Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

Joint associations of HDL cholesterol and ApoA-I with hospital-recorded osteoporosis

Yuying Fu
City University of Hong Kong
Jiazhen Zheng
The Hong Kong University of Science and Technology (Guangzhou)
Qing-Wen Ren
u3007493@connect.hku.hk

University of Hong Kong

Research Article

Keywords: HDL cholesterol, apolipoprotein A-I, osteoporosis, UK Biobank, longitudinal study, mutual adjustment

Posted Date: November 25th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-11604466/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License

Additional Declarations: No competing interests reported.

Joint associations of HDL cholesterol and ApoA-I with hospital-recorded osteoporosis

Yuying Fu [1], Jiazhen Zheng [3], Qing-Wen Ren [2, *]

*: Corresponding author, u3007493@connect.hku.hk

1. Department of Biomedical Sciences, City University of Hong Kong, Hong Kong, China. Email: yuyingfu3-c@my.cityu.edu.hk

2. Cardiology Division, Department of Medicine, The University of Hong Kong, Queen Mary Hospital, Hong Kong, China.

3. Bioscience and Biomedical Engineering Thrust, Systems Hub, The Hong Kong University of Science and Technology - Guangzhou Campus, Guangzhou, China.

Abstract

Background

High-density lipoprotein cholesterol (HDL-C) and apolipoprotein A-I (ApoA-I) describe related components of circulating lipoproteins. We examined whether their associations with hospital-recorded osteoporosis differ when modelled individually and jointly.

Methods

This exploratory UK Biobank analysis included 409,271 participants without baseline osteoporosis and with complete exposure and adjustment data. Follow-up ended on 31 March 2017. Cause-specific Cox models estimated hazard ratios (HRs) per standard deviation (SD), adjusting for demographic, lifestyle, treatment and lipid variables. We examined concentration-response curves, follow-up intervals, joint categories and sensitivity to additional skeletal risk factors. The osteoporosis focus was selected after an initial broader skeletal analysis.

Results

During 3,280,415 person-years, 4,725 hospital-recorded osteoporosis events occurred. In separate multivariable models, HRs were 1.05 (95% confidence interval [CI] 1.01–1.09) for HDL-C and 1.00 (0.96–1.03) for ApoA-I. Mutual adjustment yielded opposing associations: HDL-C HR 1.35 (1.24–1.48) and ApoA-I HR 0.78 (0.72–0.85). The biomarkers were strongly correlated (r = 0.9185). Both associations showed nonlinearity and departures from proportional hazards. HDL-C HRs across 0–2, 2–5 and beyond 5 years were 1.46, 1.42 and 1.29; corresponding ApoA-I HRs were 0.68, 0.74 and 0.83. Opposing directions persisted after extended skeletal and health adjustment (HRs 1.24 and 0.84), after additional heel bone-density adjustment (1.27 and 0.84), and among women after adjustment for menopause and hormone replacement therapy (1.27 and 0.81). Exploratory subgroup models showed stronger conditional estimates in men, with HRs of 1.81 for HDL-C and 0.66 for ApoA-I; the joint sex interaction q value was 0.023.

Conclusion

HDL-C and ApoA-I showed opposing conditional associations with hospital-recorded osteoporosis, with variation by concentration and follow-up time. These findings support joint characterization of HDL-related biomarkers in further skeletal research. Strong collinearity, selective diagnosis capture, residual confounding and the exploratory endpoint selection preclude causal interpretation. Direct functional measurements and independent validation are needed before assigning distinct biological roles or considering clinical use.

Keywords: HDL cholesterol; apolipoprotein A-I; osteoporosis; UK Biobank; longitudinal study; mutual adjustment.

Introduction

Circulating lipids are increasingly studied in relation to skeletal health. In UK Biobank, Xiong and colleagues reported associations of multiple lipid traits with fractures and osteoporosis, including higher risks at elevated concentrations of HDL cholesterol (HDL-C) and apolipoprotein A.[1] Subsequent work examined HDL particle characteristics in relation to fractures.[2] Associations have also been reported in older populations outside UK Biobank, including ASPREE and the Cardiovascular Health Study.[3,4,5] These observations establish a clinical context for investigating HDL-related measures, while leaving uncertainty about which dimensions of their variation are associated with skeletal disease.

HDL-C and apolipoprotein A-I (ApoA-I) are closely related but do not measure the same quantity. HDL-C quantifies cholesterol carried by HDL, whereas ApoA-I measures a major HDL-associated protein. Their shared variation can make single-exposure associations look similar even when the variation not shared between the biomarkers has a different relationship with an outcome. Conversely, mutual adjustment can generate large conditional coefficients through statistical suppression or correlated measurement error. A joint model therefore addresses a different question from a model containing either biomarker alone.

Distinguishing concentration from function is essential to interpreting this approach. Cardiovascular research has shown that cholesterol efflux capacity can provide information distinct from HDL-C concentration.[6] However, neither an ApoA-I measurement nor an HDL-C coefficient adjusted for ApoA-I directly measures cholesterol efflux, particle size or other HDL functions. Evidence for opposing statistical associations is therefore a basis for further phenotyping, not evidence that one HDL component damages bone while another protects it.

We investigated the individual and joint associations of baseline HDL-C and ApoA-I with subsequent hospital-recorded osteoporosis in UK Biobank. The analysis focused on the change in estimates with mutual adjustment, the concentration-response pattern and variation across follow-up. Sensitivity analyses assessed additional skeletal risk factors, heel bone mineral density (BMD), recorded medication and hormonal factors. This osteoporosis-focused report was developed after reviewing a broader skeletal-outcome analysis, and its findings are presented as exploratory. The objective was to characterize the observed conditional associations and their stability, rather than to establish a causal effect or a clinical prediction tool.

Methods

Study design and population

UK Biobank is a longitudinal research resource that recruited approximately 500,000 adults in 2006–2010 and collected baseline questionnaire, physical and biological measurements with subsequent linkage to health records.[7] We analysed the available baseline extract and hospital outcomes through 31 March 2017. Reporting was informed by STROBE and RECORD.[8,9] UK Biobank operates within an established research ethics and participant-consent framework.[10]

The analysis file contained 502,506 valid baseline records. Participants with osteoporosis recorded on or before baseline, an undated qualifying diagnosis or baseline self-reported osteoporosis were excluded. Positive follow-up, paired HDL-C and ApoA-I measurements and complete primary adjustment covariates were required. The final cohort comprised 409,271 participants (Figure 1). The endpoint and original model specifications had been documented in a local exploratory analysis plan; the selection of osteoporosis as the focus of this report occurred after examining the broader results. Neither the original work nor this focused extension was prospectively registered. The initial endpoint hierarchy and full companion results are retained in the Supplementary Materials. New osteoporosis subgroup analyses were specified before fitting the subgroup models.

Exposure and covariate measurement

HDL-C and apolipoprotein A were obtained from baseline fields 30760 and 30630, respectively. We refer to the latter as ApoA-I. According to UK Biobank assay documentation, HDL-C was measured by enzyme immunoinhibition and apolipoprotein A by immunoturbidimetry on a Beckman Coulter AU5800 platform.[11,12] Units were mmol/L and g/L. Nonpositive lipid measurements were treated as missing. Exposures were standardized using available baseline values before endpoint-specific exclusions: one SD was 0.3823 mmol/L for HDL-C and 0.2707 g/L for ApoA-I. These fixed SDs were retained in all models and subgroups.

Primary adjustment variables were age, recorded sex, BMI, smoking status, alcohol use, Townsend deprivation index, ethnicity, lipid-lowering medication, assessment centre, LDL-C and log-transformed triglycerides. Smoking and alcohol use were categorical. Ethnicity was represented as White or other in the implemented model. Negative questionnaire nonresponse codes were treated as missing.

Additional sensitivity variables were falls in the preceding year, fracture in the preceding five years, International Physical Activity Questionnaire activity category, vitamin D, albumin, log(1 + C-reactive protein), creatinine, maximum available grip strength from the two hands, and selected recorded bone medications or potentially systemic steroids. Medication records did not establish adherence, duration or consistently establish administration route. Explicitly local steroid preparations and combination products were excluded from the selected steroid code list. Heel ultrasound-derived BMD was used in an additional subset. Among women, menopause status and ever-use of hormone replacement therapy were examined. Field definitions and medication lists are retained in the accompanying analysis files.

Hospital osteoporosis ascertainment

The endpoint was the first hospital-recorded osteoporosis diagnosis after baseline, defined by ICD-10 M80, M81 or M82, or ICD-9 733.0. This definition includes osteoporosis with pathological fracture, osteoporosis without pathological fracture and osteoporosis associated with other diseases. It is not restricted to primary postmenopausal osteoporosis. Baseline self-reported osteoporosis was identified using illness code 1309 in field 20002.

ICD-10 diagnosis arrays were paired with their corresponding first-recorded diagnosis dates using fields 41270 and 41280; ICD-9 arrays used fields 41271 and 41281. Codes and dates were matched by array index, and the earliest qualifying date determined endpoint status. Follow-up began at baseline and ended at the earliest osteoporosis diagnosis, death, recorded loss to follow-up or 31 March 2017. A qualifying diagnosis on the censoring date was counted as an event. Follow-up time was expressed in years using 365.25 days per year.

The endpoint is a newly recorded hospital diagnosis. Primary-care records and systematic densitometric ascertainment were not used, and the first code should not be interpreted as the biological onset of disease. Death before a qualifying diagnosis was treated as censoring in cause-specific hazard models and as a competing event in descriptive cumulative-incidence estimation.

Association models

Cox models used time since baseline and Efron's handling of ties. Model 1 included a single lipid, sex and age modelled using a natural cubic spline with three degrees of freedom. Model 2 added BMI with three spline degrees of freedom, smoking, alcohol, Townsend index, ethnicity, lipid medication, LDL-C and log triglycerides, and stratified the baseline hazard by assessment centre. Model 3 included HDL-C and ApoA-I simultaneously with the same adjustment set. All three models used the identical complete-case cohort. HRs and 95% CIs were reported per SD.

We quantified Pearson correlation and the two-exposure variance-inflation measure $1/(1 - r^2)$. This was a descriptive measure of correlation between the exposures, not a full covariate-adjusted variance-inflation factor. A joint two-degree-of-freedom Wald test assessed the pair of lipid coefficients. The jointly adjusted HRs represent contrasts conditional on the other lipid and covariates, rather than total effects of increasing the biomarkers.

Concentration response and time variation

Natural cubic splines with three degrees of freedom were fitted simultaneously for both lipids. For each exposure, a likelihood-ratio test compared this model with a model in which only that exposure was linear, giving a two-degree-of-freedom test of nonlinearity. Curves used each exposure's cohort median as reference while the other lipid was held at its median. Displayed values spanned the 5th–95th percentiles among participants whose other standardized lipid lay within 0.25 SD of its median, to reduce unsupported joint contrasts. The models used all complete cases. Confidence bands were pointwise 95% CIs.

Proportional-hazards diagnostics used scaled Schoenfeld residuals.[13] We estimated separate joint models for 0–2 years, 2–5 years and more than 5 years. Participants entered an interval only if alive, under observation and free of a recorded endpoint at its start, and were censored at the interval end where applicable. Subtracting the common entry time within an interval gave an equivalent Cox risk-set representation. These analyses describe variation across follow-up and do not remove selection arising from survival or prior disease. Different nominal P values across intervals were not treated as a test of temporal heterogeneity.

Sensitivity and subgroup analyses

Sensitivity analyses used two- and five-year landmark risk sets, excluded baseline hospital-recorded cancer, excluded reported lipid medication, or excluded selected recorded bone drugs and steroids. Baseline cancer exclusion did not use complete cancer-registry ascertainment. Extended adjustment included the skeletal and health variables listed above. Models before and after additional adjustment were compared within identical complete-data subsets. A further paired comparison added heel BMD to the extended model. Among women with available data, paired models examined menopause and hormone replacement therapy. Re-exclusion of baseline osteoporosis was not presented as a separate sensitivity analysis because it duplicates the disease-free entry criterion.

The new subgroup analyses covered sex, age below versus at least 60 years, and BMI below versus at least 25 kg/m². Each stratum-specific model included both lipids and the primary adjustment set, omitting sex when analysing a single sex. Pooled models tested both lipid-by-subgroup interaction terms jointly with two degrees of freedom. Pooled tests assumed common adjustment-covariate effects across strata, whereas separate subgroup models allowed those effects to differ. All predefined subgroup estimates were retained irrespective of significance.

Supplementary analyses and reporting

Sex-specific baseline medians defined four joint categories. Thresholds for HDL-C and ApoA-I were 1.236 mmol/L and 1.405 g/L in men, and 1.549 mmol/L and 1.610 g/L in women; values equal to the threshold were high. Category models used Model 2 covariates and the low/low category as reference. The cut points are descriptive, not validated clinical thresholds. Unadjusted Aalen–Johansen cumulative-incidence curves accounted for competing death and included numbers at risk through eight years. These descriptive curves were not covariate-adjusted and did not include confidence bands.

Complete-case analysis was used without imputation. Baseline characteristics, missingness and available characteristics of included and excluded participants were described. Tests were two-sided, and the focused manuscript's association estimates and P values are exploratory. The original broader analysis used Holm adjustment for its two primary hip-fracture coefficients and Benjamini–Hochberg correction across 12 single-lipid screening tests.[14,15] Those families and their results remain unchanged in the supplementary record. The three new osteoporosis interaction tests additionally have Benjamini–Hochberg q values within that three-test family; this does not correct for selecting the endpoint after examining results. There was no a priori sample-size calculation, external validation or assessment of incremental clinical prediction. Analyses used R 4.2.1 and survival 3.5-7.

Results

Participants and recorded osteoporosis events

Of 502,506 valid baseline records, 9,860 were excluded for prevalent, self-reported or undated osteoporosis. Among the remaining 492,646 participants, 73,568 lacked paired HDL-C and ApoA-I measurements and a further 9,807 lacked primary adjustment covariates, leaving 409,271 participants (Figure 1). Baseline characteristics are summarized in Table 1. Median age was 58 years (interquartile range 50–63), and 217,958 participants (53.3%) were women. Median HDL-C was 1.40 mmol/L and ApoA-I was 1.51 g/L. Increasing HDL-C quartiles were accompanied by lower BMI and triglycerides and higher ApoA-I. Recorded lipid medication use decreased from 25.5% in the lowest quartile to 12.0% in the highest.

During 3,280,415 person-years, 4,725 hospital-recorded osteoporosis events occurred, corresponding to 1.44 events per 1,000 person-years. Median observed follow-up was 8.13 years. There were 13,319 deaths before a recorded osteoporosis endpoint. HDL-C and ApoA-I were strongly correlated (r = 0.9185), with a two-exposure variance-inflation measure of 6.39. Missingness and selection comparisons are reported in Supplementary Table S1, and exposure scaling and correlation in Supplementary Table S2.

Opposing associations after mutual adjustment

In age- and sex-adjusted models, the HR per SD was 1.07 (95% CI 1.04–1.10) for HDL-C and 1.02 (0.99–1.05) for ApoA-I. In Model 2, including lifestyle, treatment and other lipid variables, the HRs were 1.05 (1.01–1.09) and 1.00 (0.96–1.03), respectively. With HDL-C and ApoA-I entered together, the HDL-C HR was 1.35 (1.24–1.48; $P = 2.48 \times 10^{-11}$), while the ApoA-I HR was 0.78 (0.72–0.85; $P = 7.96 \times 10^{-10}$) (Table 2). The joint Wald statistic was 44.57 with two degrees of freedom ($P < 0.001$).

Thus, the opposing pattern emerged in the conditional model and was substantially stronger than the single-exposure associations. The relative changes describe model-specific hazard contrasts; they are not absolute-risk differences or estimated effects of interventions on HDL-C or ApoA-I.

Concentration response and follow-up intervals

Nonlinearity tests were positive for HDL-C ($P = 0.023$) and ApoA-I ($P < 0.001$). Within the central conditional support displayed in Figure 2, the estimated HDL-C curve increased and the ApoA-I curve decreased. These plots do not depict the full exposure distribution, and the nonlinearity tests concern the models fitted to all complete cases (Supplementary Table S3).

Exposure-specific proportional-hazards tests gave $P = 0.006$ for HDL-C and $P = 0.001$ for ApoA-I. Consequently, full-follow-up HRs should be read as working summaries rather than constant associations. During 0–2 years, 638 osteoporosis events occurred and HRs were 1.46 (95% CI 1.14–1.86) for HDL-C and 0.68 (0.55–0.85) for ApoA-I. During 2–5 years, 1,477 events occurred, with corresponding HRs of 1.42 (1.21–1.66) and 0.74 (0.65–0.85). Beyond five years, 2,610 events occurred and HRs were 1.29 (1.15–1.46) and 0.83 (0.75–0.92) (Figure 3; Supplementary Table S4). The opposing direction was present in each period, with estimates numerically closer to unity later in follow-up.

Stability across alternative adjustment sets

Opposing conditional associations persisted across the examined sensitivity models (Table 3; Figure 4). After excluding participants with baseline hospital-recorded cancer, HRs were 1.34 (95% CI 1.22–1.47) and 0.79 (0.73–0.86). Among 337,631 participants reporting no lipid-lowering medication, with 3,624 events, HRs were 1.23 (1.11–1.36) and 0.82 (0.75–0.89). Excluding recorded bone drugs and selected steroids yielded HRs of 1.31 (1.19–1.45) and 0.80 (0.73–0.87).

The extended-adjustment subset included 315,241 participants and 3,269 events. Before additional skeletal and health adjustment, HRs were 1.32 (95% CI 1.18–1.47) and 0.80 (0.72–0.88); after adjustment, they were 1.24 (1.11–1.38) and 0.84 (0.77–0.92). In 178,811 participants with heel BMD data

and 1,918 events, the corresponding extended-model HRs before BMD adjustment were 1.31 (1.15–1.51) and 0.81 (0.71–0.91). Adding heel BMD gave HRs of 1.27 (1.10–1.46) and 0.84 (0.74–0.95).

Among 217,081 women with hormonal information and 3,847 events, HRs after adjustment for menopause and hormone replacement therapy were 1.27 (95% CI 1.15–1.40) for HDL-C and 0.81 (0.74–0.88) for ApoA-I. Similar before-and-after estimates within each restricted subset suggest that the observed directions were not explained entirely by adding these measured factors. They do not exclude residual confounding or establish mediation-independent effects.

Subgroup and supplementary analyses

Both biomarkers retained opposing directions in every specified sex, age and BMI stratum (Table 4; Supplementary Figure S2). Among men, the HDL-C HR was 1.81 (95% CI 1.44–2.28) and the ApoA-I HR was 0.66 (0.54–0.81); corresponding estimates in women were 1.28 (1.16–1.41) and 0.81 (0.74–0.88). Joint interaction tests gave P = 0.016 for sex, P = 0.130 for age and P < 0.001 for BMI. Within the three-test correction family, q values were 0.023, 0.130 and <0.001, respectively. The joint tests do not attribute interaction to either individual biomarker, and these post hoc subgroup results require independent validation.

The joint median-category analysis gave HRs of 0.88 (95% CI 0.78–1.00) for low HDL-C/high ApoA-I, 1.11 (0.98–1.26) for high HDL-C/low ApoA-I and 1.02 (0.95–1.09) for high/high, relative to low/low (Supplementary Table S5). These broad categories did not reproduce the magnitude of the continuous conditional contrasts and should not be interpreted as validated clinical risk groups. Unadjusted cumulative incidence with competing death is shown in Supplementary Figure S1. The complete original lipid screen and companion skeletal-outcome results are retained in Supplementary Tables S6–S7 and the original supplementary report.

Discussion

In this exploratory UK Biobank analysis of 409,271 participants, HDL-C and ApoA-I showed opposing associations with subsequent hospital-recorded osteoporosis when entered in the same model. A one-SD higher HDL-C concentration was associated with a higher cause-specific hazard, whereas a one-SD higher ApoA-I concentration was associated with a lower hazard conditional on the other biomarker and measured covariates. The pattern was not constant across the exposure range or follow-up, making the concentration-response and interval-specific estimates integral to its interpretation.

The main observation is the difference between individual and joint biomarker models. An individually modelled ApoA-I concentration had little association with the endpoint after adjustment, yet its conditional coefficient was inverse when HDL-C was also included. The HDL-C coefficient likewise became more positive. Reporting only the joint coefficients would obscure this change in the statistical question. Single-exposure models capture shared variation; mutual adjustment isolates the residual component conditional on the other lipid. The results therefore support investigating the joint distribution of HDL-related measures, rather than treating either concentration as an isolated functional readout.

Previous UK Biobank work provides important context. Xiong and colleagues reported associations of elevated HDL-C and apolipoprotein A with osteoporosis and fractures, using a broader exposure set, longer follow-up and primary-care information for osteoporosis ascertainment.[1] Another UK Biobank publication examined particle size and subclasses in relation to fractures.[2] The present analysis addresses a narrower conditional-association question within the same research resource and should not be regarded as independent replication of those studies. Differences in case ascertainment, follow-up and adjustment further limit direct comparison of effect sizes. The results add a description of how two strongly correlated measurements behave jointly.

A biological interpretation is plausible but remains untested. Direct functional phenotyping has already proved informative in cardiovascular research, where cholesterol efflux capacity can carry information distinct from HDL-C.[5] However, the current extract did not measure cholesterol efflux, HDL particle number, particle size or protein composition. It would be premature to infer that high HDL-C identifies

dysfunctional HDL or that ApoA-I protects the skeleton. Those hypotheses require direct assays and an independent study design capable of separating composition, function and confounding.

Statistical explanations also remain credible. HDL-C and ApoA-I correlated at approximately 0.92, so jointly adjusted contrasts depend on relatively limited unshared variation. Suppression, measurement error and unmeasured common causes can contribute to opposite coefficients in such a setting. The categorical analysis provides an additional caution: dividing each exposure at its sex-specific median yielded modest and imprecise category contrasts. Categorization loses information and changes the estimand, but these results do not support interpreting the continuous coefficients as a ready-made discordance phenotype with clinical thresholds.

Several sensitivity analyses make a single measured explanation less likely. Adjustment for falls, prior fractures, activity, nutritional and inflammatory markers, renal function and grip strength attenuated the conditional estimates, but did not reverse their directions. Further heel-BMD adjustment also preserved the opposing pattern. This finding indicates persistence after accounting for the available BMD measure, not proof of a pathway independent of bone density. Ultrasound-derived heel BMD is an imperfect measure of skeletal vulnerability, and the smaller measured subset may be selected. The analysis was not a formal mediation study.

The hormonal and medication analyses similarly address specific measured factors without excluding treatment-related confounding. Among women, adjustment for menopause and ever-use of hormone replacement therapy had little effect within the available-data subset. Results were also present among participants who did not report lipid-lowering medication and after excluding selected bone-drug and steroid records. Restriction itself changes the population and cannot establish that the association is unaffected by treatment.

The temporal findings are particularly relevant to reverse causation and disease detection. The association magnitudes were numerically smaller after five years, although both directions persisted. A newly recorded osteoporosis diagnosis may follow years of unrecognized disease, changes in activity, nutritional status or clinical contact. Early associations can therefore reflect disease processes that preceded the baseline assay. Conversely, later attenuation can arise from exposure changes, regression dilution or a changing risk set. The exposure-specific proportional-hazards departures justify reporting the interval estimates alongside the full-period summaries.

Hospital ascertainment defines the clinical scope of the findings. A first osteoporosis code depends on testing, referral, hospital contact and documentation, as well as underlying disease. The code set also includes osteoporosis with pathological fracture and secondary osteoporosis. It does not provide comprehensive community incidence or establish when skeletal deterioration began. The present associations may therefore involve both disease and its recognition. These diagnosis-based findings alone do not establish fracture prevention benefits.

The subgroup extension suggests that the conditional pattern may vary by sex and BMI. Estimates were more pronounced in men, and the joint sex interaction remained below a q value of 0.05 within the three new interaction tests. This finding is compatible with heterogeneity but does not identify its source. Different case ascertainment, treatment, hormonal factors or unmeasured composition could contribute. They should not be interpreted as a validated sex-specific biological mechanism or used to define clinical thresholds.

The study benefits from a large cohort, 4,725 events, consistently defined exposure scales and identical participants in the single-exposure and joint primary models. It examines nonlinearity and time variation rather than relying solely on a single adjusted HR. Paired subset analyses distinguish additional adjustment from selection into smaller samples. These features allow the observed association pattern to be evaluated against its model assumptions and alternative specifications.

There are nonetheless major limits to inference. This report's endpoint focus was selected after viewing earlier results, and its P values should not be interpreted as those of an independent confirmatory test. Complete-case analysis excluded more than 83,000 otherwise eligible participants, without an established missing-at-random mechanism. UK Biobank has a healthy-volunteer profile that may affect

representativeness and selection.[16] Single baseline lipid measurements do not capture subsequent treatment or exposure changes. Lifestyle, ethnicity and medication variables were incomplete or coarse, and residual confounding remains possible. Strong exposure correlation, selective hospital ascertainment, nonlinearity and proportional-hazards departures all affect interpretation. Follow-up ending in 2017 also limits comparison with newer data releases. No external validation, genetic causal analysis, direct HDL functional assay or clinical prediction assessment was performed.

The findings justify a focused next step: replicate the joint association in an independent cohort using the same exposure definitions, test alternative ascertainment of osteoporosis and obtain direct particle or functional measurements. Repeated lipid measurements would help separate stable differences from changes accompanying disease recognition. Any proposed clinical application would additionally require evaluation of absolute risk, calibration and incremental value beyond established skeletal risk factors. Statistical significance alone does not meet those requirements.

In conclusion, HDL-C and ApoA-I showed opposing conditional associations with hospital-recorded osteoporosis in UK Biobank. The pattern persisted across several alternative adjustment sets and participant restrictions, while varying with concentration and follow-up time. Joint biomarker characterization offers a useful hypothesis for further skeletal research, but these exploratory observations do not establish opposing biological effects or a basis for lipid-directed osteoporosis prevention.

Data availability

Individual-level UK Biobank data are available through its approved researcher access process and applicable conditions. Aggregate numerical results, endpoint definitions and medication code lists accompany this report. Participant-level records are not included in the results package.

Code availability

The package includes the original analysis code, new osteoporosis baseline and subgroup code, figure and document builders, software session information and aggregate outputs. Reproduction requires authorized access to the documented UK Biobank inputs. Numerical consistency checks are not equivalent to independent replication of all models.

Acknowledgments

The UK Biobank received ethical approval from the North West Multicenter Research Ethics Committee (REC reference for UK Biobank 11/NW/0382) and participants provided written informed consent. The UK Biobank application number for this study is 97089.

Ethics Declaration

All procedures performed in this study were in accordance with the Declaration of Helsinki.

Research Funding

This research received no external funding.

Human Ethics and Consent to Participate

Human Ethics and Consent to Participate declarations: not applicable.

Clinical Trial Number

Clinical trial number: not applicable.

Consent to Publish declaration

Consent to Publish declaration: not applicable.

References

1. Xiong, X. et al. Associations of serum lipid traits with fracture and osteoporosis: a prospective cohort study from the UK Biobank. J. Cachexia Sarcopenia Muscle 15, 2669–2683 (2024). https://doi.org/10.1002/jcsm.13611

2. UK Biobank. Publication 19400: Relationship of high-density lipoprotein cholesterol, average size, and subclasses by size with the risk of fractures. UK Biobank publication record for Precision Nutrition (2025). https://bb30.ndph.ox.ac.uk/showcase/pub.cgi?id=19400 (accessed 19 September 2026).

3. Hussain, S. M., Ebeling, P. R., Barker, A. L., Beilin, L. J., Tonkin, A. M. & McNeil, J. J. Association of plasma high-density lipoprotein cholesterol level with risk of fractures in healthy older adults. JAMA Cardiol. 8, 268–272 (2023). https://doi.org/10.1001/jamacardio.2022.5124

4. Barzilay, J. I. et al. The association of lipids and lipoproteins with hip fracture risk: the Cardiovascular Health Study. Am. J. Med. 135, 1101–1108.e1 (2022). https://doi.org/10.1016/j.amjmed.2022.05.024

5. Jamal, S. A., West, S. L. & Miller, P. D. Fracture risk assessment in patients with chronic kidney disease. Osteoporos. Int. 23, 1191‑1198 (2012). https://doi.org/10.1007/s00198-011-1781-0

6. Rohatgi, A. et al. HDL cholesterol efflux capacity and incident cardiovascular events. N. Engl. J. Med. 371, 2383–2393 (2014). https://doi.org/10.1056/NEJMoa1409065

7. Sudlow, C. et al. UK Biobank: an open access resource for identifying the causes of a wide range of complex diseases of middle and old age. PLoS Med. 12, e1001779 (2015). https://doi.org/10.1371/journal.pmed.1001779

8. von Elm, E. et al. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. PLoS Med. 4, e296 (2007). https://doi.org/10.1371/journal.pmed.0040296

9. Benchimol, E. I. et al. The REporting of studies Conducted using Observational Routinely-collected health Data (RECORD) statement. PLoS Med. 12, e1001885 (2015). https://doi.org/10.1371/journal.pmed.1001885

10. UK Biobank. Ethics. https://www.ukbiobank.ac.uk/about-us/how-we-work/ethics/ (accessed 19 September 2026).

11. UK Biobank. Data-Field 30760: HDL cholesterol. https://biobank.ndph.ox.ac.uk/ukb/field.cgi?id=30760 (accessed 19 September 2026).

12. UK Biobank. Data-Field 30630: Apolipoprotein A. https://biobank.ndph.ox.ac.uk/ukb/field.cgi?id=30630 (accessed 19 September 2026).

13. Grambsch, P. M. & Therneau, T. M. Proportional hazards tests and diagnostics based on weighted residuals. Biometrika 81, 515–526 (1994). https://doi.org/10.1093/biomet/81.3.515

14. Holm, S. A simple sequentially rejective multiple test procedure. Scand. J. Stat. 6, 65–70 (1979). https://www.jstor.org/stable/4615733

15. Benjamini, Y. & Hochberg, Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. J. R. Stat. Soc. B 57, 289–300 (1995). https://doi.org/10.1111/j.2517-6161.1995.tb02031.x

16. Fry, A. et al. Comparison of sociodemographic and health-related characteristics of UK Biobank participants with those of the general population. Am. J. Epidemiol. 186, 1026–1034 (2017). https://doi.org/10.1093/aje/kwx246

Table 1 Baseline characteristics of the osteoporosis cohort

Characteristic	Overall	HDL Q1	HDL Q2	HDL Q3	HDL Q4
Participants	409,271	101,799	102,434	102,341	102,697
Age, years	58.00 (50.00-63.00)	57.00 (49.00-63.00)	57.00 (50.00-63.00)	58.00 (50.00-63.00)	58.00 (51.00-63.00)
Women	217,958 (53.3%)	54,440 (53.5%)	54,557 (53.3%)	54,444 (53.2%)	54,517 (53.1%)
BMI, kg/m2	26.78 (24.19-29.93)	28.97 (26.12-32.46)	27.41 (24.86-30.44)	26.22 (23.88-28.99)	24.94 (22.82-27.40)
HDL-C, mmol/L	1.40 (1.17-1.67)	1.04 (0.94-1.19)	1.34 (1.16-1.44)	1.56 (1.34-1.68)	1.88 (1.64-2.06)
ApoA-I, g/L	1.51 (1.35-1.70)	1.27 (1.17-1.37)	1.44 (1.34-1.55)	1.58 (1.47-1.69)	1.81 (1.67-1.98)
Triglycerides, mmol/L	1.49 (1.05-2.15)	2.04 (1.45-2.88)	1.65 (1.19-2.29)	1.37 (1.01-1.89)	1.10 (0.84-1.49)
LDL-C, mmol/L	3.52 (2.95-4.12)	3.36 (2.77-3.96)	3.57 (2.98-4.18)	3.60 (3.03-4.20)	3.54 (3.00-4.13)
Townsend index	-2.17 (-3.66-0.46)	-1.82 (-3.47-1.09)	-2.17 (-3.65-0.45)	-2.32 (-3.73-0.16)	-2.33 (-3.76-0.10)
Current smoker	42,994 (10.5%)	14,065 (13.8%)	10,765 (10.5%)	9,378 (9.2%)	8,786 (8.6%)
Current drinker	377,487 (92.2%)	88,832 (87.3%)	94,066 (91.8%)	96,019 (93.8%)	98,570 (96.0%)
Lipid medication	71,640 (17.5%)	25,964 (25.5%)	18,532 (18.1%)	14,823 (14.5%)	12,321 (12.0%)
Recorded bone drug	2,976 (0.7%)	561 (0.6%)	678 (0.7%)	730 (0.7%)	1,007 (1.0%)
Recorded steroid	3,472 (0.8%)	747 (0.7%)	774 (0.8%)	844 (0.8%)	1,107 (1.1%)
Vitamin D, nmol/L	46.60 (32.30-62.10)	42.90 (29.40-58.40)	46.00 (32.00-61.30)	48.20 (33.70-63.40)	49.70 (34.70-65.10)
Heel BMD, g/cm2	0.53 (0.46-0.62)	0.54 (0.46-0.62)	0.53 (0.46-0.62)	0.53 (0.46-0.62)	0.52 (0.45-0.61)
Osteoporosis during follow-up	4,725 (1.2%)	1,154 (1.1%)	1,071 (1.0%)	1,137 (1.1%)	1,363 (1.3%)

Continuous variables are median(IQR); categorical variables are n(%). Quartiles use sex-specific cut points. Vitamin D and heel BMD use available data; missingness in Supplementary Table S1. Osteoporosis events are follow-up outcomes. Steroid codes include selected preparations of uncertain route.

Table 2 Individual and joint associations with osteoporosis

Model	N / events	HDL-C HR (95% CI)	ApoA-I HR (95% CI)
Age and sex	409,271 / 4,725	1.07 (1.04-1.10) P <0.001	1.02 (0.99-1.05) P 0.281
Multivariable single exposure	409,271 / 4,725	1.05 (1.01-1.09) P 0.014	1.00 (0.96-1.03) P 0.776
Multivariable joint exposures	409,271 / 4,725	1.35 (1.24-1.48) P <0.001	0.78 (0.72-0.85) P <0.001

HRs per fixed baseline SD: 0.3823 mmol/L HDL-C, 0.2707 g/L ApoA-I. Identical complete cases. Model 1 adjusts age spline and sex; Model 2 adds BMI spline, smoking, alcohol, Townsend index, ethnicity, lipid medication, LDL-C, log triglycerides; assessment-centre strata. Model 3 includes both exposures. P values nominal, exploratory. Correlation r=0.9185; two-exposure VIF=6.39. Opposing coefficients are conditional associations, not functional effects. Nonlinearity and proportional-hazards departures mean full-period HRs are working summaries.

Table 3 Sensitivity of the joint osteoporosis associations

Analysis	N	Events	HDL-C HR	ApoA-I HR
M3 joint	409,271	4,725	1.35 (1.24-1.48)	0.78 (0.72-0.85)
2-year lag	406,621	4,087	1.34 (1.22-1.47)	0.80 (0.73-0.87)
5-year lag	399,850	2,610	1.29 (1.15-1.46)	0.83 (0.75-0.92)
No baseline hospital cancer	390,682	4,328	1.34 (1.22-1.47)	0.79 (0.73-0.86)
No reported lipid medication	337,631	3,624	1.23 (1.11-1.36)	0.82 (0.75-0.89)
No recorded bone drugs or steroids	403,628	4,122	1.31 (1.19-1.45)	0.80 (0.73-0.87)
Extended subset before adjustment	315,241	3,269	1.32 (1.18-1.47)	0.80 (0.72-0.88)
Extended bone and health adjustment	315,241	3,269	1.24 (1.11-1.38)	0.84 (0.77-0.92)
BMD subset before adjustment	178,811	1,918	1.31 (1.15-1.51)	0.81 (0.71-0.91)
Plus heel BMD	178,811	1,918	1.27 (1.10-1.46)	0.84 (0.74-0.95)
Women hormonal subset before adjustment	217,081	3,847	1.27 (1.16-1.40)	0.81 (0.74-0.88)
Women plus menopause and HRT	217,081	3,847	1.27 (1.15-1.40)	0.81 (0.74-0.88)

Each model includes both lipids. Extended adjustment: falls, prior fracture, activity, vitamin D, albumin, log(1+CRP), creatinine, grip strength, recorded bone drugs/steroids. Heel BMD added to extended model. Paired subset rows isolate adjustment changes within identical samples. Redundant no-baseline-osteoporosis model omitted (main cohort already excludes). Exact P values in numeric/Table3_sensitivity.csv. All estimates observational, exploratory.

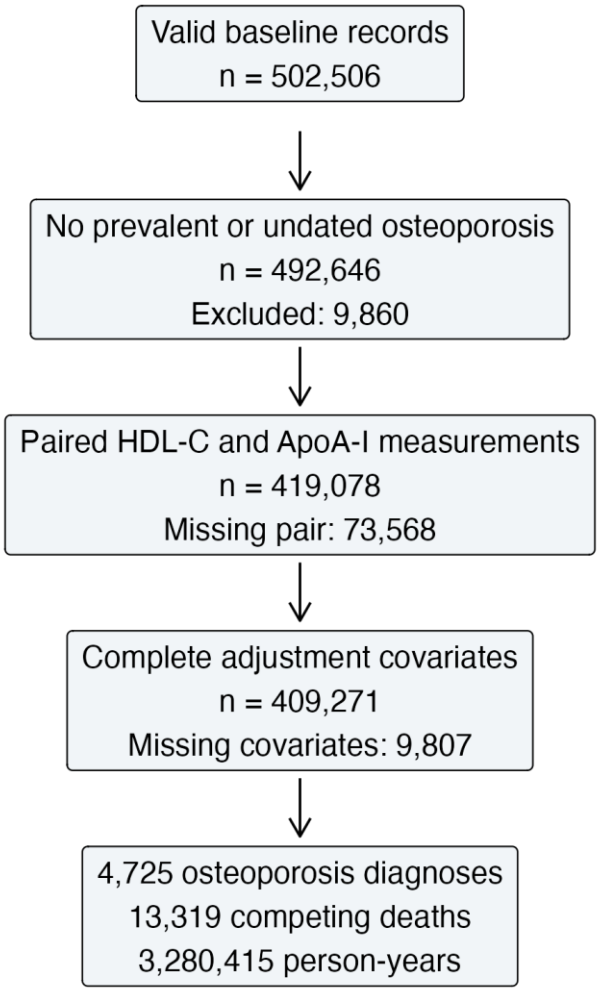
Table 4 Exploratory subgroup associations with osteoporosis

Subgroup	N / events	HDL-C HR	ApoA-I HR	Joint P	Joint q
Men	191,313 / 853	1.81 (1.44-2.28)	0.66 (0.54-0.81)	0.016	0.023
Women	217,958 / 3,872	1.28 (1.16-1.41)	0.81 (0.74-0.88)	0.016	0.023
Age <60	233,864 / 1,469	1.40 (1.19-1.64)	0.78 (0.68-0.90)	0.130	0.130
Age >=60	175,407 / 3,256	1.32 (1.19-1.47)	0.79 (0.72-0.86)	0.130	0.130
BMI <25	133,359 / 2,021	1.29 (1.13-1.47)	0.78 (0.69-0.87)	<0.001	<0.001
BMI >=25	275,912 / 2,704	1.48 (1.30-1.67)	0.77 (0.69-0.85)	<0.001	<0.001

All specified strata shown. HRs per fixed baseline SD, both lipids in each model. Interaction tests jointly assess both lipid-by-subgroup terms (2 df); domain-level P and q repeated across strata. Benjamini-Hochberg correction restricted to three interaction tests; does not correct broader exploratory selection. Pooled tests assume common adjustment-covariate effects; separate strata allow them to differ. Full-period working estimates; proportional hazards not separately established per stratum.

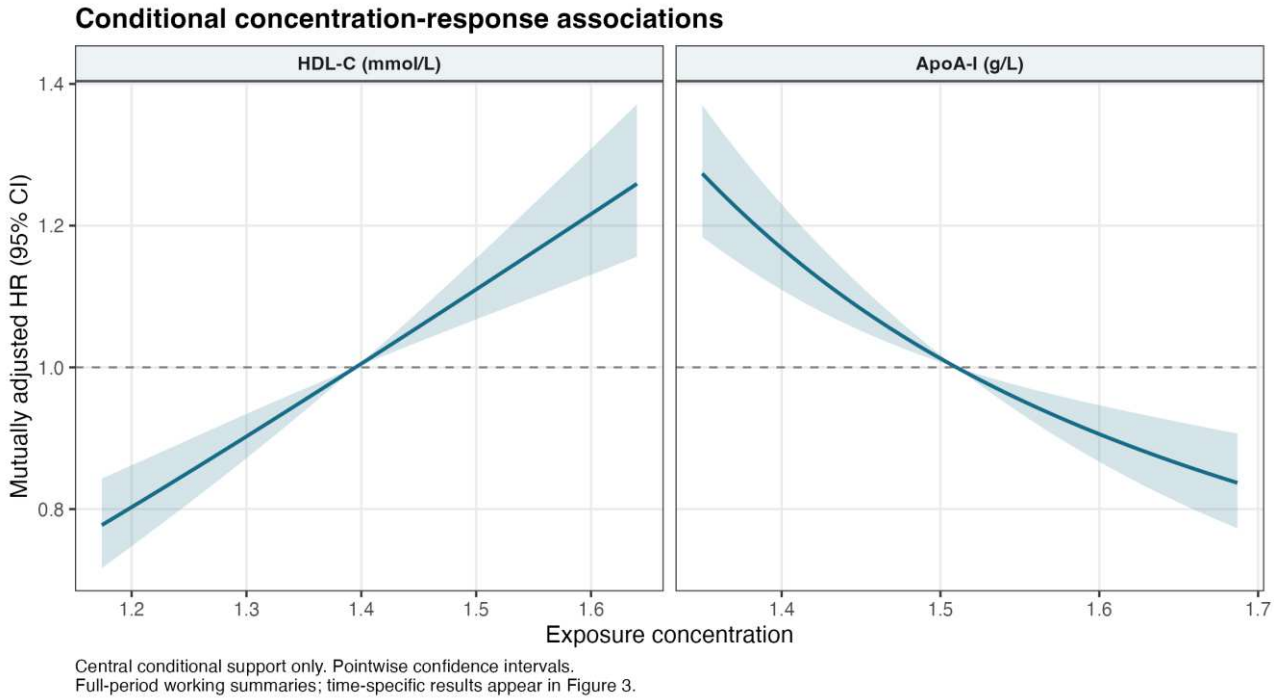
Figure 1 Osteoporosis cohort construction

Hospital-recorded osteoporosis cohort



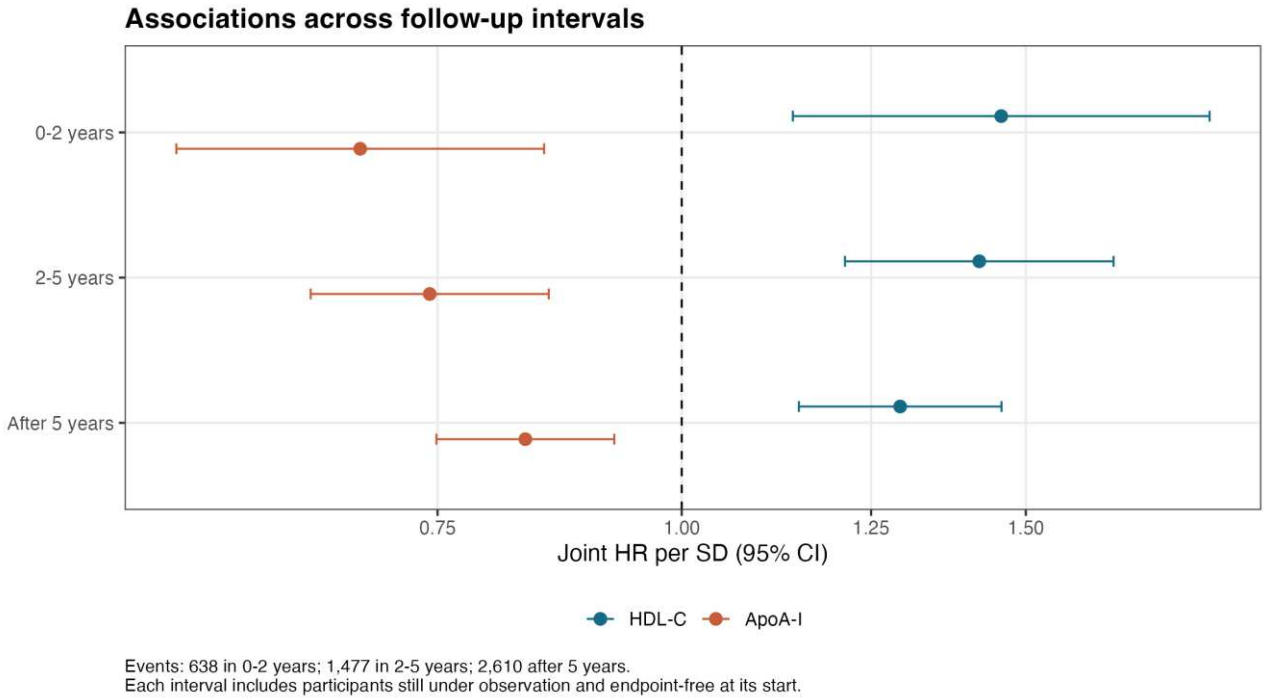
Excludes baseline self-reported osteoporosis and qualifying hospital diagnoses on/before baseline or undated. No eligible participant had nonpositive follow-up. Competing deaths before recorded osteoporosis endpoint. Administrative follow-up to 31 March 2017.

Figure 2 Conditional concentration response



Central conditional support; pointwise confidence intervals. Full-period working summaries; time-specific results in Figure 3. Both exposures use 3-df natural cubic splines with Model 2 covariates. Each contrast referenced to cohort median, other exposure fixed at median. Displayed 5th–95th percentile range conditional on other standardized exposure within 0.25 SD of its median. Model fitting uses full cohort. Bands pointwise 95% CIs. Nonlinearity P=0.023 HDL-C, P<0.001 ApoA-I. Curves working full-period summaries, not constant-time effects or evidence about unshown tails.

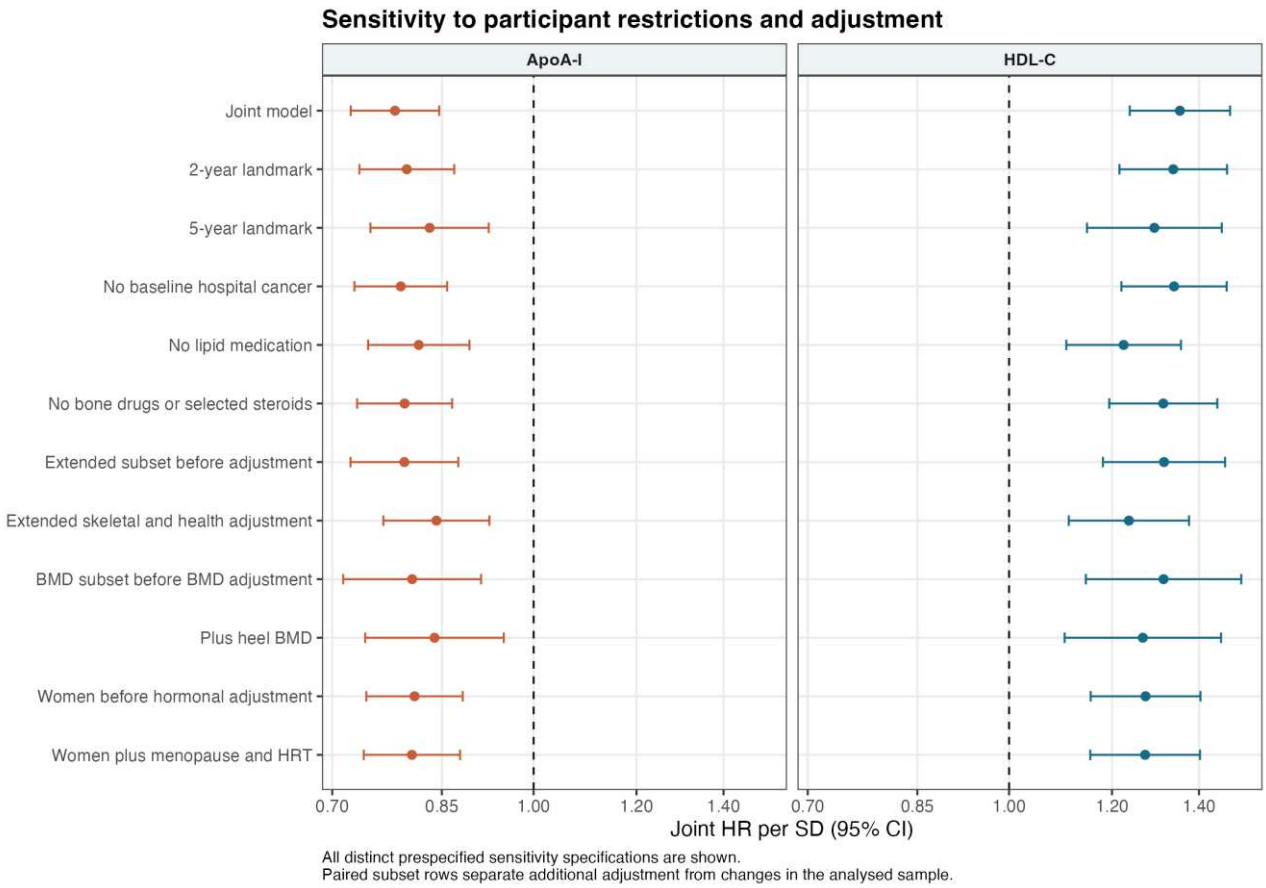
Figure 3 Associations across follow up intervals



Events: 638 in 0-2 years; 1,477 in 2-5 years; 2,610 after 5 years.
Each interval includes participants still under observation and endpoint-free at its start.

Mutually adjusted cause-specific HRs per SD, 95% CIs. Three risk sets include participants under observation and endpoint-free at 0, 2, 5 years. Exposure-specific PH tests: P=0.006 HDL-C, P=0.001 ApoA-I. Numerical attenuation over time descriptive; comparison of nominal significance not a heterogeneity test. Full counts/estimates in Supplementary Table S4.

Figure 4 Sensitivity analyses of the joint associations



Sensitivity to participant restrictions and adjustment. All distinct prespecified sensitivity specifications shown. Paired subset rows separate additional adjustment from changes in analysed sample. All distinct sensitivity specifications displayed, including paired models before/after additional adjustment. Forest plot corresponds to Table 3. Restriction to participants without recorded medication does not establish absence of treatment confounding. Persistence after heel-BMD adjustment does not establish independent mechanism or formal mediation effect.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Supplementary.docx