



Low Nonfasting Triglycerides and Reduced All-Cause Mortality: A Mendelian Randomization Study

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BACKGROUND: Increased nonfasting plasma triglycerides marking increased amounts of cholesterol in remnant lipoproteins are important risk factors for cardiovascular disease, but whether lifelong reduced concentrations of triglycerides on a genetic basis ultimately lead to reduced all-cause mortality is unknown. We tested this hypothesis.

METHODS: Using individuals from the Copenhagen City Heart Study in a mendelian randomization design, we first tested whether low concentrations of nonfasting triglycerides were associated with reduced all-cause mortality in observational analyses ($n = 13\,957$); second, whether genetic variants in the triglyceride-degrading enzyme lipoprotein lipase, resulting in reduced nonfasting triglycerides and remnant cholesterol, were associated with reduced all-cause mortality ($n = 10\,208$).

RESULTS: During a median 24 and 17 years of 100% complete follow-up, 9991 and 4005 individuals died in observational and genetic analyses, respectively. In observational analyses compared to individuals with nonfasting plasma triglycerides of 266–442 mg/dL (3.00–4.99 mmol/L), multivariably adjusted hazard ratios for all-cause mortality were 0.89 (95% CI 0.78–1.02) for 177–265 mg/dL (2.00–2.99 mmol/L), 0.74 (0.65–0.84) for 89–176 mg/dL (1.00–1.99 mmol/L), and 0.59 (0.51–0.68) for individuals with nonfasting triglycerides <89 mg/dL (<1.00 mmol/L). The odds ratio for a genetically derived 89-mg/dL (1-mmol/L) lower concentration in nonfasting triglycerides was 0.50 (0.30–0.82), with a corresponding observational hazard ratio of 0.87 (0.85–0.89). Also, the odds ratio for a genetically derived 50% lower concentration in nonfasting triglycerides was 0.43 (0.23–0.80), with a corresponding observational hazard ratio of 0.73 (0.70–0.77).

CONCLUSIONS: Genetically reduced concentrations of nonfasting plasma triglycerides are associated with reduced all-cause mortality, likely through reduced amounts of cholesterol in remnant lipoproteins.

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Increased nonfasting plasma triglycerides marking increased amounts of cholesterol in remnant lipoproteins are increasingly being recognized as an important risk factor for ischemic vascular disease (1–13). On the basis of this evidence, it may therefore seem obvious that low triglycerides will lead to reduced all-cause mortality, but this has not been documented at present. Neither randomized intervention trials nor mendelian randomization studies have previously addressed this question. An answer to this question is nevertheless important, as general practitioners and other clinicians are unlikely to increase therapeutic targeting of increases in triglycerides before such evidence exists.

A parallel situation was treatment of high cholesterol before publication of the 4S trial (Scandinavian Simvastatin Survival Study) (14), because before that statin trial many other studies had documented reduced cardiovascular disease as a consequence of cholesterol-lowering therapy, but not reduced all-cause mortality. When the 4S trial on this scientific background suddenly documented reduced all-cause mortality after LDL cholesterol reduction, clinical practice was changed worldwide; that is, cholesterol-lowering therapy was accepted as a key treatment for preventing cardiovascular disease.

We tested the hypothesis that genetically low concentrations of nonfasting triglycerides marking reduced amounts of cholesterol in remnant lipoproteins are associated with reduced all-cause mortality. Using a mendelian randomization design circumventing con-

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founding (15, 16), we first tested whether low nonfasting triglyceride concentrations were associated with reduced all-cause mortality in observational analyses; second, whether variants in the triglyceride-degrading enzyme lipoprotein lipase, causing reduced concentrations of nonfasting triglycerides and remnant cholesterol, were associated with reduced all-cause mortality in genetic analyses. We chose well-known variants in the lipoprotein lipase (*LPL*)⁵ gene (1–3), because lipoprotein lipase is the most important enzyme in the metabolism of triglycerides in plasma (17) and variants in this gene are therefore probably the best genetic instruments reflecting lifelong low concentrations of triglycerides. Importantly, strategies to increase lipoprotein lipase activity to reduce triglyceride concentrations are being developed (18, 19), and studies like the one described here may thus help clarify whether such a strategy is likely to be successful.

Methods

PARTICIPANTS

The Copenhagen City Heart Study is a prospective study of the general population initiated in 1976–1978 with follow-up examinations in 1981–1983, 1991–1994, and 2001–2003 (4). Participants were randomly selected on the basis of the national Danish Civil Registration System to reflect the adult Danish population ages 20–100 years. Data were obtained from a questionnaire, a physical examination, and blood samples at each examination. The study was approved by Herlev Hospital, Copenhagen University Hospital, and a Danish ethics committee and was conducted according to the Declaration of Helsinki. Written informed consent was obtained from all participants.

In observational analyses, to examine the association between low plasma concentrations of nonfasting triglycerides and all-cause mortality, we included 13 957 individuals from the 1976–1978 examination with measurements available (4). In genetic analyses, to examine the association between genetic variants in *LPL*, plasma nonfasting triglycerides, and all-cause mortality, we included 10 208 individuals from the 1991–1994 and the 2001–2003 examinations with genotypes for all 4 genetic variants in *LPL*; 6696 of these individuals had also participated in the 1976–1978 examination and were therefore also included in observational analyses.

GENETIC ANALYSES

In the 1991–1994 examination, we genotyped participants for S447X (rs328), D9N (rs1801177), N291S (rs268), and G188E (rs118204057) in *LPL*, as described (1, 3, 20–21). In the 2001–2003 examination, we used TaqMan-based assays (Applied Biosystems) for genotyping. TaqMan assays included positive controls genotyped using the original methods. Participants were also genotyped for 4 noncoding polymorphisms in *CRP* (C-reactive protein, pentraxin-related) (rs1205, rs3091244, rs1130864, and rs3093077) with TaqMan-based assays (22, 23). These polymorphisms affect plasma concentrations of C-reactive protein (CRP) and were included in genetic analyses as a negative control on all-cause mortality (23). Finally, participants were genotyped for 3 polymorphisms in *TRIB1* (tribbles pseudokinase 1) (rs2954029), *GCKR* [glucokinase (hexokinase 4) regulator] (rs1260326), and *APOA5* (apolipoprotein A-V) (rs651821) that also reduce plasma nonfasting triglycerides (13), and these were included in sensitivity analyses. All genotype distributions were in Hardy–Weinberg equilibrium ($P \geq 0.3$).

NONFASTING PLASMA TRIGLYCERIDES

Plasma triglyceride concentrations were measured in the nonfasting state with standard hospital assays. In observational analyses, baseline triglyceride concentrations from the 1976–1978 examination were grouped into 5 categories [<89 mg/dL (<1.00 mmol/L), 89–176 mg/dL (1.00–1.99 mmol/L), 177–265 mg/dL (2.00–2.99 mmol/L), 266–442 mg/dL (3.00–4.99 mmol/L), and ≥ 443 mg/dL (≥ 5.00 mmol/L)]. We preplanned cutoffs at each 89-mg/dL (1-mmol/L) decrease as done previously (4); however, to achieve a large enough reference group for statistically meaningful comparisons, we grouped individuals with moderately increased triglyceride concentrations of 266–442 mg/dL (3.00–4.99 mmol/L) into 1 group to serve as reference. Also, because individuals with severely increased triglycerides [≥ 443 mg/dL (≥ 5.00 mmol/L)] may represent a special phenotype, it was not meaningful to let this group serve as the reference group. In genetic analyses, baseline concentrations of triglycerides from the 1991–1994 and the 2001–2003 examinations were used to examine the association between genetic variants in *LPL* and plasma nonfasting triglycerides.

ALL-CAUSE MORTALITY

Information on death from any cause was obtained from the national Danish Civil Registration System. Mortality from cardiovascular disease were deaths due to ischemic heart disease (WHO, *International Classification of Diseases*, revision 8 and revision 10: codes

⁵ Human genes: *LPL*, lipoprotein lipase; *CRP*, C-reactive protein, pentraxin-related; *TRIB1*, tribbles pseudokinase 1; *GCKR*, glucokinase (hexokinase 4) regulator; *APOA5*, apolipoprotein A-V; *APOC3*, apolipoprotein C-III.

410-414 and I20-I25) and/or ischemic cerebrovascular disease (codes 431-438 and I60-I69, G45) obtained from the national Danish Causes of Death Registry, or deaths within 28 days of a hospital admission with the diagnoses listed above as obtained from the national Danish Patient Registry. Participants were followed by use of the unique Central Person Register number from study entry to end of follow-up in May 2011. Follow-up was 100% complete; that is, we did not lose track of even a single individual.

COVARIATES

Body mass index was calculated as measured weight (kilograms) divided by measured height (meters) squared. Hypertension was use of antihypertensive medication, systolic blood pressure ≥ 140 mmHg, and/or diastolic blood pressure ≥ 90 mmHg. Diabetes was self-reported disease, use of insulin or oral hypoglycemic agents, and/or nonfasting plasma glucose concentrations > 198 mg/dL (> 11 mmol/L). Smokers were current smokers. Physical inactivity was leisure time activity < 4 h weekly and predominantly sedentary work. Alcohol intake was self-reported and converted to grams of alcohol per day. Plasma total cholesterol, HDL cholesterol, and CRP were measured by use of standard hospital assays; in the 1976–1978 examination, only triglycerides and total cholesterol were measured, that is, HDL and LDL cholesterol were not measured. In the 1991–1994 and 2001–2003 examinations, LDL cholesterol was calculated by use of the Friedewald equation when plasma triglycerides were < 155 mg/dL (≤ 4.0 mmol/L) and otherwise measured directly by use of a standard hospital assay. Lipid-lowering therapy was self-reported and although the type was not specified, the majority of individuals were likely by use of statins as this is the most commonly used type of lipid-lowering therapy in Denmark. The rates of missing values for covariates were 1% or below. The missing indicator method was used to account for missing information for categorical covariates. For continuous covariates, missing values were imputed by use of linear regression analysis with age and sex as predictors.

STATISTICAL ANALYSES

We used STATA/SE 12.0. ANOVA models were used to compare means for continuous covariates and Pearson χ^2 test to compare frequencies for categorical covariates.

In observational analyses, to examine the association between nonfasting triglyceride concentrations and all-cause mortality, we used Cox proportional hazard models with delayed entry at examination to estimate hazard ratios with 95% CIs. Proportionality of hazards over time was judged by visual inspection of

cumulative hazard logarithm plots against age; no violations were observed. Models were adjusted for age (as timescale) and sex and multivariably for other cardiovascular risk factors such as hypertension, smoking, alcohol intake, physical inactivity, total cholesterol, and use of lipid-lowering medication; a priori we did not adjust risk estimates for body mass index and diabetes, because these covariates may be part of the biological pathway linking low triglyceride concentrations to reduced all-cause mortality. However, if we adjusted for these additional covariates, results were similar to those presented. We estimated hazard ratios for all-cause mortality for a 89-mg/dL (1-mmol/L) decrease in triglycerides by analyzing triglycerides as a continuous variable, and \log_2 -transformed triglycerides to estimate the hazard ratio for a 50% reduction. Combined effects of measurement errors, longer-term fluctuations, and within-person changes, also known as regression dilution, led to underestimation of the true association of a risk factor with disease outcome. To accommodate this, hazard ratios including CIs in observational analyses were corrected for regression dilution bias by use of a nonparametric method (24). For this correction, we used a subset of individuals ($n = 6696$) with replicate measurements of nonfasting triglycerides from the 1976–1978 examination, and approximately 15 years later in the 1991–1994 examination, to compute a regression dilution ratio for triglycerides of 0.58. This correction helps avoid underestimation of risk estimates but does not affect levels of statistical significance.

In genetic analyses, to examine the effect of *LPL* genotypes on plasma nonfasting triglycerides, the genotypes with the highest concentrations served as reference. To obtain maximum statistical power for all-cause mortality, we first examined all possible genotype combinations and grouped each participant according to number of triglyceride-decreasing alleles (G for S447X, G for D9N, A for N291S, and G for G188E). The group with the lowest number of these alleles and thus the highest concentrations of triglycerides served as reference. ANOVA between groups was performed by use of nonparametric methods owing to the nonnormal distribution of triglycerides. To examine the association between genotypes and all-cause mortality, we used Kaplan–Meier curves and the log-rank trend test to compare probabilities of survival as a function of age. For the test for trend, groups based on number of triglyceride-decreasing alleles were coded 1, 2, 3, etc. Additionally, Cox proportional hazard models were used to estimate hazard ratios for all-cause mortality according to number of triglyceride-decreasing alleles. Models were adjusted for age (as timescale), sex, hypertension, smoking, alcohol intake, physical inactivity, total cholesterol, and use of lipid-lowering medication;

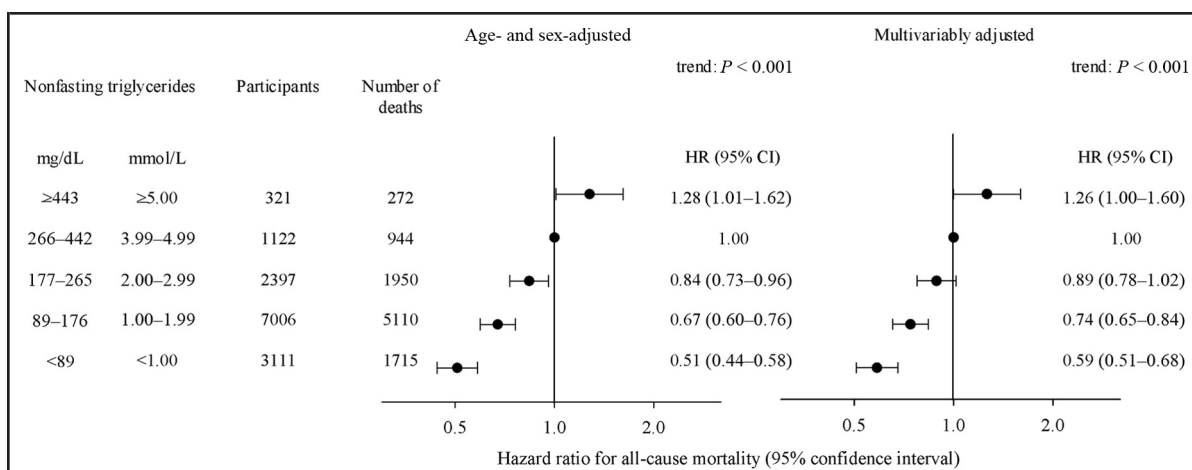


Fig. 1. Hazard ratios for all-cause mortality by decreasing concentrations of nonfasting plasma triglycerides in 13 957 individuals from the Copenhagen City Heart Study.

Hazard ratios were adjusted for age (as timescale) and sex and multivariably for age (as timescale), sex, hypertension, smoking status, alcohol intake, and use of lipid-lowering medication. HR, hazard ratio. P values are for trend.

in sensitivity analysis, we also adjusted for HDL cholesterol concentrations. As described in Zacho et al. (22), the observed hazard ratio for a 50% reduction in triglycerides was used to predict theoretical risk of all-cause mortality associated with the changes in nonfasting triglyceride concentrations caused by the number of triglyceride-decreasing alleles. In analyses using *CRP*, *TRIB1*, *GCKR*, and *APOA5* genotypes, the analytical strategy was similar.

To estimate the association of genetically low triglycerides with reduced all-cause mortality, we applied the general theory of instrumental variable analyses using the generated unweighted allele score as a marker of nonfasting triglycerides (25). We performed 2-stage least squares regression with a second-stage logistic regression to estimate a genetically derived odds ratio for a 89-mg/dL (1-mmol/L) and a 50% lower concentration of nonfasting triglycerides (25). With a weighted allele score or the multiplicative generalized method of moments estimator, results were similar to those presented.

Results

Baseline characteristics of the 13 957 individuals included in observational analyses and the 10 208 individuals included in genetic analyses are shown in Supplemental Table 1, which accompanies the online version of this article at <http://www.clinchem.org/content/vol60/issue5>. In observational analyses, participants entered the study at the 1976–1978 examination ($n = 13\ 957$), and during a median 24 years of follow-up, 9991 died. In genetic analyses, participants entered

the study in 1991–1994 ($n = 9114$) or in 2001–2003 ($n = 1094$), and during a median of 17 years of follow-up, 4005 died.

NONFASTING TRIGLYCERIDES AND ALL-CAUSE MORTALITY

There was a stepwise reduced risk of all-cause mortality by decreasing concentrations of nonfasting plasma triglycerides (Fig. 1). Compared to individuals with nonfasting triglyceride concentrations of 266–442 mg/dL (3.00–4.99 mmol/L), age- and sex-adjusted hazard ratios were 0.84 (95% CI 0.73–0.96) for 177–265 mg/dL (2.00–2.99 mmol/L), 0.67 (0.60–0.76) for 89–176 mg/dL (1.00–1.99 mmol/L), and 0.51 (0.44–0.58) for individuals with nonfasting triglycerides < 89 mg/dL (< 1.00 mmol/L). Corresponding multivariably adjusted hazard ratios were 0.89 (0.78–1.02), 0.74 (0.65–0.84), and 0.59 (0.51–0.68), respectively. For individuals with nonfasting triglycerides ≥ 443 mg/dL (≥ 5.00 mmol/L) vs 266–442 mg/dL (3.00–4.99 mmol/L), the hazard ratio for all-cause mortality was 1.28 (1.01–1.62) in the sex- and age-adjusted model and 1.26 (1.00–1.60) in the multivariably adjusted model.

LPL GENOTYPES AND NONFASTING TRIGLYCERIDES

Minor allele frequencies were 0.10 for S447X, 0.01 for D9N, 0.02 for N291S, and 0.0004 for G188E (pairwise correlations, all: $r^2 < 1\%$). Lower concentrations of nonfasting triglycerides were observed for the S447X polymorphism (CG and GG genotypes vs CC genotype, 11% and 22% decrease), the D9N polymorphism (AA/AG vs GG, 9% decrease), the N291S polymor-

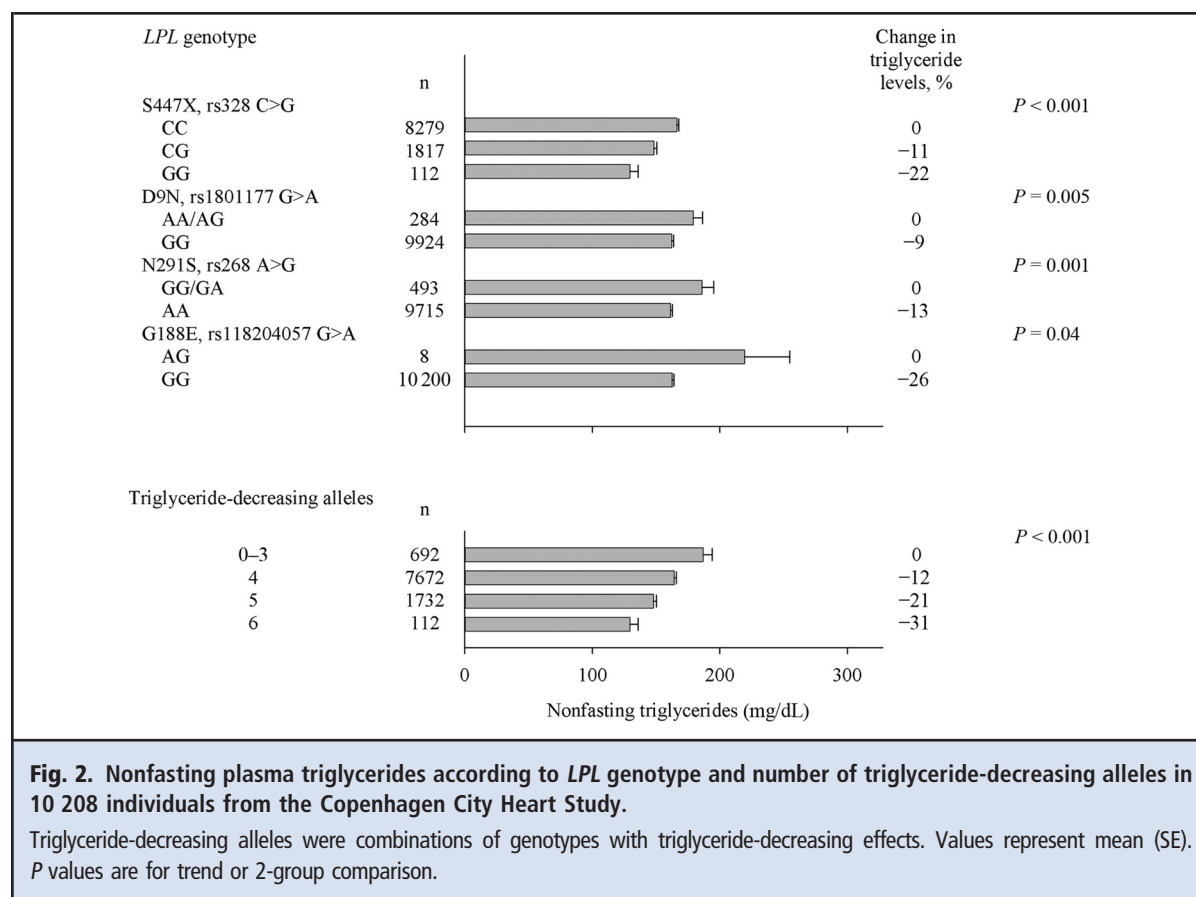


Fig. 2. Nonfasting plasma triglycerides according to LPL genotype and number of triglyceride-decreasing alleles in 10 208 individuals from the Copenhagen City Heart Study.

Triglyceride-decreasing alleles were combinations of genotypes with triglyceride-decreasing effects. Values represent mean (SE). P values are for trend or 2-group comparison.

phism (GG/GA vs AA, 13% decrease), and the G188E mutation (AG vs GG, 26% decrease) (Fig. 2). When combining genotypes by number of triglyceride-decreasing alleles, an increasing number of alleles resulted in a decrease in nonfasting triglycerides of up to 31%. Corresponding values were 23% lower concentrations of remnant cholesterol, 15% higher concentrations of HDL cholesterol, and unaffected LDL cholesterol concentrations (see online Supplemental Fig. 1).

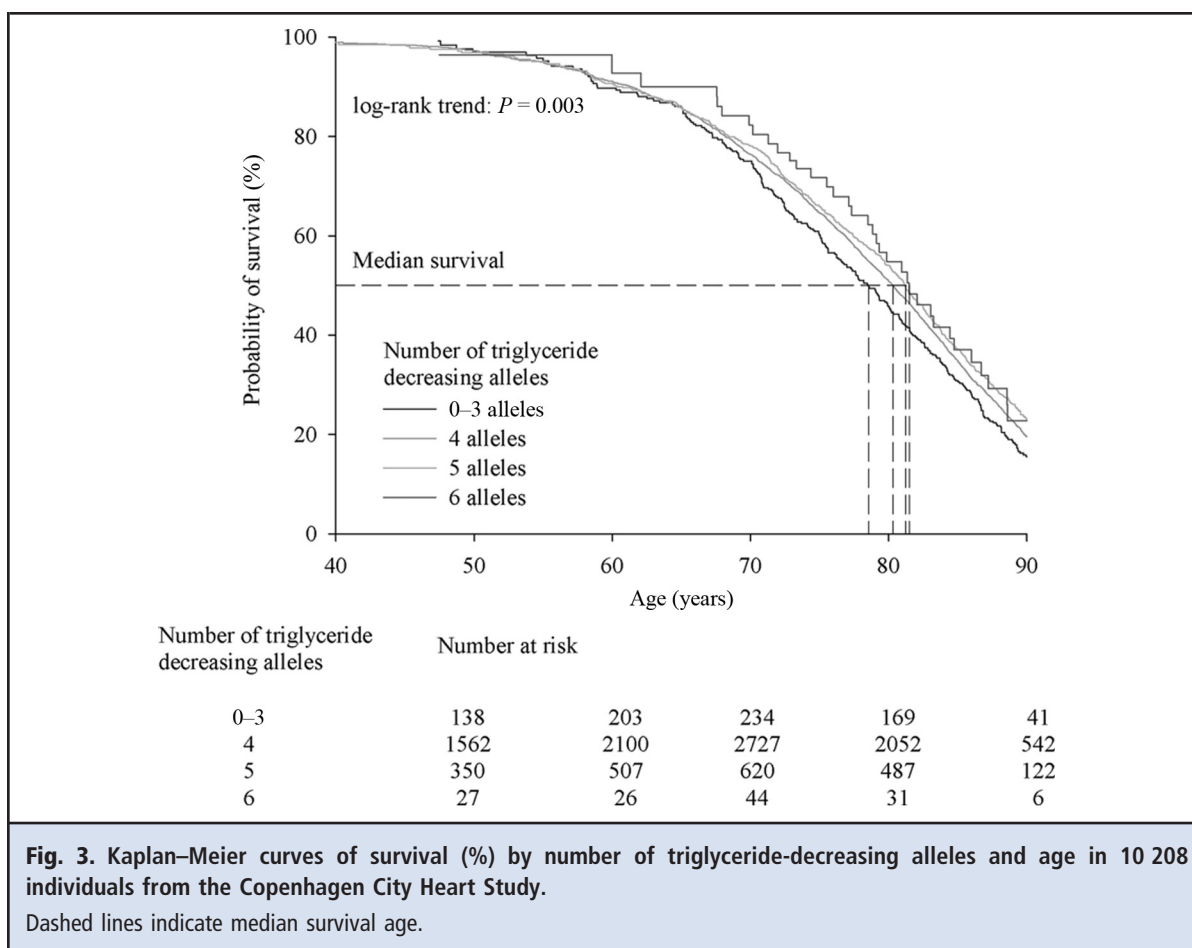
Among all participants, 3031 individuals had 3 separate measurements of nonfasting plasma triglycerides, namely from the examinations in 1976–1978, 1991–1994, and 2001–2003. At all 3 examinations spanning 25 years, concentrations of nonfasting triglycerides were lowest in those with the largest number of triglyceride-decreasing alleles (see online Supplemental Fig. 2). This demonstrates that low concentrations of nonfasting plasma triglycerides from genetic variations in LPL are lifelong.

LPL GENOTYPES AND ALL-CAUSE MORTALITY

The probability of surviving increased by number of triglyceride-decreasing alleles (log-rank trend: P =

0.004) (Fig. 3). Median survival age was 78 years in individuals with 0–3 triglyceride decreasing alleles, 80 years in individuals with 4 alleles, and 81 years in individuals with 5 or 6 alleles.

On the basis of assuming that a decrease in nonfasting triglycerides via reduced remnant cholesterol is causally associated with reduced all-cause mortality, lifelong decreased concentrations due to genetic variants should confer a similar reduced risk of death compared to observational estimates. On the basis of this assumption and similar to that done previously (22), we estimated that the decrement in nonfasting triglycerides due to number of triglyceride-decreasing alleles would theoretically predict a hazard ratio for all-cause mortality of 0.94 (0.93–0.95) for 4 alleles, 0.90 (0.88–0.92) for 5 alleles, and 0.86 (0.83–0.88) for 6 alleles, respectively, vs 0–3 alleles (Fig. 4, upper panel). Importantly, we observed corresponding hazard ratios of 0.86 (0.76–0.97), 0.81 (0.71–0.93), and 0.77 (0.55–1.08) by increasing number of triglyceride-decreasing alleles; that is, the observed risks for all-cause mortality by triglyceride-decreasing alleles were larger than those theoretically predicted. To test our method, we examined the association between CRP genotypes and risk of



all-cause mortality, as a negative control (23). Number of CRP-decreasing alleles was associated with a decrement in plasma CRP concentrations of up to 28%, resulting in a theoretically predicted risk of all-cause mortality of 0.89 (0.87–0.90) (Fig. 4, lower panel). However, the observed hazard ratios for all-cause mortality by CRP-decreasing alleles did not differ from 1.0.

Various cardiovascular risk factors were equally distributed among participants grouped according to number of triglyceride-decreasing alleles (see online Supplemental Table 2). This indicates that genotype was not confounded by conventional cardiovascular risk factors.

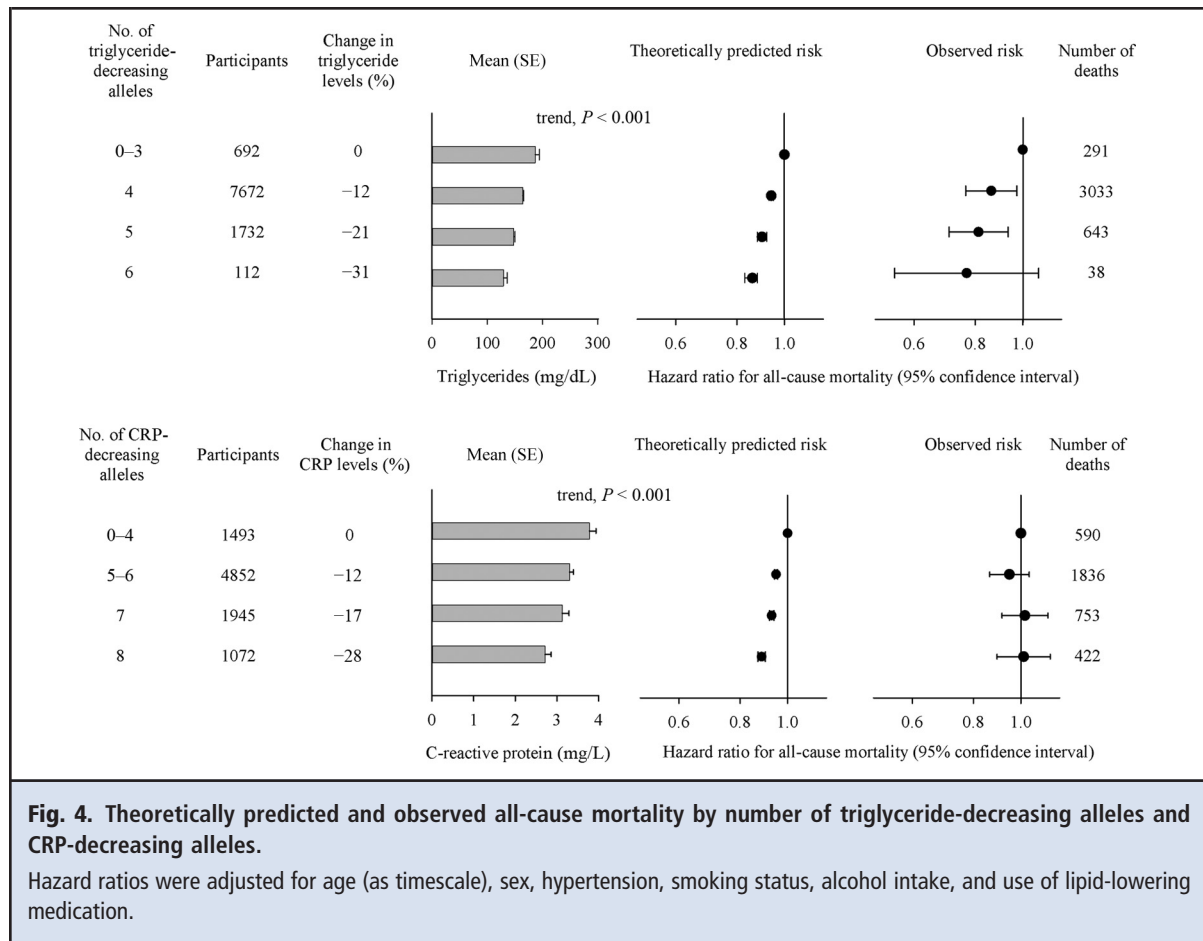
GENETIC VS OBSERVATIONAL ASSOCIATIONS

In instrumental variable analyses, the odds ratio for a genetically derived 89-mg/dL (1-mmol/L) lower concentration in nonfasting plasma triglycerides was 0.50 (0.30–0.82) ($f = 15$, $r^2 = 0.7\%$) (Fig. 5); the corresponding observational hazard ratio was 0.87 (0.85–0.89). Also, the corresponding odds ratio for a genetically derived 50% lower concentration in nonfasting

plasma triglycerides was 0.43 (0.23–0.80), whereas the observational hazard ratio was 0.73 (0.70–0.77).

SENSITIVITY ANALYSES

When all-cause mortality was stratified into mortality from cardiovascular disease and mortality from other causes, results from observational and genetic analyses were largely similar to those presented for all-cause mortality (see online Supplemental Figs. 3 and 4). Also, including adjustment for HDL cholesterol concentrations in genetic analyses, results were similar (see online Supplemental Fig. 5). Finally, we examined 3 other genetic variants in *TRIB1*, *GCKR*, and *APOA5* that also affect concentrations of nonfasting triglycerides (see online Supplemental Fig. 6). Hazard ratios for all-cause mortality for these 3 variants alone and in combination with the 4 *LPL* genotypes are shown in online Supplemental Fig. 7. Risk estimates for all-cause mortality were similarly reduced in all genotype combinations; however, CIs were wider in analyses including only *TRIB1*, *GCKR*, and *APOA5* genotypes, explained by lower statistical power due to the relatively small num-



ber of individuals in the reference group. Online Supplemental Fig. 7 also illustrates that the chosen genetic variants in *LPL* are optimal genetic instruments to examine the effect of low concentrations of triglycerides, because of the inclusion of the common gain-of-function variant S447X that results in a large enough reference group for a statistically meaningful comparison.

Discussion

The principal novel finding of this study is that low concentrations of nonfasting plasma triglycerides from genetic variations in the triglyceride-degrading enzyme lipoprotein lipase are associated with reduced all-cause mortality, likely through reduced amounts of cholesterol in remnant lipoproteins. This is a valuable observation for the design of future randomized intervention trials aimed at lowering triglycerides in those with increased concentrations.

Mechanistically, these findings are easy to understand, as lipoprotein lipase hydrolyses triglycerides

contained in chylomicrons and very-low-density lipoprotein (17). Thus, homozygosity for rare deleterious mutations in *LPL* leads to the chylomicronemia syndrome characterized by severely increased triglycerides with excessive accumulation of chylomicrons and very large very-low-density lipoproteins in plasma. This syndrome rarely leads to cardiovascular disease, most likely explained by plasma lipoprotein particles being too large to enter into the arterial intima (26, 27). However, more common variants in *LPL*, such as those examined in this study, lead to moderately decreased or increased triglycerides with chylomicron remnants and very-low-density lipoprotein remnants present in plasma. These cholesterol-containing smaller remnant lipoproteins are able to penetrate the intima (28) and appear to be preferentially trapped within the arterial wall (29), promoting atherosclerosis. Thus, lower concentrations of triglycerides reflecting low amounts of cholesterol in remnant lipoproteins may prevent the development of atherosclerosis and ultimately reduce all-cause mortality, as seen in our study. In support, a genome-wide association study in the inbred Old Or-

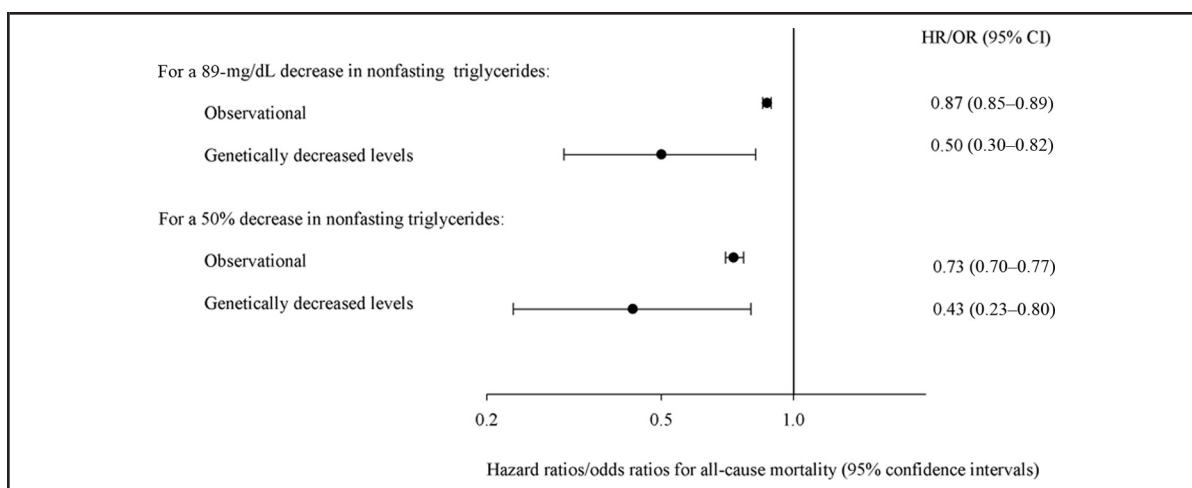


Fig. 5. Genetic and observational risk estimates for an 89-mg/dL (1-mmol/L) decrease and a 50% decrease of nonfasting plasma triglycerides.

Instrumental variable analyses were used to estimate genetically derived odds ratios. Hazard ratios were adjusted for age (as timescale), sex, hypertension, smoking status, alcohol intake, and use of lipid-lowering medication. HR, hazard ratio; OR, odds ratio.

der Amish identified heterozygosity for a null mutation (R19X) in *APOC3* (apolipoprotein C-III) resulting in lower triglycerides and a decrease in coronary artery calcification as a marker for subclinical atherosclerosis (8), suggesting that lifelong deficiency of apolipoprotein C-III promoting triglyceride hydrolysis may have a cardioprotective effect. Our study suggests that this apparent cardioprotective effect of lifelong low triglycerides translates into reduced all-cause mortality.

The similar reduction in mortality from cardiovascular disease and mortality from other causes could be a result of cardiovascular deaths that have not been registered correctly in the Danish registries. Since 1990, autopsies in Denmark have become rare, and consequently the cause of death in most cases is based on the attending physicians' best guess postmortem, naturally leading to some degree of misclassification. Therefore, in our primary analyses, we used all-cause mortality with the most statistical power, with no misclassification, and with 100% complete ascertainment in Denmark owing to each person having a Central Person Registration number. Nevertheless, it is reassuring that low concentrations of nonfasting plasma triglycerides are associated with reduced all-cause and cardiovascular mortality alike.

Some limitations of our study must be considered in evaluating our results. The inverse association between triglycerides and HDL cholesterol concentrations in plasma likely represents the most important pleiotropic effects of the genetic variants in *LPL* (3); however, although observational studies have found an association between high concentrations of HDL cho-

lesterol and reduced risk of ischemic heart disease (9), this association has not been confirmed in genetic studies (30–32), indicating that this relationship is not causal. Also, increased cholesterol in triglyceride-rich or remnant lipoproteins is causally associated with increased risk of ischemic heart disease as well as with low-grade inflammation (12–13, 33) independent of reduced HDL cholesterol concentrations (13). Furthermore, adjustment for HDL concentrations in the present genetic analyses gave similar results to those presented. It is therefore most likely that our results are explained by the triglyceride- and remnant cholesterol-lowering effect of the *LPL* genotypes rather than the parallel increase in HDL cholesterol. A more thorough understanding of the variability in genetic effects on different lipid subfractions and metabolites may be useful in the design of future mendelian randomization studies to fully understand the biological processes leading to atherosclerosis and cardiovascular disease. Also, participants prescribed with lipid-lowering medication late into follow-up may have confounded results.

Lifestyle intervention and current best treatment are aimed at lowering LDL cholesterol, reducing blood pressure, and preventing thrombotic events, thus reducing risk of cardiovascular disease in patients. However, persistent residual risk even among individuals with optimal treatment and hypertriglyceridemia seems to be implicated in this excess risk (34). Indeed, subgroup analyses in randomized trials show that among those with increased triglycerides, lowering of triglycerides lead to reduced cardiovascular disease

(34–36). Genetic studies are generally free from confounding and reverse causation and may provide insights in the beneficial effects of lifelong low triglycerides. Indeed, lifelong low triglycerides resulting from genetic polymorphisms are associated with a decrease in coronary artery calcification as a marker for subclinical atherosclerosis (8) and ultimately also seem to reduce all-cause mortality, as seen in the present study. These results highlight the need for clinical trials of new drugs targeted at reducing cholesterol in remnant lipoproteins, perhaps through modulation of lipoprotein lipase activity, to reduce residual cardiovascular risk.

Author Contributions: All authors confirmed they have contributed to the intellectual content of this paper and have met the following 3 requirements: (a) significant contributions to the conception and design,

acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; and (c) final approval of the published article.

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