Age- and sex-specific lipoprotein profiles in general and cardiometabolic population cohorts

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Summary

Background Nuclear magnetic resonance (NMR) spectroscopy enables the characterisation of lipoprotein sub-particles, providing a more detailed lipid profile than the conventional lipid measurements, with potential clinical relevance, particularly in cardiovascular disease (CVD), which remains the leading cause of mortality worldwide. Nonetheless, for clinical implementation, it is essential to first determine the normal variation of lipoprotein parameters by age and sex.

Methods This cross-sectional study analysed a large dataset of 31,275 serum or plasma samples from five different countries using the B.I.LISA™ NMR-based platform, quantifying 112 lipoprotein parameters, including subclass size and concentration. Lipoprotein parameters from specific cohorts were fitted to a Quantile Generalised Additive Model (QGAM) to calculate the different percentiles as a function of age and sex.

Findings A sub-cohort of individuals belonging to non-oriented cohorts (27,470 individuals) showed that lipoprotein parameters exhibit distinct sex- and age-dependent patterns, with inflection points observed around 44 and 60 years in women and around 60 years in men, aligning with known ageing acceleration models. The sub-cohort of 3021 individuals showing cardiometabolic risk factors was used to evaluate the effect of obesity, hypertension and diabetes in the lipoprotein distribution. Finally, we analysed the lipoprotein parameters that align with SCORE2 (a well-known CVD risk predictor) in an age- and sex-dependent manner. Many NMR-derived parameters effectively distinguish between low and high/very high CVD risk profiles, with very low-density (VLDL)-associated parameters demonstrating the highest sensitivity across a broad age range.

Interpretation Our findings provide reference values for NMR-derived lipoprotein parameters by age and sex, enabling their accurate interpretation in the context of cardiovascular disease risk stratification.

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Keywords: Lipoproteins; Lipids; NMR spectroscopy; Ageing; Cardiovascular disease risk; SCORE2

Introduction

Cardiovascular disease (CVD) encompasses a range of conditions that affect the heart and blood vessels, including coronary artery disease, heart failure, arrhythmias, and stroke.[1] Atherosclerosis is the leading cause of CVD, and circulating lipoproteins are widely used to predict cardiovascular diseases, forming part of various risk scoring systems.[2] While medical practice continues to rely on these lipoprotein parameters for predicting cardiovascular events, their utility is limited, as many reported incidents occur in individuals with normal lipoprotein levels,[3] likely due to the multifactorial nature of CVD.[4] NMR spectroscopy distinguishes these lipoprotein sub-particles and analyses their lipid composition in blood, thereby improving cardiovascular risk prediction.[5,6] This additional granularity possesses clinical value; for instance, NMR can identify atherogenic LDL particles in patients who have low LDL-C levels, constituting a new tool able to capture hidden risks still not detected by standard tests.[7]

The use of NMR-derived lipoprotein parameters as biomarkers of CVD risk or in any other clinical application requires the knowledge of the reference values for the general population including an understanding of how these parameters are influenced by age and sex. Several studies have addressed this issue, reporting sex-based variations in lipoproteins.[8–11] However, some cohorts were not specifically designed to adequately investigate age dependence,[8–10] while others were not large enough to analyse the age influence independently.[11] A comprehensive study of the lipoprotein data available in the UK Biobank, determined using the Nightingale Health© methodology,[12] successfully integrated lipoprotein data with other metabolic information to predict the risk for over 700 pathologies.[13] Despite the importance of this study, its analysis did not directly address age-related alteration in lipoprotein parameter and is limited to the examination of a national database.

Here, we have created a large international cohort of 31,275 individuals generated by merging several independent cohorts from five different countries and specifically designed to cover all the age ranges (over 18 y. o.) with statistical power. The goal is to use NMR spectroscopy, to analyse lipoprotein variability in relation to age and sex using NMR spectroscopy and to evaluate possible age ranges associated with non-linear age-related change. In the absence of longitudinal, lifetime-spanning data, we will model the age-related evolution of lipoprotein parameters using large cross-sectional

Research in context

Evidence before this study

On April 24, 2025, we conducted a search of the PubMed database for peer-reviewed articles using the terms ("lipoprotein" AND "NMR" AND ("cohort" OR "population")), which returned 306 results. A second search using the terms ("lipoprotein" AND "NMR" AND ("age" OR "ageing")) yielded 270 results. The two searches exhibited substantial overlap, with 44% of the identified articles appearing in both datasets. From these results, only 36 and 29 entries, respectively, were original research articles that quantified lipoprotein concentrations in large cohorts using NMR spectroscopy. Most of these studies focused on cardiovascular disease (CVD) risk assessment based on lipoprotein profiles, including six studies with very large cohorts exceeding 30,000 participants. Only a few studies addressed sex-based differences in lipoproteins (one study) or explored lipoprotein composition in specific population segments such as older adults (two studies).

Added value of this study

The study aimed to achieve an unbiased classification of lipoprotein parameters, as measured by NMR spectroscopy, in relation to sex and age. By analysing a large cohort from the general population, comprising over 27,000 participants, we ensured robust statistical power across all age groups and for both sexes. To the best of our knowledge, this type of analysis for establishing reference values is entirely unprecedented. Furthermore, the identification of inflection points reveals age ranges characterised by non-linear changes in lipoprotein profiles, consistent with findings in other molecules but not previously reported for these metabolites. Notably, while most existing studies have concentrated on stratifying CVD risk using lipoprotein biomarkers, they often overlook the natural age-related dynamics of lipoproteins as a potential confounding factor.

Implications of all the available evidence

Our study provides a set of reference values for the age- and sex-specific evolution of lipoproteins, derived from experimental measurements obtained via NMR spectroscopy in a large cohort of individuals representing the general population. Trajectory analysis revealed patterns of non-linear lipoprotein changes across specific age ranges, particularly those associated with advancing age. The newly reported reference values have proven instrumental in enhancing the interpretive power of lipoprotein sub-particles in CVD risk stratification, offering a more nuanced understanding of their role in clinical assessment.

cohorts. In this analysis, the effect of risk factors such as obesity, hypertension and diabetes will be considered. Finally, the sub-cohort of individuals with high risk of CVD will be compared to the rest of the cohort to find NMR-derived parameters that are sensitive to CVD risk factors in an age- and sex-dependent manner.

Methods

Cohorts design

The study included individuals from southern Europe (Portugal, Spain, and Italy), Austria and Western Australia. Individual cohort descriptions are provided in the Supplementary Materials and Methods (Supplementary S1) while the general characteristics (self-reported sex, age, body mass index, etc) for the individual cohorts are described in the Supplementary Tables S1–S10. This resulted in data from 31,275 individuals (42.9% women) with a mean age of 48.4 ± 12.4 years. A small portion of the samples (less than 7%) correspond to repeated donations from the same individuals. However, they are treated as independent samples due to the long interval between donations (more than 2 years).

Blood collection and serum/plasma preparation

Venous blood was collected from fasting participants, and serum or plasma samples (hereafter referred to as serum) were processed and stored at –80 °C for subsequent analysis. Serum/plasma samples were handled according to standardised operating protocols, as previously described.[14] Both serum and plasma matrices can be used for the NMR-based lipoprotein analysis, yielding equivalent results.[15]

NMR spectroscopy

^{1}H NMR spectra were acquired using both, Avance IIIHD and Neo IVDr 600 MHz spectrometers equipped with BBI probes and integrated with Bruker SampleJet™ robots, maintaining a cooling temperature of 5 °C. Prior to analysis, quantitative calibration was performed following the protocol of Dona et al.[16] For each sample, a ^{1}H 1D NMR spectrum with solvent pre-saturation was acquired (32 scans, 98,304 data points, 18,028.85 Hz spectral width), with each test lasting approximately 4.5 min.

Lipoprotein profiling using B.I.LISA™

Detailed lipoprotein profiles were generated using the B.I.LISA™ technique, which quantifies 112 parameters for each serum sample. Quantification or estimation was based on $–CH_2$ (δ = 1.25 ppm) and $–CH_3$ (δ = 0.8 ppm) peak values from the 1D spectrum, normalised using the Bruker QuantRef™ tool within the Topspin™ platform. A Partial Least Squares (PLS-2) regression model was employed for analysis. Lipoprotein subclasses included very-low-density lipoprotein (VLDL; δ = 0.950–1.006 kg/L), intermediate-density lipoprotein (IDL; δ = 1.006–1.019 kg/L), low-density lipoprotein (LDL; δ = 1.019–1.063 kg/L), and high-density lipoprotein (HDL; δ = 1.063–1.210 kg/L). Further fractionation of VLDL, LDL and HDL was based

on density, as detailed in Supplementary Table S11. A comprehensive breakdown of lipoprotein annotations is provided in Supplementary Table S12.

Evaluation of risk factors

Biochemical and anthropometrical data from participants, together with their medical records, were used to extract CVD risk and its associated risk factors: obesity, glucose intolerance or diabetes and hypertension. Since we are measuring lipoproteins, we did not consider dyslipidaemia as a risk factor.[17] Definitions for the inclusion criteria for each risk factor are listed in Supplementary Table S13. In addition, the cohorts were classified as non-oriented cohorts (NOC), meaning that health status was not considered in the inclusion criteria, in contrast to the cardiovascular risk cohorts (CVR), which were enriched with patients exhibiting risk factors associated with cardiovascular disease. The NOC cohorts included BCP, SPBB, DDM, BPH, BHAS, BioAg LA2, and EBIc, comprising a total of 27,470 samples, whereas the CVR cohorts LV, SPBB and BPM comprised 3805 samples in total.[17]

For the 28,855 donors with enough clinical data available (sex, age, smoking status, systolic blood pressure, total cholesterol, HDL cholesterol and region of residence), alternative risk scores were also calculated. The SCORE2/SCORE2-OP risk from the European Society of Cardiology was calculated using age, self-reported sex, smoking status, systolic blood pressure, diabetes, total cholesterol, HDL cholesterol, and risk region.[18] Individuals with high or very high 10-year risk ($\geq$2.5–7.5%, depending on age) were used as a comparison versus individuals with low-to moderate 10-year risk according to SCORE2.

Statistics

Exploratory data analysis of lipoprotein data

Principal Component Analysis (PCA) was performed on lipoprotein concentration data to visually inspect overall patterns, assess cohort comparability, and identify potential outliers. The analysis was used solely for exploratory purposes and did not inform statistical testing.

Descriptive statistics

Continuous variables are reported as median (interquartile range) if not normally distributed, as assessed by the Kolmogorov–Smirnov test; normally distributed variables are presented as mean ± standard deviation. Categorical variables are expressed as counts (percentages).

Effect size estimation

Different effect size measures were used depending on the nature and interpretability of the comparison. Cohen's d was applied to quantify standardised mean differences, useful for assessing the magnitude of group separation relative to within-group variability. Log_2 fold-changes were used to express proportional differences in concentration between groups, offering direct interpretability in relative terms. Odds ratios were employed to evaluate the association between lipoprotein levels and cardiovascular risk classification, reflecting how concentration relates to estimated risk.

Statistical significance testing

Group comparisons for continuous variables were performed using the Wilcoxon rank-sum test. Categorical variables were compared using Fisher's exact test. All tests were two-sided. P-values were adjusted for multiple comparisons using the Benjamini–Hochberg false discovery rate (FDR) method, with statistical significance defined as FDR-adjusted $P < 0.05$.

Correlation analysis

Spearman's rank correlation was used to assess monotonic relationships between variables.

Modelling age-related trajectories from cross-sectional data

To approximate age-related trajectories in lipoprotein concentrations from cross-sectional data, Quantile Generalized Additive Models (QGAMs) were fitted separately for each lipoprotein. The models estimated smooth, sex-specific quantiles (2.5th, 25th, 50th, 75th, and 97.5th percentiles) as a function of age. The QGAM formula included a thin plate spline for age with sex-specific smooths and a main effect for sex. Thin plate splines were chosen for their flexibility and stability in modelling smooth, non-linear trends without requiring pre-specification of knot locations. To visually validate the QGAM estimates, empirical quantiles were also computed within ±2.5-year age windows, providing a direct reference from the raw data for comparison. An excerpt of the R code used to fit the QGAM models, along with an explanation, is provided in Supplementary S2 of the Supplementary Materials.

Quantifying age-related distributional shifts in lipoproteins

To assess the magnitude of age-related changes in lipoprotein concentrations, we computed a generalised version of Cohen's d that compares full distributions rather than just means. Traditional Cohen's d assumes normality and focuses on mean differences, which may not capture skewness or changes in variability often present in lipoprotein data across age. To address this, synthetic distributions were generated from estimated quantiles (2.5th, 25th, 50th, 75th, and 97.5th percentiles), allowing effect size estimates that reflect shifts in distribution shape and spread. Pairwise generalised d values were computed for all age combinations, with positive values indicating increases and negative values indicating decreases. Results were summarised in sex-specific heatmaps, enabling structured visualisation of distributional changes across the lifespan. Effect sizes were categorised into nine levels: < –1 (very large negative), –1 to –0.8 (large negative), –0.8 to –0.5 (moderate negative), –0.5 to –0.2

(small negative), –0.2 to 0.2 (no effect), 0.2 to 0.5 (small positive), 0.5 to 0.8 (moderate positive), 0.8 to 1 (large positive), and >1 (very large positive). An excerpt of the R code used to calculate generalised Cohen's d, along with an explanation, is provided in Supplementary S3 of the Supplementary Materials.

Missing data management

Analyses were performed using a complete case approach, including only participants with available data for the variables involved in each analysis. However, since this method implicitly assumes that data are missing completely at random, we conducted a sensitivity analysis to evaluate the potential impact of missingness (Supplementary S4). In this analysis, we first identified the variables affected by missing data and quantified their proportion across the dataset. When the proportion of missing data exceeded 5%, we applied multiple imputations using the mice package in R. Predictive mean matching (*pmm*) was used to preserve the original variable distributions and avoid implausible imputed values. Twenty imputed datasets were generated with 10 iterations each, using sex, age, BMI, diabetes, dyslipidaemia, hypertension, and smoking status as predictors. Results obtained from the imputed datasets were compared with the complete case estimates to assess the robustness of the findings.

Software

All analyses were performed using R language (4.3.1) with RStudio (2025.02.10), using the following R packages: tidyverse (2.0.0), factoextra (1.0.7), dplyr (1.1.4), RiskScorescvd (0.2.0), writexl (1.4.2), rstatix (0.7.2), corrplot (0.92), tidyr (1.3.0), ggplot2 (3.5.1), mgcv (1.9-1), gridExtra (2.3), viridis (0.6.5), reshape2 (1.4.4), proxy (0.4-27), circlize (0.4.16), RColorBrewer (1.1-3), qgam (1.3.4), ComplexHeatmap (2.18.0), CompareGroups (4.9.1), ggforestplot (0.1.0), pheatmap (1.0.12), ggrepel (0.9.4) and mice (3.18).

Ethics

The complete ethical statements for each cohort participating in this study are available in Supplementary S1 on the Supplementary Materials.

Role of funders

Funding sources are described on the Supplementary S5 on the Supplementary Materials Funding sources had no role in the design of this study, and did not have any role during its execution, analyses, interpretation of the data, or decision to submit results.

Results

Cohort characteristics and model construction

Lipoprotein analysis included 31,275 individuals aged 20–90 years (42.9% women) from eight non-targeted cohorts and two targeted cohort across Europe (Portugal, Spain, Austria, Italy) and Western Australia (Fig. 1A). Principal Component Analysis (PCA) was used to assess cohort heterogeneity, revealing no significant bias related to cohort origin (Supplementary Figure S1).

Participants were stratified into two groups based on the inclusion criteria (see Evaluation of Risk Factors in Methods section). This separation led to a total of 27,470 individuals representing the general population (mean age 47.4 years, 42.8% women) and 3805 individuals representing the CVD risk population (CVR, mean age 56.1 years, 43.7% women). As expected, the two groups exhibited significant differences in serum biochemical profiles (Table 1), reflecting the systemic nature of the associated risk factors.

The descriptive model of lipoprotein parameter variation with age and sex was developed using serum or plasma samples from the NOC cohorts (8 cohorts, black names in Fig. 1A, 27,470 samples) and the B.I. LISA™ model, which reports 112 parameters, including lipoprotein particle and sub-particle sizes and compositions. Parameters derived from standard clinical analyses (e.g., HDL-C, LDL-C, total cholesterol and TG) show excellent agreement with NMR-derived parameters (including the NOC and CVR cohorts, R values > 0.88) (Fig. 1B). The low proportion of missing values in the clinical parameters (≤7.7%) had no meaningful impact on the observed associations (Supplementary S4). Fig. 2A shows representative parameters uniquely accessible through the B.I.LISA™ model, with equivalent plots for the entire set of parameters shown in Supplementary Figure S2. Experimental data were visualised as age- and sex-specific trends, with median (50th percentile) values represented by grey dashed lines and interquartile ranges (25th–75th percentiles) by solid grey lines. Below 60 years of age, trends are relatively smooth for both sexes, but increased variability is observed in older individuals, likely due to smaller sample sizes and age-related metabolic dysregulation. Despite this, sample sizes remain sufficient to maintain statistical power (Supplementary Table S14).

To address the scattered data, we decided to generate a smooth model of the age evolution of the lipoprotein parameters. Specifically, lipoprotein parameters were fitted to a QGAM model to estimate smooth, sex-specific quantiles as a function of age, with results depicted in Fig. 2A and Supplementary Figure S2. The model uses solid lines for median values and coloured areas for interquartile ranges, while quantile values (Q2.5, Q25, Q50, Q75, and Q97.5) are listed in Supplementary Tables S15–S16. This smoothed model is particularly useful for observing age-related trends in lipoprotein parameters. To quantify the magnitude of these changes, we employed the generalised Cohen's d method, which enables comparisons of entire

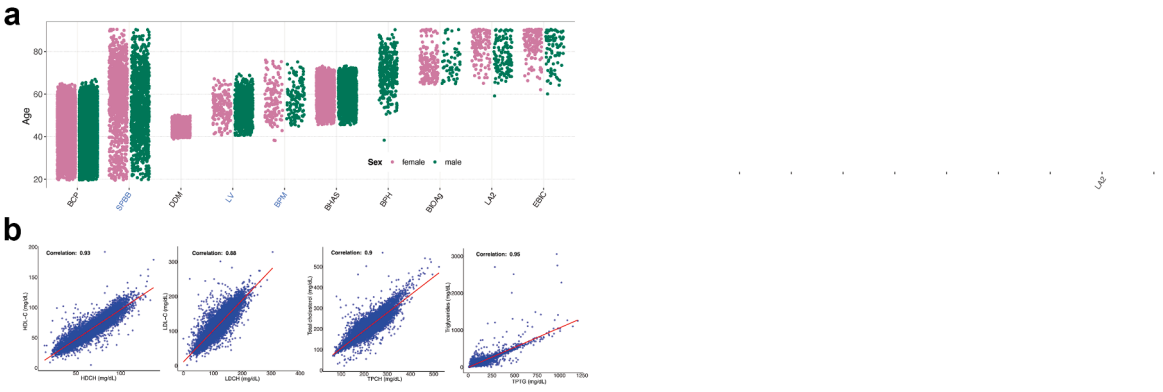

Fig. 1: (A) Distribution of sample donors as a function of age, origin and sex. Cohorts: BCP, Basque Country population Cohort (n = 21,733); SPBB, Spanish Biobanks cohort (n = 2491); DDM, DDM-Madrid cohort (n = 913); LV, Liver Bible cohort (n = 1035); BPM, BioPersMed cohort (n = 279); BHAS, Busselton Healthy Ageing Study (n = 3792); BPH, benign prostate hyperplasia cohort; BIOAg, Biosilver cohort (n = 225); LA2, LA2 cohort (n = 248); EBIC, EBI cohort (n = 244). Cohorts with the name in black/blue correspond to the NOC/CVR categories. (B) Comparison of the main lipoprotein parameters as determined by NMR spectroscopy (abscise axis) or by standard clinical analysis (ordinate axis) for all the samples under consideration (n = 31,275).

distributions to evaluate its changes. Fig. 2B shows Cohen's distributions for representative lipoprotein parameters, with equivalent distributions for all parameters provided in Supplementary Figure S3. In Fig. 2B, a positive (negative) change in the median value of a parameter with increasing age is shown in red (blue) colours, with the colour intensity reflecting the magnitude of the change. While the NMR dataset is complete, the associated general characteristics contain missing data (5.9–7.7%) and imputation analysis show small but noticeable differences for the QGAM models of the clinical parameters in the extreme quantiles (2.5th and 97.5th, Supplementary S4).

A key finding was the presence of inflection points, ages at which the rate of change in lipoprotein parameters shifted markedly. These points, identified through first derivative analysis of the QGAM model, are clinically significant as they may indicate periods of accelerated metabolic change. Fig. 2C shows the resulting histogram for the inflection points found for all the lipoprotein parameters in both, women and men models. In women, many parameters show inflection points around 44 and 60 years of age, indicating that lipoproteins also follow a nonlinear trajectory, with accelerated changes at specific ages. A less robust cluster is observed around age 72 in women. In men, inflection points primarily centre around age 60, with no significant lipoprotein remodelling observed around age 44 or 72.

Age- and sex-dependent changes in lipoprotein composition within the NOC cohorts were also analysed. Fig. 3A shows a heatmap comparing men and women, while Fig. 3B illustrates changes in particle number and composition for major lipoprotein types (HDL, IDL, LDL, and VLDL) as a function of sex and age, with equivalent plots for all the parameters provided in Supplementary Figure S4. Ageing is associated with an overall increase in atherogenic risk, reflected by rising TG content across most lipoprotein classes. In HDL particles, TG increases were offset by decreases in CH and FC. IDL shows persistent increases in men until age 60, followed by fluctuations, while levels stabilised in women after menopause. IDL particles also become richer in TG but poorer in phospholipids. VLDL particle numbers increase with age in women but stabilised in men after adulthood, with TG content decreasing and CH and FC levels rising. LDL concentrations generally trend upward with age, accompanied by increased TG content. These age-related shifts reflect TG enrichment and alter lipoprotein profiles, underscoring an elevated risk for atherosclerotic disease in older adults.

A particularly significant period in women's lives is the menopause transition. To assess its potential impact on lipoprotein distribution, we analysed lipoprotein parameters across four distinct groups: premenopausal women (aged 40–45 years, n = 1963), postmenopausal women (aged 55–60 years, n = 1045), and, for comparison, men in the same age ranges (40–45 years, n = 2987; 55–60 years, n = 1669). Among women, 57 out of 127 parameters showed medium or large effect sizes (Cohen's $d > 0.5$) (Supplementary Figure S5). These included the ApoB-100/ApoA-1 ratio (ABA1), several HDL-4 particle parameters (excluding triglycerides), and most IDL-related measures. Within the LDL subclasses, parameters from LDL-1 to LDL-6 exhibited menopause-related differences, again excluding triglycerides. Furthermore, total LDL parameters and multiple total particle measures (e.g., TBPN, TPA2, TPAB, TPCH, TPTG) were also affected.

	NOC				CVR			
	Women (N = 11,755)	Men (N = 15,715)	P-value	N	Women (N = 1664)	Men (N = 2141)	P-value	N
Age (years)	48 (42–56)	46 (38–54)	<0.0001	27,470	46 (40–52)	59 (50–66)	<0.0001	3805
Age range			<0.0001	27,470			<0.0001	3805
20–30	729 (6.2%)	1555 (9.9%)			98 (5.89%)	74 (3.46%)		
30–40	1823 (15.5%)	3382 (21.5%)			81 (4.87%)	144 (6.73%)		
40–50	4493 (38.2%)	5227 (33.3%)			200 (12.02%)	570 (26.62%)		
50–60	3094 (26.3%)	3869 (24.6%)			431 (25.90%)	767 (35.82%)		
60–70	1172 (10%)	1236 (7.9%)			601 (36.12%)	411 (19.20%)		
70–80	203 (1.7%)	301 (1.9%)			195 (11.72%)	133 (6.21%)		
80–90	241 (2.1%)	145 (0.9%)			58 (3.49%)	42 (1.96%)		
BMI (kg/m^2)	24.94 (20.3–29.57)	26.24 (22.51–29.97)	<0.0001	27,470	27.51 (22.1–32.92)	28.33 (24.29–32.38)	<0.0001	3805
Smoker	1780 (15.90%)	2735 (18.01%)	<0.0001	26,378	32 (10.53%)	80 (8.81%)	0.4356	1212
Drink alcohol:			<0.0001	21,721			<0.0001	169
Never	1826 (21.74%)	1413 (10.61%)			60 (43.17%)	4 (13.33%)		
Social drinker	6174 (73.50%)	10,241 (76.88%)			16 (11.51%)	10 (33.33%)		
Only during meals	332 (3.95%)	1157 (8.69%)			62 (44.60%)	15 (50.00%)		
Several times a day	68 (0.81%)	510 (3.83%)			1 (0.72%)	1 (3.33%)		
Total cholesterol (mg/dL)	197 (173–223)	196 (173–221)	0.1116	25,767	243 (199–293)	209 (177–246)	<0.0001	3103
HDL-cholesterol (mg/dL)	65.8 (57–77)	54 (46–64)	<0.0001	25,753	59 (49–71.6)	44 (38–53)	<0.0001	3102
Non-HDL-cholesterol (mg/dL)	128 (105–154.68)	139.21 (116–166)	<0.0001	25,753	181 (140–227)	163.9 (132–197)	<0.0001	3102
LDL-cholesterol (mg/dL)	107 (88.2–128.4)	116.6 (96.6–138.6)	<0.0001	20,875	119 (99.6–142)	121.8 (101–142.6)	0.6018	1348
Remnant cholesterol (mg/dL)	14 (10.8–18.4)	16.8 (12.8–23)	<0.0001	20,861	26.6 (18–34)	30.8 (20.4–39.2)	<0.0001	1307
Triglycerides (mg/dL)	74 (57–101)	90 (67–130)	<0.0001	26,015	116 (83.1–171)	151 (99–209)	<0.0001	3407
SCORE2/SCORE2OP:			<0.0001	25,753			<0.0001	3102
Very high risk	515 (4.85%)	7792 (51.47%)			280 (22.93%)	1539 (81.82%)		
High risk	102 (0.96%)	583 (3.85%)			37 (3.03%)	46 (2.45%)		
Low-moderate risk	9996 (94.19%)	6765 (44.68%)			904 (74.04%)	296 (15.74%)		

Categorical variables are presented as *n* (%), and continuous variables as median (25th–75th percentiles) for those not normally distributed, as determined by the Kolmogorov–Smirnov test. Categorical variables were analysed using Fisher's exact test, while continuous variables were evaluated using the Wilcoxon signed-rank test. P-values were adjusted using the false discovery rate (FDR) method.

Table 1: **Baseline characteristics of men and women in the NOC and CVR cohorts.**

In contrast, the comparison between younger and older men revealed significant changes in only one parameter (LDTG), suggesting that lipoprotein metabolism alterations in this age range are substantially more pronounced in women, consistent with the biological effects of menopause.

Relationship between lipoprotein parameters and risk factors associated to CVD

To evaluate the isolated effects of key cardiovascular risk factors on lipoprotein levels, we applied quantile normalisation based on age- and sex-specific reference models. Using QGAM-derived distributions, each individual's lipoprotein values were converted to their corresponding quantile positions within the expected distribution for their age and sex. These reference models included five quantiles (2.5th, 25th, 50th, 75th, and 97.5th percentiles), and intermediate values were assigned quantile positions via linear interpolation. Values outside this range were assigned quantile scores of 0 (below P2.5) or 1 (above P97.5). This transformation homogenised the cohort, removing the confounding effects of age and sex. For each risk group (diabetes, obesity, hypertension), we then calculated the average deviation of quantile positions from the median (0.5), allowing us to quantify the impact of each risk factor on lipoprotein distribution independently, as shown in Fig. 4. Individuals with diabetes, hypertension or obesity exhibit strong associations between TGs in HDL particles and these conditions (Fig. 4). In contrast, the other components of large- and small-dense HDL demonstrate a protective effect against the evaluated conditions. VLDL- and IDL-associated parameters always increase in the presence of risk factors. Small dense LDL particles show a similar trend, showing a positive association with the risk factors, while large LDL seems to have a protective factor.

Finally, we investigated the effect of any type of dyslipidaemia treatment on the lipoprotein profile by comparing two cohorts: individuals with dyslipidaemia according to the WHO definition (i.e., triglycerides >150 mg/dL; HDL-C <34.75 mg/dL in men or <38.61 mg/dL in women) who are not receiving treatment (n = 2414), and individuals undergoing medication (n = 1553). Consistent with their known mechanism of action,[19–21] statins and equivalent drugs

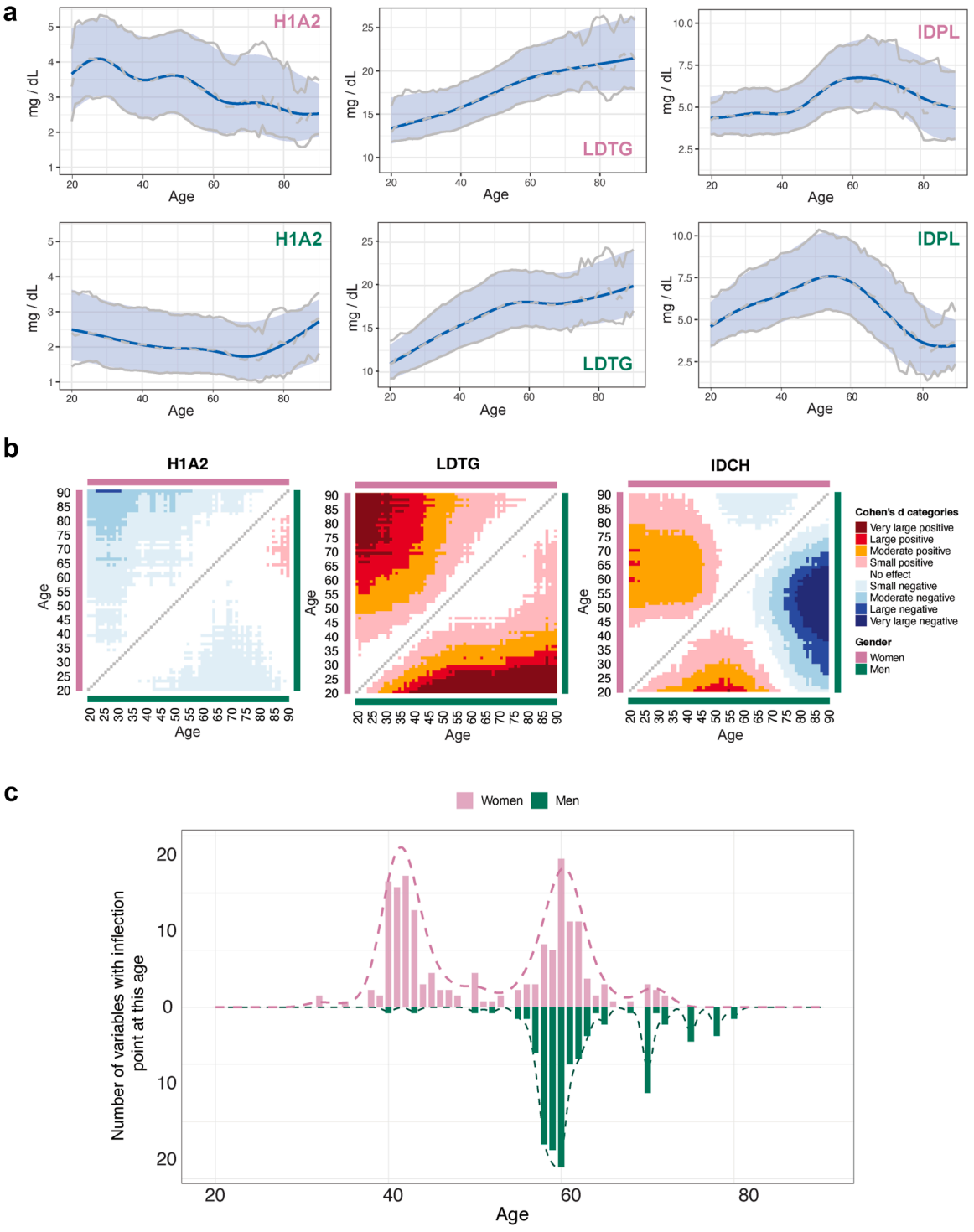

Fig. 2: (A) Selected examples of lipoprotein distribution for the NOC cohorts as a function of age and sex, as indicated. Grey lines represent the raw experimental values for the median (dashed line) or the Q25 and Q75 quantiles (solid lines). The equivalent values obtained with the QGAM model are shown with the blue solid line (median) and the blue shaded area (Q25-Q75 interquartile range). (B) Cohen's d values for the same selected examples, with positive values (warm colours) indicating increased lipoprotein levels with age and negative values (cold colours) indicating decreased levels. The upper/lower triangle of the plot represents women/men, as indicated by the colour code (purple/green). (C) Histogram on the number of inflexion points observed in the age evolution plots of the lipoprotein parameters as a function of age and obtained from the analysis of the 112 lipoprotein parameters. The accumulation of inflexion points at a given age is a readout for non-linear age ranges. When necessary, plots use the colours purple/green to refer to women/men.

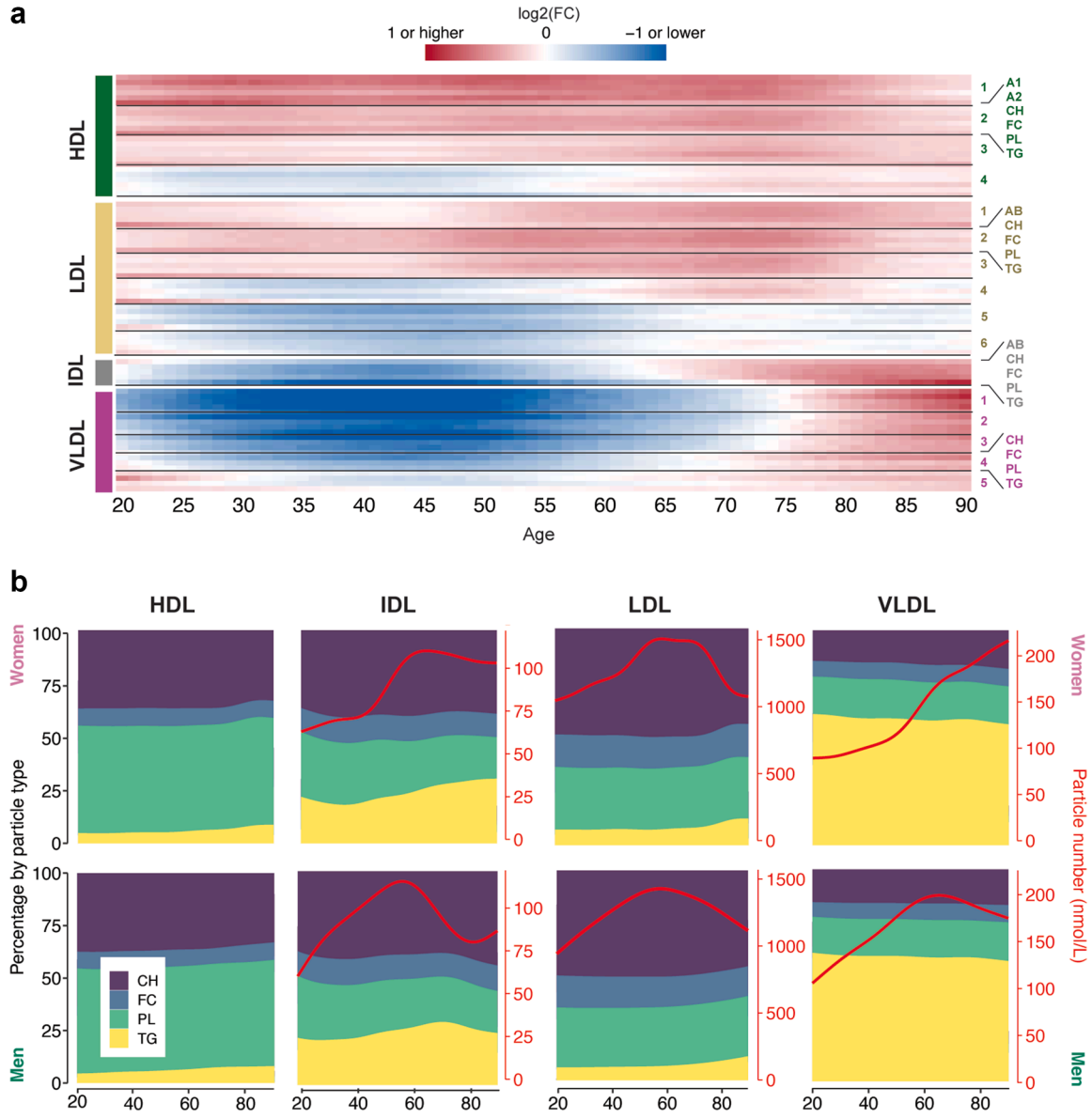

Fig. 3: (A) Heatmap to compare the sex variations of lipoprotein parameters. Blue/red colours indicate higher/lower concentration in women. The black horizontal lines separate the different sub-fractions (including CH, PL, TG, FC and the corresponding apolipoproteins). (B) Age and sex evolution of the main NMR-based lipoprotein parameters. The relative distribution of the lipoprotein composition (including CH, FC, PL and TG) is indicated by the colour code, as shown in the legend and quantified in the left ordinate axis. The total number of the particle (right ordinate axis) is shown with solid red lines when available.

tend to reduce LDL and VLDL parameter levels, while HDL-associated parameters remain unchanged or show a slight increase (Supplementary Figure S6). These results seem to be robust and not significantly affected by imputing untreated records (Supplementary S4).

Relationship between lipoprotein parameters and CVD risk prediction

To investigate the potential value of lipoprotein parameters in predicting CVD risk, we compared individuals with increasing CVD risk according to the SCORE2/SCORE2OP risk estimator. Specifically, we compared people at high or very high risk (n = 8537) with people at low-to-moderate risk (n = 12,614). Odds ratios (OR) were estimated separately for each lipoprotein, and for each age and sex group, using logistic regression models where the risk group was the dependent variable, the auto-scaled lipoprotein value was the independent variable, and BMI was included as confounding factor. The reported OR correspond

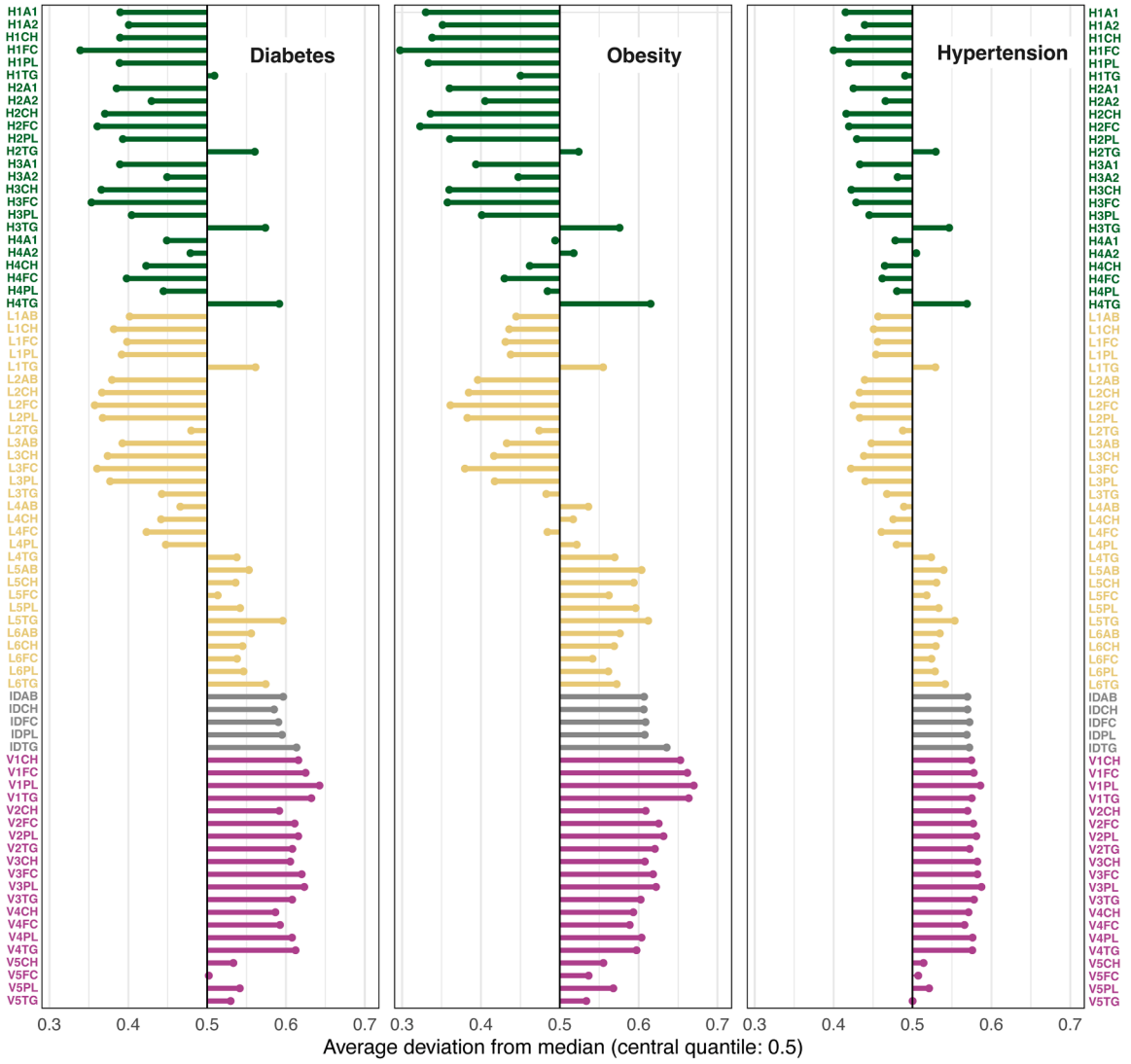

Fig. 4: Deviation of lipoprotein quantile positions from the population median in individuals with diabetes, obesity, and hypertension. Each dot represents the average absolute deviation from the median quantile (0.5) for a given lipoprotein, in individuals with the specified risk factor. Lipoprotein levels were previously normalised using sex- and age-specific QGAM-derived quantile models. This transformation enables the comparison of lipoprotein distributions across individuals independently of age and sex. Higher deviations indicate stronger divergence from the expected distribution, suggesting a specific influence of the risk factor on that lipoprotein subclass. Lipoproteins are grouped and colour-coded by density class: HDL (green), LDL (yellow), IDL (grey), and VLDL (magenta).

to the coefficient associated with the lipoprotein variable.

The discriminative power of lipoprotein parameters is visualised as heatmaps in Supplementary Figure S7 and the imputation analysis in Supplementary S4. In this heatmap, ages where lipoprotein values are highlighted indicate a statistically significant difference in discrimination between the low-to-moderate and high/very high SCORE2 profiles (i.e., adjusted P-value <0·05). The colour coding represents the direction of change: red indicates an increase in the likelihood of belonging to the intermediate/high SCORE2 population as the parameter increases (OR > 1), whereas blue indicates a decreased likelihood (OR < 1).

Many lipoprotein sub-particles effectively differentiate between low-to-moderate and high/very high SCORE2 profiles across a broad age range, although their discriminative ability varies by parameter and sex. While some lipoprotein parameters such as H2TG in men or L1PL in women show complete or significant overlap between the SCORE2 groups, others such as H1PL in women and V1FC in men have distinct, non-

overlapping associations with age. Parameters associated with HDL particles tend to decrease more sharply with age in the CVD-risk-associated cohort for both men and women and are associated with a protective effect against cardiometabolic deterioration, except for HDTG, which increases more steadily with age in this group. IDL and VLDL parameters generally show higher values in unhealthy groups. In contrast, LDL-associated parameters exhibit a dual behaviour: large buoyant LDL sub-particles (L1–L3) either remain unchanged or decrease in the high/very high SCORE2 group, while small dense LDL sub-particles (L4–L6) increase in unhealthy cohorts.

At older ages (>65 years), the sample sizes for individuals with high CVD risk scores based on SCORE2 become more limited, requiring cautious interpretation. However, it is evident that lipoprotein subparticles have limited value for CVD risk stratification at very advanced ages (>80 years). Despite this, VLDL lipoprotein particles and, in women, small and intermediate-dense HDL-associated parameters remain informative for CVD risk assessment in older populations. Notably, these NMR-based lipoprotein parameters demonstrate utility across a wider age range than conventional clinical markers such as LDL-C or HDL-C.

Discussion

Here we conducted a statistical analysis on a large dataset of lipoprotein parameters obtained using the B.I.LISA™ model. This analysis provides new insights into the dynamic nature of these particles across the adult lifespan and highlights the complex interplay between age, sex, and metabolic health.

The quantile values calculated for lipoprotein parameters reveal distinct evolutionary patterns influenced by sex and metabolic health, in good agreement with a previous study employing the same experimental methodology.[9] Notably, our analysis of inflection points in the different life spans of healthy individuals demonstrates that lipoproteins are sensitive to nonlinear ageing processes. To the best of our knowledge, this observation, based only on lipoprotein analysis, is unprecedented, and these findings align with previous studies using multiple molecular markers, reinforcing the generality of these observations across diverse cohorts.[22,23] In women, an inflection points clusters around age 44, consistent with perimenopause metabolic changes and related changes in weight.[24] This period may represent a critical window for intervention, as hormonal shifts during menopause are known to exacerbate lipid dysregulation and increase CVD risk. On the other hand, the inflection point around age 60, which is common to men and women, may indicate a distinct phase of ageing, where immunosenescence may be accelerated by cardiometabolic disease risk factors such as obesity.[25] The efficiency of HDL to promote reverse cholesterol transport is reduced in T cells from elderly compared to younger individuals, consistent with an age threshold for progression of cardiometabolic disease.[26]

By classifying donors based on CVD risk using general characteristics and various criteria, we evaluated the discriminatory power of NMR-derived lipoprotein parameters in characterising CVD risk and its associated risk factors. Some NMR-derived lipoprotein parameters perform better than the clinical parameters in the CVD risk stratification. Interestingly, individuals at risk for CVD or with metabolic dysfunction (e.g., diabetic patients, hypertense and those with obesity) exhibit lipoprotein profiles resembling those of healthy older adults, suggesting shared metabolic pathways between ageing and disease. As expected, the predictive value varies by parameter and HDL demonstrate a protective effect against the evaluated conditions in line with a previously reported NMR-based hypertension study in the Finnish population[27] and supported by many other prospective studies.[28,29] VLDL-associated parameters emerge as the most effective in distinguishing between healthy and unhealthy individuals across a broad age range. This result aligns well with the Dutch population cohort characterisation,[11] and with other studies that show that elevated VLDL levels are strongly associated with CVD risk, particularly in individuals undergoing metabolic syndrome.[30,31] Additionally, an excess of VLDL secretion has been associated with the metabolic-associated steatotic liver disease subtype that results in elevated CVD risk.[32]

At the lipoprotein-composition level, ageing is associated with increased TG content within HDL and LDL particles, contributing to a more atherogenic lipid profile in older adults. This effect is particularly pronounced in individuals at risk for CVD but is also observed in the general population. These age-related lipid shifts may be driven by metabolic and hormonal changes, including decreased lipoprotein lipase (LPL) activity and altered sex hormone levels.[33,34] Reduced LPL activity, especially in skeletal muscle and adipose tissue, impairs the clearance of TG-rich lipoproteins such as VLDL, leading to TG enrichment in LDL and HDL particles.[35] This process is further exacerbated by increased cholesterol ester (CE) transfer protein activity, which promotes the exchange of TG and CE between lipoprotein classes.[36]

This study has several limitations. Its cross-sectional design prevents the assessment of longitudinal changes in lipoprotein profiles or the calculation of hazard ratios for metabolic health outcomes. Future research should incorporate longitudinal studies to validate these findings and explore causal relationships. Additionally, some age and sex subgroups may lack sufficient statistical power to draw definitive conclusions. We have acknowledged these limitations in our analysis to

prevent data overinterpretation. Finally, while the study includes over 31K individuals from diverse cohorts across Southern Europe and Western Australia, most participants are of Caucasian descent. Further validation studies incorporating individuals from diverse ethnic backgrounds would be highly beneficial.

In summary, this study provides a comprehensive analysis of age- and sex-related variations in lipoproteins and lipoprotein sub-particles, and their implications for CVD risk assessment. The reference values here provided are essential for the future integration of NMR-derived lipoprotein parameters into clinical practice, for example, to monitor the effects of dietary or drug interventions, and to assess the impact of surgical procedures. Moreover, advances in technology now allow lipoprotein quantification using Benchtop spectrometers,[37] facilitating the rapid and affordable clinical translation of NMR-based lipoprotein analysis. Future longitudinal studies and validation in diverse populations will be crucial to fully establish the clinical utility of these biomarkers and further enhance precision medicine approaches for CVD prevention and management.

Contributors

Conceptualisation: OM, JMM, SCL; Data curation: JW, EH, JH, MLH, BBY, FC, AC, MU-U, AS, NO, MLS, SM, MPS-F, DF-B, MRG-F, MJZB, BO-P, NV, CH, HH, VL, ACe, PN, SL, BG-V, AD, MB, RG-R, RC; Formal analysis: RG-R, RC; Funding acquisition: OM, JMM, LV, ACa, TM, IV; Investigation: JN, JW, JH, MLH, BBY, TD, MU-U, AS, NO, MLS, SM, MPS-F, DF-B, MRG-F, MJZB, BO-P, NV, CH, HH, VL, PN, SL, AD, MB, RC; Methodology: OM, NE, TD, AD, MB, RG-R, RC; Order: OM, JMM, JN, JW, EH, JH, MLH, BBY, SCL, FC, LV, NE, CC, MS, HS, AC, TD, ACa, MU-U, TM, IV, AS, NO, MLS, EA, GH, SM, MPS-F, AMS, DF-B, MRG-F, MJZB, BO-P, NV, CH, HH, VL, ACe, PN, SL, BG-V, AD, MB, RG-R, RC; Project administration: OM, NE, BG-V; Resources: JN, JW, EH, JH, MLH, BBY, FC, LV, CC, MS, HS, AC, TD, ACa, MU-U, TM, IV, AS, NO, MLS, MPS-F, DF-B, MRG-F, MJZB, BO-P, NV, CH, HH, VL, ACe, PN, BG-V; Software: CC, MS, HS, TD, RG-R, RC; Supervision: OM, JMM, NE; Visualisation: OM, RC; Writing—original draft: OM, JMM, NE, RC, RG-R; Writing—review & editing: All authors. All authors read and approved the final version of the manuscript. OM, RC and RG-R had verified the underlying data.

Data sharing statement

The data used in this analysis are available to bona fide researchers by application to each of the participating cohorts, see Supplementary Material, Supplementary S5 for more details.

Declaration of interests

JMM and OM as well as other authors have an agreement with Bruker to provide free B.I. methods access for IVDr based quantification of serum metabolites and lipoproteins (B.I. QUANT-PS™ and B.I. LISA™).

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.ebiom.2025.106021.

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