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Extreme high high-density lipoprotein cholesterol is paradoxically associated with high mortality in men and women: two prospective cohort studies

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Aims High-density lipoprotein (HDL) cholesterol concentrations are inversely associated with cardiovascular disease and mortality across a range of concentrations, but genetic evidence suggest that extreme high concentrations may paradoxically lead to more cardiovascular disease. We tested the hypothesis that extreme high concentrations of HDL cholesterol are associated with high all-cause mortality in men and women.

Methods and results A total of 52 268 men and 64 240 women were included from the two prospective population-based studies, the Copenhagen City Heart Study and the Copenhagen General Population Study. During 745 452 person-years of follow-up, number of deaths from any cause were 5619 (mortality rate, 17.1/1000 person-years (95% confidence interval (CI): 16.7–17.6)) in men and 5059 (mortality rate, 12.1/1000 person-years (11.8–12.4)) in women. The association between HDL cholesterol concentrations and all-cause mortality was U-shaped for both men and women, with both extreme high and low concentrations being associated with high all-cause mortality risk. The concentration of HDL cholesterol associated with the lowest all-cause mortality was 1.9 mmol/L (95% CI: 1.4–2.0) (73 mg/dL (54–77)) in men and 2.4 mmol/L (1.8–2.5) (93 mg/dL (69–97)) in women. When compared with the groups with the lowest risk, the multifactorially adjusted hazard ratios for all-cause mortality were 1.36 (95% CI: 1.09–1.70) for men with HDL cholesterol of 2.5–2.99 mmol/L (97–115 mg/dL) and 2.06 (1.44–2.95) for men with HDL cholesterol ≥3.0 mmol/L (116 mg/dL). For women, corresponding hazard ratios were 1.10 (0.83–1.46) for HDL cholesterol of 3.0–3.49 mmol/L (116–134 mg/dL) and 1.68 (1.09–2.58) for HDL cholesterol ≥3.5 mmol/L (135 mg/dL).

Conclusion Men and women in the general population with extreme high HDL cholesterol paradoxically have high all-cause mortality. These findings need confirmation in other studies.

Keywords Lipids • Lipoproteins • HDL • Mortality • General population • Epidemiology

Introduction

Through observational studies it has been established that high-density lipoprotein (HDL) cholesterol is inversely associated with both cardiovascular disease and mortality across a wide range of concentrations.[1–3] This association does however not appear to be causal, as raising HDL cholesterol pharmacologically has not proven beneficial in randomized clinical trials,[4] and has even paradoxically been associated with increased mortality in one study.[5]

Furthermore, certain genetic variants associated with higher concentrations of HDL cholesterol have been associated paradoxically with high risk of cardiovascular disease.[6–8] One of these studies refers to elevated HDL cholesterol associated with a polymorphism of the *CETP* gene leading to low activity of the cholesteryl ester transfer

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protein (CETP),[6] however, in a subsequent study on the same cohort another genetic deficiency of CETP activity was associated with reduced (not increased) cardiovascular risk.[9] Most recently, a loss of function mutation in a major HDL receptor, Scavenger Receptor B1 (SCARB1), which lead to high concentrations of HDL cholesterol, was associated with high risk of coronary heart disease.[10] Despite these indications that extreme high HDL cholesterol concentrations might not be associated with low morbidity and mortality, the association between extreme high HDL cholesterol and mortality is not well-described. This is in part because only few individuals have extremely high HDL cholesterol concentrations[11] and therefore often are grouped together with individuals with only modestly high concentrations, in for example quintiles.[1] If those with extreme high HDL cholesterol have high mortality, like those with low concentrations, this could have implications for clinical risk assessment in those with extreme high concentrations of HDL cholesterol, and consequences for development of compounds aimed at increasing HDL concentrations.

We tested the hypothesis that men and women with extreme high HDL cholesterol have high all-cause mortality, using two separate studies of the Danish general population. Furthermore, we determined the concentration of HDL cholesterol associated with the lowest all-cause mortality.

Methods

A detailed description of endpoints, covariates and statistical analyses is given in the Supplementary material online, *Appendix (Methods)*.

Study cohorts

White individuals of Danish descent (according to the Danish Civil Registration System, that is, the person and both parents were born in Denmark and were Danish citizens) were included from two studies of the Danish general population. Written informed consent was obtained from all. Studies were conducted in accordance with the Declaration of Helsinki and approved by local institutional reviews boards and Danish Ethics Committees (KF-100.2039/91 and H-KF-01-144/01).

The Copenhagen City Heart Study (CCHS)

Individuals aged 20–100 years were invited randomly from the general population of Copenhagen using information from the Danish Civil Registration System. Information on health, lifestyle etc. was obtained from questionnaires, which were reviewed with an investigator on the day of attendance, and from a physical examination. Blood was drawn for biochemical measurements. For this study 9387 individuals from the 1991 to 1994 examination of the CCHS were included (participation rate, 61%).[12]

The Copenhagen General Population Study (CGPS)

The study was initiated in 2003 with ongoing recruitment similar to that of the CCHS, but from a geographically separate area around Copenhagen. No individuals were included in both studies. Examinations were conducted as described for the CCHS. 107 121 individuals were included from the CGPS (participation rate, 43%).[13]

Endpoints

Death from any cause was ascertained using the Danish Civil Registration System and the cause of death was retrieved from the Danish Register of Causes of Death. For this study, cause of death was classified into three major categories, as done previously.[14] If one of the three first ranked causes of death was cancer (ICD-8:140-209, ICD-10:C00-C97) it was classified as cancer related. The remaining deaths were classified as cardiovascular if one of the three first ranked causes of death was cardiovascular disease (ICD-8:390-458, ICD-10:I00-I99), or classified as other mortality if neither a cancer nor a cardiovascular diagnosis were among the three first ranked causes of death. Secondary endpoints in the form of ischaemic heart disease (ICD-8:410-414, ICD-10:I20-I25), myocardial infarction (ICD-8:410, ICD-10:I21-I22) and ischaemic stroke (ICD8:431-438, ICD10:I60-I69 + G45) were ascertained in the national Danish Patient Registry.

Laboratory analyses

Blood samples were collected non-fasting.[15] HDL cholesterol and triglycerides were measured using colorimetric assays (Konelab). LDL cholesterol was calculated using the Friedewald equation when triglycerides were below 4 mmol/L (352 mg/dL) or otherwise measured directly (Konelab).

Covariates

Covariates for statistical adjustment were chosen a priori according to known associations with all-cause mortality and HDL cholesterol and included: age, study, body mass index, current smoking, cumulative tobacco smoking, alcohol consumption, physical activity, systolic blood pressure, diabetes mellitus, lipid-lowering therapy, LDL cholesterol, triglycerides, birth year, and menopausal status and hormone replacement therapy for women.

Statistical analyses

Statistical analyses were performed using Stata 13.1. Missing information on covariates was imputed based on age and sex using multivariable linear regression for continuous variables, whereas categorical variables where assigned a separate category.

The association between HDL cholesterol and endpoints was examined using Cox proportional hazards regression with 95% confidence intervals (CI) and age as the underlying time scale (referred to as age adjustment) with delayed entry (left truncation) and censoring at emigration ($n = 478$ (0.4%)) or end of follow-up. As women on average have higher concentrations of HDL cholesterol, analyses were conducted separately for men and women.

In primary analyses, the association between mortality and HDL cholesterol on a continuous scale was examined using restricted cubic splines incorporated in Cox proportional hazards models. The concentration of HDL cholesterol associated with the lowest mortality was the concentration with the lowest hazard ratio in the spline Cox regression. 95% CIs were non-parametric from bootstrap estimation with 5000 repetitions, as done previously.[14]

The association between extreme high concentrations of HDL cholesterol and mortality was further examined using a priori selected groups of clinically meaningful HDL cholesterol concentrations. These were concentration cut points with 0.5 mmol/L intervals starting from 1.0 mmol/L. For men the highest group was ≥3.0 mmol/L, and as women on average have higher HDL cholesterol than men, the highest group for women was ≥3.5 mmol/L. Individuals were also divided into predefined groups based on percentiles. Reference groups were the ones containing the concentration of HDL cholesterol associated with the lowest risk of death determined using multifactorially adjusted spline regression.

Risk estimates and confidence intervals were corrected for regression dilution bias using a non-parametric method,[16] as done previously.[17,18] Regression dilution is a phenomenon where random measurement error and long-term fluctuations in the exposure variable will lead to

underestimation of the actual association. Using measurements of HDL cholesterol from 4196 individuals from the CCHS, who attended both the 1991–94 examination and the 2001–03 examination and did not use lipid-lowering therapy at either time point, a regression dilution ratio of 0.73 was calculated for HDL cholesterol.

Results

A total of 52 268 men and 64 240 women were included from the CGPS and CCHS combined and were followed for 745 452 person-years. Median follow-up was 6.0 years (range: 0–23), with 5.7 years (0–11), and 19.9 years (0–23) for the CGPS and CCHS, respectively. During follow-up, 5619 men and 5059 women died from any cause giving crude mortality rates of 17.1 (95% CI: 16.7–17.6) and 12.1 (11.8–12.4) per 1000 person-years. Baseline characteristics for men and women separately are shown in *Table 1* and further divided by study and concentration cut points in Supplementary material online, *Tables S2, S3, S4*, and *S5*.

The distribution of HDL cholesterol concentrations in the general population was wider for women compared with the distribution in men, and on average women had higher HDL cholesterol (*Figure 1*). The distributions were similar in the two studies (Supplementary material online, *Figure S1*).

HDL cholesterol and all-cause mortality

The association between HDL cholesterol on a continuous scale and all-cause mortality was U-shaped for men and women, as both low and high concentrations were associated with high all-cause mortality (*Figure 2*). The association between extreme high HDL cholesterol and high mortality was most pronounced for men. The concentration of HDL cholesterol associated with the lowest all-cause mortality in men was 1.9 mmol/L (95% CI: 1.4–2.0 mmol/L) (73 mg/dL (54–77 mg/dL)) in age and study adjusted analyses, 1.9 mmol/L (1.4–2.0 mmol/L) (73 mg/dL (54–77 mg/dL)) in age, study, and triglyceride adjusted analyses, and 1.9 mmol/L (1.1–2.0 mmol/L) (73 mg/dL (42–77 mg/dL)) in multifactorially adjusted analyses. For women the corresponding concentrations of HDL cholesterol associated with the lowest all-cause mortality were 2.4 mmol/L (1.8–2.5 mmol/L) (93 mg/dL (69–97 mg/dL)), 2.4 mmol/L (1.8–2.5 mmol/L) (93 mg/dL (69–97 mg/dL)), and 2.4 mmol/L (1.7–6.2 mmol/L) (93 mg/dL (66–239 mg/dL)), respectively. For the multifactorially adjusted analyses in women the upper CI limit could not be definitively determined.

Similar to restricted cubic spline analyses, a U-shaped association between HDL cholesterol and all-cause mortality was seen in both sexes using concentration cut points (*Figure 3*). Men with HDL cholesterol of 2.5–2.99 mmol/L (97–115 mg/dL) and ≥3.0 mmol/L (116 mg/dL) had multifactorially adjusted hazard ratios of 1.36 (95% CI: 1.09–1.70) and 2.06 (1.44–2.95) when compared with men in the reference group (1.5–1.99 mmol/L (58–76 mg/dL)). Men with HDL cholesterol <1.0 mmol/L (39 mg/dL) had a corresponding hazard ratio of 1.27 (1.12–1.45). Women with HDL cholesterol of 3.0–3.49 mmol/L (116–134 mg/dL), ≥3.5 mmol/L (135 mg/dL) and <1.0 mmol/L (39 mg/dL) had hazard ratios for all-cause mortality of 1.10 (0.83–1.46), 1.68 (1.09–2.58) and 1.78 (1.43–2.22), respectively, when compared with the reference group (2.0–2.49 mmol/L (77–96 mg/dL)).

Table 1 Baseline characteristics of men and women

	Men	Women
Individuals	52 268 (45%)	64 240 (55%)
Age, years	58 (48–68)	57 (47–67)
Body mass index, kg/m^2	26.3 (24.2–28.8)	24.7 (22.3–27.9)
Current smokers	10 922 (21%)	12 111 (19%)
Cumulative tobacco consumption, pack-years[a]	21 (10–38)	13 (5–26)
Alcohol consumption, units/week[b]	11 (6–20)	6 (2–11)
Physical inactivity[c]	23 318 (45%)	34 272 (54%)
Systolic blood pressure, mmHg	142 (130–156)	136 (122–152)
Diabetes mellitus	2845 (5%)	2074 (3%)
Lipid-lowering therapy	6895 (13%)	5980 (9%)
Post-menopausal	—	43 431 (68%)
Hormone replacement therapy	—	7190 (11%)
HDL cholesterol		
mmol/L	1.4 (1.1–1.7)	1.7 (1.4–2.1)
mg/dL	52 (42–64)	67 (55–81)
LDL cholesterol		
mmol/L	3.2 (2.6–3.9)	3.2 (2.6–3.9)
mg/dL	124 (100–151)	124 (100–151)
Triglycerides		
mmol/L	1.6 (1.1–2.4)	1.2 (0.9–1.8)
mg/dL	143 (99–211)	108 (77–157)

Values are median (inter-quartile range) or number of individuals (%). The number of participants varies slightly according to availability of the variables (data is without imputation).
HDL, high-density lipoprotein; LDL, low-density lipoprotein.
[a]Current and former smokers only.
[b]1 unit~ 12 g of alcohol.
[c]<4 h physical activity in leisure time per week.

In percentile-based analyses, multifactorially adjusted hazard ratios for all-cause mortality for the top percentile (100th) were 1.94 (1.47–2.55) for men and 1.36 (0.99–1.85) for women, when compared with men and women in the reference groups (81st–95th percentile).

HDL cholesterol and cause-specific mortality

A U-shaped association between HDL cholesterol and cardiovascular mortality was also seen for both men and women (*Figure 4*). For cancer mortality, lower concentrations of HDL cholesterol were associated with high risk in both sexes. Risk of other mortality showed a U-shaped association with HDL cholesterol in men, but no association in women. These findings were confirmed when using concentration cut points (Supplementary material online, *Figure S2*). The extreme high HDL group in both men and women had high cardiovascular mortality with multifactorially adjusted hazard ratios of 2.53 (95% CI: 1.24–5.18) and 2.89 (1.33–6.24), respectively, compared with men and women in the reference groups. For cancer

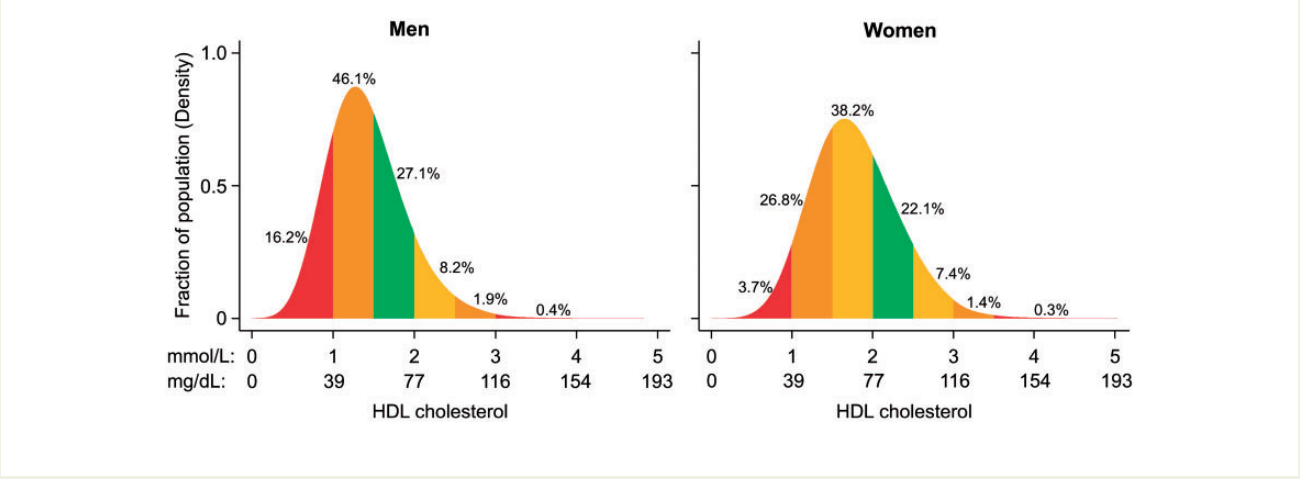


Figure 1 Distribution of HDL cholesterol concentrations in men and women in the general population. Based on 52 268 men and 64 240 women from the Copenhagen General Population Study and the Copenhagen City Heart Study combined. HDL, high-density lipoprotein.

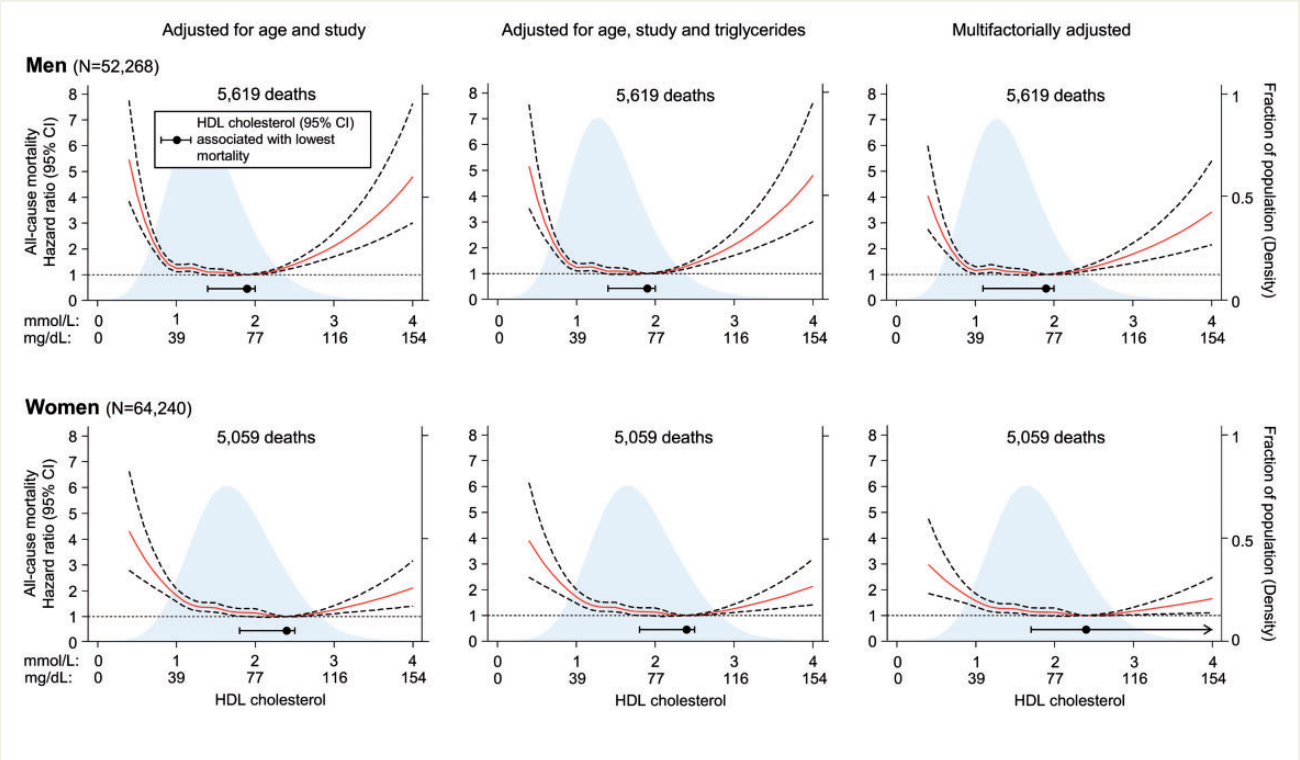


Figure 2 HDL cholesterol on a continuous scale and risk of all-cause mortality in the general population. Based on 52 268 men and 64 240 women from the Copenhagen General Population Study and the Copenhagen City Heart Study combined. Hazard ratio (solid line) and 95% confidence interval (dashed lines) from Cox regression using restricted cubic splines. Multifactorial adjustment was for age, study, body mass index, current smoking, cumulative tobacco smoking, alcohol consumption, physical activity, systolic blood pressure, diabetes mellitus, lipid-lowering therapy, LDL cholesterol, triglycerides, birth year, and menopausal status and hormone replacement therapy for women. The concentration of HDL cholesterol associated with lowest mortality was used as reference. Graphs were truncated at 0.4 and 4.0 mmol/L due to limited number of individuals and events outside these cutpoints. The light blue area indicates the distribution of HDL cholesterol concentrations in men and women. CI, confidence interval; HDL, high-density lipoprotein.

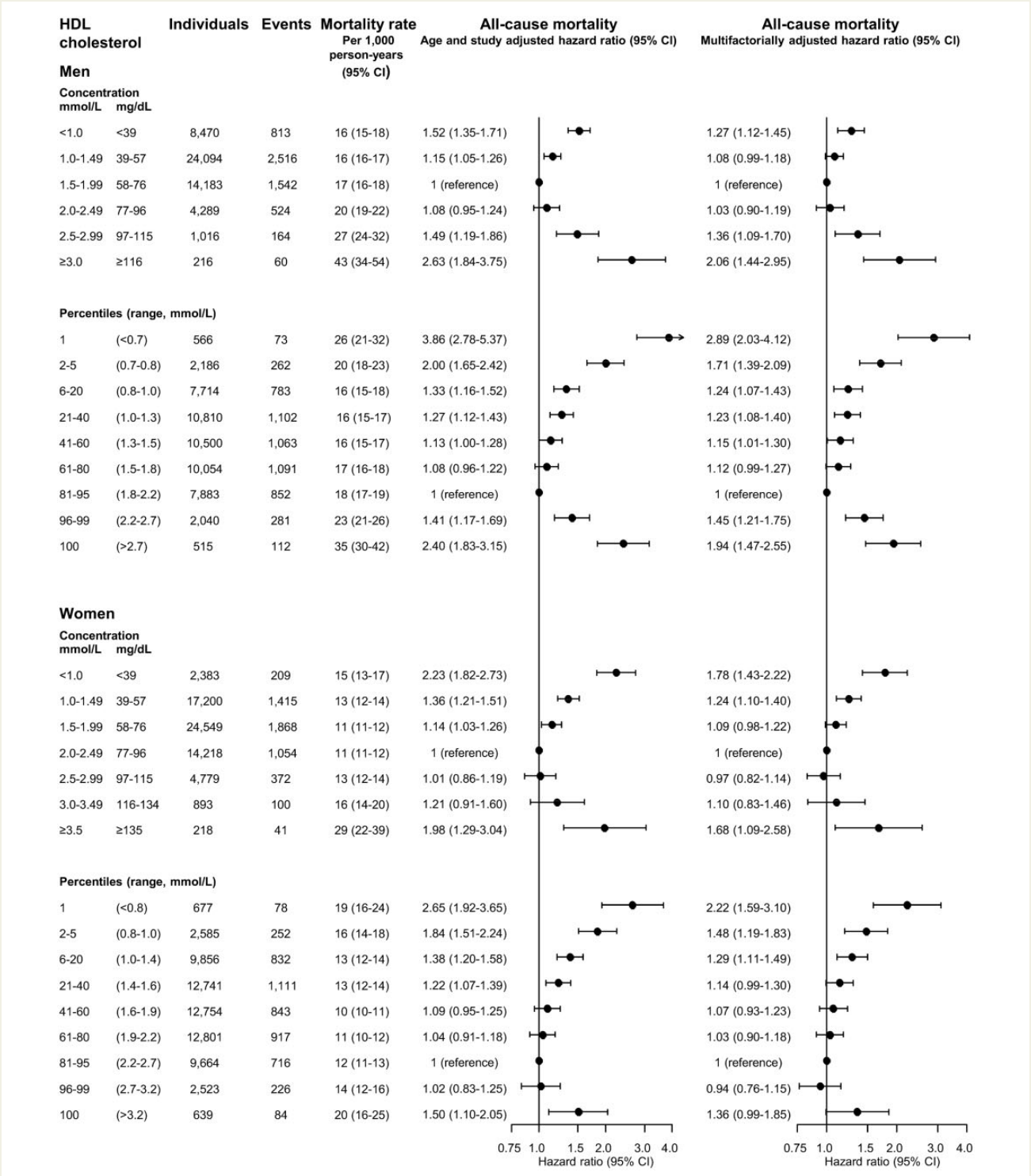


Figure 3 HDL cholesterol in categories based on concentration cutpoints and percentiles, and risk of all-cause mortality in the general population. Based on 52 268 men and 64 240 women from the Copenhagen General Population Study and the Copenhagen City Heart Study combined. Hazard ratios from Cox regression were adjusted for age and study or multifactorially for age, study, body mass index, current smoking, cumulative tobacco smoking, alcohol consumption, physical activity, systolic blood pressure, diabetes mellitus, lipid-lowering therapy, LDL cholesterol, triglycerides, birth year, and menopausal status and hormone replacement therapy for women. Reference groups were the one containing HDL cholesterol concentrations associated with lowest all-cause mortality determined by multifactorially adjusted spline regressions. CI, confidence interval; HDL, high-density lipoprotein.

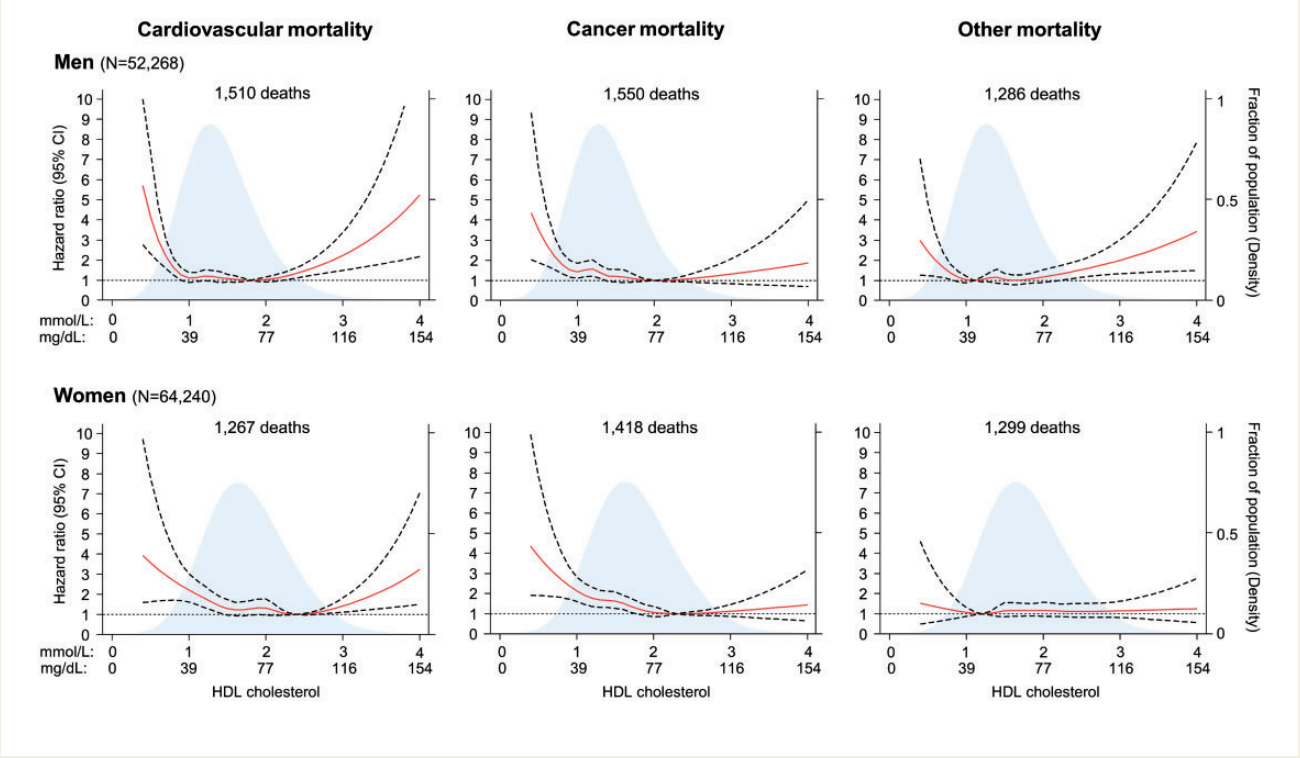


Figure 4 HDL cholesterol on a continuous scale and risk of cardiovascular, cancer, and other mortality in the general population. Based on 52 268 men and 64 240 women from the Copenhagen General Population Study and the Copenhagen City Heart Study combined. Hazard ratio (solid line) and 95% confidence interval (dashed lines) were from Cox regression using restricted cubic splines. Analyses were multifactorially adjusted for age, study, body mass index, current smoking, cumulative tobacco smoking, alcohol consumption, physical activity, systolic blood pressure, diabetes mellitus, lipid-lowering therapy, LDL cholesterol, triglycerides, birth year, and menopausal status and hormone replacement therapy for women. The HDL cholesterol concentration associated with the lowest cause specific mortality was used as reference. Graphs were truncated at 0.4 and 4.0 mmol/L due to limited number of individuals and events outside these cutpoints. Total number of deaths from the three categories does not add up to the total number of deaths by any cause, as not all deaths was classified according to cause, as described in Methods. The light blue area indicates the distribution of HDL cholesterol concentrations in men and women. CI, confidence interval; HDL, high-density lipoprotein.

mortality corresponding hazard ratios were 1.76 (0.88–3.53) and 1.33 (0.56–3.19), and for other mortality 1.90 (0.95–3.82) and 1.51 (0.66–3.46), for men and women, respectively.

HDL cholesterol and cardiovascular disease

For cardiovascular disease endpoints ischaemic heart disease and myocardial infarction, a high risk was observed for low concentrations of HDL cholesterol (*Figure 5*). There was a plateau around HDL cholesterol of 1.5 mmol/L (58 mg/dL) and 2.0 mmol/L (77 mg/dL) for men and women, respectively, with no further decrease in risk with concentrations of HDL cholesterol higher than that. No significant increase in these risks were observed with extreme high concentrations of HDL cholesterol. For ischaemic stroke similar patterns of association were observed.

Sensitivity analyses

Results from complete-case analyses including only individuals with complete information on all covariates were similar to analyses using the full dataset (compare *Figure 3* with Supplementary material online, *Figure S3*). Individuals included in the main analyses were combined from two independent studies; however, results were similar for the two studies separately (compare *Figure 3* with Supplementary material online, *Figure S4*). Hence, we were able to obtain confirmation of the results in two independent studies.

Results for the extreme high HDL cholesterol group vs. the reference group were robust across different strata of risk factors for all-cause mortality and medication use affecting all-cause mortality (Supplementary material online, *Figures S5* and *S6*). However, number of individuals and events were small in some extreme groups in stratified analyses generating risk estimates with wide CIs. There was, however, no clear evidence for statistical interaction in the stratified analyses.

To assess possible effects of reverse causation from severe disease likely leading to death and high HDL cholesterol, we examined association with all-cause mortality for the extreme high HDL vs. the reference group excluding individuals with less than 1 to 5 years of follow-up (Supplementary material online, *Figure S7*). Overall hazard ratios were similar, except for a slight attenuation for women when excluding those with less than 4 and 5 years of follow-up, indicating that results were not caused by reverse causation. Analyses of cause-

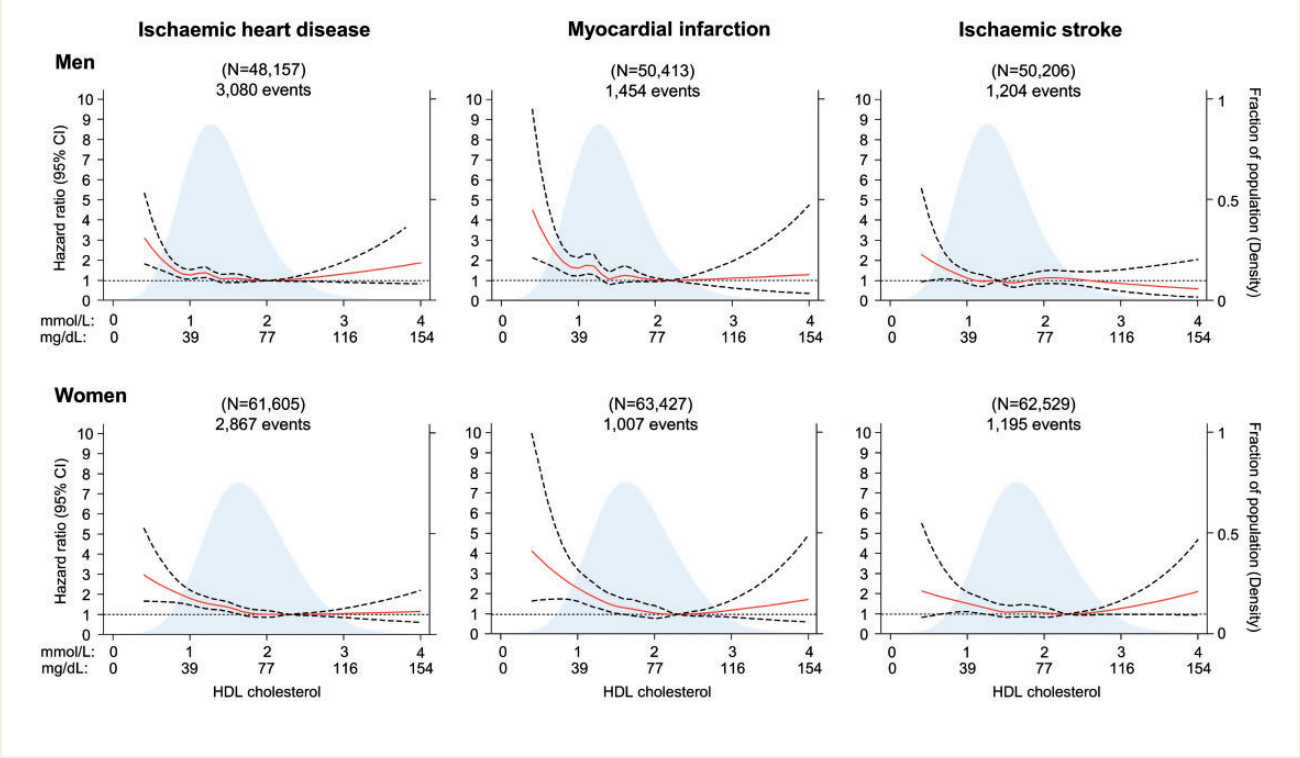


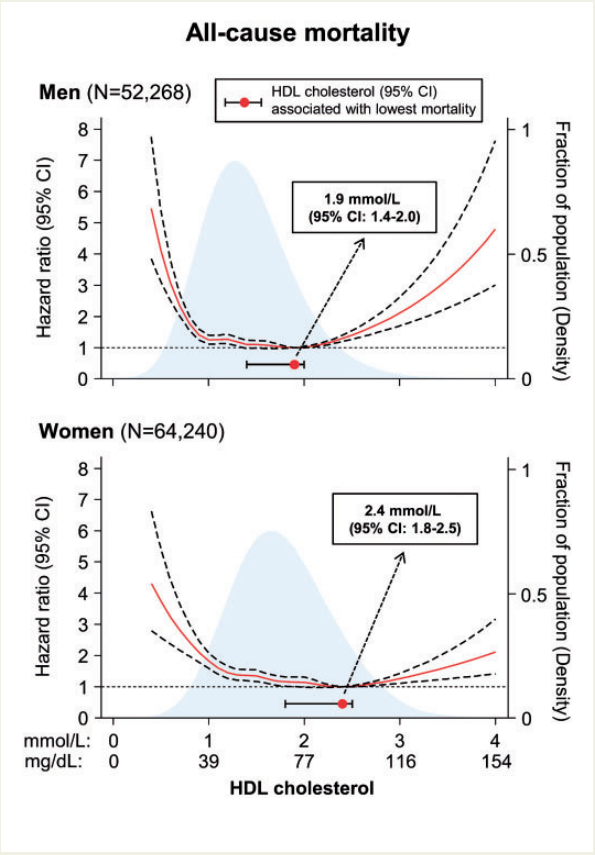
Figure 5 HDL cholesterol on a continuous scale and risk of ischaemic heart disease, myocardial infarction, and ischaemic stroke in the general population. Based on men and women from the Copenhagen General Population Study and the Copenhagen City Heart Study combined. Number of individuals included in the different analyses varies, as individuals with events before baseline were excluded for the three endpoints, respectively. Hazard ratio (solid line) and 95% confidence interval (dashed lines) were from Cox regression using restricted cubic splines. Analyses were multifactorially adjusted for age, study, body mass index, current smoking, cumulative tobacco smoking, alcohol consumption, physical activity, systolic blood pressure, diabetes mellitus, lipid-lowering therapy, LDL cholesterol, triglycerides, birth year, and menopausal status and hormone replacement therapy for women. The HDL cholesterol concentration associated with the lowest risk was used as reference, except for the endpoint ischaemic stroke in men, where the median value was used as the lowest risk was observed at the extreme high end. Graphs were truncated at 0.4 and 4.0 mmol/L due to limited number of individuals and events outside these cutpoints. The light blue area indicates the distribution of HDL cholesterol concentrations in men and women. CI, confidence interval; HDL, high-density lipoprotein.

specific mortality using underlying cause of death resulted in more deaths being classified as other mortality, however, results were similar to the primary analyses (compare *Figure 4* with Supplementary material online, *Figure S8*).

Discussion

In this study of 116 508 individuals from the general population, the association between HDL cholesterol and all-cause mortality was U-shaped, with both extreme high and low HDL cholesterol concentrations being associated with high mortality (*Summarizing Figure*). The same was observed in men with moderately elevated HDL cholesterol. The HDL cholesterol concentration associated with the lowest risk of all-cause mortality was 1.9 mmol/L (73 mg/dL) for men, and 2.4 mmol/L (93 mg/dL) for women, which are novel findings.

A possible explanation for the association between extreme high HDL cholesterol and higher mortality is that extreme high concentrations often are due to genetic variants.[19] Genetic variants may have detrimental effects causing high risk of disease and death, which is the case for certain mutations in *CETP*, *ABCA1*, *LIPC*, and *SCARB1*, which are associated with both high risk of coronary heart disease and high concentrations of HDL cholesterol.[6–8,10] These studies, done mainly in the Copenhagen City Heart Study, only overlap with 10–20% of individuals included in the present study, where the majority of individuals were from the Copenhagen General Population Study. The observed associations in this study could also be an epiphenomenon where there is a pathophysiologic abnormality, perhaps genetic, which increases risk in ways we do not understand and also increases HDL, suggesting that the physiology of HDL is complex and perhaps not well understood. Conformation and functional properties of the HDL particle may also be altered in individuals with extreme high HDL cholesterol and an alternative hypothesis could be that in individuals with extreme high HDL cholesterol, the functionality of HDL may be compromised such that HDL no longer function normally but rather cause harm. Another possible explanation includes differences in risk factors associated with both high HDL cholesterol and mortality. Although our multifactorially adjusted analyses included the most important risk factors for all-cause mortality,[20] residual confounding cannot be discarded completely. Whether the



Summarizing Figure HDL cholesterol and risk of all-cause mortality in the general population. Based on 52 268 men and 64 240 women from the Copenhagen General Population Study and the Copenhagen City Heart Study combined. Hazard ratio (solid line) and 95% confidence interval (dashed lines) from age and study adjusted Cox regression using restricted cubic splines. The concentration of HDL cholesterol associated with lowest mortality was used as reference. The light blue area indicates the distribution of HDL cholesterol concentrations in men and women. CI, confidence interval; HDL, high-density lipoprotein.

association between extreme high HDL cholesterol and increased mortality is causal is an important unresolved question in relation to the findings in this study.

Most observational studies investigating the association between HDL cholesterol and mortality have categorized individuals in larger groups, such as quintiles, and focus has been on low concentrations of HDL cholesterol, thereby failing to elucidate associations at extreme high concentrations. Two recently published studies based on routinely collected health data support our findings, as they indicated that the association between HDL cholesterol and mortality is not linear over the entire concentration range of HDL cholesterol.[21,22] Specifically, in the study by Ko *et al.* age-standardized all-cause mortality was lowest in the strata of HDL cholesterol from 51 to 70 mg/dL (1.3–1.8 mmol/L; 7.2/1000 person-years) in men and from 61 to 70 mg/dL (1.6–1.8 mmol/L; 5.6/1000 person-years) in women, and all-cause mortality increased to 12.1/1000 person-years in men with HDL cholesterol >90 mg/dL (>2.3 mmol/L) and to 6.8/1000 person-years in women with HDL cholesterol >90 mg/dL (>2.3 mmol/L). The study by Bowe *et al.* showed a similar U-shaped association between all-cause mortality and HDL cholesterol in men, with the lowest risk at HDL cholesterol of 30–50 mg/dL (0.8–1.3 mmol/L). However, both studies were based on selected populations as individuals were included because they had a previous contact with the health care system that included obtainment of a lipid panel, and either focused on men and the association between HDL cholesterol and mortality as modified by kidney function, or excluded individuals with cardiovascular disease and other comorbidities. Hence, results of these studies are not necessarily representative for the general population.

Results from this study may have clinical implications for individuals with extreme high concentrations of HDL cholesterol. First, when HDL cholesterol is used for risk assessment, clinicians should be aware that those with extreme high HDL cholesterol may be a high-risk group for all-cause mortality. Current guidelines recommend measuring HDL cholesterol for risk estimation and before initiation of lipid-lowering therapy.[23] Our results indicate that the common belief that, the higher the concentration of HDL cholesterol the better, does not hold for extreme high concentrations, as the relationship between HDL cholesterol and all-cause mortality was not linear over the entire range of HDL concentrations. Secondly, if the association between extreme high HDL cholesterol and high risk of death is causal, these findings would add to the uncertainty regarding elevating HDL cholesterol pharmacologically to extreme high concentrations. Some of the developed CETP inhibitors can increase HDL cholesterol to extreme high concentrations,[24] that in this study were associated with higher mortality. Interestingly, the development of the CETP inhibitor torcetrapib was discontinued as it increased mortality, although this could be due to off-target effects.[5] However, the present findings point toward an alternative explanation, namely that extreme high HDL cholesterol could in itself be the cause of high mortality.

As this study was observational, it could not determine if the association between extreme high HDL cholesterol and higher mortality was causal. The association may be due to unmeasured confounding or reverse causation. However, results from analyses excluding individuals dying within the first 1 to 5 years of follow-up did not seem to indicate reverse causation. Another limitation was the relative rarity of extremely high HDL concentrations; however, the ~2.5% of men with the highest HDL cholesterol were all at increased risk of mortality. Despite the very large number of individuals included in this study, numbers in extreme high HDL cholesterol groups were small, limiting statistical power, especially in stratified analyses and in analyses of cause-specific mortality, and affecting model fit at these extremes. Finally, as we only included white individuals of Danish descent, results may not necessarily be generalizable to other geographical regions or ethnicities; however, we are unaware of studies indicating that the present findings would not apply elsewhere and in other ethnicities.

A primary strength of the present study is the large number of prospectively recruited individuals from the general population. Second, we investigated the full range of HDL cholesterol concentrations, using both splines and categories based on concentrations and percentiles. Third, we had detailed information on several confounders with known effect on mortality. Fourth, use of the Danish registries ensured complete follow-up and complete information on death. Last, in the main analyses, data from two studies was combined to obtain maximum statistical power; however, results were similar in the

two studies separately. This indicates that the findings are robust, as they were confirmed in two independent cohorts, with inclusion of individuals in two different time periods.

Conclusions

The association between HDL cholesterol concentrations and all-cause mortality was U-shaped, and men and women from the general population with extreme high HDL cholesterol had high all-cause mortality. This was most pronounced in men, and for cardiovascular mortality. These findings need confirmation in future studies.

Supplementary material

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

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Conflict of interest: none declared.

References

1. Emerging Risk Factors Collaboration, Di Angelantonio E, Sarwar N, Perry P, Kaptoge S, Ray KK, Thompson A, Wood AM, Lewington S, Sattar N, Packard CJ, Collins R, Thompson SG, Danesh J. Major lipids, apolipoproteins, and risk of vascular disease. *Jama* 2009;**302**:1993–2000.
2. Wilson PW, Abbott RD, Castelli WP. High density lipoprotein cholesterol and mortality. The Framingham Heart Study. *Arteriosclerosis* 1988;**8**:737–741.
3. Pekkanen J, Linn S, Heiss G, Suchindran CM, Leon A, Rifkind BM, Tyroler HA. Ten-year mortality from cardiovascular disease in relation to cholesterol level among men with and without preexisting cardiovascular disease. *N Engl J Med* 1990;**322**:1700–1707.
4. Keene D, Price C, Shun-Shin MJ, Francis DP. Effect on cardiovascular risk of high density lipoprotein targeted drug treatments niacin, fibrates, and CETP inhibitors: meta-analysis of randomised controlled trials including 117,411 patients. *Bmj* 2014;**349**:g4379.
5. Barter PJ, Caulfield M, Eriksson M, Grundy SM, Kastelein JJ, Komajda M, Lopez-Sendon J, Mosca L, Tardif JC, Waters DD, Shear CL, Revkin JH, Buhr KA, Fisher MR, Tall AR, Brewer B, Investigators I. Effects of torcetrapib in patients at high risk for coronary events. *N Engl J Med* 2007;**357**:2109–2122.
6. Agerholm-Larsen B, Nordestgaard BG, Steffensen R, Jensen G, Tybjaerg-Hansen A. Elevated HDL cholesterol is a risk factor for ischemic heart disease in white women when caused by a common mutation in the cholesteryl ester transfer protein gene. *Circulation* 2000;**101**:1907–1912.
7. Andersen RV, Wittrup HH, Tybjaerg-Hansen A, Steffensen R, Schnohr P, Nordestgaard BG. Hepatic lipase mutations, elevated high-density lipoprotein cholesterol, and increased risk of ischemic heart disease: the Copenhagen City Heart Study. *J Am Coll Cardiol* 2003;**41**:1972–1982.
8. Frikke-Schmidt R, Nordestgaard BG, Jensen GB, Steffensen R, Tybjaerg-Hansen A. Genetic variation in ABCA1 predicts ischemic heart disease in the general population. *Arterioscler Thromb Vasc Biol* 2008;**28**:180–186.
9. Johannsen TH, Frikke-Schmidt R, Schou J, Nordestgaard BG, Tybjaerg-Hansen A. Genetic inhibition of CETP, ischemic vascular disease and mortality, and possible adverse effects. *J Am Coll Cardiol* 2012;**60**:2041–2048.
10. Zanoni P, Khetarpal SA, Larach DB, Hancock-Cerutti WF, Millar JS, Cuchel M, DerOhannessian S, Kontush A, Surendran P, Saleheen D, Trompet S, Jukema JW, De Craen A, Deloukas P, Sattar N, Ford I, Packard C, Majumder A, Alam DS, Di Angelantonio E, Abecasis G, Chowdhury R, Erdmann J, Nordestgaard BG, Nielsen SF, Tybjaerg-Hansen A, Schmidt RF, Kuulasmaa K, Liu DJ, Perola M, Blankenberg S, Salomaa V, Mannisto S, Amouyel P, Arveiler D, Ferrieres J, Muller-Nurasyid M, Ferrario M, Kee F, Willer CJ, Samani N, Schunkert H, Butterworth AS, Howson JM, Peloso GM, Stitziel NO, Danesh J, Kathiresan S, Rader DJ. Consortium CHDE, Consortium CAE, Global Lipids Genetics C. Rare variant in scavenger receptor BI raises HDL cholesterol and increases risk of coronary heart disease. *Science* 2016;**351**:1166–1171.
11. Wilkins JT, Ning H, Stone NJ, Criqui MH, Zhao L, Greenland P, Lloyd-Jones DM. Coronary heart disease risks associated with high levels of HDL cholesterol. *J Am Heart Assoc* 2014;**3**:e000519.
12. 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.
13. Nordestgaard BG, Palmer TM, Benn M, Zacho J, Tybjaerg-Hansen A, Davey Smith G, Timpson NJ. The effect of elevated body mass index on ischemic heart disease risk: causal estimates from a Mendelian randomisation approach. *PLoS Med* 2012;**9**:e1001212.
14. Afzal S, Tybjaerg-Hansen A, Jensen GB, Nordestgaard BG. Change in body mass index associated with lowest mortality in Denmark, 1976-2013. *Jama* 2016;**315**:1989–1996.
15. Nordestgaard BG, Langsted A, Mora S, Kolovou G, Baum H, Bruckert E, Watts GF, Sypniewska G, Wiklund O, Boren J, Chapman MJ, Cobbaert C, Descamps OS, von Eckardstein A, Kamstrup PR, Pulkki K, Kronenberg F, Remaley AT, Rifai N, Ros E, Langlois M. European Atherosclerosis Society, the European Federation of Clinical Chemistry, Laboratory Medicine joint consensus initiative. Fasting is not routinely required for determination of a lipid profile: clinical and laboratory implications including flagging at desirable concentration cut-points-a joint consensus statement from the European Atherosclerosis Society and European Federation of Clinical Chemistry and Laboratory Medicine. *Eur Heart J* 2016;**37**:1944–1958.
16. Clarke R, Shipley M, Lewington S, Youngman L, Collins R, Marmot M, Peto R. Underestimation of risk associations due to regression dilution in long-term follow-up of prospective studies. *Am J Epidemiol* 1999;**150**:341–353.
17. Jorgensen AB, Frikke-Schmidt R, Nordestgaard BG, Tybjaerg-Hansen A. Loss-of-function mutations in APOC3 and risk of ischemic vascular disease. *N Engl J Med* 2014;**371**:32–41.
18. Nordestgaard BG, Benn M, Schnohr P, Tybjaerg-Hansen A. Nonfasting triglycerides and risk of myocardial infarction, ischemic heart disease, and death in men and women. *Jama* 2007;**298**:299–308.
19. Motazacker MM, Peter J, Treskes M, Shoulders CC, Kuivenhoven JA, Hovingh GK. Evidence of a polygenic origin of extreme high-density lipoprotein cholesterol levels. *Arterioscler Thromb Vasc Biol* 2013;**33**:1521–1528.
20. World Health Organization. *Global Health Risks: Mortality and Burden of Disease Attributable to Selected Major Risks*. Geneva, Switzerland: World Health Organization; 2009.
21. Ko DT, Alter DA, Guo H, Koh M, Lau G, Austin PC, Booth GL, Hogg W, Jackevicius CA, Lee DS, Wijeysundera HC, Wilkins JT, Tu JV. High-density lipoprotein cholesterol and cause-specific mortality in individuals without previous cardiovascular conditions. The CANHEART Study. *J Am Coll Cardiol* 2016;**68**:2073–2083.
22. Bowe B, Xie Y, Xian H, Balasubramanian S, Az M, Al-Aly Z. High density lipoprotein cholesterol and the risk of all-cause mortality among U.S. veterans. *Clin J Am Soc Nephrol* 2016;**11**:1784–1793.
23. Piepoli MF, Hoes AW, Agewall S, Albus C, Brotons C, Catapano AL, Cooney MT, Corra U, Cosyns B, Deaton C, Graham I, Hall MS, Hobbs FD, Lochen ML, Lollgen H, Marques-Vidal P, Perk J, Prescott E, Redon J, Richter DJ, Sattar N, Smulders Y, Tiberi M, van der Worp HB, van Dis I, Verschuren WM. Authors/Task Force M. 2016 European Guidelines on cardiovascular disease prevention in clinical practice: The Sixth Joint Task Force of the European Society of Cardiology and Other Societies on Cardiovascular Disease Prevention in Clinical Practice (constituted by representatives of 10 societies and by invited experts)Developed with the special contribution of the European Association for Cardiovascular Prevention & Rehabilitation (EACPR). *Eur Heart J* 2016;**37**:2315–2381.
24. Krishna R, Anderson MS, Bergman AJ, Jin B, Fallon M, Cote J, Rosko K, Chavez-Eng C, Lutz R, Bloomfield DM, Gutierrez M, Doherty J, Bieberdorf F, Chodakewitz J, Gottesdiener KM, Wagner JA. Effect of the cholesteryl ester transfer protein inhibitor, anacetrapib, on lipoproteins in patients with dyslipidaemia and on 24-h ambulatory blood pressure in healthy individuals: two double-blind, randomised placebo-controlled phase I studies. *Lancet* 2007;**370**:1907–1914.