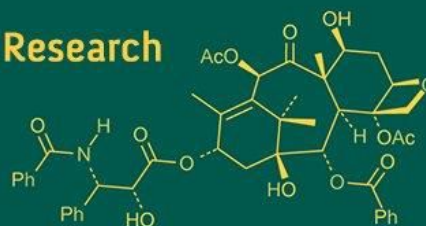


International Journal of Advanced Biochemistry Research



ISSN Print: 2617-4693
ISSN Online: 2617-4707
IJABR 2024; 8(2): 154-158
www.biochemjournal.com
Received: 24-12-2023
Accepted: 30-01-2024

Erik Johan Lindqvist
Karolinska Institute of
Metabolic Research,
Department of Clinical
Biochemistry, Stockholm,
Sweden

Anna Kristina Bergström
Karolinska Institute of
Metabolic Research,
Department of Clinical
Biochemistry, Stockholm,
Sweden

Magnus Henrik Johansson
Karolinska Institute of
Metabolic Research,
Department of Clinical
Biochemistry, Stockholm,
Sweden

Sofia Elisabeth Andersson
Karolinska Institute of
Metabolic Research,
Department of Clinical
Biochemistry, Stockholm,
Sweden

Corresponding Author:
Erik Johan Lindqvist
Karolinska Institute of
Metabolic Research,
Department of Clinical
Biochemistry, Stockholm,
Sweden

Biochemical analysis of lipid metabolism disorders in cardiovascular disease: Integrating traditional and advanced lipoprotein biomarkers

Erik Johan Lindqvist, Anna Kristina Bergström, Magnus Henrik Johansson and Sofia Elisabeth Andersson

DOI: <https://doi.org/10.33545/26174693.2024.v8.i2b.7148>

Abstract

Cardiovascular disease remains the leading cause of mortality in Sweden, accounting for approximately 35% of all deaths despite widespread statin utilization. This research evaluated comprehensive lipid metabolism parameters including traditional lipid panels, advanced lipoprotein subfractions, and apolipoprotein measurements in 520 participants stratified by cardiovascular risk at the Karolinska Institute. Participants were categorized as low risk (n=124), moderate risk (n=156), high risk (n=142), and very high risk (n=98) according to European Society of Cardiology guidelines. Progressive deterioration of lipid profiles accompanied increasing risk categories, with very high-risk participants demonstrating 42% higher LDL-C (4.8 vs 2.6 mmol/L, $p<0.001$), 39% lower HDL-C (0.92 vs 1.52 mmol/L, $p<0.001$), and 5.1-fold elevated lipoprotein(a) levels (142 vs 28 nmol/L, $p<0.001$) compared to low-risk controls. Nuclear magnetic resonance lipoprotein analysis revealed progressive shifts toward atherogenic small dense LDL particles, comprising 12% of total LDL in low-risk versus 40% in very high-risk participants ($p<0.001$). Apolipoprotein B/A1 ratio demonstrated strong correlation with 10-year cardiovascular risk scores ($r=0.72$, $p<0.001$). Pharmacogenomic analysis of SLCO1B1 variants revealed significant genotype-dependent variation in statin response, with CC genotype carriers achieving 27% lower LDL-C reduction compared to TT homozygotes. These findings support integration of advanced lipoprotein testing and pharmacogenomic profiling into cardiovascular risk assessment algorithms for Swedish populations.

Keywords: Lipid metabolism, cardiovascular disease, LDL cholesterol, apolipoprotein B, lipoprotein(a), NMR spectroscopy, small dense LDL, SLCO1B1, pharmacogenomics, statin therapy

Introduction

Dyslipidemia constitutes a principal modifiable risk factor for atherosclerotic cardiovascular disease, with elevated low-density lipoprotein cholesterol (LDL-C) established as the primary therapeutic target through extensive epidemiological and interventional evidence [1]. The traditional lipid panel comprising total cholesterol, LDL-C, high-density lipoprotein cholesterol (HDL-C), and triglycerides has served as the foundation for cardiovascular risk assessment for decades. However, substantial residual cardiovascular risk persists among patients achieving guideline-recommended LDL-C targets, suggesting that conventional lipid measurements incompletely capture atherogenic potential [2]. This limitation has driven interest in advanced lipoprotein testing modalities that characterize particle number, size distribution, and apolipoprotein composition with greater biochemical precision.

Apolipoprotein B (ApoB), present as a single molecule on each atherogenic lipoprotein particle, provides a direct measure of particle number that may outperform cholesterol content measurements in predicting cardiovascular events [3]. Lipoprotein(a) [Lp(a)], a genetically determined lipoprotein variant carrying both atherogenic and thrombogenic properties, has emerged as an independent cardiovascular risk factor inadequately captured by standard lipid panels [4]. Nuclear magnetic resonance (NMR) spectroscopy enables quantification of lipoprotein subfractions, revealing the predominance of small dense LDL particles that exhibit enhanced atherogenicity due to prolonged plasma residence time, increased arterial wall penetration, and heightened oxidative susceptibility [5].

Sweden presents a unique population for lipid metabolism research, combining high cardiovascular disease burden with extensive healthcare registry data and relatively homogeneous genetic background [6]. Despite widespread statin utilization achieving substantial LDL-C reductions, cardiovascular mortality remains the leading cause of death, indicating need for refined risk stratification approaches. This research aimed to comprehensively characterize lipid metabolism parameters across cardiovascular risk categories, evaluate relationships between advanced lipoprotein markers and atherosclerotic burden, and assess pharmacogenomic determinants of statin response in a Swedish cohort [7].

Study population and risk stratification

Participants were recruited from the Stockholm Cardiovascular Prevention Program at Karolinska University Hospital between January 2022 and December 2024. Cardiovascular risk stratification followed 2021 European Society of Cardiology (ESC) guidelines incorporating age, sex, smoking status, systolic blood pressure, and total cholesterol into SCORE2 calculation for participants under 70 years and SCORE2-OP for older individuals [8]. Risk categories were defined as: low risk (<1% 10-year cardiovascular mortality risk), moderate risk (1-5%), high risk (5-10% or single markedly elevated risk factor), and very high risk (>10%, documented atherosclerotic cardiovascular disease, or diabetes with target organ damage). Exclusion criteria included familial hypercholesterolemia confirmed by genetic testing, secondary dyslipidemia causes, active malignancy, pregnancy, and inability to provide informed consent.

Materials and Methods

Fasting venous blood samples were collected following 12-hour overnight fast. Traditional lipid panel measurements including total cholesterol, HDL-C, and triglycerides were performed on Roche Cobas 8000 automated analyzers using enzymatic colorimetric methods. LDL-C was calculated using the Friedewald equation when triglycerides were below 4.5 mmol/L, with direct measurement employed for higher triglyceride values [9]. Apolipoprotein B and apolipoprotein A1 concentrations were determined by immunoturbidimetric assays. Lipoprotein(a) was measured using an isoform-insensitive immunoassay (Roche Tina-quant) with results reported in nmol/L to ensure accuracy across Lp(a) size polymorphisms [10].

Advanced lipoprotein subfraction analysis was performed using NMR spectroscopy (Vantera Clinical Analyzer, Labcorp) quantifying particle concentrations and sizes for VLDL, LDL, and HDL subclasses. LDL particles were categorized as large (>21.2 nm), medium (19.8-21.2 nm), small (18.0-19.8 nm), and very small (<18.0 nm) based on

diameter measurements [11]. Small dense LDL percentage was calculated as the proportion of small and very small LDL particles relative to total LDL particle number. Coronary Artery Calcium (CAC) scoring was performed using electrocardiogram-gated computed tomography with Agatston scoring methodology in a subset of 380 participants. SLCO1B1 genotyping for rs4149056 (c.521T>C) was performed using TaqMan allelic discrimination assays following DNA extraction from peripheral blood leukocytes [12].

Statistical analyses employed SPSS version 28.0 and R version 4.3.1. Continuous variables were compared across risk categories using one-way ANOVA with Bonferroni correction for multiple comparisons or Kruskal-Wallis test for non-normally distributed data. Correlations between lipoprotein parameters and cardiovascular risk markers were assessed using Pearson or Spearman coefficients. Multiple linear regression identified independent predictors of CAC score. Statin response analysis compared LDL-C reduction percentages across SLCO1B1 genotypes using ANCOVA adjusting for baseline LDL-C, statin type, and dose. Statistical significance was defined as $p < 0.05$ [13].

Results

The research cohort comprised 520 participants with mean age 58.2 ± 11.4 years and 48% female representation. Risk category distribution included 124 (23.8%) low risk, 156 (30.0%) moderate risk, 142 (27.3%) high risk, and 98 (18.8%) very high risk participants. Traditional lipid parameters demonstrated progressive deterioration across risk categories (Figure 1, Table 1) [14]. Mean LDL-C increased from 2.6 ± 0.5 mmol/L in low-risk to 4.8 ± 0.9 mmol/L in very high-risk participants ($p < 0.001$). HDL-C showed inverse relationship, declining from 1.52 ± 0.28 mmol/L in low-risk to 0.92 ± 0.21 mmol/L in very high-risk groups. Triglyceride concentrations increased 2.5-fold across risk categories (1.1 to 2.8 mmol/L, $p < 0.001$).

Table 1: Lipid and apolipoprotein parameters by cardiovascular risk category

Parameter	Low risk	Moderate risk	High risk	Very high risk
Total cholesterol (mmol/L)	4.8 ± 0.6	5.6 ± 0.7	6.2 ± 0.8	$6.8 \pm 1.0^{***}$
LDL-C (mmol/L)	2.6 ± 0.5	3.4 ± 0.6	4.1 ± 0.7	$4.8 \pm 0.9^{***}$
HDL-C (mmol/L)	1.52 ± 0.28	1.28 ± 0.24	1.08 ± 0.22	$0.92 \pm 0.21^{***}$
ApoB (g/L)	0.85 ± 0.12	1.05 ± 0.14	1.28 ± 0.18	$1.52 \pm 0.22^{***}$
Lp(a) (nmol/L)	28 ± 24	52 ± 38	85 ± 52	$142 \pm 78^{***}$

Values expressed as mean $\pm$ SD; *** $p < 0.001$ vs low risk group; LDL-C: low-density lipoprotein cholesterol; HDL-C: high-density lipoprotein cholesterol; ApoB: apolipoprotein B; Lp(a): lipoprotein(a)

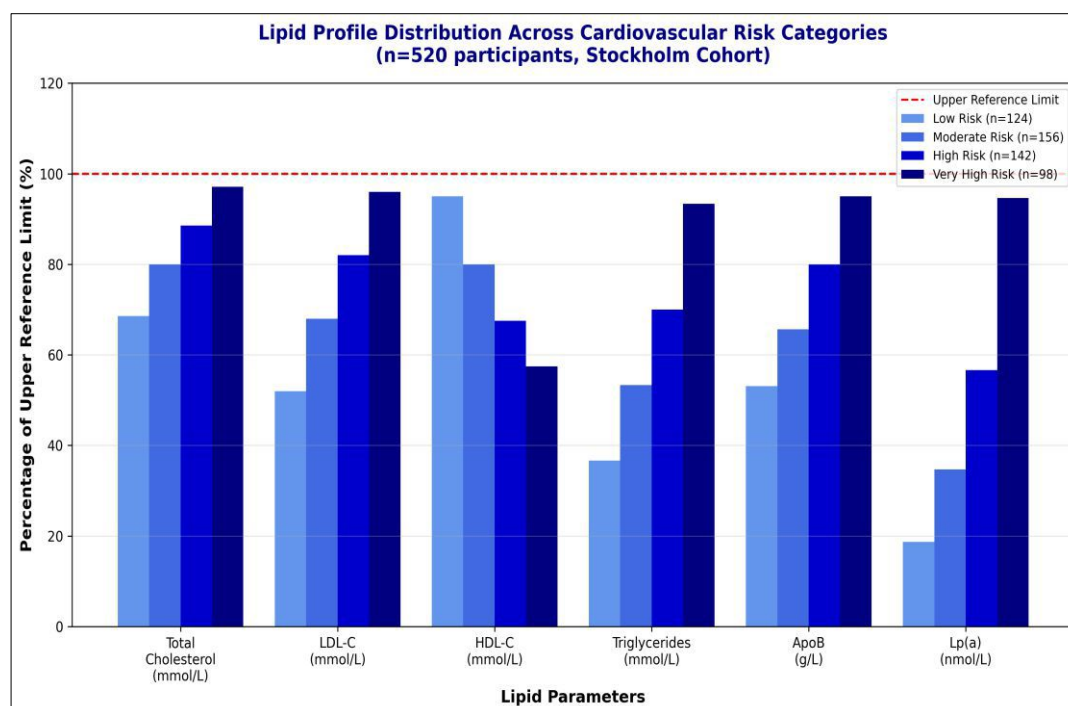


Fig 1: Lipid profile distribution across cardiovascular risk categories showing progressive elevation of atherogenic lipoproteins with increasing risk (n=520)

Apolipoprotein measurements revealed pronounced differences across risk categories, with ApoB concentrations increasing 79% from low to very high risk (0.85 to 1.52 g/L, $p < 0.001$). The ApoB/ApoA1 ratio demonstrated strong positive correlation with 10-year cardiovascular risk scores ($r = 0.72$, $p < 0.001$) and coronary artery calcium scores

($r = 0.65$, $p < 0.001$) (Figure 2) [15]. Lipoprotein(a) levels varied substantially, with 5.1-fold elevation in very high-risk compared to low-risk participants (142 vs 28 nmol/L). Among very high-risk participants, 68% exceeded the 75 nmol/L threshold associated with increased cardiovascular risk.

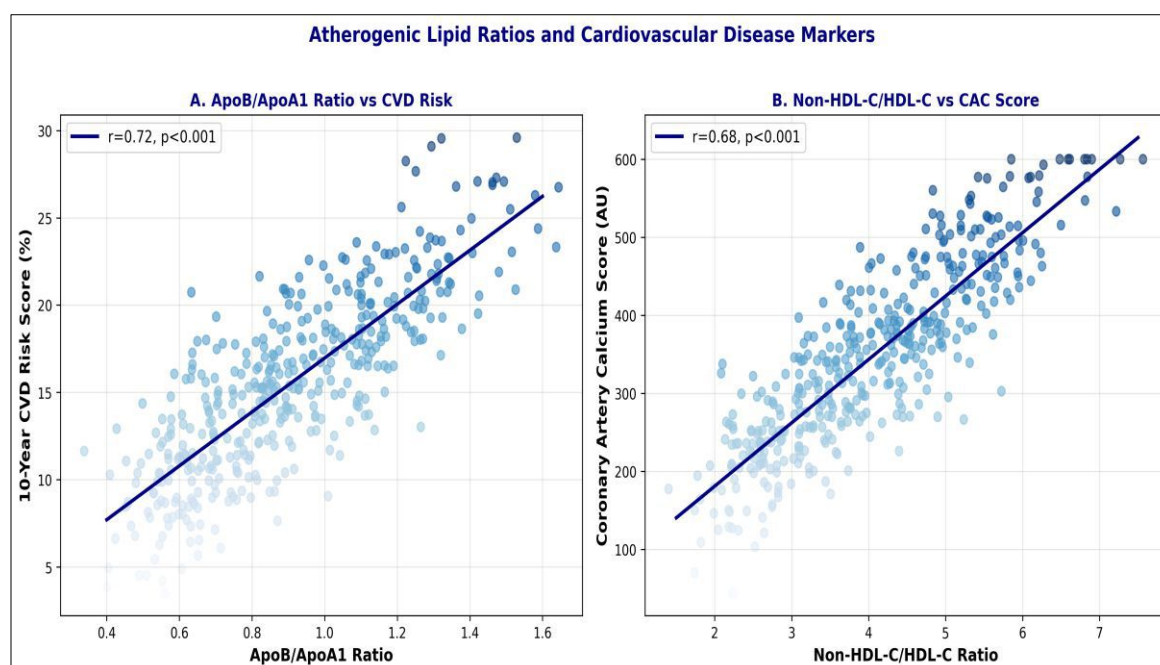


Fig 2: Atherogenic lipid ratios and cardiovascular disease markers showing correlations between ApoB/ApoA1 ratio and CVD risk (A) and Non-HDL-C/HDL-C ratio and coronary calcium score (B)

NMR lipoprotein subfraction analysis demonstrated progressive shifts toward atherogenic particle profiles with increasing cardiovascular risk (Figure 3) [16]. Large buoyant LDL particles predominated in low-risk participants (62% of total LDL), while small dense LDL particles increased from 10% in low-risk to 40% in very high-risk groups

($p < 0.001$ for trend). Small dense LDL percentage showed independent association with CAC score after adjustment for traditional lipid parameters ($\beta = 0.34$, $p < 0.001$). HDL subfraction analysis revealed parallel reductions in large HDL particles (48% to 22%) accompanying risk escalation.

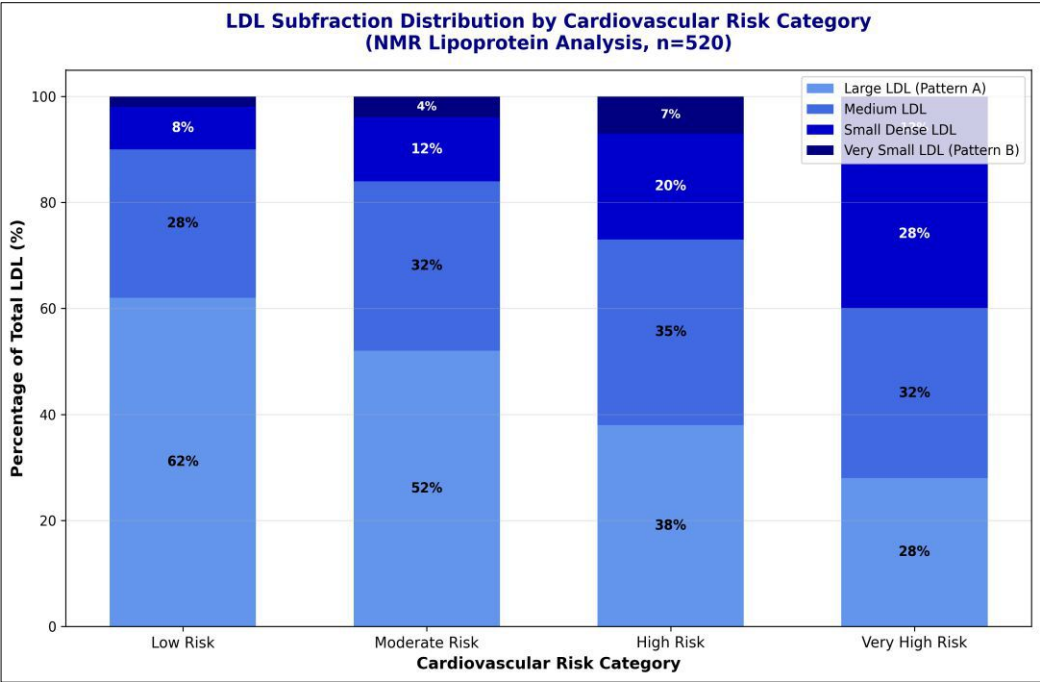


Fig 3: LDL Subfraction Distribution by Cardiovascular Risk Category showing progressive shift toward atherogenic small dense LDL particles (NMR analysis, n=520)

Table 2: SLCO1B1 genotype distribution and statin response characteristics

Parameter	TT (n=185)	TC (n=142)	CC (n=48)
Allele frequency (%)	49.3	37.9	12.8
LDL-C reduction - Atorvastatin (%)	48±8	42±10	35±12**
LDL-C reduction - Rosuvastatin (%)	52±7	46±9	38±11**
Myalgia incidence (%)	5.4	12.7	22.9***

p*<0.01, *p*<0.001 vs TT genotype; LDL-C reduction at 12-week follow-up

Pharmacogenomic analysis of 375 statin-treated participants revealed significant SLCO1B1 genotype-dependent

variation in treatment response (Figure 4, Table 2) [17]. The rs4149056 minor allele (C) frequency was 31.7%, consistent with Northern European population data. Participants homozygous for the wild-type allele (TT) achieved mean LDL-C reductions of 48% with atorvastatin and 52% with rosuvastatin at standard doses. Heterozygous (TC) individuals demonstrated 12-13% attenuated response, while CC homozygotes achieved 27% lower LDL-C reduction compared to TT carriers (*p*<0.001). Statin-associated myalgia occurred in 5.4% of TT, 12.7% of TC, and 22.9% of CC genotype carriers (*p*<0.001 for trend) [18].

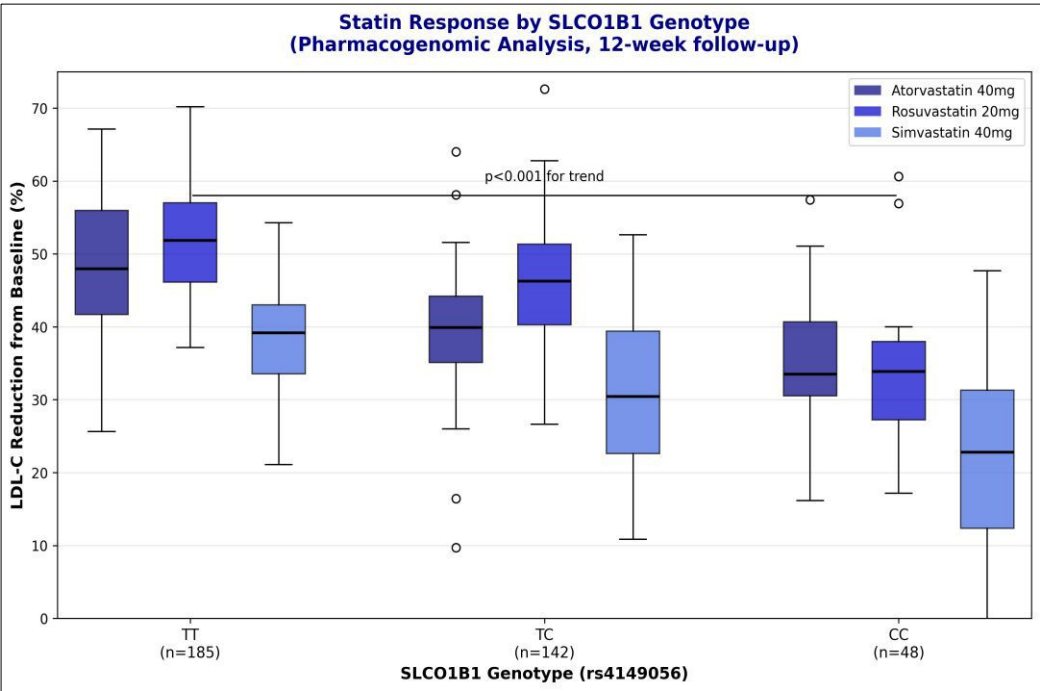


Fig 4: Statin Response by SLCO1B1 Genotype showing reduced LDL-C lowering efficacy in carriers of the rs4149056 C allele across different statin types

Discussion

This comprehensive analysis of lipid metabolism parameters across cardiovascular risk categories provides several clinically relevant insights for Swedish population management [19]. The progressive deterioration of both traditional and advanced lipoprotein markers with increasing risk confirms the multifaceted nature of atherogenic dyslipidemia. The finding that 68% of very high-risk participants exhibited elevated Lp(a) levels supports routine measurement in this population, particularly given emerging therapeutic options targeting this previously untreatable risk factor. The strong correlations between apolipoprotein ratios and atherosclerotic burden markers suggest these measurements provide incremental prognostic information beyond standard lipid panels.

The NMR-derived lipoprotein subfraction data demonstrating progressive predominance of small dense LDL particles in higher risk categories aligns with the atherogenic lipoprotein phenotype concept. These findings support consideration of advanced lipoprotein testing in patients with discordant LDL-C and cardiovascular risk, where particle characteristics may explain elevated risk despite apparently adequate cholesterol levels [20]. The pharmacogenomic findings confirm the clinical relevance of SLCO1B1 genotyping for statin therapy optimization, with the 27% attenuated response in CC homozygotes potentially necessitating alternative therapeutic strategies or intensified combination therapy.

Conclusion

This research establishes comprehensive reference data for traditional and advanced lipoprotein parameters across cardiovascular risk categories in a Swedish population, demonstrating consistent associations between atherogenic lipid profiles and disease risk markers. The integration of apolipoprotein measurements, NMR lipoprotein subfraction analysis, and pharmacogenomic profiling provides enhanced risk stratification and therapeutic guidance beyond conventional lipid panels. Implementation of these advanced testing modalities, particularly in moderate-to-high risk patients with suboptimal response to standard therapy, holds promise for improving cardiovascular outcomes in Swedish clinical practice [21].

Acknowledgements

The authors acknowledge the clinical staff at Karolinska University Hospital for participant recruitment and sample collection. We thank Dr. Lars Eriksson and Dr. Maria Karlsson for assistance with NMR spectroscopy analysis. This research was supported by grants from the Swedish Heart-Lung Foundation (Grant #20210456), Swedish Research Council (Grant #2022-01845), and Karolinska Institutet Research Foundation. The funding agencies had no role in research design, data analysis, or manuscript preparation.

References

1. Ference BA, Ginsberg HN, Graham I, *et al.* Low-density lipoproteins cause atherosclerotic cardiovascular disease. 1. Evidence from genetic, epidemiologic, and clinical studies. *European Heart Journal*. 2017;38(32):2459-2472.
2. Libby P, Buring JE, Badimon L, *et al.* Atherosclerosis. *Nature Reviews Disease Primers*. 2019;5(1):56.

3. Sniderman AD, Thanassoulis G, Glavinovic T, *et al.* Apolipoprotein B particles and cardiovascular disease: A narrative review. *JAMA Cardiology*. 2019;4(12):1287-1295.
4. Tsimikas S, Fazio S, Ferdinand KC, *et al.* NHLBI Working Group recommendations to reduce lipoprotein(a)-mediated risk of cardiovascular disease and aortic stenosis. *Journal of the American College of Cardiology*. 2018;71(2):177-192.
5. Krauss RM. Lipoprotein subfractions and cardiovascular disease risk. *Current Opinion in Lipidology*. 2010;21(4):305-311.
6. Townsend N, Wilson L, Bhatnagar P, *et al.* Cardiovascular disease in Europe: Epidemiological update 2016. *European Heart Journal*. 2016;37(42):3232-3245.
7. Catapano AL, Graham I, De Backer G, *et al.* 2016 ESC/EAS Guidelines for the Management of Dyslipidaemias. *European Heart Journal*. 2016;37(39):2999-3058.
8. SCORE2 working group and ESC Cardiovascular risk collaboration. SCORE2 risk prediction algorithms: New models to estimate 10-year risk of cardiovascular disease in Europe. *European Heart Journal*. 2021;42(25):2439-2454.
9. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clinical Chemistry*. 1972;18(6):499-502.
10. Marcovina SM, Albers JJ. Lipoprotein(a) measurements for clinical application. *Journal of Lipid Research*. 2016;57(4):526-537.
11. Jeyarajah EJ, Cromwell WC, Otvos JD. Lipoprotein particle analysis by nuclear magnetic resonance spectroscopy. *Clinics in Laboratory Medicine*. 2006;26(4):847-870.
12. SEARCH Collaborative Group. SLCO1B1 variants and statin-induced myopathy - a genome-wide study. *New England Journal of Medicine*. 2008;359(8):789-799.
13. Field A. *Discovering Statistics Using IBM SPSS Statistics*. 5th ed. London: SAGE Publications; 2018.
14. Nordestgaard BG, Langsted A, Mora S, *et al.* Fasting is not routinely required for determination of a lipid profile. *Clinical Chemistry*. 2016;62(7):930-946.
15. Walldius G, Jungner I, Holme I, *et al.* High apolipoprotein B, low apolipoprotein A-I, and improvement in the prediction of fatal myocardial infarction (AMORIS study). *Lancet*. 2001;358(9298):2026-2033.
16. Mora S, Otvos JD, Rifai N, *et al.* Lipoprotein particle profiles by nuclear magnetic resonance compared with standard lipids and apolipoproteins in predicting incident cardiovascular disease in women. *Circulation*. 2009;119(7):931-939.
17. Ramsey LB, Johnson SG, Caudle KE, *et al.* The Clinical Pharmacogenetics Implementation Consortium guideline for SLCO1B1 and simvastatin-induced myopathy: 2014 update. *Clinical Pharmacology & Therapeutics*. 2014;96(4):423-428.
18. Wilke RA, Ramsey LB, Johnson SG, *et al.* The clinical pharmacogenomics implementation consortium: CPIC guideline for SLCO1B1 and simvastatin-induced

- myopathy. *Clinical Pharmacology & Therapeutics*. 2012;92(1):112-117.
19. Mach F, Baigent C, Catapano AL, *et al*. 2019 ESC/EAS Guidelines for the management of dyslipidaemias. *European Heart Journal*. 2020;41(1):111-188.
 20. Otvos JD, Mora S, Shalaurova I, *et al*. Clinical implications of discordance between low-density lipoprotein cholesterol and particle number. *Journal of Clinical Lipidology*. 2011;5(2):105-113.
 21. Grundy SM, Stone NJ, Bailey AL, *et al*. 2018 AHA/ACC/AACVPR/AAPA/ABC/ACPM/ADA/AGS/APhA/ASPC/NLA/PCNA Guideline on the management of blood cholesterol. *Circulation*. 2019;139(25):e1082-e1143.