ESC European Society of Cardiology

European Heart Journal (2023) **44**, 1432–1445
https://doi.org/10.1093/eurheartj/ehac822

CLINICAL RESEARCH
Dyslipidaemias

Elevated remnant cholesterol, plasma triglycerides, and cardiovascular and non-cardiovascular mortality

Benjamin N. Wadström [1,2,3], Kasper M. Pedersen [1,2,3], Anders B. Wulff [1,2,3], and Børge G. Nordestgaard [1,2,3,4]*

[1]Department of Clinical Biochemistry, Herlev and Gentofte Hospital, Copenhagen University Hospital, Borgmester Ib Juuls Vej 73, elevator 7, DK-2730, Herlev, Denmark; [2]The Copenhagen General Population Study, Herlev and Gentofte Hospital, Copenhagen University Hospital, Herlev, Denmark; [3]Department of Clinical Medicine, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark; and [4]The Copenhagen City Heart Study, Frederiksberg Hospital, Copenhagen University Hospital, Frederiksberg, Denmark

Received 10 May 2022; revised 29 November 2022; accepted 23 December 2022; online publish-ahead-of-print 12 January 2023

See the editorial comment for this article 'What is 'remnant cholesterol'?', by P.E. Stürzebecher *et al.*, https://doi.org/10.1093/eurheartj/ehac783.

Abstract

Aims Cholesterol carried in triglyceride-rich lipoproteins, also called remnant cholesterol, is being increasingly acknowledged as an important causal risk factor for atherosclerosis. Elevated remnant cholesterol, marked by elevated plasma triglycerides, is associated causally with an increased risk of atherosclerotic cardiovascular disease. The association with cause-specific mortality is, however, unclear. The aim of this study was to test the hypothesis that elevated remnant cholesterol and plasma triglycerides are associated with increased mortality from cardiovascular disease, cancer, and other causes.

Methods and results Using a contemporary population-based cohort, 87 192 individuals from the Copenhagen General Population Study aged 20–69 years at baseline in 2003–2015 were included. During up to 13 years of follow-up, 687 individuals died from cardiovascular disease, 1594 from cancer, and 856 from other causes, according to the National Danish Causes of Death Registry. In individuals with remnant cholesterol ≥1.0 mmol/L (≥39 mg/dL; 22% of the population) compared with those with levels <0.5 mmol/L (<19 mg/dL), multivariable-adjusted mortality hazard ratios were 2.2 (95% confidence interval 1.3–3.5) for cardiovascular disease, 1.0 (0.7–1.3) for cancer, and 2.1 (1.4–3.3) for other causes. Exploratory analysis of the cause of death subcategories showed corresponding hazard ratios of 4.4 (1.6–11) for ischemic heart disease, 8.4 (2.0–34) for infectious diseases, and 9.1 (1.9–43) for endocrinological diseases. Results for plasma triglycerides >2 vs. <1 mmol/L (>177 vs. <89 mg/dL) were similar.

Conclusion Remnant cholesterol of ≥1 mmol/L (39 mg/dL), present in 22% of the population, and plasma triglycerides of ≥2 mmol/L (177 mg/dL), present in 28% of the population, were associated with two-fold mortality from cardiovascular and other causes, but not from cancer. This novel finding should be confirmed in other cohorts.

* Corresponding author. Tel: +45 3868 3297, Email: boerge.nordestgaard@regionh.dk

Structured Graphical Abstract

Key Question

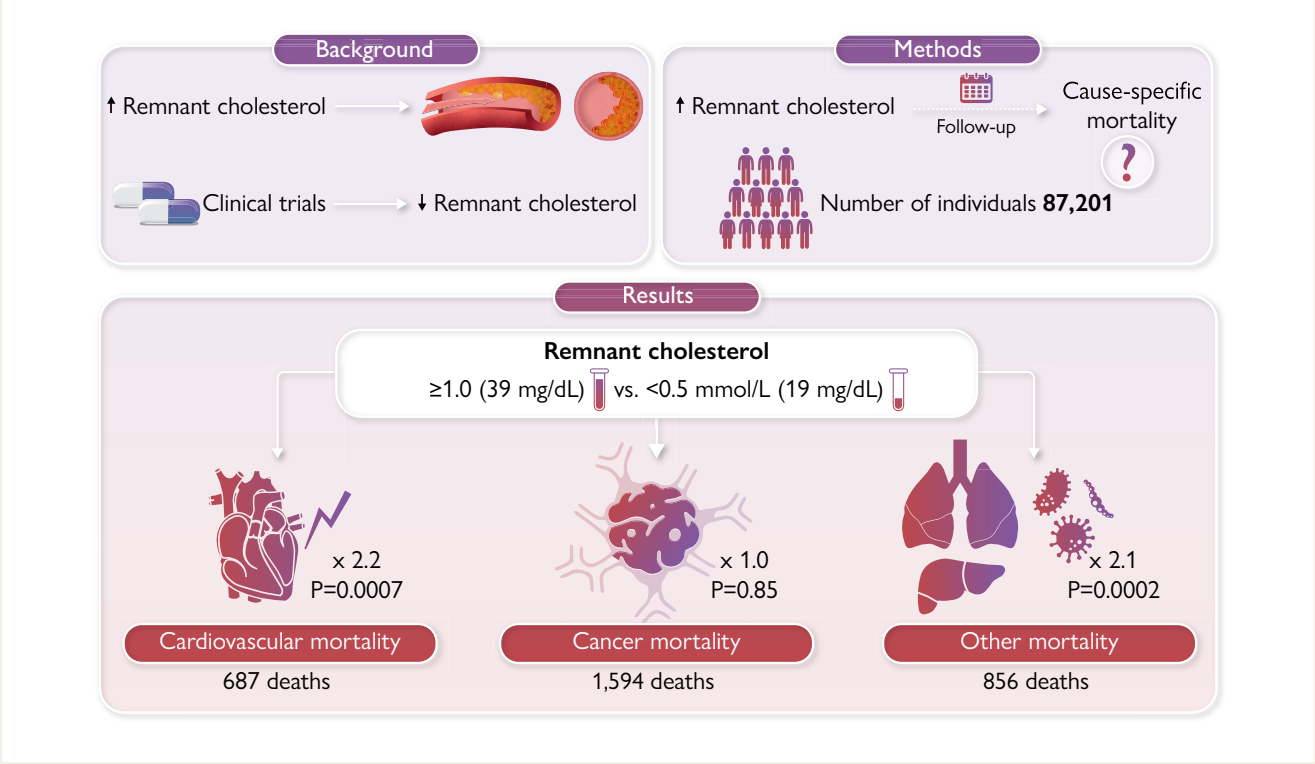
Is cholesterol carried in triglyceride-rich lipoproteins, also called remnant cholesterol, associated with increased mortality from cardiovascular disease, cancer, and other causes?

Key Finding

Remnant cholesterol above 1 mmol/L (39 mg/dL), observed in 22% of the population, was associated with 2-fold mortality from cardiovascular and other causes, but not from cancer.

Take Home Message

Large randomized trials should investigate if remnant cholesterol-lowering therapy without increases in LDL cholesterol or apolipoprotein B reduces all-cause and cause-specific mortality in addition to atherosclerotic cardiovascular disease.



The previously unknown association between elevated remnant cholesterol and cause-specific mortality was investigated in the Copenhagen General Population Study. Results are hazard ratios from Cox regression adjusted for age, sex, LDL cholesterol, systolic blood pressure, smoking status, cumulative smoking, time since last meal, and birth year. LDL, low-density lipoprotein; No., number; vs., versus. Parts of the figure were drawn using pictures from Servier Medical Art. Servier Medical Art by Servier is licensed under a Creative Commons Attribution 3.0 Unported License.

Keywords Cause-specific mortality • Low-density lipoprotein • Triglyceride-rich lipoprotein • Very-low-density lipoprotein • Intermediate-density lipoprotein • Atherosclerosis

Introduction

Remnant cholesterol, also called triglyceride-rich lipoprotein cholesterol, comprises cholesterol carried in very-low-density lipoproteins and chylomicron remnants. Alongside low-density lipoprotein (LDL) cholesterol, remnant cholesterol is emerging as a causal risk factor in the development of atherosclerotic cardiovascular disease.[1]

Drugs that lower remnant cholesterol (or plasma triglycerides, a commonly used marker for remnant cholesterol) are currently being tested for the prevention of atherosclerotic cardiovascular disease in large randomized controlled trials. REDUCE-IT (Reduction of Cardiovascular Events With Icosapent Ethyl–Intervention Trial) found that a purified omega-3 fatty acid, compared with mineral oil, decreased the risk of major ischemic events after lowering triglycerides by 20%.[2] Meanwhile, the somewhat similar STRENGTH (Long-Term Outcomes Study to Assess STatin Residual Risk Reduction With EpaNova in HiGh CV Risk PatienTs With Hypertriglyceridemia) was terminated due to futility despite 19% triglyceride reduction.[3] Also, the PROMINENT (Pemafibrate to Reduce Cardiovascular OutcoMes by Reducing Triglycerides IN patiENts With diabesTes) trial, testing a selective

peroxisome proliferator-activated receptor α modulator (SPPARMα) was also recently terminated due to futility despite a 26% reduction in plasma triglycerides.[4]

Finally, several other drug companies are considering yet other drugs with the aim of reducing atherosclerotic cardiovascular disease through the reduction of plasma triglycerides, triglyceride-rich lipoproteins, and remnant cholesterol, including inhibition of apolipoprotein C3 and angiopoietin-related protein 3.[5] It is thus possible that therapies for lowering remnant cholesterol and plasma triglycerides could become increasingly clinically available, and associations of elevated remnant cholesterol and plasma triglycerides with cause-specific mortality could potentially suggest important new indications and/or contraindications for remnant cholesterol and plasma triglyceride-lowering therapy.

It is unknown whether elevated remnant cholesterol and plasma triglycerides are associated with increased mortality from cardiovascular disease, cancer, and/or other diseases. While an association between elevated remnant cholesterol and plasma triglycerides with increased cardiovascular disease mortality seems likely, an association with cancer mortality is also possible, as elevated levels of triglyceride-rich lipoproteins have been causally associated with decreased risk of breast cancer.[6] Furthermore, triglyceride-rich lipoprotein levels may also be causally associated with several non-cardiovascular and non-cancer diseases.[7]

Using the Copenhagen General Population Study, we tested the hypothesis that elevated remnant cholesterol and plasma triglycerides are associated with increased mortality from cardiovascular disease, cancer, and other causes.

Methods

Study cohorts

We included 87 192 individuals aged 20–69 years from the contemporary Copenhagen General Population Study as the primary cohort and as secondary cohort 11 743 individuals aged 20–69 years from the historic Copenhagen City Heart Study, the latter to yield absolute incidences for a high-risk population without statin use at baseline and with a high smoking prevalence.[8] Individuals aged 70–100 years were excluded due to violation of the proportional hazards assumption (further explained in the statistical analyses section). No individual appeared in both studies. Both studies were approved by local institutional review boards and Danish ethical committees (H-KF-01–144/01 and KF-100.2039/91) and were conducted according to the Declaration of Helsinki. All participants signed written informed consent. The Danish Data Protection Agency does not allow open access to our data; however, upon reasonable request to the corresponding author, additional analyses can be performed.

The Copenhagen General Population Study is an ongoing prospective cohort initiated in 2003–15, consisting of individuals aged 20–100 years. Individuals were selected and invited from the Danish Civil Registration System to reflect the adult White Danish general population. At baseline, participants completed a questionnaire, underwent a physical examination, and had blood drawn for biochemical analyses.

The Copenhagen City Heart Study is an ongoing prospective study of the adult White general population initiated in 1976–78 (see Supplementary material online, *Methods*). The recruitment procedure and baseline examinations were like those of the Copenhagen General Population study.

Endpoints

Information on death was obtained from the Danish Civil Registration System, which records vital status for all individuals living in Denmark. Information on cause of death was obtained from the National Danish Register of Causes of Death, based on mandatory reporting of death certificates by the individuals' general practitioner, attending hospital physician, or following autopsy.[9] Cause of death was classified into three major categories. If one of the three first-ranked causes of death were a cardiovascular disease diagnosis (ICD-8: 3900–4589, 7820–7829, ICD-10: I00–I99), the death was classified as death from cardiovascular disease. Remaining deaths were classified as death from cancer if any of the three first-ranked causes was a cancer diagnosis (ICD-8: 1400–2399, ICD-10: C00–C99), and all other deaths were classified as death from other causes (see Supplementary material online, *Table S1*, *Figure S1*).

Information on vital status was available until the end of 2018, and information on the cause of death until the end of 2016. By using the unique Central Person Registration number provided to everyone in Denmark at birth or immigration and linking it with the national registries, no person was lost to follow-up; individuals were followed until death, emigration ($n = 441$ for the Copenhagen General Population Study and $n = 59$ for the Copenhagen City Heart Study), or end of follow-up, whichever occurred first.

Laboratory methods

Non-fasting plasma triglycerides, total cholesterol, high-density lipoprotein (HDL) cholesterol, glucose, creatinine, high-sensitivity C-reactive protein, apolipoprotein B, and blood leukocytes were measured using standard hospital assays. LDL cholesterol was calculated using the Friedewald equation,[10] unless triglycerides were >4 mmol/L (352 mg/dL), where it was measured directly. Remnant cholesterol was calculated as total cholesterol minus LDL cholesterol minus HDL cholesterol in the Copenhagen General Population Study. This means that in individuals with triglycerides ≤4 mmol/L (352 mg/dL), remnant cholesterol was triglycerides (in mmol/L) divided by 2.2, as calculated by the Friedewald equation, while at triglycerides >4 mmol/L remnant cholesterol was total cholesterol minus measured LDL cholesterol minus measured HDL cholesterol. Remnant cholesterol was also calculated using the Martin–Hopkins equation[11] and the Sampson–NIH equation[12] for sensitivity analysis. In the Copenhagen City Heart Study, there were no measurements of LDL or HDL cholesterol at the baseline examination in 1976–78. Therefore, remnant cholesterol was calculated using the Friedewald equation. Directly measured remnant cholesterol was from the re-examination of the Copenhagen General Population Study and measured using a newly developed Denka (TRL-C) assay on standard hospital equipment.[13,14] Statin treatment is often given to individuals at high cardiovascular risk and on average decreases LDL cholesterol, remnant cholesterol, and plasma triglycerides by approximately 40, 20, and 20%, respectively.[15,16] To correct for this, LDL cholesterol values of individuals taking statins at baseline were divided by 0.6, and remnant cholesterol and plasma triglyceride values by 0.8. The estimated glomerular filtration rate was calculated from creatinine, sex, and age using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation.[17]

Other covariates

Smoking, medication, time since last meal, diet, alcohol intake, income, and education were self-reported. Systolic blood pressure and weight and height for calculation of body mass index were measured at the baseline examination. The use of lipid-lowering drugs was mainly statins (see Supplementary material online, *Table S2*) and only 3% of individuals not reporting the use of lipid-lowering drugs had lipid-lowering drugs prescribed before baseline in data based on all individuals in the Copenhagen General Population Study (see Supplementary material online, *Table S3*). Time since last meal was hours since blood sampling categorized as 0–1, 1–2, 2–3, 3–4, 4–5, 5–6, 6–7, 7–8, or >8 h. Fasting was >8 h. Impaired kidney function was estimated glomerular filtration rate <60 mL/min/1.73 m^2. Income was yearly household income recorded in six brackets. High household income was the two highest brackets. Education was ranging from 0 to ≥14 years of school attendance. Higher education was ≥13 years. Atherosclerotic cardiovascular disease was ischemic heart disease or ischemic stroke in the national Danish Patient Registry, and diabetes was either a diagnosis in the

national Danish Patient Registry or diagnosed at the baseline examination (see Supplementary material online, *Methods*). High dietary guideline adherence was determined according to a food frequency questionnaire.[18] Cancer diagnoses were obtained from the national Danish Cancer Registry, in addition to the national Danish Patient Registry, according to ICD-7 or ICD-10. Cancer was defined as the diagnosis of any cancer, except for non-melanoma skin cancer.

Statistical analyses

We used R, version 3.6.1. For more details on statistical analyses, please see Supplementary material online, *Methods*.

The proportional hazards assumption for Cox regression and Fine and Gray competing risk regression was examined using Schoenfeld residuals and log(time) plots. For all-cause mortality, cardiovascular mortality, and other mortality, there were violations of proportional hazards. However, after excluding individuals aged 70–100 years, there were no violations. This well-known phenomenon[19] led us to focus on results for those aged 20–69 years; however, we also tested the associations in individuals aged 70–100 years.

Associations of remnant cholesterol and plasma triglycerides on continuous scales with all-cause and cause-specific mortality were plotted using Cox regression with restricted cubic splines. The associations of LDL cholesterol with all-cause mortality, and of non-HDL cholesterol, apolipoprotein B, and LDL cholesterol with cause-specific mortality were plotted for comparison. The numbers of knots were chosen according to Akaike's information criterion and placed at default locations.

Entry age-adjusted age (age with left truncation, also termed delayed entry) was used as a timescale in all prospective analyses. Left truncation adjusts for age at baseline (entry age) in a non-parametric way (i.e. does not require statistical modeling) and eliminates survival-time bias from individuals entering the study.[20] It, therefore, yields less biased probabilities of survival and is considered the most appropriate model for epidemiological studies;[21] however, if the time on the study used was the timescale with age adjustment at the baseline, results were similar to those reported.

Cumulative mortality curves using the Aalen–Johansen estimator were plotted according to clinically relevant remnant cholesterol and plasma triglyceride groups. Deaths from the two remaining causes were used as competing risks for cause-specific mortality. Using identical groups, Cox regressions were done for all-cause and cause-specific mortality.

Multivariable adjustment was done for the potential confounders age, sex, systolic blood pressure, smoking status, cumulative smoking, time since last meal, birth year, and LDL cholesterol (for remnant cholesterol and plasma triglyceride analyses) or remnant cholesterol (for LDL cholesterol analyses). Variables located within the biological pathway from elevated remnant cholesterol and plasma triglycerides to mortality, i.e. body mass index, waist circumference, diabetes, statin use, atherosclerotic cardiovascular disease, adherence to dietary guidelines, and alcohol consumption were purposely not adjusted for in main analyses. Information on covariates was 99.5% complete and missing values were imputed using a single imputation based on predictive mean matching; however, results were similar in complete case analyses.

Interactions between continuous remnant cholesterol and plasma triglycerides and covariates on mortality endpoints were tested for by introducing two-factor interaction terms. Remnant cholesterol and plasma triglyceride groups were numerically ordered, and a χ^2 Wald test was used to yield *P*-values for trend and interaction. Multivariable adjusted cumulative mortalities at age 75 years from cardiovascular disease, cancer, and other causes were each estimated using a Fine and Gray competing risk model.

Random measurement error biases regression coefficients toward lower estimates (=regression dilution bias). Correction for measurement error was done in all analyses using regression calibration,[22] except for the interaction analyses, as the measurement error may not be the same in different subgroups. The regression calibration was done using two repeat measurements 10 years apart from the Copenhagen City Heart Study.

Sensitivity analyses were done to test for the impact of (i) using time on study as a timescale, (ii) adjustment for variables within the biological pathway, (iii) adjustment for comorbidities, (iv) competing risk, by using Fine and Gray competing risk regression, (v) not correcting for measurement error, (vi) reverse causation, by excluding individuals with <1–5 years of follow-up, (vii) using the Martin–Hopkins and the Sampson–NIH remnant cholesterol equations, (viii) remnant cholesterol and LDL cholesterol values half corrected and uncorrected for statin use, (ix) adjustment for HDL cholesterol, and (x) only using first-ranked cause of death. For other mortality, additional sensitivity analyses were done (i) censoring the cause of death subcategories 'unclassified' and 'remaining' and (ii) with additional adjustment for atherosclerotic cardiovascular disease either before baseline or during follow-up.

Results

Among 87 192 individuals from the Copenhagen General Population Study followed for up to 15 years (median 9.7 years), 4252 died. Individuals were followed for up to 13 years (median 7.9 years) for cause-specific mortality, during which time 687 died from cardiovascular causes, 1594 from cancer, and 856 from other causes. Baseline characteristics according to remnant cholesterol levels are shown in *Table 1*, according to plasma triglyceride levels in Supplementary material online, *Table S4*, and according to the cause of death in Supplementary material online, *Table S5*. In the re-examination of 23 220 individuals in the Copenhagen General Population Study, calculated remnant cholesterol as used in this study was highly positively correlated to directly measured remnant cholesterol (see Supplementary material online, *Figure S2*).

Remnant cholesterol and plasma triglycerides on continuous scales

Higher levels of remnant cholesterol and plasma triglycerides were associated with increased all-cause mortality (*Figure 1*), while LDL cholesterol had a U-shaped association with all-cause mortality, as reported previously.[23] For cause-specific mortality, higher levels of remnant cholesterol and plasma triglycerides were associated with increased cardiovascular and other mortality, whereas no association with cancer mortality was observed (*Figure 2*). In contrast, associations for non-HDL cholesterol, apolipoprotein B, and LDL cholesterol with cause-specific mortality were U-shaped (see Supplementary material online, *Figure S3*), analogous to previously reported corresponding associations for LDL cholesterol.[23]

Clinically relevant groups according to remnant cholesterol and plasma triglyceride levels

Groups with incrementally higher levels of remnant cholesterol and plasma triglycerides had stepwise higher cumulative all-cause, cardiovascular, and other mortality, while there was no convincing evidence that similar incrementally higher levels were associated with higher cumulative mortality from cancer (*Figure 3*). The cumulative all-cause mortality at age 75 years was 12.6% for individuals with remnant cholesterol <0.5 mmol/L (<19 mg/dL), 14.3% for 0.5–0.99 mmol/L (19–38 mg/dL), 16.5% for 1–1.49 mmol/L (39–58 mg/dL), and 20.4% for individuals with remnant cholesterol ≥1.5 mmol/L (≥58 mg/dL). The corresponding cumulative mortalities were 2.5, 3.4, 3.5, and 5.8% for cardiovascular mortality, 7.2, 7.4, 7.6, and 9.6% for cancer mortality, and 3.4, 4.1, 5.1, and 5.8% for other mortality, respectively. For individuals with plasma triglycerides levels <1 mmol/L (89 mg/dL), 1–1.99 mmol/L (89–176 mg/dL), 2–2.99 mmol/L (177–265 mg/dL),

Table 1 Baseline characteristics of individuals from the Copenhagen General Population Study

	Remnant cholesterol, mmol/L (mg/dL)			
	<0.5 (<19)	0.5–0.99 (19–38.9)	1.0–1.49 (39–57.9)	≥1.5 (≥58)
Number of individuals	30 236	37 852	13 134	5970
Men	8903 (29%)	17 437 (46%)	8052 (61%)	4185 (70%)
Age, years	51 (44–60)	55 (47–63)	56 (49–63)	56 (48–63)
Remnant cholesterol, mmol/L	0.4 (0.3–0.4)	0.7 (0.6–0.8)	1.2 (1.1–1.3)	1.7 (1.6–2.0)
Remnant cholesterol, mg/dL	15 (12–17)	27 (23–31)	46 (42–51)	67 (62–78)
Plasma triglycerides, mmol/L	0.8 (0.7–1.0)	1.5 (1.3–1.8)	2.7 (2.4–3.0)	4.1 (3.6–5.2)
Plasma triglycerides, mg/dL	74 (61–86)	135 (114–161)	236 (213–266)	360 (321–461)
LDL cholesterol, mmol/L	3.0 (2.4–3.5)	3.5 (2.9–4.1)	3.7 (3.0–4.3)	3.6 (3.0–4.4)
LDL cholesterol, mg/dL	115 (94–135)	135 (112–159)	142 (116–168)	140 (116–170)
Current smokers	4089 (14%)	7047 (19%)	2928 (22%)	1657 (28%)
Cumulative smoking, pack-years	0 (0–11)	3 (0–18)	7 (0–25)	13 (0–30)
Systolic blood pressure, mmHg	132 (120–146)	138 (125–152)	142 (130–156)	144 (132–158)
Fasting	384 (1%)	320 (1%)	96 (1%)	43 (1%)
Within biological pathway				
Body mass index, kg/m^2	24 (22–26)	26 (24–29)	28 (25–30)	29 (26–32)
Waist circumference, cm	81 (74–89)	90 (82–98)	96 (89–104)	100 (93–108)
Diabetes	463 (2%)	1109 (3%)	766 (6%)	591 (10%)
Statin use[a]	1084 (4%)	3325 (9%)	1956 (15%)	1473 (25%)
ASCVD	776 (3%)	1668 (4%)	879 (7%)	529 (9%)
High dietary guideline adherence	9559 (32%)	10 496 (28%)	2913 (22%)	1166 (20%)
Alcohol, g/week	84 (36–156)	96 (36–180)	108 (48–204)	120 (48–228)
Biomarkers and lipids				
hsCRP, mg/L	1.2 (0.7–1.6)	1.4 (0.9–2.2)	1.6 (1.1–2.7)	1.7 (1.2–2.9)
Leukocytes, 10^9/L	6.6 (5.7–7.7)	7.0 (6.0–8.2)	7.4 (6.3–8.6)	7.6 (6.5–8.9)
eGFR, mL/min/1.73 m^2	85 (75–95)	82 (72–93)	82 (72–92)	83 (73–93)
Non-HDL cholesterol, mmol/L	3.3 (2.8–3.9)	4.2 (3.6–4.9)	4.9 (4.3–5.6)	5.6 (4.9–6.5)
Non-HDL cholesterol, mg/dL	129 (108–152)	162 (138–188)	190 (164–218)	218 (190–252)
Apolipoprotein B, mg/dL	85 (73–99)	109 (94–126)	135 (117–156)	164 (139–192)
Comorbidities and socioeconomic factors				
Cancer	1260 (4%)	1894 (5%)	688 (5%)	316 (5%)
Impaired kidney function	982 (3%)	2058 (5%)	788 (6%)	351 (6%)
High household income	17 080 (56%)	18 671 (49%)	6011 (46%)	2503 (42%)
Higher education	8006 (26%)	7899 (21%)	2352 (18%)	926 (16%)

Values are median (interquartile range) for continuous variables and *number* (%) for categorical variables. ASCVD, atherosclerotic cardiovascular disease, defined as ischemic heart disease or ischemic stroke at baseline; eGFR, estimated glomerular filtration rate; hsCRP, high-sensitivity C-reactive protein; HDL, high-density lipoprotein; LDL, low-density lipoprotein.
[a]Statin use indicates the use of lipid-lowering drugs, which were mostly statins.

and ≥3 mmol/L (≥266 mg/dL), corresponding cumulative mortalities were 12.5, 13.8, 16.2, and 19.3 for all-cause mortality, 2.4, 3.0, 3.8, and 6.3% for cardiovascular mortality, 7.2, 7.3, 7.9, and 8.2% for cancer mortality, and 3.6, 3.7, 4.2, and 6.4% for other mortality, respectively.

After multivariable adjustment, there was stepwise higher mortality in groups with higher levels of remnant cholesterol for all-cause, cardiovascular, and other mortality (*Figure 4*); however, there was no association with cancer mortality. For plasma triglycerides, results were

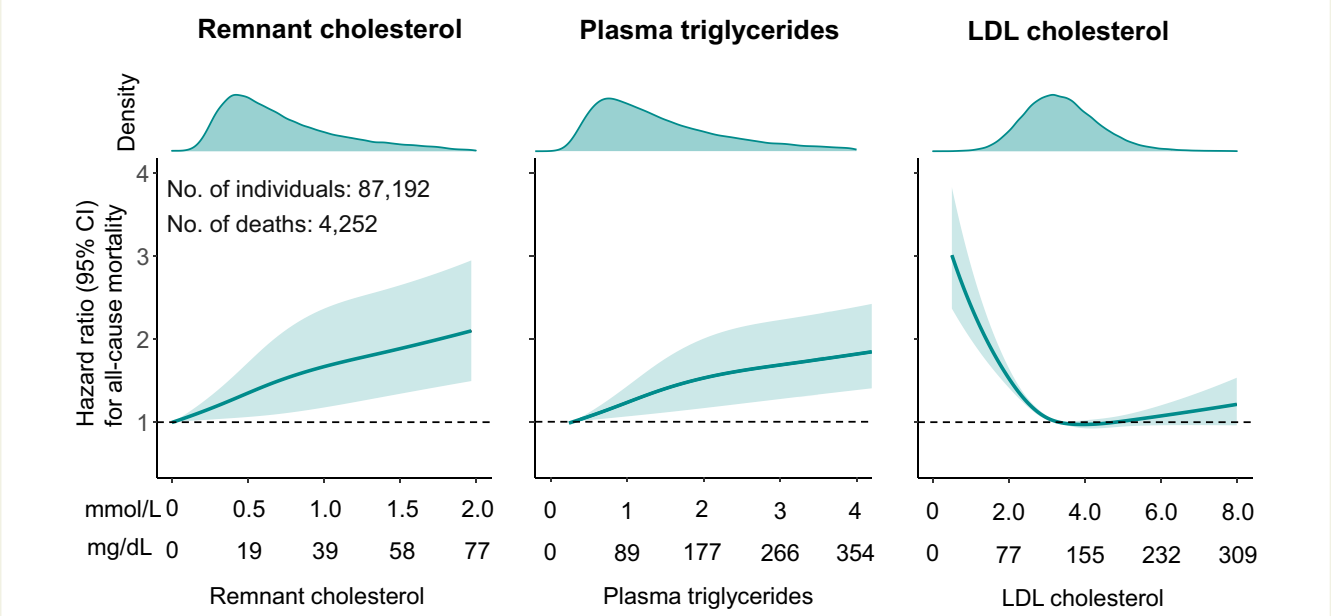


Figure 1 All-cause mortality as functions of remnant cholesterol, plasma triglycerides, and LDL cholesterol on continuous scales in the Copenhagen General Population Study. Cox regression using restricted cubic splines: when the shaded area for the 95% CI no longer touches the reference hazard ratio 1.0 (dotted black line), hazard ratios are significantly increased. Adjusted for age, sex, LDL cholesterol (remnant cholesterol and plasma triglyceride analyses) or remnant cholesterol (LDL cholesterol analyses), smoking status, cumulative smoking in pack-years, systolic blood pressure, time since last meal, and birth year. The reference was 0.01 mmol/L (4 mg/dL) for remnant cholesterol, 0.30 mmol/L (27 mg/dL) for plasma triglycerides, and the median value of 3.4 mmol/L (131 mg/dL) for LDL cholesterol. CI, confidence interval; LDL, low-density lipoprotein; No., number.

similar, but other mortality was only markedly increased in the group with ≥3 mmol/L (≥266 mg/dL). Multivariable adjusted hazard ratios for individuals with remnant cholesterol ≥1.5 mmol/L (≥58 mg/dL) compared with individuals with remnant cholesterol <0.5 mmol/L (<19 mg/dL) were 1.5 (95% confidence interval: 1.2–2.0) for all-cause mortality, 2.6 (1.4–4.7) for cardiovascular mortality, 1.1 (0.7–1.6) for cancer mortality, and 2.4 (1.4–4.2) for other mortality (*Figure 4*). For individuals with plasma triglycerides ≥3 mmol/L (≥266 mg/dL) compared with those with plasma triglycerides <1 mmol/L (<89 mg/dL), corresponding hazard ratios were 1.6 (95% confidence interval: 1.2–2.0) for all-cause mortality, 2.4 (1.4–4.3) for cardiovascular mortality, 1.1 (0.7–1.5) for cancer mortality, and 2.5 (1.5–4.1) for other mortality (*Figure 4*). For individuals with remnant cholesterol ≥1 mmol/L (≥39 mg/dL; 22% of the population) compared with individuals with remnant cholesterol <0.5 mmol/L (<19 mg/dL), corresponding hazard ratios were 1.3 (1.1–1.6), 2.2 (1.3–3.5), 1.0 (0.7–1.34), and 2.1 (1.4–3.3), respectively (*Figure 5*). Results for individuals with plasma triglycerides ≥2 mmol/L (≥177 mg/dL; 28% of the population) compared with individuals with plasma triglycerides <1 mmol/L (<89 mg/dL) were similar (see Supplementary material online, *Figure S4*). In individuals aged 70 years or older, there were no associations between remnant cholesterol groups and all-cause or cause-specific mortality (see Supplementary material online, *Figure S5*).

In subcategory analyses taking 19 multiple comparisons into account (Bonferroni correction: $P = 0.05/19 = 0.003$), elevated remnant cholesterol was associated with increased mortality from ischemic heart disease and infectious diseases at $P < 0.05$, while there was a borderline association with increased mortality from endocrinological diseases (*Figure 5*). Multivariable adjusted hazard ratios were 4.4 (1.6–11) for ischemic heart disease, 8.4 (2.0–34) for infectious diseases, and 9.1 (1.9–43) for endocrinological diseases. Elevated plasma triglycerides were not associated with increased mortality from any individual subcategory after Bonferroni correction for multiple comparisons (see Supplementary material online, *Figure S4*).

Sensitivity analyses

Results were consistent across different strata (*Figure 6* and Supplementary material online, *Figure S6*), with no convincing statistical evidence of interaction after taking multiple comparisons into account. Results were similar when using time on study as timescale, when using Fine and Gray competing risk regression (see Supplementary material online, *Figure S7*), when excluding individuals with <1–5 years of follow-up (see Supplementary material online, *Figure S8*), when adjusting for alcohol consumption and diet, income, or education (see Supplementary material online, *Figure S9*), and when adjusting for cancer or impaired kidney function (see Supplementary material online, *Figure S10*). Results lost statistical significance when additionally adjusting for variables in the biological pathway (see Supplementary material online, *Figure S7*); results were attenuated after individual adjustment for body mass index, diabetes, and statin use (see Supplementary material online, *Figure S11*), and lost significance after adjustment for waist circumference (see Supplementary material online, *Figure S9*). Results also lost significance when adjusting for HDL cholesterol (see Supplementary material online, *Figure S12*), which is a marker of average remnant cholesterol over time.[24] Cardiovascular mortality results were attenuated when only using the first-ranked cause of death (see Supplementary material online, *Figure S13*). When using remnant cholesterol and LDL cholesterol values with half the correction or uncorrected for statin use, results were similar (see Supplementary

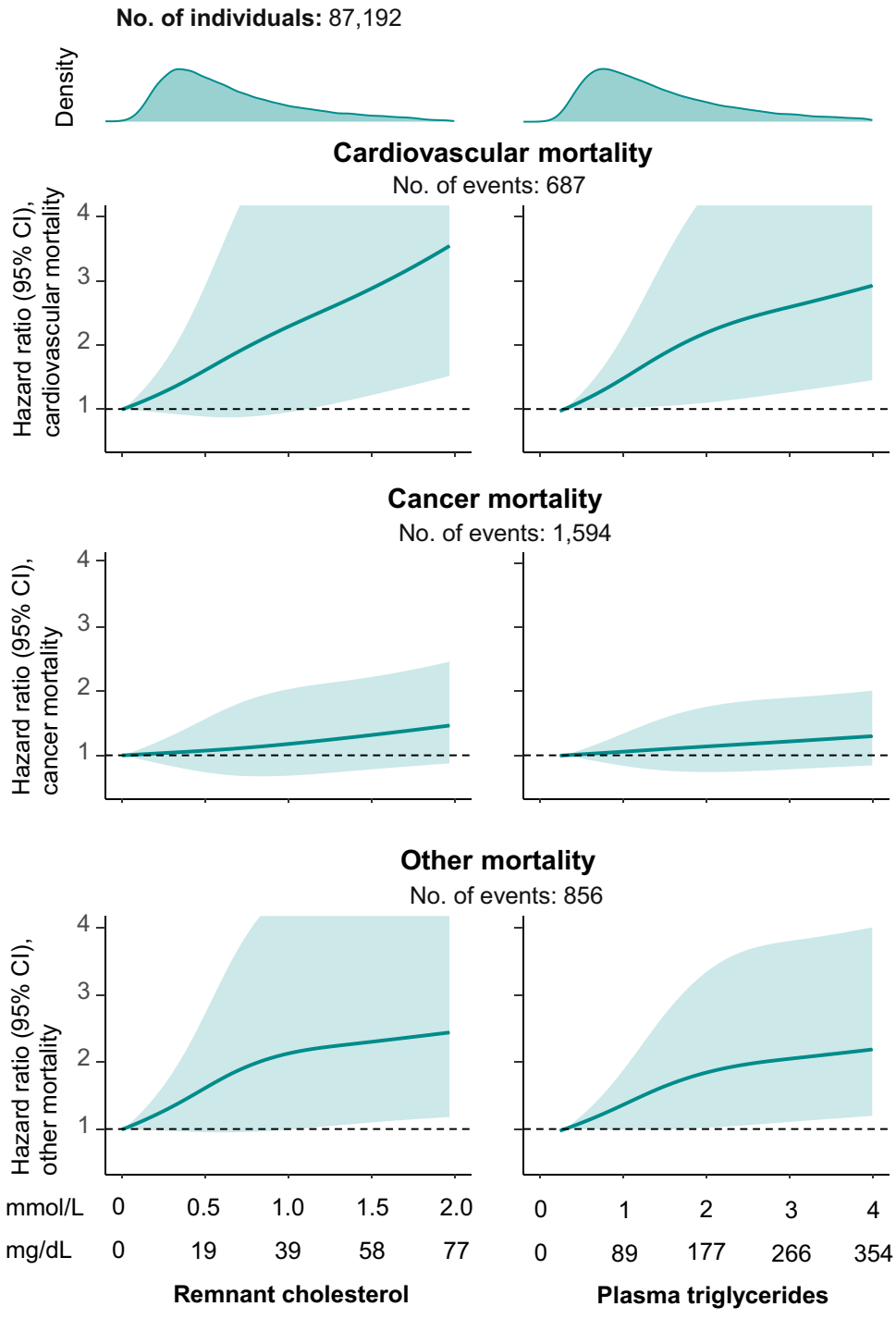


Figure 2 Cause-specific mortality as functions of remnant cholesterol and plasma triglycerides on continuous scales in the Copenhagen General Population Study. Cox regression using restricted cubic splines: when the shaded area for the 95% CI no longer touches the reference hazard ratio 1.0 (dotted black line), hazard ratios are significantly increased. Adjusted for age, sex, LDL (low-density lipoprotein) cholesterol, smoking status, cumulative smoking in pack-years, systolic blood pressure, time since last meal, and birth year. The reference was 0.01 mmol/L (4 mg/dL) for remnant cholesterol and 0.30 mmol/L (27 mg/dL) for plasma triglycerides. CI, confidence interval; No., number.

material online, *Figure S14*), and when using the Martin–Hopkins or the Sampson–NIH equations for remnant cholesterol and LDL cholesterol calculations, associations were slightly strengthened (see Supplementary material online, *Figure S15*). For other mortality, estimates were similar also when censoring unclassified and uncommon cause of death subcategories and when adjusting for an atherosclerotic cardiovascular disease before baseline or during follow-up (see Supplementary material online, *Figure S16*).

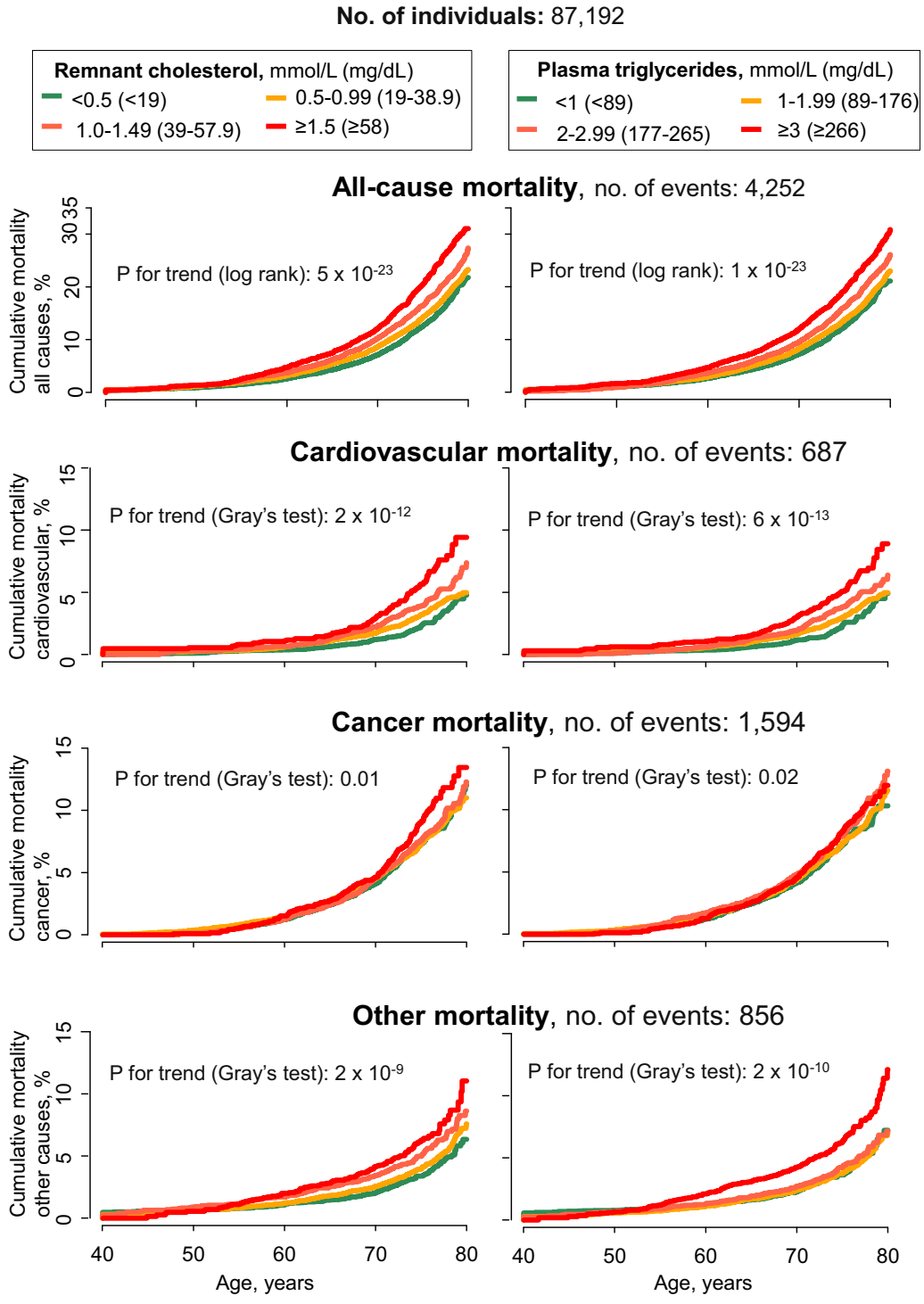


Figure 3 Cumulative all-cause and cause-specific mortality according to higher remnant cholesterol and plasma triglycerides in clinically relevant groups in the Copenhagen General Population Study. Modeled with Aalen-Johansen estimator, where death from the two remaining causes was competing risks in the estimation of cause-specific mortalities. All-cause mortality had follow-up until the end of 2018, while remaining cumulative mortalities had follow-up until the end of 2016. No., number.

Cumulative mortality

Groups with incrementally higher levels of remnant cholesterol had stepwise higher multivariable adjusted estimated cumulative mortality at age 75 from cardiovascular and other causes, but only a slight irregular trend toward higher cumulative cancer mortality (*Figure 7* and Supplementary material online, *Figure S17*). This was independently confirmed in the Copenhagen City Heart Study with individuals examined in 1976–78, though with a clearer trend for cancer mortality and

Cause of death	mmol/L	mg/dL	No. of individuals	No. of events	No. of events per 10,000 person-years	Multivariable adjusted hazard ratio (95% CI)	P for trend
	Remnant cholesterol						
All causes	<0.5	<19	30,236	1,021	36	1.0	
	0.5-0.99	19-38.9	37,852	1,896	52	1.1 (1.0-1.3)	0.0001
	1.0-1.49	39-57.9	13,134	860	67	1.3 (1.1-1.6)	
	≥1.5	≥58	5,970	475	80	1.5 (1.2-2.0)	
Cardiovascular	<0.5	<19	30,236	134	6	1.0	
	0.5-0.99	19-38.9	37,852	298	10	1.4 (0.9-2.1)	0.0002
	1.0-1.49	39-57.9	13,134	161	16	2.0 (1.2-3.4)	
	≥1.5	≥58	5,970	94	20	2.6 (1.4-4.7)	
Cancer	<0.5	<19	30,236	427	19	1.0	
	0.5-0.99	19-38.9	37,852	713	25	1.0 (0.8-1.3)	0.72
	1.0-1.49	39-57.9	13,134	297	29	1.0 (0.7-1.4)	
	≥1.5	≥58	5,970	157	33	1.1 (0.7-1.6)	
Other	<0.5	<19	30,236	185	8	1.0	
	0.5-0.99	19-38.9	37,852	382	13	1.6 (1.1-2.4)	0.0002
	1.0-1.49	39-57.9	13,134	183	18	2.1 (1.3-3.3)	
	≥1.5	≥58	5,970	106	22	2.4 (1.4-4.2)	
	Plasma triglycerides						
All causes	<1	<89	24,364	782	34	1.0	
	1-1.99	89-176	38,828	1,851	50	1.2 (1.0-1.4)	3×10^{-5}
	2-2.99	177-265	14,553	915	65	1.4 (1.1-1.7)	
	≥3	≥266	9,447	704	77	1.6 (1.2-2.0)	
Cardiovascular	<1	<89	24,364	102	6	1.0	
	1-1.99	89-176	38,828	285	10	1.4 (0.8-2.2)	0.0005
	2-2.99	177-265	14,553	165	15	2.0 (1.1-3.4)	
	≥3	≥266	9,447	135	18	2.4 (1.4-4.3)	
Cancer	<1	<89	24,364	322	18	1.0	
	1-1.99	89-176	38,828	714	24	1.1 (0.8-1.4)	0.59
	2-2.99	177-265	14,553	335	29	1.2 (0.9-1.7)	
	≥3	≥266	9,447	223	30	1.1 (0.7-1.5)	
Other	<1	<89	24,364	157	9	1.0	
	1-1.99	89-176	38,828	362	12	1.3 (0.9-1.9)	0.0005
	2-2.99	177-265	14,553	166	15	1.3 (0.8-2.0)	
	≥3	≥266	9,447	171	23	2.5 (1.5-4.1)	

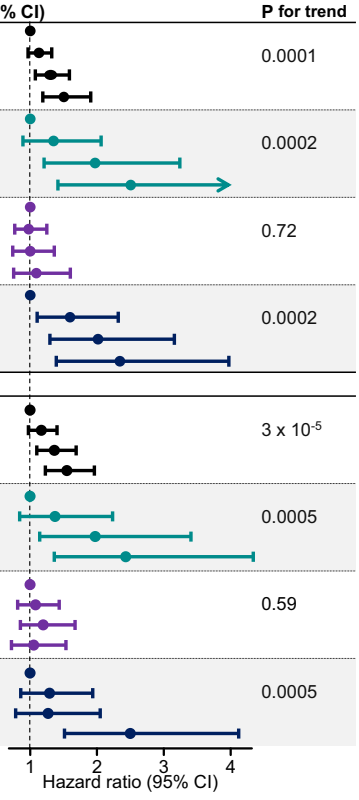


Figure 4 All-cause and cause-specific mortality according to higher remnant cholesterol and plasma triglycerides in clinically relevant groups in the Copenhagen General Population Study. Cox regression adjusted for age, sex, LDL (low-density lipoprotein) cholesterol, smoking status, cumulative smoking in pack-years, time since last meal, systolic blood pressure, and birth year. All-cause mortality had follow-up until the end of 2018, while cause-specific mortality had follow-up until the end of 2016. CI, confidence interval; No., number.

overall higher cumulative mortality estimates (*Figure 7* and Supplementary material online, *Figure S17*). Results for groups with incrementally higher levels of plasma triglyceride were similar (see Supplementary material online, *Figure S18*).

Discussion

This study of 87 192 individuals with up to 13 years of follow-up showed consistently that elevated levels of remnant cholesterol found in 22% of the population were associated with two-fold increased mortality from cardiovascular disease and other causes, but not from cancer (*Structured Graphical Abstract*); with similar results for plasma triglycerides >2 mmol/L (177 mg/dL) present in 28% of the population. We previously observed that elevated remnant cholesterol is associated with increased risk of peripheral artery disease,[25] ischemic heart disease,[13,26–30] and ischemic stroke[31] observationally and/or causally through human genetics; however, it has hitherto been unknown whether elevated remnant cholesterol and plasma triglycerides also are associated with increased cause-specific mortality. Consequently, the results from the current study are novel.

Potential explanations for our findings

The association between elevated remnant cholesterol and increased mortality from cardiovascular disease could mechanistically be explained by remnant cholesterol *per se* causing atherosclerosis. Like LDL particles, remnant lipoproteins can enter the arterial intima, where they get trapped and cause atherosclerosis due to their cholesterol content.[32] In this context, earlier studies have shown causal, genetic associations between elevated remnant cholesterol and ischemic heart disease and myocardial infarction,[26,27] conditions with high mortality.

The association with increased mortality from other causes is less easy to explain since the relationship between elevated remnant cholesterol and plasma triglycerides and most non-cardiovascular diseases is unclear. In our study, the relationship was nominally attributed to increased mortality from all other mortality subcategories, except for mental disorders and respiratory diseases, and especially to infectious and endocrinological diseases.

Hypertriglyceridemia has been causally associated with an increased risk of severe COVID-19 infection,[33] perhaps through lowering of immunoglobulin G levels,[34] indirectly making it plausible that hypertriglyceridemia may also be linked to increased mortality from other infectious diseases. In contrast, while low HDL cholesterol was causally associated with an increased risk of infections in the UK Biobank, hypertriglyceridemia was not.[35] The causal mechanism underlying the association between elevated remnant cholesterol and increased infectious mortality, therefore, needs to be further investigated.

Hypertriglyceridemia is known to cause inflammation and increase the risk of acute pancreatitis.[36,37] As an explanation for our observed association between elevated remnant cholesterol and increased

Cause of death	ICD-10 codes	No. of individuals	No. of events	No. of events per 10,000 person-years by remnant cholesterol levels <0.5 mmol/L (<19 mg/dL)	No. of events per 10,000 person-years by remnant cholesterol levels ≥1.0 mmol/L (≥39 mg/dL)	Multivariable adjusted hazard ratio (95%CI) for ≥1.0 vs. <0.5 mmol/L	P for comparison
All causes	N/A	87,192	4,252	36.0	71.3	1.3 (1.1-1.6)	0.0004
All cardiovascular	I00-99	87,192	687	5.9	16.7	2.2 (1.3-3.5)	0.0007
Myocardial infarction		87,192	97	0.5	2.7	4.7 (1.1-19)	0.03*
Ischemic heart disease		87,192	195	1.2	5.6	4.4 (1.6-11)	0.002
Ischemic stroke		87,192	89	1.1	2.2	1.5 (0.5-5.0)	0.43
Other cardiovascular		87,192	403	3.7	9.0	1.8 (0.9-3.2)	0.06
All cancer	C00-99		1,594	18.8	29.8	1.0 (0.7-1.3)	0.85
Pulmonary		87,192	384	4.2	7.4	0.6 (0.3-1.2)	0.17
Colorectal		87,192	159	1.8	2.5	1.2 (0.4-3.2)	0.69
Breast (women only)		48,615	152	2.9	7.1	2.6 (1.0-6.9)	0.04*
Prostate (men only)		38,577	65	1.8	2.0	1.0 (0.2-4.9)	0.96
Other cancer		87,192	836	10.3	16.1	1.0 (0.7-1.5)	0.84
All other		87,192	856	8.2	19.0	2.1 (1.4-3.3)	0.0002
Infectious	A00-B99	87,192	89	0.6	2.6	8.4 (2.0-34)	0.002
Hematologic	D00-99	87,192	35	0.2	1.1	16 (1.8-144)	0.01*
Endocrinological	E00-99	87,192	73	0.4	2.3	9.1 (1.9-43)	0.005*
Mental disorders	F00-99	87,192	93	0.9	1.5	0.7 (0.2-2.6)	0.64
Neurological	G00-99	87,192	84	0.8	1.7	1.8 (0.5-6.8)	0.36
Respiratory	J00-99	87,192	264	2.8	4.9	1.1 (0.5-2.4)	0.70
Digestive	K00-99	87,192	115	1.1	2.6	2.1 (0.7-6.3)	0.19
External	V00-Y99	87,192	116	1.1	2.4	2.8 (0.8-9.2)	0.08
Unclassified	R00-99	87,192	267	2.5	6.6	2.4 (1.1-5.0)	0.02*
Uncommon	N/A	87,192	51	0.4	1.1	2.2 (0.3-12.)	0.37

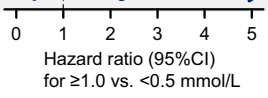


Figure 5 All-cause and cause-specific mortality by subcategory according to high remnant cholesterol in clinically relevant groups in the Copenhagen General Population Study. Cox regression adjusted for age, sex, LDL (low-density lipoprotein) cholesterol, smoking status, cumulative smoking in pack-years, systolic blood pressure, time since last meal, and birth year. All-cause mortality had follow-up until the end of 2018, and cause-specific mortality had follow-up until the end of 2016. A cause of death subcategory was assigned to a death if an ICD-10 code included in the subcategory was registered as any of the three first-ranked causes of death. This means that any single death could be included in the analysis of up to three different cause of death subcategories within each cause of death. In each analysis, all remaining causes of death were treated as censored. *After Bonferroni correction for 19 multiple comparisons of subcategories, the $P = 0.05$ equivalent threshold for significance is $0.05/19 = 0.003$. CI, confidence interval; ICD, International Classification of Diseases; No., number.

mortality from endocrinological diseases, it could thus be hypothesized that the increased inflammation from hypertriglyceridemia causes endocrinological diseases through inflammatory pathways, exemplified by the release of toxic-free fatty acids in the pancreas.[5] Free fatty acids may damage pancreatic beta-cells and increase insulin resistance,[38] which could lead to worse glycemic control and increased mortality in individuals with diabetes.

No causal associations between hypertriglyceridemia and risk of cancers were found in the UK Biobank,[39] supporting the lack of a causal role for elevated remnant cholesterol/hypertriglyceridemia in cancer mortality. Nevertheless, the relationship between elevated remnant cholesterol/hypertriglyceridemia and many non-cardiovascular diseases has not been extensively studied; our findings, therefore, warrant confirmation and further exploration in additional populations.

We observed that the association between elevated remnant cholesterol and other mortality was attenuated after adjustment for body mass index, waist circumference, and diabetes. These are factors that increase remnant cholesterol and plasma triglyceride levels but also have other pathophysiological effects, which could explain part of the association with other mortality. However, it is also likely that elevated remnant cholesterol and plasma triglycerides (or some other component of triglyceride-rich remnant particles) mediates the increased mortality from these factors and that adjustment for body mass index, waist circumference, and diabetes, therefore, obscures an actual causal association between elevated triglyceride-rich remnant particles and other mortality. The association could also be due to confounding by atherosclerotic cardiovascular disease; however, by defining all deaths with any cardiovascular disease diagnosis as cardiovascular deaths, we aimed to minimize this issue. Furthermore, the association between elevated remnant cholesterol and other mortality was not attenuated when adjusting for atherosclerotic cardiovascular disease or statin use (a marker of high atherosclerotic cardiovascular disease risk or even presence at atherosclerotic cardiovascular disease), suggesting the absence or minor influence of confounding from atherosclerotic cardiovascular disease.

In contrast to remnant cholesterol and plasma triglycerides, LDL cholesterol levels had a U-shaped association with all-cause mortality. The association between low LDL cholesterol and increased mortality was previously explained by higher mortality from cancer and other causes in our cohort.[23] In other words, cancer and other causes of death likely lead to low LDL cholesterol due to wasting before death occurs, so-called reverse causation. Interestingly, such reverse

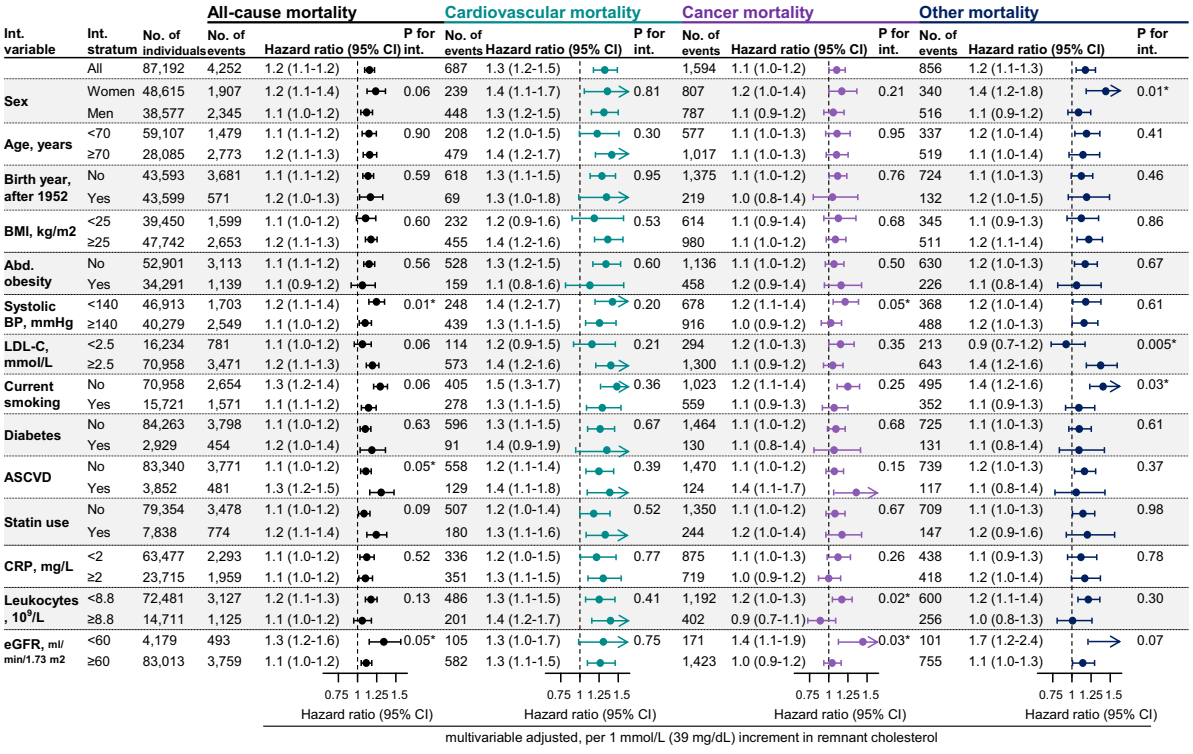


Figure 6 All-cause and cause-specific mortality as functions of 1 mmol/l (39 mg/dL) higher remnant cholesterol in different strata in the Copenhagen General Population Study. Cox regression adjusted for age, sex, LDL cholesterol, smoking status, cumulative smoking in pack-years, time since last meal, systolic blood pressure, and birth year. Estimates are not corrected for measurement error. All-cause mortality had follow-up until the end of 2018, while cause-specific mortality had follow-up until the end of 2016. *After Bonferroni correction for 56 multiple comparisons, the $P = 0.05$ equivalent threshold for significance is $0.05/56 = 0.0009$. Abd., abdominal obesity, indicated by waist circumference ≥80 cm in women and ≥90 cm in men; ASCVD, atherosclerotic cardiovascular disease; BP, blood pressure; CI, confidence interval; CRP, C-reactive protein; eGFR, estimated glomerular filtration rate; Int, interaction; LDL-C, low-density lipoprotein cholesterol; No., number.

causation does not seem to affect the association between low levels of remnant cholesterol and plasma triglycerides with all-cause or cause-specific mortality, as demonstrated in the present study. Taken together, the present study clearly demonstrates a differential relationship between remnant cholesterol and plasma triglycerides with cause-specific mortality (direct relationship for cardiovascular and other mortality) compared with U-shaped relationships for LDL cholesterol, non-HDL cholesterol (=remnant cholesterol + LDL cholesterol), and apolipoprotein B.

The adjusted cumulative mortality estimates by remnant cholesterol and plasma triglyceride levels were higher in the Copenhagen City Heart Study examined in 1976–78 than in the Copenhagen General Population Study examined in 2003–15, and might resemble the risk of contemporary populations with a higher risk factor burden (e.g. smoking)[40] and more limited access to modern healthcare (e.g. statin treatment and coronary artery revascularization).

Previous studies

Observational and causal, genetic associations between elevated remnant cholesterol and atherosclerotic cardiovascular diseases have been found repeatedly.[26,27,29,31,41] In Danish individuals examined in 1976–78 and followed until 2007, elevated triglyceride levels were associated with increased cardiovascular, cancer, and other mortality.[42] The differential result for cancer mortality compared to the present study may be explained by less accurate classification of causes of death, and by differences in cancer diagnosis and treatment in the earlier decades. Partly supporting the present findings, a study of 5729 individuals from Italy observed that elevated remnant cholesterol, calculated from plasma triglycerides, was associated with increased cardiovascular mortality; however, this was not the case for cancer or other mortality.[43] The absence of association between elevated remnant cholesterol and other mortality might be due to lower power (271 deaths from other causes vs. 856 such deaths in the present study) and a different context in terms of diseases and diagnoses.

Strengths

This population-based study used a large cohort with a long follow-up period. Furthermore, the statistical power was high due to a large number of events collected from nationwide registers without losses to follow-up. Also, remnant cholesterol and plasma triglyceride levels were from standard laboratory measurements, which makes the results easily translatable to real-world clinical settings. Finally, the wide collection of baseline information made extensive adjustment and stratification possible, which clarifies and strengthens the results.

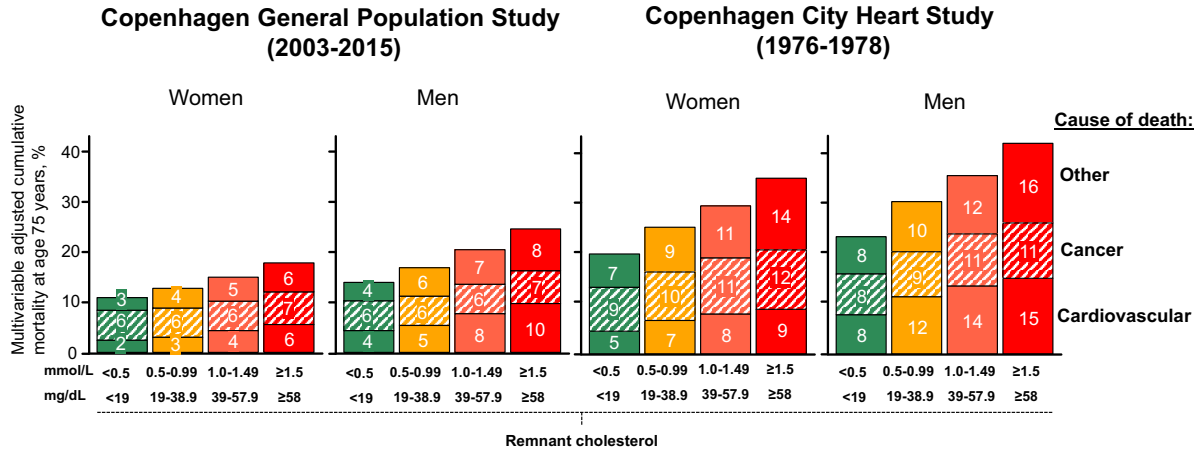


Figure 7 Adjusted cumulative cause-specific mortality at age 75 years in the Copenhagen General Population Study and the Copenhagen City Heart Study. Fine and Gray competing risk regression with the two remaining causes of death as competing risks. Estimates were adjusted to birth year 75 years before the end of follow-up, LDL cholesterol (Copenhagen General Population Study), total cholesterol (Copenhagen City Heart Study), cumulative smoking in pack-years, systolic blood pressure to median values, and to non-current smoking. Cardiovascular, cancer, and other mortality were modeled together, as three competing events.

Limitations

As observational studies like the present study can never fully exclude confounding and reverse causation, we cannot draw conclusions about causality; even after extensive adjustment, there could be residual confounding. Even though we excluded individuals with <1–5 years of follow-up to disprove reverse causation and estimates were similar, we cannot completely rule out reverse causation as individuals may live for an even longer time span with a disease before dying from it.

The use of calculated remnant cholesterol may be considered a limitation of our study, as it is highly dependent on triglyceride levels, that is, in the main analyses remnant cholesterol was triglycerides in mmol/L divided by 2.2 when triglycerides were ≤4 mmol/L (352 mg/dL), while at triglycerides >4 mmol/L remnant cholesterol was total cholesterol minus measured LDL cholesterol minus measured HDL cholesterol. However, calculated and directly measured remnant cholesterol are closely correlated[14,44] as also demonstrated in the present study, and for clinical use, calculation of remnant cholesterol can be easily performed from available lipid profile measurements at no extra cost; in fact, all lipid profiles for hospitals and general practice in the Copenhagen region of Denmark now automatically calculate and report remnant cholesterol levels. Furthermore, blood samples were collected in the non-fasting state, in which some remnant cholesterol and plasma triglycerides are held in chylomicron remnant particles. Some may argue that non-fasting samples is a limitation, while we believe it is a strength as it better captures the average remnant cholesterol and plasma triglyceride levels in plasma during a 24-h period. Although we adjusted for time since the last meal, it is nevertheless possible that results would have been slightly different in fasting individuals.

We lack data for the effect of statins on cholesterol and plasma triglyceride levels on an individual level, and correction for statin use was therefore done assuming an identical effect of statins on remnant cholesterol, plasma triglycerides, and LDL cholesterol levels in all individuals. Even though sensitivity analysis with different levels of correction for statin use did not change the overall conclusions, we cannot know which level of correction is the most appropriate; both over-correction and under-correction may lead to some bias due to statin use.

Another potential limitation is the validity of the cause of death diagnoses used. Additionally, the statistical power was low for the analysis of the cause of death subcategories, and these findings should be regarded as highly exploratory. However, our key question was the associations with cardiovascular, cancer, and other mortality, where power was higher. Finally, our study is limited by only including White individuals aged 20–69 years, the latter because of violation of statistical assumptions in those aged 70–100 years. However, younger individuals on average have more life years to gain from interventions, why they arguably are of more interest in mortality studies. The generalizability of our results is also potentially limited by the homogenous cohort, which is exclusive of White Danish descent; however, to our knowledge, there are no studies yet that would indicate any ethnic differences in the ability of elevated remnant cholesterol or plasma triglycerides to drive morbidity and mortality.

Clinical context and future studies

Drugs lowering triglyceride-rich remnant lipoproteins are currently being investigated in large clinical trials, with even more studies on the horizon aiming at inhibition of apolipoprotein C3 and angiopoietin-related protein 3;[5] promising results with extensive lowering of remnant cholesterol and plasma triglycerides have already been reported for some of these drugs in development.[45–47] Findings regarding associations between elevated remnant cholesterol and plasma triglycerides with cause-specific mortality are potentially important for determining additional indications for current and future remnant cholesterol and plasma triglyceride-lowering therapies. Our results should therefore be confirmed and further explored in other populations, and be investigated by methods that can infer causality, that is, Mendelian randomization studies and randomized controlled trials.

Conclusions

Remnant cholesterol ≥1 mmol/L (39 mg/dL) found in 22% of the population, and plasma triglycerides ≥2 mmol/L (177 mg/dL), present in 28% of the population, are associated with two-fold mortality from

cardiovascular and other causes, but not from cancer. These novel findings should be confirmed in other populations. Large, randomized trials should investigate if remnant cholesterol-lowering therapy without increases in LDL cholesterol or apolipoprotein B can reduce all-cause and cause-specific mortality.

Supplementary data

Supplementary data is available at *European Heart Journal online*.

Acknowledgements

We thank the staff and participants of the Copenhagen City Heart Study and the Copenhagen General Population Study for their valuable contributions.

Funding

The study was supported by the Independent Research Fund Denmark (grant number 1030-00168B), the Novo Nordisk Foundation (grant NNF21OC0071977), the Johan and Lise Boserup Fund, the Aase and Ejnar Danielsen Fund, and Herlev and Gentofte Hospital, Copenhagen University Hospital, Denmark. The funding sources had no role in the study design, in the writing of the report, or in the decision to submit the article for publication. The researchers were independent of the funders and all authors had full access to all data in the study and can take responsibility for the integrity of the data and the accuracy of the data analysis.

Conflicts of interest: B.G.N. reports consultancies and talks sponsored by AstraZeneca, Sanofi, Regeneron, Akcea, Amgen, Kowa, Denka, Amarin, Novartis, Novo Nordisk, Esperion, and Silence Therapeutics. There are no financial or other conflicts of interest for B.N.W., K.M.P., or A.B.W.

Data availability

The Danish Data Protection Agency does not allow open access to our data; however, upon reasonable request to the corresponding author, additional analyses can be performed.

References

1. Nordestgaard BG, Varbo A. Triglycerides and cardiovascular disease. *Lancet* 2014:**384**:626–635.
2. Bhatt DL, Steg PG, Miller M, Brinton EA, Jacobson TA, Ketchum SB, *et al.* Cardiovascular risk reduction with icosapent ethyl for hypertriglyceridemia. *N Engl J Med* 2019;**380**:11–22.
3. Nicholls Stephen J, Lincoff A. Michael, Garcia Michelle *et al.* Effect of High-Dose Omega-3 Fatty Acids vs Corn Oil on Major Adverse Cardiovascular Events in Patients at High Cardiovascular Risk. *JAMA* 2020;**324**:2268. (http://dx.doi.org/10.1001/jama.2020.22258)
4. Das Pradhan A, Glynn RJ, Fruchart JC, MacFadyen JG, Zaharris ES, Everett BM, *et al.* Triglyceride lowering with pemafibrate to reduce cardiovascular risk. *N Engl J Med* 2022; **387**:1923–1934.
5. Nordestgaard BG, Nicholls SJ, Langsted A, Ray KK, Tybjaerg-Hansen A. Advances in lipid-lowering therapy through gene-silencing technologies. *Nat Rev Cardiol* 2018;**15**:261–272.
6. Tan VY, Bull CJ, Biernacka KM, Teumer A, Richardson TG, Sanderson E, *et al.* Investigation of the interplay between circulating lipids and IGF-I and relevance to breast cancer risk: an observational and Mendelian randomization study. *Cancer Epidemiol Biomarkers Prev* 2021;**30**:2207–2216.
7. Park JK, Bafna S, Forrest IS, Duffy Á, Marquez-Luna C, Petrazzini BO, *et al.* Phenome-wide Mendelian randomization study of plasma triglycerides and 2,600 disease traits. *medRxiv* 2022:2022.07.21.22277900.
8. Varbo A, Nordestgaard BG. Nonfasting triglycerides, low-density lipoprotein cholesterol, and heart failure risk: two cohort studies of 113 554 individuals. *Arterioscler Thromb Vasc Biol* 2018;**38**:464–472.
9. Helweg-Larsen K. The Danish register of causes of death. *Scand J Public Health* 2011;**39**:26–29.
10. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem* 1972;**18**:499–502.
11. Martin SS, Blaha MJ, Elshazly MB, Toth PP, Kwiterovich PO, Blumenthal RS, *et al.* Comparison of a novel method vs the friedewald equation for estimating low-density lipoprotein cholesterol levels from the standard lipid profile. *JAMA* 2013;**310**:2061–2068.
12. Sampson M, Ling C, Sun Q, Harb R, Ashmaig M, Warnick R, *et al.* A new equation for calculation of low-density lipoprotein cholesterol in patients with normolipidemia and/or hypertriglyceridemia. *JAMA Cardiol* 2020;**5**:540–548.
13. Varbo A, Nordestgaard BG. Directly measured vs. Calculated remnant cholesterol identifies additional overlooked individuals in the general population at higher risk of myocardial infarction. *Eur Heart J* 2021;**42**:4833–4843.
14. Varbo A, Freiberg JJ, Nordestgaard BG. Remnant cholesterol and myocardial infarction in normal weight, overweight, and obese individuals from the Copenhagen general population study. *Clin Chem* 2018;**64**:219–230.
15. Karlson BW, Palmer MK, Nicholls SJ, Lundman P, Barter PJ. A VOYAGER meta-analysis of the impact of statin therapy on low-density lipoprotein cholesterol and triglyceride levels in patients with hypertriglyceridemia. *Am J Cardiol* 2016;**117**:1444–1448.
16. Grundy SM, Stone NJ, Bailey AL, Beam C, Birtcher KK, Blumenthal RS, *et al.* 2018 Aha/ACC/AACVPR/AAPA/ABC/ACPM/ADA/AGS/APhA/ASPC/NLA/PCNA guideline on the management of blood cholesterol: a report of the American college of cardiology/American heart association task force on clinical practice guidelines. *Circulation* 2019; **139**:e1082–ee143.
17. Levey AS, Stevens LA, Schmid CH, Zhang YL, Castro AF, 3rd, Feldman HI, *et al.* A new equation to estimate glomerular filtration rate. *Ann Intern Med* 2009;**150**:604–612.
18. Kjeldsen EW, Thomassen JQ, Rasmussen KL, Nordestgaard BG, Tybjaerg-Hansen A, Frikke-Schmidt R. Impact of diet on ten-year absolute cardiovascular risk in a prospective cohort of 94 321 individuals: a tool for implementation of healthy diets. *Lancet Reg Health Eur* 2022;**19**:100419.
19. Hernan MA. The hazards of hazard ratios. *Epidemiology* 2010;**21**:13–15.
20. Cologne J, Hsu WL, Abbott RD, Ohishi W, Grant EJ, Fujiwara S, *et al.* Proportional hazards regression in epidemiologic follow-up studies: an intuitive consideration of primary time scale. *Epidemiology* 2012;**23**:565–573.
21. Pencina MJ, Larson MG, D'Agostino RB. Choice of time scale and its effect on significance of predictors in longitudinal studies. *Stat Med* 2007;**26**:1343–1359.
22. Rosner B, Spiegelman D, Willett WC. Correction of logistic regression relative risk estimates and confidence intervals for random within-person measurement error. *Am J Epidemiol* 1992;**136**:1400–1413.
23. Johannesen CDL, Langsted A, Mortensen MB, Nordestgaard BG. Association between low density lipoprotein and all cause and cause specific mortality in Denmark: prospective cohort study. *BMJ* 2020;**371**:m4266.
24. Langsted A, Jensen AMR, Varbo A, Nordestgaard BG. Low high-density lipoprotein cholesterol to monitor long-term average increased triglycerides. *J Clin Endocrinol Metab* 2020;**105**:dgz265.
25. Wadstrom BN, Wulff AB, Pedersen KM, Jensen GB, Nordestgaard BG. Elevated remnant cholesterol increases the risk of peripheral artery disease, myocardial infarction, and ischaemic stroke: a cohort-based study. *Eur Heart J* 2022;**43**:3258–3269.
26. Varbo A, Benn M, Tybjaerg-Hansen A, Jorgensen AB, Frikke-Schmidt R, Nordestgaard BG. Remnant cholesterol as a causal risk factor for ischemic heart disease. *J Am Coll Cardiol* 2013;**61**:427–436.
27. Jorgensen AB, Frikke-Schmidt R, West AS, Grande P, Nordestgaard BG, Tybjaerg-Hansen A. Genetically elevated non-fasting triglycerides and calculated remnant cholesterol as causal risk factors for myocardial infarction. *Eur Heart J* 2013;**34**:1826–1833.
28. Varbo A, Benn M, Tybjaerg-Hansen A, Nordestgaard BG. Elevated remnant cholesterol causes both low-grade inflammation and ischemic heart disease, whereas elevated low-density lipoprotein cholesterol causes ischemic heart disease without inflammation. *Circulation* 2013;**128**:1298–1309.
29. Balling M, Afzal S, Varbo A, Langsted A, Davey Smith G, Nordestgaard BG. Vldl cholesterol accounts for one-half of the risk of myocardial infarction associated with apoB-containing lipoproteins. *J Am Coll Cardiol* 2020;**76**:2725–2735.
30. Langsted A, Madsen CM, Nordestgaard BG. Contribution of remnant cholesterol to cardiovascular risk. *J Intern Med* 2020;**288**:116–127.
31. Varbo A, Nordestgaard BG. Remnant cholesterol and risk of ischemic stroke in 112,512 individuals from the general population. *Ann Neurol* 2019;**85**:550–559.
32. Nordestgaard BG. Triglyceride-rich lipoproteins and atherosclerotic cardiovascular disease: new insights from epidemiology, genetics, and biology. *Circ Res* 2016;**118**:547–563.
33. Yoshikawa M, Asaba K, Nakayama T. Estimating causal effects of atherogenic lipid-related traits on COVID-19 susceptibility and severity using a two-sample Mendelian randomization approach. *BMC Med Genomics* 2021;**14**:269.
34. Richardson TG, Fang S, Mitchell RE, Holmes MV, Davey Smith G. Evaluating the effects of cardiometabolic exposures on circulating proteins which may contribute to severe SARS-CoV-2. *EBioMedicine* 2021;**64**:103228.
35. Trinder M, Walley KR, Boyd JH, Brunham LR. Causal inference for genetically determined levels of high-density lipoprotein cholesterol and risk of infectious disease. *Arterioscler Thromb Vasc Biol* 2020;**40**:267–278.

36. Moens SJ B, Verweij SL, Schnitzler JG, Stiekema LCA, Bos M, Langsted A, *et al.* Remnant cholesterol elicits arterial wall inflammation and a multilevel cellular immune response in humans. *Arterioscler Thromb Vasc Biol* 2017;**37**:969–975.
37. Hansen SEJ, Madsen CM, Varbo A, Tybjaerg-Hansen A, Nordestgaard BG. Genetic variants associated with increased plasma levels of triglycerides, via effects on the lipoprotein lipase pathway, increase risk of acute pancreatitis. *Clin Gastroenterol Hepatol* 2021; **19**:1652–60.e6.
38. Oh YS, Bae GD, Baek DJ, Park EY, Jun HS. Fatty acid-induced lipotoxicity in pancreatic beta-cells during development of type 2 diabetes. *Front Endocrinol (Lausanne)* 2018;**9**: 384.
39. Carter P, Vithayathil M, Kar S, Potluri R, Mason AM, Larsson SC, *et al.* Predicting the effect of statins on cancer risk using genetic variants from a Mendelian randomization study in the UK Biobank. *Elife* 2020;**9**:e57191.
40. Roth GA, Mensah GA, Johnson CO, Addolorato G, Ammirati E, Baddour LM, *et al.* Global burden of cardiovascular diseases and risk factors, 1990–2019: update from the GBD 2019 study. *J Am Coll Cardiol* 2020;**76**:2982–3021.
41. Castaner O, Pinto X, Subirana I, Amor AJ, Ros E, Hernaez A, *et al.* Remnant cholesterol, not LDL cholesterol, is associated with incident cardiovascular disease. *J Am Coll Cardiol* 2020;**76**:2712–2724.
42. Langsted A, Freiberg JJ, Tybjaerg-Hansen A, Schnohr P, Jensen GB, Nordestgaard BG. Nonfasting cholesterol and triglycerides and association with risk of myocardial infarction and total mortality: the Copenhagen city heart study with 31 years of follow-up. *J Intern Med* 2011;**270**:65–75.
43. Bonfiglio C, Leone CM, Silveira LVA, Guerra R, Misciagna G, Caruso MG, *et al.* Remnant cholesterol as a risk factor for cardiovascular, cancer or other causes mortality: a competing risks analysis. *Nutr Metab Cardiovasc Dis* 2020;**30**:2093–2102.
44. Duran EK, Aday AW, Cook NR, Buring JE, Ridker PM, Pradhan AD. Triglyceride-rich lipoprotein cholesterol, small dense LDL cholesterol, and incident cardiovascular disease. *J Am Coll Cardiol* 2020;**75**:2122–2135.
45. Witztum JL, Gaudet D, Freedman SD, Alexander VJ, Digenio A, Williams KR, *et al.* Volanesorsen and triglyceride levels in familial chylomicronemia syndrome. *N Engl J Med* 2019;**381**:531–542.
46. Graham MJ, Lee RG, Brandt TA, Tai LJ, Fu W, Peralta R, *et al.* Cardiovascular and metabolic effects of ANGPTL3 antisense oligonucleotides. *N Engl J Med* 2017;**377**:222–232.
47. Dewey FE, Gusarova V, Dunbar RL, O'Dushlaine C, Schurmann C, Gottesman O, *et al.* Genetic and pharmacologic inactivation of ANGPTL3 and cardiovascular disease. *N Engl J Med* 2017;**377**:211–221.