PON1 and specific inhibition of PON1 present in HDL, that the resistance of HDL to lipid peroxidation was due to its PON1 component.

To test our theory that HDL by virtue of its PON1 component might have a wider role by providing a system to protect cell membranes against oxidative damage, we determined the concentration of PON1 in experimental blister fluid as a surrogate for interstitial fluid (37). PON1 concentration was approximately one fifth that in serum and, although it was still associated with apoA1, the ratio between the two had decreased which was interpreted as likely to be due to sequestration of PON1 by the tissues. Later James' group in Geneva showed that PON1 could exchange between HDL and outer cell membranes where it decreased cellular susceptibility to loss of function induced by oxidising conditions (38). This fitted well with our earlier immunohistochemical study of atheroma in the human aorta (39). ApoA1, clusterin (apoJ), and PON1 were found to be present in healthy coronary arteries, staining with increasing intensity as atheroma progressed (39).

It would be wrong to create the impression that the concept that PON1 can explain the anti-oxidative activity of HDL has not been without criticism. Firstly, a persisting effect of HDL to prevent LDL oxidation even when no PON1 activity can be detected, for example in the presence of EDTA (40) and secondly, failure of highly purified or recombinant PON1 to protect LDL against lipid peroxidation (41–43) have been interpreted as denying the theory. These assertions have been challenged by direct experimentation (44). Furthermore, the evidence that PON1 activity was absent due to inhibition by EDTA was based on measurements made using phenyl acetate as the substrate (40). Hydrolysis of phenyl acetate by PON1 is highly Ca^{2+}-dependent whereas PON1 anti-oxidant activity can persist even in the presence of EDTA (45). Also, in experiments where highly purified or recombinant PON1 did not protect LDL against oxidative modification, it can be argued that in purifying PON1 to a high degree, whether from serum or tissue culture fluid, it is extremely difficult to maintain a lipid environment in which the conformation of PON1 necessary for its anti-oxidant activity can be maintained (41). Water-soluble PON1 mutants are even less likely to interact with the lipid environment physiologically to provide hydrolytic activity against highly hydrophobic substrates. Interestingly too, recombinant PON1 has cytotoxic properties (44) most likely due to its PAFAH-like activity in producing lysophosphatidyl choline (46), which, when it occurs outside the safe environment of HDL, is intensely damaging to tissues.

PON gene family and PON1 polymorphisms

Whilst the major paraoxonase in serum is PON1, it was found that there are two other members of its family whose genes cluster on chromosome 7 (47). Paraoxonase 2 (PON2) is a widely distributed, highly expressed intracellular enzyme, which contributes to the intracellular anti-oxidant defences. Paraoxonase 3 (PON3) is another member of the paraoxonase family located on HDL, but at much lower concentration than PON1. It has very limited arylesterase and practically no organophosphatase activity. As lactonases, the substrate specificity of PON1 and PON3 overlap, but differ by degree with PON3 showing a preference for higher molecular mass lactones.

Paraoxonase 1 is highly polymorphic and it was found even before the advent of gene sequencing technology that at least one of these polymorphisms conferred variation in activity to different substrates (48). This variation in activity did not apply when phenyl acetate, which has a high molar rate of hydrolysis compared to other substrates including paraoxon. In the case of paraoxon, however, the frequency distribution of PON1 activity in Europid population revealed that almost half have a low activity, around 8–9% high activity and the rest form an intermediate peak. The heritability of these activities led to the discovery that PON1 was allelic with

low activity and high activity homozygotes in Hardy-Weinberg equilibrium with heterozygotes. By the early 1990's sequencing of PON1 and *PON1* revealed that this difference in activity was largely determined by a substitution of glycine (Q) for arginine (R) at position 192 resulting in a 192Q isoenzyme with low PON1 activity and a 192R isoenzyme with high activity. This gene variant is also known as rs662. It should be noted that the activity of these isoenzymes was reversed with some substrates other than paraoxon, such as diazoxon (49). Although naturally humans are unlikely to be exposed to paraoxon or diazoxon, these examples suggested the polymorphism may have evolved to permit a population to withstand a wider range of toxins than would otherwise be the case (see later). The prevalence of 192R and Q alloenzymes varies in different populations and tends to reflect their typical hydrolytic activity towards paraoxon (48).

In 1995 Ruiz and colleagues reported that in type 2 diabetes the 192R isoenzyme of PON1 was associated with coronary heart disease (50). Initially this seemed counter-intuitive, because the hydrolytic activity of this isoenzyme, at least with paraoxon as substrate, is higher than that of 192Q. The explanation proved to be that, per mg of HDL protein, 192R was slightly less effective than the 192Q alloenzyme in protecting LDL against lipid peroxidation (51, 52). In other words, the anti-atherosclerotic activity of PON1 is greater for the isoenzyme which is less effective in hydrolysing paraoxon. None the less the 192R isoenzyme does have anti-oxidative activity and, if present at high concentration, it will protect perhaps more than in an individual expressing the 192Q polymorphism at lower concentration. Although the polymorphism at position 192 does not affect PON1 serum concentration, others for example at position 55 (53) and in the promoter region (54) do and there are a huge number of epigenetic and acquired factors, including diabetes, inflammatory disorders, infections and nutrition, reviewed elsewhere (55, 56) that contribute to variation in concentration and activity which in different individuals can vary by as much as 16-fold and 40-fold, respectively (57).

Epidemiology: Mendelian randomisation and serum PON1 activities

In epidemiological studies EDTA plasma is typically stored, but to test the association between PON1 activity and atherosclerosis when phenyl acetate, paraoxon, diazoxon, or a lactone are employed as substrate, serum is required, because all these activities are highly Ca^{2+}-dependent (1). Genotyping, however, which can be done on any stored material likely to yield DNA, has since the 1990's become increasingly easy. We were amongst the groups whose results were negative (58) with respect to an association between the Q allele and ASCVD risk, but others reported positively. By 2001 our meta-analysis showed a weak association between the Q allele and ASCVD, which was approximately what would be expected from our experiments (51, 52). In a subsequent meta-analysis by Wheeler et al., producing similar findings (59), it was considered that the association could also be explained by publication bias, with which we agreed (58). However, these authors concluded that their findings made it unlikely that PON1 was critical in atherogenesis. This was based on the unrealistic assumption that a gene coding for variation in PON1 activity towards paraoxon could provide results interpretable according to the principles of Mendelian randomisation. For this to be the case the influence of the 192 variants on atherosclerosis should have been similar in magnitude to its effect on *in vitro* paraoxon hydrolysis. The conclusion from meta-analysis of the 192 polymorphisms must be that it does not deny the hypothesis that low PON1 is associated with atherosclerosis: either the 192 genotype contributes nothing (publication bias) or it is supportive. Subsequently, meta-analyses, some without publication bias (60) have continued to show a small contribution of the 192Q allele to ASCVD risk, most obviously when diabetes is also present (60–62).

After the initial case-control study showing an association between PON1 activity and myocardial infarction (6), we performed another study in which it was found that serum PON1 activity was already low in samples taken within 2 h of the onset of symptoms of acute myocardial infarction (63). There followed other case-control studies in which serum PON1 activity was, as expected, more closely related to the presence of ASCVD than PON1 genotypes (58, 64). The critical epidemiological test of whether PON1 activity was relevant to future atherosclerosis, however, came with reports of prospective studies. Serum PON1 was found to be independently associated with the likelihood of future ASCVD events, generally contributing to variation in risk with a similar magnitude to established risk factors, including HDL cholesterol (65–70). The first prospective results came from the Caerphilly Heart Study (65) of middle-aged men (**Figure 2**). Kunutsor et al. performed a meta-analysis (70) of these results combined with those of an additional five reports (65–69). There were 15,064 participants and 2,958 incidences of ASCVD. In three studies paraoxon was employed as substrate (65, 66, 69) and in three phenyl acetate (67, 68, 70). The age-adjusted pooled relative risk for ASCVD per 1SD higher PON1 activity was a 0.87, which was highly statistically significant. There was no evidence of publication bias. The literature in general does not reveal a close correlation between PON1 activity and HDL cholesterol or apoA1. Kunutsor et al. (70), however, did not find that PON1 activity could contribute more than HDL cholesterol to a multivariate equation to predict the likelihood of future ASCVD events. That could have been, because individual variation in serum PON1 activity is greater than for HDL for cholesterol and thus regression dilution bias would favour HDL cholesterol. The potentially greater biological significance of PON1 and discordance between HDL cholesterol and PON1 was emphasised by the report of Corsetti et al. of decreased PON1 activity in people with premature ASCVD despite high HDL cholesterol levels (71). In the meta-analysis by Kunutsor et al. (70) PON1 measured as aryl esterase activity (phenyl acetate hydrolysis), which is unaffected by the 192 polymorphism, predicted ASCVD more strongly than paraoxonase (paraoxon hydrolysis) activity. PON1 activity predicted new ASCVD events particularly strongly in people with established ASCVD, such as those who had undergone coronary revascularisation. The study by Bhattacharyya et al. (72) was of particular interest in the latter context. They studied 1,399 people who underwent coronary angiography at the Cleveland Clinic. PON1 measured both as aryl esterase and paraoxonase activity was strongly inversely associated with the incidence of new ASCVD event over a minimum follow-up of 3 years (**Figure 3**). In a subgroup of 150 participants matched for the PON1 192 polymorphism (equal numbers with the QQ, QR, and RR) serum PON1 activity was strongly correlated ($P < 0.001$) with concentrations of fatty acid oxidation products (hydroxyeicosatetraenoic acid, HETE; hydroxyloctadecadienoic acid, HODE, and 8-isoprostane prostaglandin F2α, 8-isoPGF$_{2\alpha}$).

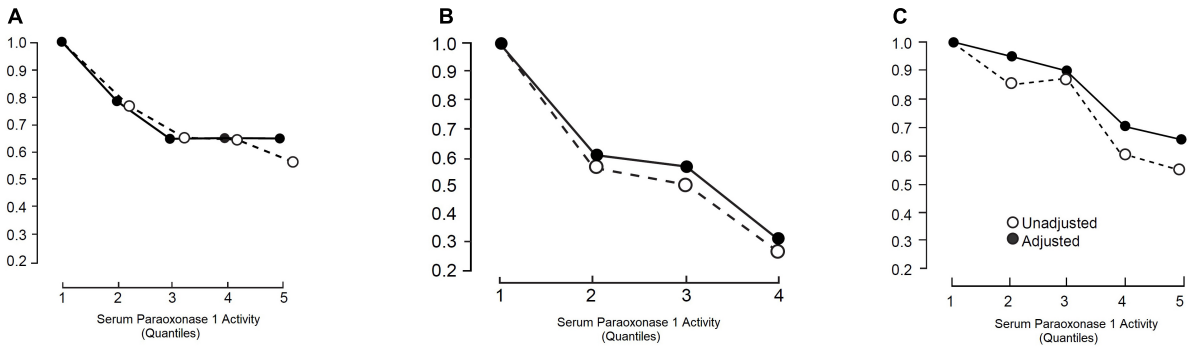


FIGURE 2
The risk of ASCVD relative to the lowest risk quartile or quintile (RR) as a function of serum paraoxonase 1 (PON1) activity studied prospectively in **(A)** Caerphilly and Speedwell (CHD only) (65), **(B)** cleveland clinic (ASCVD and all-cause mortality) (72), and **(C)** meta-analysis by Kunutsor et al. (ASCVD) (70). Closed circles are RR unadjusted for other risk factors and open circles after adjustment for some of these (see references for details).

Because of the theory that PON1 has evolved as a lactonase (see later), it has been proposed that use of a lactone substrate, such as homocysteine thiolactone or dihydroxycoumarin (73, 74) might provide a more biologically relevant estimate of PON1 activity. There is as yet no prospective epidemiological evidence that the lactonase activity of PON1 provides a superior indicator of ASCVD risk (75). The significance of PON1 lactonase activity in human disease is emergent territory (76). Specifically, in the case of atherosclerosis some evidence suggests homocysteine thiolactone, which, like glucose and lipid peroxides, can bind to apoB, may be associated with ASCVD incidence (77). Urinary homocysteine thiolactone excretion has been reported to be increased in people with low serum PON1, particularly in carriers of the PON1-192R allele (73).

Evolution and biological plausibility

Polymorphisms of PON1, by broadening the range of organophosphate neurotoxin resistance, will increase the survival of an exposed population without the need to await a new mutation with greater detoxifying properties, albeit at the expense of individuals with the less favourable variant (78). Darwinian evolution takes vastly longer and extinction may occur before a more successful mutation occurs. In this context, we have reported that agricultural workers involved in sheep-dipping with diazinon (active metabolite diazinoxon) are less likely to experience neuropsychiatric symptoms if they possess the 192Q PON1 allele associated with higher serum PON1 measured as diazoxonase activity (79). The organophosphates which led to the discovery of PON1 were synthetic, but a vast array of organophosphates is naturally produced. The habitat of the earliest hominids was on the shores of the great lakes of Africa, where cyanobacteria (blue-green algae) (80), which can produce large quantities of neurotoxic organophosphates, at times, would have threatened human survival. However, modern man has been present for a mere 6 million years, not long enough to explain the evolution of the paraoxonase family of proteins, the ancestral protein for which may have existed hundreds of millions or even billions of years ago. It is likely that PON1 evolved from PON2, its intracellular relative (49). Beyond that we know that paraoxonases are not homologous to serine esterases, carboxyesterases, or arylesterases and thus do not have similar ancestry (49). The capacity to synthesise cholinesterases and to respond to acetyl choline dates to before metazoa emerged and the evolution of any recognisable nervous system (81). The potential for organophosphate toxicity must have been present for at least as long. Organophosphates produced in the anaerobic conditions around deep sea hydrothermal vents must have been incorporated into the earliest life forms. So it may not be too fanciful to consider that the ancestral protein giving rise to paraoxonases may have existed many aeons ago and have long had a role in organophosphate metabolism. Other examples of enzymes with organophosphatase activity conserved across the domains of living organisms are: diisopropylfluorophosphatase (DFPase) (eukaryocyte squid) (82), organophosphate hydrolase, organophosphate acid anhydrolase and phosphotriesterase (bacteria) (83) and SsoPox an organophosphatase/lactonase from *Sulfolobus solfataricus* (archea) (84). Of these the structure of rePON1 resembles that of squid DFPase. Both are six-bladed propellers with each blade consisting of four β-sheets. Moreover, in both structures two calcium ions can be found in their central tunnel (82).

With the advent of photosynthetic organisms an atmosphere rich in oxygen was created and thus the scene was set for the evolution of life with more rapid metabolism (energised by oxidative respiratory chain phosphorylation) than could be sustained by simple glycolysis. However, simultaneously the necessity for protection against the toxicity of oxygen also became essential. Paraoxonases and the other anti-oxidative enzymes would have contributed to that (85).

Elias and Tawfik in a fascinating review have strongly argued that paraoxonases may have evolved, not as esterases, but as lactonases with a promiscuous esterase activity (86). Although not related in other aspects of their structure, their active site has features more in common with lactonases than esterases. Many single-celled organisms signal to each other by producing lactones, such as *N*-acylhomoserine lactones, usually when their colony size has reached some critical point (quorum sensing), altering expression of genes regulating such processes as bioluminescence, biofilm formation, virulence factor expression, and motility. Just as for a hormone to excite rather than inhibit there must be a process to destroy it after receptor binding (ironically, for example, acetyl choline and acetyl cholinesterase), so a lactonase could have a role in quorum sensing. PON1 has the capacity to metabolise homocysteine thiolactone, which has been implicated in atherogenesis (87).

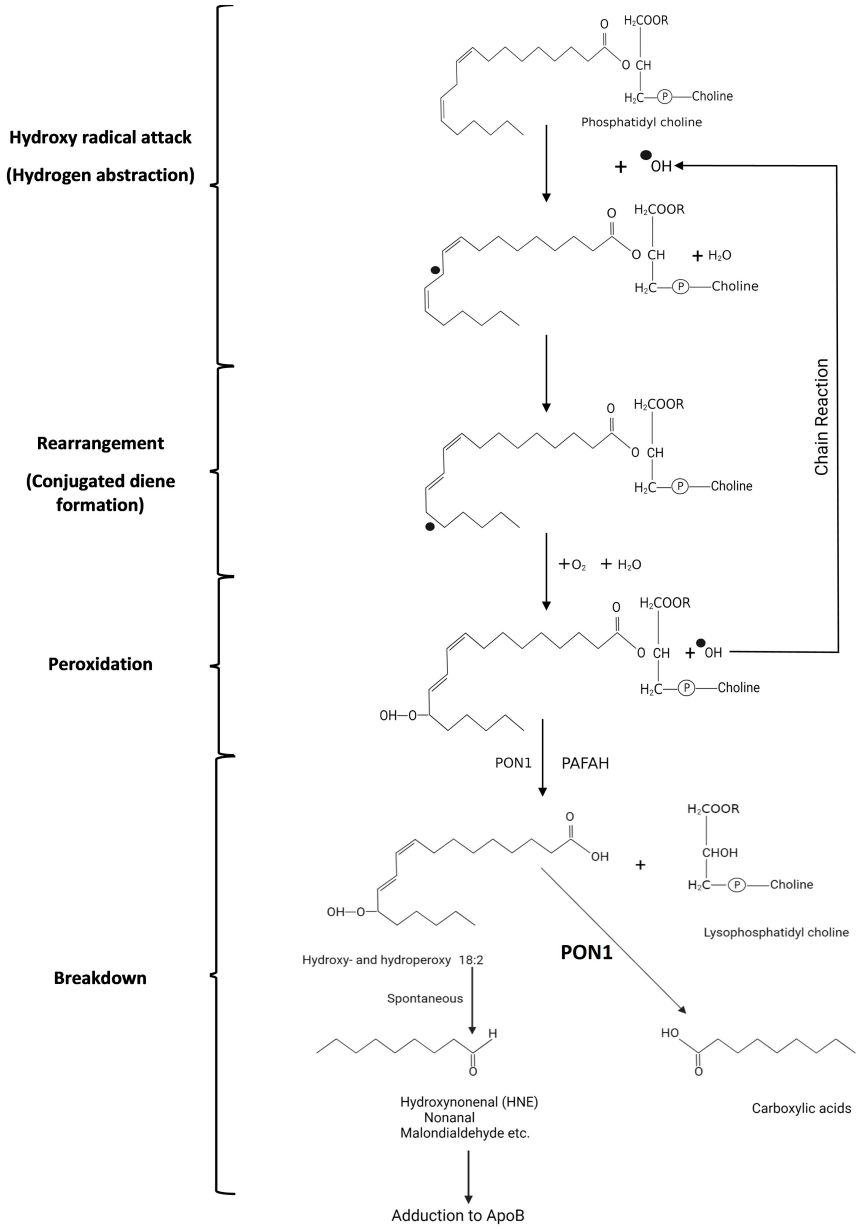


FIGURE 3
Symbolic representation of the peroxidation of phosphatidyl choline with linoleate in Sn2 by reactive oxygen species (hydroperoxy radical in this example). A superscript dot preceding an atom indicates an unpaired electron. (1) Hydrogen abstraction from the hydrocarbon chain leads to (2) rearrangement in which the double bonds between carbon 6 and 9 are no longer separated by 2 single bonds but by only one (conjugated diene formation). (3) Addition of oxygen then leads to peroxy radical formation with the potential for initiating a chain reaction and (4) spontaneous breakdown to aldehydes and ketones which can adduct to apolipoprotein B of LDL altering its receptor-binding. When PON1 in HDL is present breakdown to carboxylic acids rather than aldehydes and ketones is believed to occur.

However, this activity is dwarfed beside that of biphenyl hydrolase-like protein, making a critical role for ancestral PON1 in that regard less likely (73, 76, 77, 88).

The view that PON1 may have a more generalised role in the immune system has been proposed by Camps et al. (89) based on their finding of an increase in chemokine (C-C motif) ligand 2 (CCL2) production in PON1 deficiency. CCL2 induces migration and infiltration of immune cells into target tissues in a range of inflammatory disorders, which could include the arterial wall.

An apparently quite different role for PON1 which might have selective advantage is its capacity to inactivate gram negative bacterial endotoxin (90). This endotoxin is a lipopolysaccharide, which introduces yet another class of substrates which PON1can hydrolyse with important biological consequences.

Thus paraoxonases appear to have diverged from other enzymes early in evolution. They display great substrate promiscuity and their primary function (organophosphatase, anti-oxidant, lactonase, lipopolysaccharidase) may have been different at various times in evolutionary history and in different classes or even orders of living organisms. Myocardial infarction was unknown before the 20th century (91). It is thus inconceivable that PON1 has evolved to combat atherosclerosis. Nonetheless it is very possible that an enzyme which has provided survival success in some other context might by virtue of its promiscuity protect against ASCVD ("wide substrate

specificity" might be better terminology than "promiscuity" when considering virtue).

Evidence from animal experiments that PON1 protects against atherosclerosis

Birds do not express serum PON1 and they are not only susceptible to organophosphate poisoning, but their HDL lacks the capacity to impede the accumulation of lipid peroxides in human LDL under oxidising conditions (92). Mammals display serum PON1 activity, although with considerable species variation (93). Experimental atherosclerosis in rodents has provided consistent evidence that PON1 infusion or over-expression can suppress atherogenesis or that inhibition or ablation of the PON1 gene promotes atherogenesis. Thus the *PON1* knockout mouse, which, like birds is susceptible to organophosphate toxicity, also produces HDL which has a diminished capacity to protect LDL against oxidative modification. It is susceptible both to atherosclerosis induced nutritionally and by apoE deficiency (35). Consistent with this, rabbits fed an atherogenic diet developed more advanced atherosclerotic lesions when PON1 activity was inhibited with nandrolone (94). As long ago as 2002 a US patent was registered (95) for the prevention of atherosclerosis by injection of a preparation of PON1 192Q isoform based on a mouse model. Over-expression of PON1 has been achieved in mouse (96–100), rabbit (101–103), and rat (104) models. Such experiments have consistently revealed decreased susceptibility to atherosclerosis and enhanced HDL functionality. Ablation or over-expression of the PON1 gene causes little if any effect on lipoprotein metabolism, unlike knock out or overexpression of major genes sometimes considered to be important in atherogenesis, such as apoA1 and LCAT. The small decrease in blood pressure in PON1 deficiency (105) would be expected to oppose rather than increase atherogenesis. Thus, a major role for PON1 in atherogenesis is the only plausible explanation for the results of animal experiments. Furthermore, in experiments to test the contribution of risk factors other than PON1 to atherosclerosis, the atherogenic diet used in, for example rabbits, would have decreased PON1 and contributed to atherogenesis from whatever other cause was being examined (106).

Physical and structural properties of PON1

For its antioxidative activity towards lipid hydroperoxides, PON1 requires an intensely hydrophobic environment. Its molecular structure contains long sequences of hydrophobic amino acids creating regions eschewing water and allowing PON1 to exist within the lipid-rich domains of HDL. The strong detergent properties of apoA1 (107) are likely to be crucial in this respect. The hydrophobicity of PON1 makes it resistant to crystallisation. Removed from its lipid environment, naturally occurring PON1 is unstable and tends to aggregate in the absence of detergents. This is true whether wild-type PON1 (wtPON1) is isolated from serum or from the culture medium of *E coli* expressing wtPON1. This has had two major effects on progress in research into the involvement of PON1 in atherosclerosis. Firstly, its structure remained the subject of speculation, which was unresolved until it was submitted to directed evolution in order to increase its solubility (108). Secondly, as methods were developed for increasing purification of wtPON1, dispute ensued about how much of its capacity to prevent the accumulation of lipid peroxides on LDL was retained (41–44).

Family shuffling of four PON1 genes (human, mouse, rabbit, and rat) resulted in many variants that could be expressed in *E. coli*. One of them produced crystals of a quality suitable for X-ray diffraction studies (108). This was the recombinant-PON1 (rePON1) G2E6 variant, which exhibits 91% homology to the wt rabbit PON1 and 86% homology to the human PON1 (109). Structural analysis using X-ray crystallography revealed the six-bladed β-propeller structure of PON1 with a central tunnel that houses two calcium ions. There is a unique addition to the β-propeller scaffold in the form of three α-helices, which are located on the top of the propeller. These helices are likely to be involved in anchoring of PON1 to HDL particles.

Each calcium ion, depending on its location within the enzyme, plays an important part in the activity of PON1. The calcium ion located closer to the tunnel entrance has a structural role that may be necessary for some conformational aspects of PON1 important for some of its substrates, such as organophosphates. It may be less critical to, say phospholipids, with which it is surrounded within HDL. The other calcium ion which lies deep in the active site cavity has a catalytic role and is important for substrate positioning and ester bond activation. Differences in the active site configuration and positioning of a calcium ion are likely to be important in explaining the differential substrate specificity of the 192 polymorphisms, but with such a wide spectrum of substrates no single mechanism may exist (110). Engineered variants with increased aqueous solubility and tagged to simplify purification have at the time of writing led to 21 rePON1 products being available from 8 suppliers. Generally, the evidence that these retain enzymic activity in initial screening has been based on phenyl acetate hydrolysis. The commercial incentive has been to produce rePON1 variants for the treatment of organophosphate poisoning or prevention. The aim has thus been to create rePON1's that are more active in hydrolysing organophosphate neurotoxins than wtPON1, which is less active than squid DFPase, a rival target for bio-engineering. The hydrophobicity of rePON1 must necessarily have been diminished. This may not impair its capacity to hydrolyse molecules such as phenyl acetate and simple organophosphates, but hydrolysis of more intensely hydrophobic long chain fatty acyl lipid substrates may be abolished.

Mechanism by which HDL and PON1 act to protect against lipid peroxide accumulation

Polyunsaturated fatty acyl groups are susceptible to peroxidation due to oxygen free radicals leaking from cells or deliberately produced in the tissue fluid by inflammatory cells (myeloperoxidase, NADPH oxidase). In the human, linoleate (C18:2) is the major circulating polyunsaturated fatty acid, typically occurring at the Sn2 position of phosphatidyl choline. The earliest phase of lipid peroxidation is hydrogen abstraction. This leads to conjugated diene formation detectable by ultraviolet spectroscopy (111). The double C = C bonds (outer orbitals occupied by an electron from a single hydrogen) in linoleate are separated by two single C-C bonds (outer orbitals

occupied by electros from two hydrogens). A conjugated diene is formed when one of the double bonds flips over, because one of its hydrogen atoms is attracted by hydroxyl radicals (OH• created by the reaction of O_2• with water) with their unpaired electrons (**Figure 3**). The newly created double bond is separated from the next double bond by a single bond (conjugated diene), an assemblage which resonates with ultraviolet light. This early phase of lipid peroxidation is opposed by chain-breaking lipid soluble antioxidants, such as α-tocopherol (vitamin E), β-carotene, and ubiquinol-10, which offer themselves as electron donors in preference to linoleate, but it is largely unaffected by PON1. The major effect of PON1 present in HDL is in decreasing the accumulation of lipid peroxides in LDL derived from these conjugated diene free radicals in the later phase of the oxidation of linoleate (21). The failure of clinical trials with, for example, vitamin E (112) is frequently seen as dismissing the oxidative modification of LDL theory of atherosclerosis. However, it is not at the early stage of conjugated diene formation that LDL is chemically modified, allowing arterial wall macrophage and smooth muscle cell receptor-mediated uptake (111). This occurs later when oxygen becomes bound to linoleate causing its decomposition firstly into, for example, 9-hydroperoxy-10,12-octodecadienoic acid (9-HPODE), and thence aldehydes (e.g., propanedial, hexanal, nonanal), unsaturated aldehydes (e.g., hexenal, nonenal) and their various hydroperoxy derivatives. It is these aldehydes which adduct to the side chains of proline and arginine residues of apoB, leading to fragmentation of the apoB molecule, which thereby becomes a ligand for macrophage and transformed smooth muscle cell scavenger receptors, such as scavenger receptor class B type 1 (SCARB1) (111, 113–119). Possibly some derivatives of oxidised polyunsaturated fatty acids have a steric resemblance to lactones (120).

For PON1 to protect LDL or cell membranes against aldehyde adduction it must come into physical contact with their phosphatidyl choline and cholesteryl ester components. In tissue fluid this may be achieved through the engagement of HDL particle with outer cell membranes (38, 121). In the circulation, particularly in humans in whom cholesterol esterification occurs on HDL due to the action of LCAT, huge amounts of phospholipid and of free and esterified cholesterol are transferred between HDL and apoB-containing lipoproteins. This process is greatly facilitated by cholesteryl ester transfer protein (CETP) and phospholipid transfer protein (PLTP) (122–124). Esterification of cholesterol by LCAT yields one molecule of the highly cytotoxic lysophosphatidyl choline for every molecule of cholesterol esterified (125). HDL retains this lysophosphatidyl choline safely until it passes through the hepatic sinusoids where it is released to hepatocytes for re-esterification.

Arriving on HDL, phosphatidyl choline with, as the consequence of oxygen free radical attack, fatty acyl hydroperoxide/conjugated dienes at Sn2, the initial phase of detoxification is likely to be the release of these fatty acyl molecules from Sn2 by the PAFAH (platelet activating factor; syn phospholipase A2, PLA2)-like activity of PON1. Quite possibly this is facilitated not at the lactonase active site deep in the catalytic tunnel of PON1, but perhaps more superficially and might even take place to some extent spontaneously. It is, however, likely that the oxidised linoleate products released can reach the deeper PON1 catalytic site (whatever its teleology) (45) where they are converted to harmless carboxylic acids as opposed to reactive aldehydes (20, 23, 126; **Figure 4**). This concept of PON1 activity has been challenged by a report that highly purified PON1 lacks both PAFAH activity and the capacity to prevent the accumulation of phosphatidylcholine oxidation products (41). Nonetheless, as has been previously discussed, PON1 divorced from HDL may not be able to hydrolyse intensely hydrophobic substrates and there is no denying the antioxidant activity of intact HDL or the evidence from gene manipulation that PON1 makes a crucial contribution to this.

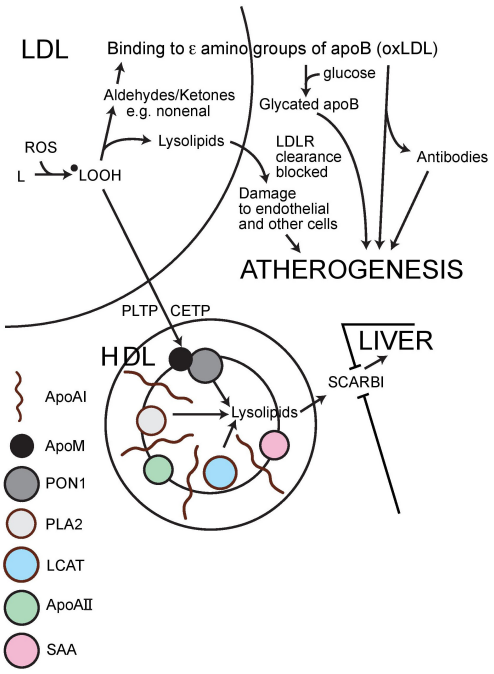


FIGURE 4
Schematic representation of the mechanism by which HDL impedes the atherogenic modification of LDL. ApoA1, apolipoprotein AI; ApoAII, apolipoprotein AII; apoB, apolipoprotein B; ApoM, apolipoprotein M; PON1, paraoxonase 1; PLA2, phospholipase A2 (syn: PAFAH, platelet activating factor hydrolase); LCAT, lecithin cholesterol acyl transferase; SAA, serum amyloid A; PLTP, phospholipid transfer protein; CETP, cholesteryl ester transfer protein; SCARB1, scavenger receptor class B1; oxLDL, oxidatively modified LDL; LDLR, LDL receptor; L, lipid; •LOOH, hydroxylipid radical; ROS, reactive oxygen species (containing oxygen with an unpaired electron giving it an outer shell resembling fluorine).

Epigenetic factors and modulators of PON1 activity

A host of diseases and nutritional factors are associated with variation in PON1 activity. These and potential mechanisms for their associations with PON1 have recently been reviewed (55, 56). Both dyslipidaemia (8, 127–134) and diabetes mellitus (8, 135–145) are associated with decreased activity (see later). The composition of HDL has a major effect on PON1 activity. In inflammation HDL has decreased PON1 activity (146–148). At the same time the apolipoprotein AI and clusterin (syn. apolipoprotein J) content of HDL also diminish whereas apoliporotein AII (apoAII) and serum amyloid A (SAA) increase (146–149). The resulting pro-inflammatory HDL has decreased antioxidant capacity. SAA increases in inflammation. Experimentally SAA can displace PON1 from HDL (148) but the mechanism for the replacement of PON1 by SAA in pro-inflammatory HDL may also involve inhibition of hepatic PON1 expression and stimulation of that of SAA (146–150). The antioxidant activity of PON1 is also limited by myeloperoxidase

(MPO), a pro-oxidant enzyme secreted by neutrophils (151–155) to kill bacteria by showering them with oxygen free radicals. MPO is taken up by HDL where it may form a complex with PON1. The anti-oxidative activity of HDL may thus reflect a balance between those two. Bearing in mind that HDL is the dominant lipoprotein in tissues, it may not be fanciful to speculate that it may operate to confine pro-oxidant activity to sites of inflammation and to limit the spread of oxygen free radicals systemically. When infection or inflammation becomes generalised, as in, say, septicaemia (90, 156) or systemic lupus erythematosus (157) PON1 activity has declined.

Diabetes and metabolic syndrome

Both type 1 and type 2 diabetes are associated with decreased PON1 activity and low PON1 activity is related to both macro- and microvascular complications (8, 135–145). Furthermore low PON1 may predispose to the development of type 2 diabetes (143, 158, 159).

It is reported that *in vitro* glycation of PON1 by incubation with glucose at high concentration decreases its activity (160, 161). This, of course, cannot explain the low serum PON1 activity in metabolic syndrome (prediabetes) before the onset of hyperglycaemia. What is of much greater interest is the occurrence of glycated apoB in the circulation. In non-diabetic people some 4% of serum apo B is glycated and in diabetes the percentage is typically twice this (162). The concentration of glycated apoB is also raised when LDL is increased even in the absence of diabetes, for example in familial hypercholesterolaemia. The concentration of glycated apoB, whether in diabetic or non-diabetic people, is higher than oxidatively modified apoB (162, 163). ApoB in the smaller, denser subfractions of LDL (SD-LDL) is more heavily glycated than in VLDL and less dense LDL (164–166). This may be because more of the apoB molecule is exposed to glucose in SD-LDL or because it has a longer residence time in the circulation or both. Glycation of apoB occurs at the arginine and proline residues to which ketones and other derivatives of lipid peroxidation adduct (167). Glycated apoB, like oxidatively modified apoB, is taken up by macrophages to form foam cells (168). *In vitro* apoB in LDL is resistant to glycation. Prolonged incubation of normal LDL with high concentrations of glucose does raise its level, but not usually to the same extent as is found in diabetes (165, 168). The explanation may be that the more highly oxidative environment *in vivo* allows prior adduction of, say aldehydes to arginine, which is then replaced by glucose (166, 169) or a more reactive derivative of glucose, such as gluconolactone or methyl glyoxal, is generated during glycolysis (170–172). Either mechanism might suggest a possible effect of HDL in protecting LDL apoB against glycation which has been reported *in vitro* with HDL from people with above median serum PON1 activity opposing glycation more than HDL from those with lower activity (173). More work is needed to explore the possibility that HDL might protect against atheroma and more specifically diabetic complications by this mechanism.

Future directions: Therapeutic and diagnostic potential

There is a plethora of nutritional studies of PON1 activity. Unsurprisingly, given the different substrates used to measure PON1 activity, the small size and the inadequate design of many, findings often appear conflicting or unconvincing. No amount of meta-analysis can provide clarification. The impression gained is that obesity is often associated with decreased PON1 activity, albeit most obviously when triglycerides are raised or diabetes is present (127, 128, 132–145, 174). It is also clear that HDL cholesterol concentration is often discordant with changes in PON1 activity. The Mediterranean diet may increase PON1 activity (175) and various fruit juices, most conspicuously pomegranate juice, can raise PON1 activity (176). Dyslipidaemia, whether due to familial hypercholesterolaemia or hypertriglyceridaemia, is associated with diminished PON1 activity (8, 127, 128, 130–134) with perhaps its most profound decreases occurring in familial dysbetalipoproteinaemia [unpublished observation]. Statin (177), fibrate (178), ezetimibe (179), probucol (128), niacin (180), and metformin (181) drugs raise serum PON1 activity whilst sulphonamides may decrease it (182).

A pharmacological approach to raising PON1 activity is attractive, but traditionally it is easier to block rather than activate enzymes. Raising HDL by CETP inhibition was ineffective in preventing atherosclerosis except by its LDL lowering effect (183). CETP may be necessary for the transfer of oxidised phospholipid and cholesteryl ester to HDL for PON1 to act on them and the HDL particles created are large (184) and not the smaller, desirable particles rich in PON1 capable of facilitating cholesterol efflux. PON1-rich HDL infusion is probably not a practical possibility, particularly as rePON1, which is easily produced may have little anti-oxidant capacity [see earlier]. Evidence suggests that HDL mimetics, some of which could be given orally, can raise PON1 activity in particles resembling physiological HDL (185, 186). It will also be important to be aware of effects on PON1 of the various antisense oligonucleotides for lowering LDL and triglycerides as they emerge. There also exists the theoretical possibility of raising PON1 activity by promotion of its gene or expression of a gain-of-function variant (but without a polar tag so that it is incorporated physiologically into HDL) (187).

Paraoxonase 1 has the potential to contribute to the clinical assessment of ASCVD risk. However, continuing uncertainties about identification of the substrate critical in its anti-atherosclerotic activity have slowed progress in that direction. Is it important, for example, to employ a long chain fatty acid peroxide or lactone rather than, say, phenyl acetate or paraoxon as the substrate in an assay? However, whilst discovery of the key substrate(s) in the mechanism by which PON1 protects against atherosclerosis is essential for our understanding of its role, this may not be critical to make use of it clinically. Alkaline phosphatase is one of the most frequently requested and informative biochemical tests in clinical practice, but its physiological role remains obscure, and the substrate used in its measurement artificial (188). Currently, measurement of PON1 hydrolytic activity has generally been more closely associated with ASCVD than PON1 protein concentration, because the specific activity of PON1 is variable, for example in diabetes (see earlier discussion). PON1 hydrolyses phenyl acetate at a much higher rate than paraoxon. The PON1 192 polymorphism does not affect the hydrolysis of phenyl acetate but does that of paraoxon. On the other hand, if the true physiological role of PON1 is its lactonase activity, then potentially there could be advantages to using lactones, such as dihydrocoumarin or homocysteine thiolactone, as assay substrates (73–75). However, this has yet to be proven. Undoubtedly too, mistakes, such as the use of plasma rather than serum and inclusion of B esterase and non-specific hydrolysis in some methods for determination of A esterase (PON1) activity, have led to some confusion. A carefully conducted laboratory investigation

using a variety of candidate substrates (189–192) linked to an epidemiological study is required.

Measurement of serum PON1 activity also provides an indication of where the HDL present in individuals is in the spectrum of pro- to anti-inflammatory and pro- to anti-atherosclerotic capacity (100, 193). Cholesterol efflux capacity is another indicator, but its measurement is more difficult and more prone to error (194). Because decreased PON1 activity is frequently associated with increased SAA in HDL the ratio of SAA concentration to PON1 activity has been proposed as better index of the type of HDL present than either measurement singly (148).

Conclusion

Serum PON1 activity is inversely associated with ASCVD incidence both in human and animal studies. Although this was discovered due to the presence on HDL of PON1 and its contribution to the anti-oxidative capacity of HDL, the lactonase activity of PON1 may also contribute to the mechanism by which it reduces ASCVD risk. PON1 provides a potential additional means of clinical ASCVD risk assessment and is an indicator of the extent to which HDL has retained its anti-atherogenic and anti-inflammatory properties. It has the potential for therapeutic exploitation.

Author contributions

PD conceptualised the study, performed the literature search, wrote the draft, and finalised the manuscript. BB and HS performed literature searches, contributed to writing, revised the draft, proofread the manuscript, and designed the figures. All authors contributed to the article and approved the submitted version.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Durrington P, Mackness B, Mackness M. Paraoxonase and atherosclerosis. *Arterioscler Thromb Vasc Biol.* (2001) 21:473–80. doi: 10.1161/01.ATV.21.4.473

2. Aldridge W. Serum esterases. II. An enzyme hydrolysing diethyl p-nitrophenyl phosphate (E600) and its identity with the A-esterase of mammalian sera. *Biochem J.* (1953) 53:117–24. doi: 10.1042/bj0530117

3. Furlong C. Paraoxonase: an historical perspective. In: Ridley A, Frampton J editors. *The paraoxonases: their role in disease development and xenobiotic metabolism.* Dordrecht: Springer (2008).

4. Draganov D, La Du B. Pharmacogenetics of paraoxonases: a brief review. *Naunyn Schmiedebergs Arch Pharmacol.* (2004) 369:78–88. doi: 10.1007/s00210-003-0833-1

5. Mackness M, Hallam S, Peard T, Warner S, Walker C. The separation of sheep and human serum "A"-esterase activity into the lipoprotein fraction by ultracentrifugation. *Comp Biochem Physiol B.* (1985) 82:675–7. doi: 10.1016/0305-0491(85)90506-1

6. McElveen J, Mackness M, Colley C, Peard T, Warner S, Walker C. Distribution of paraoxon hydrolytic activity in the serum of patients after myocardial infarction. *Clin Chem.* (1986) 32:671–3. doi: 10.1093/clinchem/32.4.671

7. Patel B, Mackness M, Harty D, Arrol S, Boot-Handford R, Durrington P. Serum esterase activities and hyperlipidaemia in the streptozotocin-diabetic rat. *Biochim Biophys Acta.* (1990) 1035:113–6. doi: 10.1016/0304-4165(90)90182-V

8. Mackness M, Harty D, Bhatnagar D, Winocour P, Arrol S, Ishola M, et al. Serum paraoxonase activity in familial hypercholesterolaemia and insulin-dependent diabetes mellitus. *Atherosclerosis.* (1991) 86:193–9. doi: 10.1016/0021-9150(91)90215-O

9. von Eckardstein A. LDL Contributes to reverse cholesterol transport. *Circ Res.* (2020) 127:793–5. doi: 10.1161/CIRCRESAHA.120.317721

10. Hessler J, Robertson A Jr, Chisolm G III. LDL-induced cytotoxicity and its inhibition by HDL in human vascular smooth muscle and endothelial cells in culture. *Atherosclerosis.* (1979) 32:213–29. doi: 10.1016/0021-9150(79)90166-7

11. Klimov A, Nikiforova A, Pleskov V, Kuz'min A, Kalashnikova N. [Protective effect of high density lipoproteins, their subfractions and lecithin-cholesterol-acyltransferases on the peroxidation modification of low density lipoproteins]. *Biokhimiia.* (1989) 54:118–23.

12. Ohta T, Takata K, Horiuchi S, Morino Y, Matsuda I. Protective effect of lipoproteins containing apoprotein A-I on Cu2+-catalyzed oxidation of human low density lipoprotein. *FEBS Lett.* (1989) 257:435–8. doi: 10.1016/0014-5793(89)81590-X

13. Parthasarathy S, Barnett J, Fong L. High-density lipoprotein inhibits the oxidative modification of low-density lipoprotein. *Biochim Biophys Acta.* (1990) 1044:275–83. doi: 10.1016/0005-2760(90)90314-N

14. Navab M, Imes S, Hama S, Hough G, Ross L, Bork R, et al. Monocyte transmigration induced by modification of low density lipoprotein in cocultures of human aortic wall cells is due to induction of monocyte chemotactic protein 1 synthesis and is abolished by high density lipoprotein. *J Clin Invest.* (1991) 88:2039–46. doi: 10.1172/JCI115532

15. Durrington P. New ideas about the role of HDL. In: Shepherd J, Packard C, Brownlie S editors. *Lipoproteins and the pathogenesis of atherosclerosis*. Amsterdam: Excerpta Medica (1991).

16. el-Saadani M, Esterbauer H, el-Sayed M, Goher M, Nassar A, Jürgens G. A spectrophotometric assay for lipid peroxides in serum lipoproteins using a commercially available reagent. *J Lipid Res.* (1989) 30:627–30. doi: 10.1016/S0022-2275(20)38354-1

17. Mackness M, Arrol S, Durrington P. Paraoxonase prevents accumulation of lipoperoxides in low-density lipoprotein. *FEBS Lett.* (1991) 286:152–4. doi: 10.1016/0014-5793(91)80962-3

18. Mackness M, Arrol S, Abbott C, Durrington P. Protection of low-density lipoprotein against oxidative modification by high-density lipoprotein associated paraoxonase. *Atherosclerosis.* (1993) 104:129–35. doi: 10.1016/0021-9150(93)90183-U

19. Mackness M, Arrol S, Abbott C, Durrington P. Is paraoxonase related to atherosclerosis. *Chem Biol Interact.* (1993) 87:161–71. doi: 10.1016/0009-2797(93)90038-Z

20. Mackness M, Durrington P. HDL, its enzymes and its potential to influence lipid peroxidation. *Atherosclerosis.* (1995) 115:243–53. doi: 10.1016/0021-9150(94)05524-M

21. Mackness M, Abbott C, Arrol S, Durrington P. The role of high-density lipoprotein and lipid-soluble antioxidant vitamins in inhibiting low-density lipoprotein oxidation. *Biochem J.* (1993) 294:829–34. doi: 10.1042/bj2940829

22. Klimov A, Gurevich V, Nikiforova A, Shatilina L, Kuzmin A, Plavinsky S, et al. Antioxidative activity of high density lipoproteins *in vivo*. *Atherosclerosis.* (1993) 100:13–8. doi: 10.1016/0021-9150(93)90063-Z

23. Watson A, Berliner J, Hama S, La Du B, Faull K, Fogelman A, et al. Protective effect of high density lipoprotein associated paraoxonase. Inhibition of the biological activity of minimally oxidized low density lipoprotein. *J Clin Invest.* (1995) 96:2882–91. doi: 10.1172/JCI118359

24. Sangvanich P, Mackness B, Gaskell S, Durrington P, Mackness M. The effect of high-density lipoproteins on the formation of lipid/protein conjugates during *in vitro* oxidation of low-density lipoprotein. *Biochem Biophys Res Commun.* (2003) 300:501–6. doi: 10.1016/S0006-291X(02)02849-8

25. Garner B, Waldeck A, Witting P, Rye K, Stocker R. Oxidation of high density lipoproteins. II. Evidence for direct reduction of lipid hydroperoxides by methionine

residues of apolipoproteins AI and AII. *J Biol Chem.* (1998) 273:6088–95. doi: 10.1074/jbc.273.11.6088

26. Hayek T, Oiknine J, Dankner G, Brook J, Aviram M. HDL apolipoprotein A-I attenuates oxidative modification of low density lipoprotein: studies in transgenic mice. *Eur J Clin Chem Clin Biochem.* (1995) 33:721–5. doi: 10.1515/cclm.1995.33.10.721

27. Arrol S, Mackness MI, Durrington PN. High density lipoprotein-associated enzymes and the prevention of lower density lipoprotein oxidation. *Eur J Lab Med.* (1995) 4:33–8.

28. Hine D, Mackness B, Mackness M. Coincubation of PON1, APO A1, and LCAT increases the time HDL is able to prevent LDL oxidation. *IUBMB Life.* (2012) 64:157–61. doi: 10.1002/iub.588

29. Neary R, Bhatnagar D, Durrington P, Ishola M, Arrol S, Mackness M. An investigation of the role of lecithin:cholesterol acyltransferase and triglyceride-rich lipoproteins in the metabolism of pre-beta high density lipoproteins. *Atherosclerosis.* (1991) 89:35–48. doi: 10.1016/0021-9150(91)90005-N

30. Soran H, Schofield J, Liu Y, Durrington P. How HDL protects LDL against atherogenic modification: paraoxonase 1 and other dramatis personae. *Curr Opin Lipidol.* (2015) 26:247–56. doi: 10.1097/MOL.0000000000000194

31. James R, Blatter Garin M, Calabresi L, Miccoli R, von Eckardstein A, Tilly-Kiesi M, et al. Modulated serum activities and concentrations of paraoxonase in high density lipoprotein deficiency states. *Atherosclerosis.* (1998) 139:77–82. doi: 10.1016/S0021-9150(98)00058-6

32. Holleboom A, Daniil G, Fu X, Zhang R, Hovingh G, Schimmel A, et al. Lipid oxidation in carriers of lecithin:cholesterol acyltransferase gene mutations. *Arterioscler Thromb Vasc Biol.* (2012) 32:3066–75. doi: 10.1161/ATVBAHA.112.255711

33. Furbee J Jr, Sawyer J, Parks J. Lecithin:cholesterol acyltransferase deficiency increases atherosclerosis in the low density lipoprotein receptor and apolipoprotein E knockout mice. *J Biol Chem.* (2002) 277:3511–9. doi: 10.1074/jbc.M109883200

34. Moore R, Navab M, Millar J, Zimetti F, Hama S, Rothblat G, et al. Increased atherosclerosis in mice lacking apolipoprotein A-I attributable to both impaired reverse cholesterol transport and increased inflammation. *Circ Res.* (2005) 97:763–71. doi: 10.1161/01.RES.0000185320.82962.F7

35. Shih D, Xia Y, Wang X, Miller E, Castellani L, Subbanagounder G, et al. Combined serum paraoxonase knockout/apolipoprotein E knockout mice exhibit increased lipoprotein oxidation and atherosclerosis. *J Biol Chem.* (2000) 275:17527–35. doi: 10.1074/jbc.M910376199

36. Aviram M, Rosenblat M, Bisgaier C, Newton R, Primo-Parmo S, La Du B. Paraoxonase inhibits high-density lipoprotein oxidation and preserves its functions. A possible peroxidative role for paraoxonase. *J Clin Invest.* (1998) 101:1581–90. doi: 10.1172/JCI1649

37. Mackness M, Mackness B, Arrol S, Wood G, Bhatnagar D, Durrington P. Presence of paraoxonase in human interstitial fluid. *FEBS Lett.* (1997) 416:377–80. doi: 10.1016/S0014-5793(97)01243-X

38. Deakin S, Bioletto S, Bochaton-Piallat M, James R. HDL-associated paraoxonase-1 can redistribute to cell membranes and influence sensitivity to oxidative stress. *Free Radic Biol Med.* (2011) 50:102–9. doi: 10.1016/j.freeradbiomed.2010.09.002

39. Mackness B, Hunt R, Durrington P, Mackness M. Increased immunolocalization of paraoxonase, clusterin, and apolipoprotein A-I in the human artery wall with the progression of atherosclerosis. *Arterioscler Thromb Vasc Biol.* (1997) 17:1233–8. doi: 10.1161/01.ATV.17.7.1233

40. Graham A, Hassall D, Rafique S, Owen J. Evidence for a paraoxonase-independent inhibition of low-density lipoprotein oxidation by high-density lipoprotein. *Atherosclerosis.* (1997) 135:193–204. doi: 10.1016/S0021-9150(97)00162-7

41. Connelly P, Draganov D, Maguire G. Paraoxonase-1 does not reduce or modify oxidation of phospholipids by peroxynitrite. *Free Radic Biol Med.* (2005) 38:164–74. doi: 10.1016/j.freeradbiomed.2004.10.010

42. Teiber J, Draganov D, La Du B. Purified human serum PON1 does not protect LDL against oxidation in the *in vitro* assays initiated with copper or AAPH. *J Lipid Res.* (2004) 45:2260–8. doi: 10.1194/jlr.M400213-JLR200

43. Draganov D, Teiber J, Speelman A, Osawa Y, Sunahara R, La Du B. Human paraoxonases (PON1, PON2, and PON3) are lactonases with overlapping and distinct substrate specificities. *J Lipid Res.* (2005) 46:1239–47. doi: 10.1194/jlr.M400511-JLR200

44. Liu Y, Mackness B, Mackness M. Comparison of the ability of paraoxonases 1 and 3 to attenuate the *in vitro* oxidation of low-density lipoprotein and reduce macrophage oxidative stress. *Free Radic Biol Med.* (2008) 45:743–8. doi: 10.1016/j.freeradbiomed.2008.05.024

45. Aviram M, Billecke S, Sorenson R, Bisgaier C, Newton R, Rosenblat M, et al. Paraoxonase active site required for protection against LDL oxidation involves its free sulfhydryl group and is different from that required for its arylesterase/paraoxonase activities: selective action of human paraoxonase allozymes Q and R. *Arterioscler Thromb Vasc Biol.* (1998) 18:1617–24. doi: 10.1161/01.ATV.18.10.1617

46. Rodrigo L, Mackness B, Durrington P, Hernandez A, Mackness M. Hydrolysis of platelet-activating factor by human serum paraoxonase. *Biochem J.* (2001) 354(Pt. 1):1–7. doi: 10.1042/bj3540001

47. Chistiakov D, Melnichenko A, Orekhov A, Bobryshev Y. Paraoxonase and atherosclerosis-related cardiovascular diseases. *Biochimie.* (2017) 132:19–27. doi: 10.1016/j.biochi.2016.10.010

48. Brophy V, Jarvik GP, Furlong CE. *Paraoxonase (PON1) in health and disease: basic and clinical aspects*. Boston: KluwerAcademic Publishers (2002).

49. La Du B, Aviram M, Billecke S, Navab M, Primo-Parmo S, Sorenson R, et al. On the physiological role(s) of the paraoxonases. *Chem Biol Interact.* (1999) 119–120:379–88. doi: 10.1016/S0009-2797(99)00049-6

50. Ruiz J, Blanché H, James R, Garin M, Vaisse C, Charpentier G, et al. Gln-Arg192 polymorphism of paraoxonase and coronary heart disease in type 2 diabetes. *Lancet.* (1995) 346:869–72. doi: 10.1016/S0140-6736(95)92709-3

51. Mackness B, Mackness M, Arrol S, Turkie W, Durrington P. Effect of the human serum paraoxonase 55 and 192 genetic polymorphisms on the protection by high density lipoprotein against low density lipoprotein oxidative modification. *FEBS Lett.* (1998) 423:57–60. doi: 10.1016/S0014-5793(98)00064-7

52. Mackness M, Arrol S, Mackness B, Durrington P. Alloenzymes of paraoxonase and effectiveness of high-density lipoproteins in protecting low-density lipoprotein against lipid peroxidation. *Lancet.* (1997) 349:851–2. doi: 10.1016/S0140-6736(05)61755-2

53. Deakin S, Leviev I, Nicaud V, Brulhart Meynet M, Tiret L, James R. Paraoxonase-1 L55M polymorphism is associated with an abnormal oral glucose tolerance test and differentiates high risk coronary disease families. *J Clin Endocrinol Metab.* (2002) 87:1268–73. doi: 10.1210/jcem.87.3.8335

54. Leviev I, Poirier O, Nicaud V, Evans A, Kee F, Arveiler D, et al. High expressor paraoxonase PON1 gene promoter polymorphisms are associated with reduced risk of vascular disease in younger coronary patients. *Atherosclerosis.* (2002) 161:463–7. doi: 10.1016/S0021-9150(01)00668-2

55. Soran H, Younis N, Charlton-Menys V, Durrington P. Variation in paraoxonase-1 activity and atherosclerosis. *Curr Opin Lipidol.* (2009) 20:265–74. doi: 10.1097/MOL.0b013e32832ec141

56. Mahrooz A, Mackness M. Epigenetics of paraoxonases. *Curr Opin Lipidol.* (2020) 31:200–5. doi: 10.1097/MOL.0000000000000687

57. Costa L, Vitalone A, Cole T, Furlong C. Modulation of paraoxonase (PON1) activity. *Biochem Pharmacol.* (2005) 69:541–50. doi: 10.1016/j.bcp.2004.08.027

58. Mackness B, Davies G, Turkie W, Lee E, Roberts D, Hill E, et al. Paraoxonase status in coronary heart disease: are activity and concentration more important than genotype? *Arterioscler Thromb Vasc Biol.* (2001) 21:1451–7. doi: 10.1161/hq0901.094247

59. Wheeler J, Keavney B, Watkins H, Collins R, Danesh J. Four paraoxonase gene polymorphisms in 11212 cases of coronary heart disease and 12786 controls: meta-analysis of 43 studies. *Lancet.* (2004) 363:689–95. doi: 10.1016/S0140-6736(04)15642-0

60. Ashiq S, Ashiq K. The role of paraoxonase 1 (PON1) gene polymorphisms in coronary artery disease: a systematic review and meta-analysis. *Biochem Genet.* (2021) 59:919–39. doi: 10.1007/s10528-021-10043-0

61. Zeng Q, Zeng J. A meta-analysis on relationship between paraoxonase 1 polymorphisms and atherosclerotic cardiovascular diseases. *Life Sci.* (2019) 232:116646. doi: 10.1016/j.lfs.2019.116646

62. Luo Z, Pu L, Muhammad I, Chen Y, Sun X. Associations of the PON1 rs662 polymorphism with circulating oxidized low-density lipoprotein and lipid levels: a systematic review and meta-analysis. *Lipids Health Dis.* (2018) 17:281. doi: 10.1186/s12944-018-0937-8

63. Ayub A, Mackness M, Arrol S, Mackness B, Patel J, Durrington P. Serum paraoxonase after myocardial infarction. *Arterioscler Thromb Vasc Biol.* (1999) 19:330–5. doi: 10.1161/01.ATV.19.2.330

64. Jarvik G, Rozek L, Brophy V, Hatsukami T, Richter R, Schellenberg G, et al. Paraoxonase (PON1) phenotype is a better predictor of vascular disease than is PON1(192) or PON1 genotype. *Arterioscler Thromb Vasc Biol.* (2000) 20:2441–7. doi: 10.1161/01.ATV.20.11.2441

65. Mackness B, Durrington P, McElduff P, Yarnell J, Azam N, Watt M, et al. Low paraoxonase activity predicts coronary events in the caerphilly prospective study. *Circulation.* (2003) 107:2775–9. doi: 10.1161/01.CIR.0000070954.00271.13

66. Birjmohun R, Vergeer M, Stroes E, Sandhu M, Ricketts S, Tanck M, et al. Both paraoxonase-1 genotype and activity do not predict the risk of future coronary artery disease; the EPIC-Norfolk prospective population study. *PLoS One.* (2009) 4:e6809. doi: 10.1371/journal.pone.0006809

67. Tang W, Hartiala J, Fan Y, Wu Y, Stewart A, Erdmann J, et al. Clinical and genetic association of serum paraoxonase and arylesterase activities with cardiovascular risk. *Arterioscler Thromb Vasc Biol.* (2012) 32:2803–12. doi: 10.1161/ATVBAHA.112.253930

68. van Himbergen T, van der Schouw Y, Voorbij H, van Tits L, Stalenhoef A, Peeters P, et al. Paraoxonase (PON1) and the risk for coronary heart disease and myocardial infarction in a general population of Dutch women. *Atherosclerosis.* (2008) 199:408–14. doi: 10.1016/j.atherosclerosis.2007.11.018

69. Troughton J, Woodside J, Yarnell J, Arveiler D, Amouyel P, Ferrières J, et al. Paraoxonase activity and coronary heart disease risk in healthy middle-aged males: the PRIME study. *Atherosclerosis.* (2008) 197:556–63. doi: 10.1016/j.atherosclerosis.2007.08.019

70. Kunutsor S, Bakker S, James R, Dullaart R. Serum paraoxonase-1 activity and risk of incident cardiovascular disease: the PREVEND study and meta-analysis of prospective population studies. *Atherosclerosis.* (2016) 245:143–54. doi: 10.1016/j.atherosclerosis.2015.12.021

71. Corsetti J, Sparks C, James R, Bakker S, Dullaart R. Low serum paraoxonase-1 activity associates with incident cardiovascular disease risk in subjects with concurrently high levels of high-density lipoprotein cholesterol and C-reactive protein. *J Clin Med.* (2019) 8:1357. doi: 10.3390/jcm8091357

72. Bhattacharyya T, Nicholls S, Topol E, Zhang R, Yang X, Schmitt D, et al. Relationship of paraoxonase 1 (PON1) gene polymorphisms and functional activity with systemic oxidative stress and cardiovascular risk. *JAMA.* (2008) 299:1265–76. doi: 10.1001/jama.299.11.1265

73. Perła-Kaján J, Borowczyk K, Głowacki R, Nygård O, Jakubowski H. Paraoxonase 1 Q192R genotype and activity affect homocysteine thiolactone levels in humans. *FASEB J.* (2018) doi: 10.1096/fj.201800346R [Epub ahead of print].

74. Murillo-González F, Ponce-Ruiz N, Rojas-García A, Rothenberg S, Bernal-Hernández Y, Cerda-Flores R, et al. PON1 lactonase activity and its association with cardiovascular disease. *Clin Chim Acta.* (2020) 500:47–53. doi: 10.1016/j.cca.2019.09.016

75. Petrič B, Kunej T, Bavec AA. Multi-omics analysis of PON1 lactonase activity in relation to human health and disease. *Omics.* (2021) 25:38–51. doi: 10.1089/omi.2020.0160

76. Jakubowski H. Homocysteine modification in protein structure/function and human disease. *Physiol Rev.* (2018) 99:555–604. doi: 10.1152/physrev.00003.2018

77. Borowczyk K, Piechocka J, Głowacki R, Dhar I, Midtun Ø, Tell G, et al. Urinary excretion of homocysteine thiolactone and the risk of acute myocardial infarction in coronary artery disease patients: the WENBIT trial. *J Inter Med.* (2019) 285:232–44. doi: 10.1111/joim.12834

78. Smith J. *The theory of evolution*. 3rd ed. Cambridge: Cambridge University Press (1975).

79. Cherry N, Mackness M, Durrington P, Povey A, Dippnall M, Smith T, et al. Paraoxonase (PON1) polymorphisms in farmers attributing ill health to sheep dip. *Lancet.* (2002) 359:763–4. doi: 10.1016/S0140-6736(02)07847-9

80. Zanchett G, Oliveira-Filho E. Cyanobacteria and cyanotoxins: from impacts on aquatic ecosystems and human health to anticarcinogenic effects. *Toxins.* (2013) 5:1896–917. doi: 10.3390/toxins5101896

81. Venter J, di Porzio U, Robinson D, Shreeve S, Lai J, Kerlavage A, et al. Evolution of neurotransmitter receptor systems. *Prog Neurobiol.* (1988) 30:105–69. doi: 10.1016/0301-0082(88)90004-4

82. Blum M, Chen J. Structural characterization of the catalytic calcium-binding site in diisopropyl fluorophosphatase (DFPase)–comparison with related beta-propeller enzymes. *Chem Biol Interact.* (2010) 187:373–9. doi: 10.1016/j.cbi.2010.02.043

83. Thakur M, Medintz I, Walper S. Enzymatic bioremediation of organophosphate compounds-progress and remaining challenges. *Front Bioeng Biotechnol.* (2019) 7:289. doi: 10.3389/fbioe.2019.00289

84. Hiblot J, Gotthard G, Chabriere E, Elias M. Characterisation of the organophosphate hydrolase catalytic activity of SsoPox. *Sci Rep.* (2012) 2:779. doi: 10.1038/srep00779

85. Lei X, Zhu J, Cheng W, Bao Y, Ho Y, Reddi A, et al. Paradoxical roles of antioxidant enzymes: basic mechanisms and health implications. *Physiol Rev.* (2016) 96:307–64. doi: 10.1152/physrev.00010.2014

86. Elias M, Tawfik D. Divergence and convergence in enzyme evolution: parallel evolution of paraoxonases from quorum-quenching lactonases. *J Biol Chem.* (2012) 287:11–20. doi: 10.1074/jbc.R111.257329

87. McCully K. Homocysteine and the pathogenesis of atherosclerosis. *Expert Rev Clin Pharmacol.* (2015) 8:211–9. doi: 10.1586/17512433.2015.1010516

88. Marsillach J, Suzuki S, Richter R, McDonald M, Rademacher P, MacCoss M, et al. Human valacyclovir hydrolase/biphenyl hydrolase-like protein is a highly efficient homocysteine thiolactonase. *PLoS One.* (2014) 9:e110054. doi: 10.1371/journal.pone.0110054

89. Camps J, Castañé H, Rodríguez-Tomàs E, Baiges-Gaya G, Hernández-Aguilera A, Arenas M, et al. On the role of paraoxonase-1 and chemokine ligand 2 (C-C motif) in metabolic alterations linked to inflammation and disease. A 2021 update. *Biomolecules.* (2021) 11:971. doi: 10.3390/biom11070971

90. Camps J, Iftimie S, García-Heredia A, Castro A, Joven J. Paraoxonases and infectious diseases. *Clin Biochem.* (2017) 50:804–11. doi: 10.1016/j.clinbiochem.2017.04.016

91. Herrick J. Landmark article (JAMA 1912). Clinical features of sudden obstruction of the coronary arteries. By James B. Herrick. *JAMA.* (1983) 250:1757–65. doi: 10.1001/jama.250.13.1757

92. Mackness B, Durrington P, Mackness M. Lack of protection against oxidative modification of LDL by avian HDL. *Biochem Biophys Res Commun.* (1998) 247:443–6. doi: 10.1006/bbrc.1998.8803

93. Meyer W, Jamison J, Richter R, Woods S, Partha R, Kowalczyk A, et al. Ancient convergent losses of paraoxonase 1 yield potential risks for modern marine mammals. *Science.* (2018) 361:591–4. doi: 10.1126/science.aap7714

94. Amani M, Darbin A, Pezeshkian M, Afrasiabi A, Safaie N, Jodati A, et al. The role of cholesterol-enriched diet and paraoxonase 1 inhibition in atherosclerosis progression. *J Cardiovasc Thorac Res.* (2017) 9:133–9. doi: 10.15171/jcvtr.2017.23

95. Radtke K. *Method of using PON-1 to decrease atheroma formation. USA patent US 6,391,298 B1*. Pittsburgh, PA: Bayer Corporation (2002).

96. Zhang C, Peng W, Wang M, Zhu J, Zang Y, Shi W, et al. Studies on protective effects of human paraoxonases 1 and 3 on atherosclerosis in apolipoprotein E knockout mice. *Gene Ther.* (2010) 17:626–33. doi: 10.1038/gt.2010.11

97. Mackness B, Quarck R, Verreth W, Mackness M, Holvoet P. Human paraoxonase-1 overexpression inhibits atherosclerosis in a mouse model of metabolic syndrome. *Arterioscler Thromb Vasc Biol.* (2006) 26:1545–50. doi: 10.1161/01.ATV.0000222924.62641.aa

98. Guns P, Van Assche T, Verreth W, Fransen P, Mackness B, Mackness M, et al. Paraoxonase 1 gene transfer lowers vascular oxidative stress and improves vasomotor function in apolipoprotein E-deficient mice with pre-existing atherosclerosis. *Br J Pharmacol.* (2008) 153:508–16. doi: 10.1038/sj.bjp.0707585

99. She Z, Zheng W, Wei Y, Chen H, Wang A, Li H, et al. Human paraoxonase gene cluster transgenic overexpression represses atherogenesis and promotes atherosclerotic plaque stability in ApoE-null mice. *Circ Res.* (2009) 104:1160–8. doi: 10.1161/CIRCRESAHA.108.192229

100. Ikhlef S, Berrougui H, Kamtchueng Simo O, Zerif E, Khalil A. Human paraoxonase 1 overexpression in mice stimulates HDL cholesterol efflux and reverse cholesterol transport. *PLoS One.* (2017) 12:e0173385. doi: 10.1371/journal.pone.0173385

101. Burillo E, Tarin C, Torres-Fonseca M, Fernandez-García C, Martinez-Pinna R, Martinez-Lopez D, et al. Paraoxonase-1 overexpression prevents experimental abdominal aortic aneurysm progression. *Clin Sci.* (2016) 130:1027–38. doi: 10.1042/CS20160185

102. Bai J, Zhou H, Yang X, Liu H, Meng Y. Inhibitory effect of the paraoxonase gene on the formation of rabbit coronary atherosclerosis. *Asian Pac J Trop Med.* (2013) 6:544–7. doi: 10.1016/S1995-7645(13)60093-0

103. Miyoshi M, Nakano Y, Sakaguchi T, Ogi H, Oda N, Suenari K, et al. Gene delivery of paraoxonase-1 inhibits neointimal hyperplasia after arterial balloon-injury in rabbits fed a high-fat diet. *Hypertens Res.* (2007) 30:85–91. doi: 10.1291/hypres.30.85

104. Wang F, Xue J, Wang D, Wang X, Lu S, Tan M. Treatment of atherosclerosis by transplantation of bone endothelial progenitor cells over-expressed paraoxonase-1 gene by recombinant adeno-associated virus in rat. *Biol Pharm Bull.* (2010) 33:1806–13. doi: 10.1248/bpb.33.1806

105. Gamliel-Lazarovich A, Abassi Z, Khatib S, Tavori H, Vaya J, Aviram M, et al. Paraoxonase1 deficiency in mice is associated with hypotension and increased levels of 5,6-epoxyeicosatrienoic acid. *Atherosclerosis.* (2012) 222:92–8. doi: 10.1016/j.atherosclerosis.2012.01.047

106. Mackness M, Boullier A, Hennuyer N, Mackness B, Hall M, Tailleux A, et al. Paraoxonase activity is reduced by a pro-atherosclerotic diet in rabbits. *Biochem Biophys Res Commun.* (2000) 269:232–6. doi: 10.1006/bbrc.2000.2265

107. Lund-Katz S, Phillips M. High density lipoprotein structure-function and role in reverse cholesterol transport. *Subcell Biochem.* (2010) 51:183–227. doi: 10.1007/978-90-481-8622-8_7

108. Harel M, Aharoni A, Gaidukov L, Brumshtein B, Khersonsky O, Meged R, et al. Structure and evolution of the serum paraoxonase family of detoxifying and anti-atherosclerotic enzymes. *Nat Struct Mol Biol.* (2004) 11:412–9. doi: 10.1038/nsmb767

109. Harel M, Berchansky A, Sussman JL, Prilusky J, Hodis E, Brumshtein B, et al. *Serum Paraoxonase*. (2020). Available online at: https://proteopedia.org/wiki/index.php/Serum_Paraoxonase (accessed September 14, 2022).

110. Karabulut S, Mansour B, Casanola-Martin G, Rasulev B, Gauld J. The hydrolysis rate of paraoxonase-1 Q and R isoenzymes: an in silico study based on *in vitro* data. *Molecules.* (2022) 27:6780. doi: 10.3390/molecules27206780

111. Esterbauer H, Gebicki J, Puhl H, Jürgens G. The role of lipid peroxidation and antioxidants in oxidative modification of LDL. *Free Radic Biol Med.* (1992) 13:341–90. doi: 10.1016/0891-5849(92)90181-F

112. Myung S, Ju W, Cho B, Oh S, Park S, Koo B, et al. Efficacy of vitamin and antioxidant supplements in prevention of cardiovascular disease: systematic review and meta-analysis of randomised controlled trials. *BMJ.* (2013) 346:f10. doi: 10.1136/bmj.f10

113. Spiteller P, Kern W, Reiner J, Spiteller G. Aldehydic lipid peroxidation products derived from linoleic acid. *Biochim Biophys Acta.* (2001) 1531:188–208. doi: 10.1016/S1388-1981(01)00100-7

114. Frankel E. *Lipid oxidation*. Sawston: Woodhead Publishing (2003).

115. Yoshida Y, Umeno A, Akazawa Y, Shichiri M, Murotomi K, Horie M. Chemistry of lipid peroxidation products and their use as biomarkers in early detection of diseases. *J Oleo Sci.* (2015) 64:347–56. doi: 10.5650/jos.ess14281

116. Ahmed Z, Ravandi A, Maguire G, Emili A, Draganov D, La Du B, et al. Multiple substrates for paraoxonase-1 during oxidation of phosphatidylcholine by peroxynitrite. *Biochem Biophys Res Commun.* (2002) 290:391–6. doi: 10.1006/bbrc.2001.6150

117. Poznyak A, Nikiforov N, Markin A, Kashirskikh D, Myasoedova V, Gerasimova E, et al. Overview of OxLDL and its impact on cardiovascular health: focus on atherosclerosis. *Front Pharmacol.* (2020) 11:613780. doi: 10.3389/fphar.2020.613780

118. Steinberg D, Witztum J. Oxidized low-density lipoprotein and atherosclerosis. *Arterioscler Thromb Vasc Biol.* (2010) 30:2311–6. doi: 10.1161/ATVBAHA.108.179697

119. Glass C, Witztum J. Atherosclerosis. The road ahead. *Cell.* (2001) 104:503–16. doi: 10.1016/S0092-8674(01)00238-0

120. Gilad D, Atiya S, Mozes-Autmazgin Z, Ben-Shushan R, Ben-David R, Amram E, et al. Paraoxonase 1 in endothelial cells impairs vasodilation induced by arachidonic

acid lactone metabolite. *Biochim Biophys Acta Mol Cell Biol Lipids.* (2019) 1864:386–93. doi: 10.1016/j.bbalip.2018.12.008

121. Berrougui H, Loued S, Khalil A. Purified human paraoxonase-1 interacts with plasma membrane lipid rafts and mediates cholesterol efflux from macrophages. *Free Radic Biol Med.* (2012) 52:1372–81. doi: 10.1016/j.freeradbiomed.2012.01.019

122. Christison J, Rye K, Stocker R. Exchange of oxidized cholesteryl linoleate between LDL and HDL mediated by cholesteryl ester transfer protein. *J Lipid Res.* (1995) 36:2017–26. doi: 10.1016/S0022-2275(20)41119-8

123. Hine D, Mackness B, Mackness M. Cholesteryl-ester transfer protein enhances the ability of high-density lipoprotein to inhibit low-density lipoprotein oxidation. *IUBMB Life.* (2011) 63:772–4. doi: 10.1002/iub.508

124. Kim D, Burt A, Ranchalis J, Vuletic S, Vaisar T, Li W, et al. PLTP activity inversely correlates with CAAD: effects of PON1 enzyme activity and genetic variants on PLTP activity. *J Lipid Res.* (2015) 56:1351–62. doi: 10.1194/jlr.P058032

125. Myant N. *The biology of cholesterol and related steroids*. Oxford: Butterworth-Heinemann (1981). doi: 10.1016/B978-0-433-22880-6.50009-6

126. Rosenblat M, Gaidukov L, Khersonsky O, Vaya J, Oren R, Tawfik D, et al. The catalytic histidine dyad of high density lipoprotein-associated serum paraoxonase-1 (PON1) is essential for PON1-mediated inhibition of low density lipoprotein oxidation and stimulation of macrophage cholesterol efflux. *J Biol Chem.* (2006) 281:7657–65. doi: 10.1074/jbc.M512595200

127. Brites F, Verona J, Schreier L, Fruchart J, Castro G, Wikinski R. Paraoxonase 1 and platelet-activating factor acetylhydrolase activities in patients with low hdl-cholesterol levels with or without primary hypertriglyceridemia. *Arch Med Res.* (2004) 35:235–40. doi: 10.1016/j.arcmed.2004.02.002

128. Inagaki M, Nakagawa-Toyama Y, Nishida M, Nakatani K, Nakaoka H, Kawase M, et al. Effect of probucol on antioxidant properties of HDL in patients with heterozygous familial hypercholesterolemia. *J Atheroscler Thromb.* (2012) 19:643–56. doi: 10.5551/jat.12807

129. Itabe H, Obama T, Kato R. The dynamics of oxidized LDL during atherogenesis. *J Lipids.* (2011) 2011:418313. doi: 10.1155/2011/418313

130. Roest M, Jansen A, Barendrecht A, Leus F, Kastelein J, Voorbij H. Variation at the paraoxonase gene locus contributes to carotid arterial wall thickness in subjects with familial hypercholesterolemia. *Clin Biochem.* (2005) 38:123–7. doi: 10.1016/j.clinbiochem.2004.10.005

131. Roest M, Rodenburg J, Wiegman A, Kastelein J, Voorbij H. Paraoxonase genotype and carotid intima-media thickness in children with familial hypercholesterolemia. *Eur J Cardiovasc Prev Rehabil.* (2006) 13:464–6. doi: 10.1097/00149831-200606000-00025

132. Rosenblat M, Ward S, Volkova N, Hayek T, Aviram M. VLDL triglycerides inhibit HDL-associated paraoxonase 1 (PON1) activity: *in vitro* and *in vivo* studies. *Biofactors.* (2012) 38:292–9. doi: 10.1002/biof.1021

133. Rozek L, Hatsukami T, Richter R, Ranchalis J, Nakayama K, McKinstry L, et al. The correlation of paraoxonase (PON1) activity with lipid and lipoprotein levels differs with vascular disease status. *J Lipid Res.* (2005) 46:1888–95. doi: 10.1194/jlr.M400489-JLR200

134. van Himbergen T, van Tits L, Hectors M, de Graaf J, Roest M, Stalenhoef A. Paraoxonase-1 and linoleic acid oxidation in familial hypercholesterolemia. *Biochem Biophys Res Commun.* (2005) 333:787–93. doi: 10.1016/j.bbrc.2005.05.176

135. Abbott C, Mackness M, Kumar S, Olukoga A, Gordon C, Arrol S, et al. Relationship between serum butyrylcholinesterase activity, hypertriglyceridaemia and insulin sensitivity in diabetes mellitus. *Clin Sci.* (1993) 85:77–81. doi: 10.1042/cs0850077

136. Abbott C, Mackness M, Kumar S, Boulton A, Durrington P. Serum paraoxonase activity, concentration, and phenotype distribution in diabetes mellitus and its relationship to serum lipids and lipoproteins. *Arterioscler Thromb Vasc Biol.* (1995) 15:1812–8. doi: 10.1161/01.ATV.15.11.1812

137. Mackness B, Mackness M, Arrol S, Turkie W, Julier K, Abuasha B, et al. Serum paraoxonase (PON1) 55 and 192 polymorphism and paraoxonase activity and concentration in non-insulin dependent diabetes mellitus. *Atherosclerosis.* (1998) 139:341–9. doi: 10.1016/S0021-9150(98)00095-1

138. Mackness B, Durrington P, Abuashia B, Boulton A, Mackness M. Low paraoxonase activity in type II diabetes mellitus complicated by retinopathy. *Clin Sci.* (2000) 98:355–63. doi: 10.1042/cs0980355

139. Mackness B, Durrington P, Boulton A, Hine D, Mackness M. Serum paraoxonase activity in patients with type 1 diabetes compared to healthy controls. *Eur J Clin Invest.* (2002) 32:259–64. doi: 10.1046/j.1365-2362.2002.00977.x

140. Wu D, Wu C, Zhong Y. The association between paraoxonase 1 activity and the susceptibilities of diabetes mellitus, diabetic macroangiopathy and diabetic microangiopathy. *J Cell Mol Med.* (2018) 22:4283–91. doi: 10.1111/jcmm.13711

141. Srikanthan K, Feyh A, Visweshwar H, Shapiro J, Sodhi K. Systematic review of metabolic syndrome biomarkers: a panel for early detection, management, and risk stratification in the west virginian population. *Int J Med Sci.* (2016) 13:25–38. doi: 10.7150/ijms.13800

142. Hashemi M, Mousavi E, Arab-Bafrani Z, Nezhadebrahimi A, Marjani A. The most effective polymorphisms of paraoxonase-1 gene on enzyme activity and concentration of paraoxonase-1 protein in type 2 diabetes mellitus patients and non-diabetic individuals: a systematic review and meta-analysis. *Diabetes Res Clin Pract.* (2019) 152:135–45. doi: 10.1016/j.diabres.2019.05.007

143. Koren-Gluzer M, Aviram M, Meilin E, Hayek T. The antioxidant HDL-associated paraoxonase-1 (PON1) attenuates diabetes development and stimulates β-cell insulin release. *Atherosclerosis.* (2011) 219:510–8. doi: 10.1016/j.atherosclerosis.2011.07.119

144. Shokri Y, Variji A, Nosrati M, Khonakdar-Tarsi A, Kianmehr A, Kashi Z, et al. Importance of paraoxonase 1 (PON1) as an antioxidant and antiatherogenic enzyme in the cardiovascular complications of type 2 diabetes: genotypic and phenotypic evaluation. *Diabetes Res Clin Pract.* (2020) 161:108067. doi: 10.1016/j.diabres.2020.108067

145. Vaisar T, Kanter J, Wimberger J, Irwin A, Gauthier J, Wolfson E, et al. High concentration of medium-sized HDL particles and enrichment in HDL paraoxonase 1 associate with protection from vascular complications in people with long-standing type 1 diabetes. *Diabetes Care.* (2020) 43:178–86. doi: 10.2337/dc19-0772

146. Barter P, Nicholls S, Rye K, Anantharamaiah G, Navab M, Fogelman A. Antiinflammatory properties of HDL. *Circ Res.* (2004) 95:764–72. doi: 10.1161/01.RES.0000146094.59640.13

147. Ribas V, Sánchez-Quesada J, Antón R, Camacho M, Julve J, Escolà-Gil J, et al. Human apolipoprotein A-II enrichment displaces paraoxonase from HDL and impairs its antioxidant properties: a new mechanism linking HDL protein composition and antiatherogenic potential. *Circ Res.* (2004) 95:789–97. doi: 10.1161/01.RES.0000146031.94850.5f

148. Kotani K, Yamada T, Gugliucci A. Paired measurements of paraoxonase 1 and serum amyloid A as useful disease markers. *Biomed Res Int.* (2013) 2013:481437. doi: 10.1155/2013/481437

149. Yang N, Qin Q. Apolipoprotein J: a new predictor and therapeutic target in cardiovascular disease? *Chin Med J.* (2015) 128:2530–4. doi: 10.4103/0366-6999.164983

150. Han C, Chiba T, Campbell J, Fausto N, Chaisson M, Orasanu G, et al. Reciprocal and coordinate regulation of serum amyloid A versus apolipoprotein A-I and paraoxonase-1 by inflammation in murine hepatocytes. *Arterioscler Thromb Vasc Biol.* (2006) 26:1806–13. doi: 10.1161/01.ATV.0000227472.70734.ad

151. Castellani L, Chang J, Wang X, Lusis A, Reynolds W. Transgenic mice express human MPO –463G/A alleles at atherosclerotic lesions, developing hyperlipidemia and obesity in –463G males. *J Lipid Res.* (2006) 47:1366–77. doi: 10.1194/jlr.M600005-JLR200

152. Liu C, Desikan R, Ying Z, Gushchina L, Kampfrath T, Deiuliis J, et al. Effects of a novel pharmacologic inhibitor of myeloperoxidase in a mouse atherosclerosis model. *PLoS One.* (2012) 7:e50767. doi: 10.1371/journal.pone.0050767

153. Huang Y, Wu Z, Riwanto M, Gao S, Levison B, Gu X, et al. Myeloperoxidase, paraoxonase-1, and HDL form a functional ternary complex. *J Clin Invest.* (2013) 123:3815–28. doi: 10.1172/JCI67478

154. Khine H, Teiber J, Haley R, Khera A, Ayers C, Rohatgi A. Association of the serum myeloperoxidase/high-density lipoprotein particle ratio and incident cardiovascular events in a multi-ethnic population: observations from the dallas heart study. *Atherosclerosis.* (2017) 263:156–62. doi: 10.1016/j.atherosclerosis.2017.06.007

155. Bacchetti T, Ferretti G, Carbone F, Ministrini S, Montecucco F, Jamialahmadi T, et al. Dysfunctional high-density lipoprotein: the role of myeloperoxidase and paraoxonase-1. *Curr Med Chem.* (2021) 28:2842–50. doi: 10.2174/0929867327999200716112353

156. Scavone D, Sgorbini M, Borges A, Oliveira-Filho J, Vitale V, Paltrinieri S. Serial measurements of Paraoxonase-1 (PON-1) activity in horses with experimentally induced endotoxemia. *BMC Vet Res.* (2020) 16:422. doi: 10.1186/s12917-020-02629-4

157. Gaál K, Tarr T, Lőrincz H, Borbás V, Seres I, Harangi M, et al. High-density lipoprotein antioxidant capacity, subpopulation distribution and paraoxonase-1 activity in patients with systemic lupus erythematosus. *Lipids Health Dis.* (2016) 15:60. doi: 10.1186/s12944-016-0229-0

158. Meneses M, Silvestre R, Sousa-Lima I, Macedo M. Paraoxonase-1 as a regulator of glucose and lipid homeostasis: impact on the onset and progression of metabolic disorders. *Int J Mol Sci.* (2019) 20:4049. doi: 10.3390/ijms20164049

159. Manandhar B, Cochran B, Rye K. Role of high-density lipoproteins in cholesterol homeostasis and glycemic control. *J Am Heart Assoc.* (2020) 9:e013531. doi: 10.1161/JAHA.119.013531

160. Mastorikou M, Mackness B, Liu Y, Mackness M. Glycation of paraoxonase-1 inhibits its activity and impairs the ability of high-density lipoprotein to metabolize membrane lipid hydroperoxides. *Diabet Med.* (2008) 25:1049–55. doi: 10.1111/j.1464-5491.2008.02546.x

161. Yu W, Liu X, Feng L, Yang H, Yu W, Feng T, et al. Glycation of paraoxonase 1 by high glucose instigates endoplasmic reticulum stress to induce endothelial dysfunction *in vivo*. *Sci Rep.* (2017) 7:45827. doi: 10.1038/srep45827

162. Tames F, Mackness M, Arrol S, Laing I, Durrington P. Non-enzymatic glycation of apolipoprotein B in the sera of diabetic and non-diabetic subjects. *Atherosclerosis.* (1992) 93:237–44. doi: 10.1016/0021-9150(92)90260-N

163. Holvoet P, Vanhaecke J, Janssens S, Van de Werf F, Collen D. Oxidized LDL and malondialdehyde-modified LDL in patients with acute coronary syndromes and stable coronary artery disease. *Circulation.* (1998) 98:1487–94. doi: 10.1161/01.CIR.98.15.1487

164. Younis N, Soran H, Sharma R, Charlton-Menys V, Greenstein A, Elseweidy M, et al. Small-dense LDL and LDL glycation in metabolic syndrome and in statin-treated and non-statin-treated type 2 diabetes. *Diab Vasc Dis Res.* (2010) 7:289–95. doi: 10.1177/1479164110383063

165. Younis N, Charlton-Menys V, Sharma R, Soran H, Durrington P. Glycation of LDL in non-diabetic people: small dense LDL is preferentially glycated both *in vivo* and *in vitro*. *Atherosclerosis.* (2009) 202:162–8. doi: 10.1016/j.atherosclerosis.2008.04.036

166. Younis N, Soran H, Pemberton P, Charlton-Menys V, Elseweidy M, Durrington P. Small dense LDL is more susceptible to glycation than more buoyant LDL in Type 2 diabetes. *Clin Sci.* (2013) 124:343–9. doi: 10.1042/CS20120304

167. Pietzsch J, Julius U. Different susceptibility to oxidation of proline and arginine residues of apolipoprotein B-100 among subspecies of low density lipoproteins. *FEBS Lett.* (2001) 491:123–6. doi: 10.1016/S0014-5793(01)02181-0

168. Younis N, Soran H, Sharma R, Charlton–Menys V, Durrington P. Lipoprotein glycation in atherogenesis. *Clin Lipidol.* (2009) 4:781–90. doi: 10.2217/clp.09.61

169. Sobal G, Menzel J, Sinzinger H. Why is glycated LDL more sensitive to oxidation than native LDL? a comparative study. *Prostaglandins Leukot Essent Fatty Acids.* (2000) 63:177–86. doi: 10.1054/plef.2000.0204

170. Rohatgi N, Nielsen T, Bjørn S, Axelsson I, Paglia G, Voldborg B, et al. Biochemical characterization of human gluconokinase and the proposed metabolic impact of gluconic acid as determined by constraint based metabolic network analysis. *PLoS One.* (2014) 9:e98760. doi: 10.1371/journal.pone.0098760

171. Schalkwijk C, Stehouwer C. Methylglyoxal, a highly reactive dicarbonyl compound, in diabetes, its vascular complications, and other age-related diseases. *Physiol Rev.* (2020) 100:407–61. doi: 10.1152/physrev.00001.2019

172. Lindsay R, Smith W, Lee W, Dominiczak M, Baird J. The effect of delta-gluconolactone, an oxidised analogue of glucose, on the nonenzymatic glycation of human and rat haemoglobin. *Clin Chim Acta.* (1997) 263:239–47. doi: 10.1016/S0009-8981(97)00067-3

173. Younis N, Soran H, Charlton-Menys V, Sharma R, Hama S, Pemberton P, et al. High-density lipoprotein impedes glycation of low-density lipoprotein. *Diab Vasc Dis Res.* (2013) 10:152–60. doi: 10.1177/1479164112454309

174. Kjellmo C, Karlsson H, Nestvold T, Ljunggren S, Cederbrant K, Marcusson-Ståhl M, et al. Bariatric surgery improves lipoprotein profile in morbidly obese patients by reducing LDL cholesterol, apoB, and SAA/PON1 ratio, increasing HDL cholesterol, but has no effect on cholesterol efflux capacity. *J Clin Lipidol.* (2018) 12:193–202. doi: 10.1016/j.jacl.2017.10.007

175. Lou-Bonafonte J, Gabás-Rivera C, Navarro M, Osada J. PON1 and mediterranean diet. *Nutrients.* (2015) 7:4068–92. doi: 10.3390/nu7064068

176. Lou-Bonafonte J, Gabás-Rivera C, Navarro M, Osada J. The search for dietary supplements to elevate or activate circulating paraoxonases. *Int J Mol Sci.* (2017) 18:416. doi: 10.3390/ijms18020416

177. Ferretti G, Bacchetti T, Sahebkar A. Effect of statin therapy on paraoxonase-1 status: a systematic review and meta-analysis of 25 clinical trials. *Prog Lipid Res.* (2015) 60:50–73. doi: 10.1016/j.plipres.2015.08.003

178. Sahebkar A, Hernández-Aguilera A, Abelló D, Sancho E, Camps J, Joven J. Systematic review and meta-analysis deciphering the impact of fibrates on paraoxonase-1 status. *Metabolism.* (2016) 65:609–22. doi: 10.1016/j.metabol.2016.01.002

179. Turfaner N, Uzun H, Balci H, Ercan M, Karter Y, Caner M, et al. Ezetimibe therapy and its influence on oxidative stress and fibrinolytic activity. *South Med J.* (2010) 103:428–33. doi: 10.1097/SMJ.0b013e3181d83374

180. Yadav R, Liu Y, Kwok S, Hama S, France M, Eatough R, et al. Effect of extended-release niacin on high-density lipoprotein (HDL) functionality, lipoprotein metabolism, and mediators of vascular inflammation in statin-treated patients. *J Am Heart Assoc.* (2015) 4:e001508. doi: 10.1161/JAHA.114.001508

181. Esteghamati A, Eskandari D, Mirmiranpour H, Noshad S, Mousavizadeh M, Hedayati M, et al. Effects of metformin on markers of oxidative stress and antioxidant reserve in patients with newly diagnosed type 2 diabetes: a randomized clinical trial. *Clin Nutr.* (2013) 32:179–85. doi: 10.1016/j.clnu.2012.08.006

182. Demir Y, Köksal Z. The inhibition effects of some sulfonamides on human serum paraoxonase-1 (hPON1). *Pharmacol Rep.* (2019) 71:545–9. doi: 10.1016/j.pharep.2019.02.012

183. Bowman L, Hopewell J, Chen F, Wallendszus K, Stevens W, Collins R, et al. Effects of anacetrapib in patients with atherosclerotic vascular disease. *N Engl J Med.* (2017) 377:1217–27.

184. Chen Y, Dong J, Zhang X, Chen X, Wang L, Chen H, et al. Evacetrapib reduces preβ-1 HDL in patients with atherosclerotic cardiovascular disease or diabetes. *Atherosclerosis.* (2019) 285:147–52. doi: 10.1016/j.atherosclerosis.2019.04.211

185. Dunbar R, Movva R, Bloedon L, Duffy D, Norris R, Navab M, et al. Oral apolipoprotein A-I mimetic D-4F lowers HDL-inflammatory index in high-risk patients: a first-in-human multiple-dose, Randomized Controlled Trial. *Clin Transl Sci.* (2017) 10:455–69. doi: 10.1111/cts.12487

186. Vakili L, Hama S, Kim J, Tien D, Safarpoor S, Ly N, et al. The effect of HDL mimetic peptide 4F on PON1. *Adv Exp Med Biol.* (2010) 660:167–72. doi: 10.1007/978-1-60761-350-3_15

187. Brandes R, Dueck A, Engelhardt S, Kaulich M, Kupatt C, De Angelis M, et al. DGK and DZHK position paper on genome editing: basic science applications and future perspective. *Basic Res Cardiol.* (2021) 116:2. doi: 10.1007/s00395-020-00839-3

188. Siller A, Whyte M. Alkaline phosphatase: discovery and naming of our favorite enzyme. *J Bone Miner Res.* (2018) 33:362–4. doi: 10.1002/jbmr.3225

189. Charlton-Menys V, Liu Y, Durrington P. Semiautomated method for determination of serum paraoxonase activity using paraoxon as substrate. *Clin Chem.* (2006) 52:453–7. doi: 10.1373/clinchem.2005.063412

190. Marsillach J, Richter R, Costa L, Furlong C. Paraoxonase-1 (PON1) status analysis using non-organophosphate substrates. *Curr Protoc.* (2021) 1:e25. doi: 10.1002/cpz1.25

191. Mohammed C, Lamichhane S, Connolly J, Soehnlen S, Khalaf F, Malhotra D, et al. A PON for all seasons: comparing paraoxonase enzyme substrates, activity and action including the role of PON3 in health and disease. *Antioxidants.* (2022) 11:590. doi: 10.3390/antiox11030590

192. Camps J, Marsillach J, Joven J. The paraoxonases: role in human diseases and methodological difficulties in measurement. *Crit Rev Clin Lab Sci.* (2009) 46:83–106. doi: 10.1080/10408360802610878

193. Loued S, Isabelle M, Berrougui H, Khalil A. The anti-inflammatory effect of paraoxonase 1 against oxidized lipids depends on its association with high density lipoproteins. *Life Sci.* (2012) 90:88–92. doi: 10.1016/j.lfs.2011.10.018

194. Anastasius M, Kockx M, Jessup W, Sullivan D, Rye K, Kritharides L. Cholesterol efflux capacity: an introduction for clinicians. *Am Heart J.* (2016) 180:54–63. doi: 10.1016/j.ahj.2016.07.005

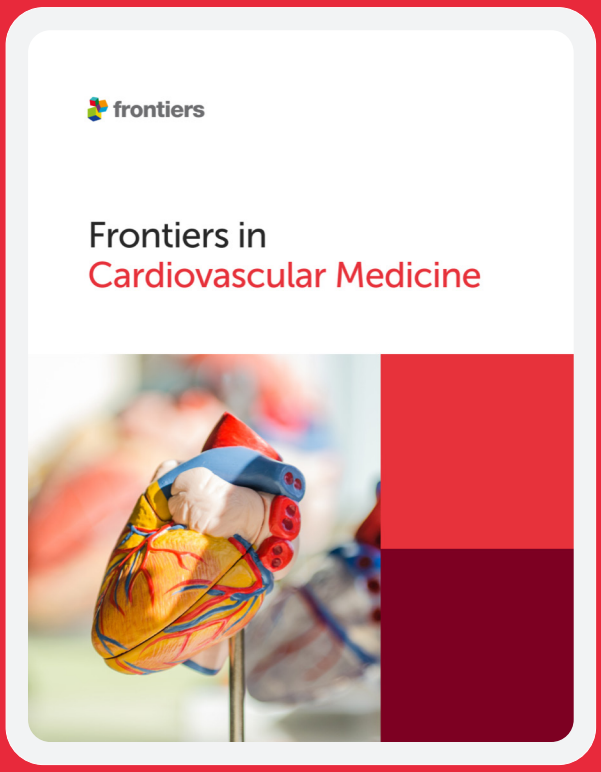
Frontiers in Cardiovascular Medicine

Innovations and improvements in cardiovascular treatment and practice

Focuses on research that challenges the status quo of cardiovascular care, or facilitates the translation of advances into new therapies and diagnostic tools.

Discover the latest Research Topics

See more →



Frontiers

Avenue du Tribunal-Fédéral 34
1005 Lausanne, Switzerland
frontiersin.org

Contact us

+41 (0)21 510 17 00
frontiersin.org/about/contact