Elevated nonfasting remnant cholesterol and low-density lipoprotein (LDL) cholesterol are causally associated with ischemic heart disease (IHD), but whether nonfasting remnant cholesterol and LDL cholesterol both cause low-grade inflammation is currently unknown.

Methods and Results—We studied 60608 individuals from the Copenhagen General Population Study, the Copenhagen City Heart Study, and the Copenhagen Ischemic Heart Disease study, of whom 10668 had IHD diagnosed between 1977 and 2011. We genotyped for variants affecting levels of nonfasting remnant cholesterol, LDL cholesterol, C-reactive protein by CRP alleles, and C-reactive protein by IL6R alleles. Using a multidirectional mendelian randomization design, we investigated possible causal associations between the lipoproteins and C-reactive protein and between the lipoproteins and IHD. A 1-mmol/L (39 mg/dL) higher level of nonfasting remnant cholesterol was associated observationally with a 37% (95% confidence interval, 35–39) higher C-reactive protein level and causally with a 28% (95% confidence interval, 10–48) higher level. For LDL cholesterol, a 1-mmol/L (39 mg/dL) higher level was associated observationally with a 7% (95% confidence interval, 6–7) higher C-reactive protein level, but we found no causal association. Likewise, higher levels of C-reactive protein did not associate causally with elevated nonfasting remnant cholesterol or LDL cholesterol. Finally, the causal risk ratio for IHD for a 1-mmol/L (39 mg/dL) higher level was 3.3 (95% confidence interval, 2.1–5.2) for nonfasting remnant cholesterol and 1.8 (95% confidence interval, 1.5–2.2) for LDL cholesterol. The causal associations for remnant cholesterol were present even in those without diabetes mellitus and obesity.

Conclusions—Elevated nonfasting remnant cholesterol is causally associated with low-grade inflammation and with IHD, whereas elevated LDL cholesterol is associated causally with IHD without inflammation. (Circulation. 2013;128:1298-1309.)

Key Words: cardiovascular diseases | genetics | lipids | lipoproteins | risk factors

Elevated nonfasting remnant cholesterol and low-density lipoprotein (LDL) cholesterol are causally associated with ischemic heart disease (IHD), but whether nonfasting remnant cholesterol and LDL cholesterol both cause low-grade inflammation is currently unknown.

Clinical Perspective on p 1309

This is a clinically important question because statin therapy lowers both LDL cholesterol and C-reactive protein (CRP) levels and because randomized statin trials have shown that lowering of both, compared with lowering of only LDL cholesterol, is associated with additional reduction of cardiovascular disease. The reason could be that statins not only inhibit the production of LDL cholesterol but also have pleiotropic anti-inflammatory effects, which could help prevent cardiovascular disease. Alternatively, because statins lower not only LDL cholesterol and CRP but also triglycerides and thus remnant cholesterol levels, this could be an additional means of reducing atherosclerosis and inflammation in the arterial wall. Remnant cholesterol is the cholesterol content of triglyceride-rich lipoproteins, composed of very low-density lipoproteins and intermediate-density lipoproteins in the fasting state and of these 2 lipoproteins together with chylomicron remnants in the nonfasting state. Remnant
cholesterol and triglycerides are 2 different types of fat and are components of the same lipoproteins, that is, remnants; therefore, levels of remnant cholesterol and triglycerides are highly correlated ($R^2=0.96$); however, because many human cells can degrade triglycerides and none can degrade cholesterol, it seems more plausible that it is the cholesterol content of remnants that is causal for atherosclerosis development, in the same way as for LDL cholesterol, by accumulation in the arterial wall.\textsuperscript{7,8}

Using a multidirectional mendelian randomization approach, we tested the hypothesis that nonfasting remnant cholesterol and LDL cholesterol are both causally associated with low-grade inflammation, marked by elevated CRP levels, and with IHD. In the mendelian randomization approach, the random assortment of alleles at conception is used to circumvent confounding and reverse causation.\textsuperscript{9}

We genotyped 60,608 white individuals of Danish descent from Copenhagen, of whom 10,668 had IHD, for genetic variants affecting levels of nonfasting remnant cholesterol, LDL cholesterol, CRP by CRP alleles, and CRP by IL6R alleles. Using knowledge from our previous studies\textsuperscript{1} to select genetic variants affecting levels of nonfasting remnant cholesterol or LDL cholesterol to address the study hypothesis, we chose genetic variants in genes encoding key enzymes, receptors, and apolipoproteins in lipoprotein metabolism or genetic variants found in genome-wide association studies to be associated with elevated triglycerides and thus with remnant cholesterol levels. To further exclude reverse causation, that is, the possibility that low-grade inflammation, marked by elevated CRP levels, may directly result in higher levels of remnant cholesterol or LDL cholesterol, we used genetic variants known to increase levels of CRP.\textsuperscript{10,11} By including both CRP and IL6R alleles, which represent different stages of the inflammatory pathway, we examined the causality of inflammation and CRP per se.

The various associations examined in this study are outlined in Figure 1. We initially examined all relationships observationally, that is, analyses prone to confounding and reverse causation, and next conducted corresponding causal analyses using genetic instruments not prone to confounding and reverse causation. First, we examined the observational association between elevated lipoproteins and elevated CRP levels (Figure 1, number 1). Second, we confirmed that the genetic variants chosen in fact directly caused elevated levels of nonfasting remnant cholesterol, LDL cholesterol, or CRP (Figure 1, number 2). Third, we examined whether elevated nonfasting remnant cholesterol or elevated LDL cholesterol is a direct cause of elevated CRP levels and vice versa (Figure 1, number 3). Fourth and fifth, we compared the observational (Figure 1, number 4) and causal (number 5) associations of elevated levels of nonfasting remnant cholesterol, LDL cholesterol, and CRP with risk of IHD.

### Methods

Studies were approved by institutional review boards and Danish ethics committees (H-KF-01-144/01, KF-100.2039/91, KF-01-144/01, KA-93125, KA-99039) and conducted according to the Declaration of Helsinki with informed consent from participants. All participants were white and of Danish descent. No individual was included in >1 study. Because of the completeness of the Danish registers, follow-up was 100% complete; that is, no individual was lost to follow-up.

### The Copenhagen General Population Study

The Copenhagen General Population Study (CGPS) is a prospective study of the Danish general population initiated in 2003 with ongoing enrollment.\textsuperscript{12} Participants of Danish descent from the greater Copenhagen area were randomly selected within age strata from the national Danish Civil Registration System to reflect the adult population 20 to 100 years of age. Data collection included a self-administered questionnaire that was reviewed by an investigator on the day of attendance, a physical examination, and blood sampling for biochemical analyses and for DNA extraction. A total of 48,250 participants were eligible for analyses, that is, had information on all genotypes, lipoprotein, and CRP levels and on end points.

### The Copenhagen City Heart Study

The Copenhagen City Heart Study (CCHS) is a prospective study of the Danish general population initiated in 1976 to 1978 with follow-up examinations in 1981 to 1983, 1991 to 1994, and 2001 to 2003.\textsuperscript{13} Participants were recruited and examined exactly as in the CGPS. Blood samples for DNA extraction and biochemical measurements were drawn at the 1991–1994 examination. A total of 7,417 participants attending the 1991–1994 examination had information on all genotypes, lipoprotein and CRP levels, and end points.

### The Copenhagen Ischemic Heart Disease Study

The Copenhagen Ischemic Heart Disease Study (CIHDS) comprises 4,941 patients from the greater Copenhagen area referred for coronary angiography to Rigshospitalet, Copenhagen University Hospital during the period of 1991 to 2011. Besides a diagnosis of IHD or myocardial infarction as described below, these patients also had stenosis/atherosclerosis on coronary angiography or a positive exercise ECG test. All participants had information on all genotypes and end points but not on lipoprotein or CRP levels.

![Figure 1](https://example.com/figure1.png)

**Figure 1.** Study design with possible associations among plasma levels of nonfasting remnant cholesterol, low-density lipoprotein (LDL) cholesterol, and C-reactive protein (CRP); genetic variants influencing the levels of these biomarkers; and ischemic heart disease. Arrows numbered 1 through 5 denote the various associations examined in this study. Two-sided arrows are for observational associations; 1-sided arrows are for causal associations indicating the causal direction. IL6 indicates interleukin 6.
Ischemic Heart Disease

Information on the diagnosis of IHD (World Health Organization International Classification of Diseases [ICD], Eighth Revision [ICD8] and 10th Revision [ICD10]; ICD8: 410–414; ICD10: I20-I25) was collected and verified from 1977 until 2011 by reviewing all hospital admissions and diagnoses entered in the national Danish Patient Registry and all causes of death entered in the national Danish Causes of Death Registry, as described. All individuals in Denmark are assigned an identification number at birth or immigration (the national Danish Civil Registration System) by which they can be tracked in the national Danish Patient Registry and the national Danish Causes of Death Registry. Therefore, these registers contain complete information on all participants, and follow-up is 100% complete for both morbidity leading to hospital contact and mortality.

Laboratory Analyses

Standard hospital colorimetric assays measured nonfasting total cholesterol, triglycerides, and high-density lipoprotein cholesterol (Boehringer Mannheim and Konelab). LDL cholesterol was calculated with the Friedewald equation when plasma triglycerides were ≤4.0 mmol/L and otherwise measured directly (Konelab). Nonfasting remnant cholesterol was nonfasting total cholesterol minus high-density lipoprotein cholesterol minus LDL cholesterol. CRP was measured as high-sensitivity CRP on plasma stored for 12 to 15 years in the CCHS and on fresh plasma samples in the CGPS using turbidimetry or nephelometry (Dako or Dade Behring). All assays were assessed daily for precision by using internal controls and 4 to 12 times yearly for accuracy with a Scandinavian external quality control program.

Genotypes

Genotyping was by TaqMan (Applied Biosystems) or by restriction enzyme assays (details available from authors). Genotypes were verified by genotyping of randomly selected samples of each variant by 2 different methods (TaqMan plus sequencing or restriction enzyme assay). Call rates for genotypes were >99.9% for all assays because of reruns.

Other Covariates

Smokers were current smokers. Hypertension was systolic blood pressure ≥140 mmHg (≥135 mmHg for diabetics), diastolic blood pressure ≥90 mmHg (≥85 mmHg for diabetics), or use of antihypertensive medication prescribed specifically for hypertension. Diabetes mellitus was self-reported disease, nonfasting plasma glucose >11.0 mmol/L, medication prescribed for diabetes mellitus, or hospitalization for or death resulting from diabetes mellitus (ICD8: 249, 250; ICD10: E10, E11, E13, E14). Body mass index was measured weight (kg) divided by measured height squared (m²). Lipid-lowering therapy, menopausal status, and hormone replacement therapy were self-reported.

Figure 2. Association of nonfasting remnant cholesterol (left) and low-density lipoprotein (LDL) cholesterol (right) with C-reactive protein (CRP) in 48250 participants from the Copenhagen General Population Study. Analyses were by linear regression with different adjustments and represent number 1 in Figure 1. Gray areas indicate 95% confidence interval (CI) of the fitted regression line.
Statistical Analysis

Data were analyzed with Stata/SE12.0. Nonnormally distributed variables were log transformed to approach normal distribution. The χ² tests evaluated Hardy-Weinberg equilibrium. Participants with CRP levels >10 mg/L were excluded from all analyses a priori because these individuals likely have concurrent diseases such as infections, inflammatory diseases, or cancer. The statistical analyses were conducted according to the order of number 1 through 5 in Figure 1.

Nonfasting Remnant Cholesterol, LDL Cholesterol, and CRP: Observational Associations

In Figure 2, linear regression examined associations of nonfasting remnant cholesterol or LDL cholesterol versus CRP levels in 48,250 participants from the CGPS. Linear regressions were unadjusted; were adjusted for age, sex, and lipid-lowering therapy; or were multivariably adjusted for age, sex, lipid-lowering therapy, smoking, hypertension, menopausal status (women only), and hormone replacement therapy (women only) because these factors may influence lipoprotein or CRP levels. Analyses in Figure 2 were purposely not adjusted for variables that in themselves through biological pathways may affect lipoprotein or CRP levels such as body mass index and diabetes mellitus.

In Figure 3 (top), linear regression examined associations between nonfasting remnant cholesterol and CRP levels stratified by sex and use of hormone replacement therapy. Multivariable adjustment was for age, lipid-lowering therapy, smoking, and hypertension.

Using interaction tests, we examined whether the association between remnant cholesterol and CRP levels was modified by sex, menopausal status in women, or use of hormone replacement therapy in postmenopausal women. Interaction was tested for by introducing a 2-factor interaction term (remnant cholesterol×modifying covariate) in the multivariably adjusted model and comparing this model to a multivariably adjusted model without the interaction term using a likelihood ratio test.

In Figure 3 (bottom), linear regression was also used to estimate associations between nonfasting remnant cholesterol and CRP levels stratified by diabetes mellitus and obesity, that is, body mass index ≤30 and >30 kg/m². Linear regressions stratified by diabetes mellitus and obesity were adjusted for age, sex, lipid-lowering therapy, smoking, hypertension, menopausal status (women only), and hormone replacement therapy (women only). Interactions between remnant cholesterol and diabetes mellitus or obesity on CRP levels were tested for in the same way as described above.

Figures 2 and 3 show fitted lines of the regressions with 95% confidence intervals (CIs), that is, linear regressions of CRP levels on remnant cholesterol or LDL cholesterol within the specific strata examined. An assumption for linear regression is normally distributed residuals, and this was evaluated by examining fitted values, and by plotting residuals against the fitted values, and by plotting residuals against fitted values. No violations were observed.
Genotypes and Levels of Lipoproteins and CRP

In Figure 4, 1-way ANOVA was used to compare lipoprotein and CRP levels as a function of genotypes in 48250 participants from the CGPS. Genotypes were combined in 3 groups: (1) nonfasting remnant cholesterol–increasing alleles (TRIB1 rs2954029, APOA5 rs651821, LPL rs1801177, LPL rs118204057, LPL rs268, and LPL rs328), (2) LDL cholesterol–increasing alleles (APOB rs5742904, LDLR W23X, LDLR W66G, LDLR W568S, and PCSK9 rs11591147), (3) CRP-increasing alleles in the CRP gene (rs1205, rs1130864, rs3091244, and rs3093077), and (4) CRP-increasing alleles in the IL6R gene (rs4537545). P values for trend were estimated by the Cuzick extension of the Wilcoxon rank-sum test.

From the genetic variants associated with increased levels of remnant cholesterol, the allele score was calculated by adding the number of remnant cholesterol–increasing alleles and combining these into 3 larger groups of 0 to 2, 3 to 4, and 5 to 7 remnant cholesterol–increasing alleles to maximize the statistical power in each group. A similar strategy was used for genetic variants associated with LDL cholesterol levels. From the 4 genetic variants in CRP, we generated all possible genotype combinations and ranked the 9 most common combinations according to increasing plasma CRP levels, as done previously. For the IL6R genotypes, only 1 genetic variant was used.

Assumptions for 1-way ANOVA include variance homogeneity and normality of the x variable, and because CRP levels are highly skewed, CRP levels were log transformed to approach a normal distribution. Effect sizes in Figure 4 therefore indicate mean values and standard errors of the mean of remnant cholesterol and LDL cholesterol, as well as geometric means and standard errors of the geometric mean of CRP in each genotype group.

Nonfasting Remnant Cholesterol, LDL Cholesterol, and CRP: Causal Associations

In Figure 5, potential causal relationships among elevated levels of nonfasting remnant cholesterol, LDL cholesterol, and CRP were estimated by 2-stage least-squares regression in an additive model in 48250 participants from the CGPS. Genotypes associated with a specific
lipoprotein or CRP, that is, nonfasting remnant cholesterol, LDL cholesterol, or CRP, were included as allele or genotype scores in first- and second-stage regressions. The strength of the instruments was evaluated by $F$ statistics from the first-stage regression in which an $F$ statistic $>10$ indicates sufficient strength to ensure the validity of the instrumental variable analysis.9 $R^2$ as a percentage was used as a measure of the contribution of genotypes to the variation in the different lipoprotein or CRP levels. Observational associations in Figure 5 were examined by unadjusted linear regressions as in Figure 2. They were added for comparison and represent the analyses in number 1 in Figure 1.

In Figure 6, causal and observational associations between elevated levels of nonfasting remnant cholesterol and CRP were estimated in 48,250 participants from the Copenhagen General Population Study. Observational and causal analyses represent numbers 1 and 3 in Figure 1, respectively. IL6R indicates interleukin 6 receptor.

Risk of IHD: Observational and Causal Associations

In Figure 7, observational odds ratios for IHD were estimated by logistic regression in the CGPS and CCHS combined (left) or the CGPS, CCHS, and CHDS combined (right) and were adjusted for age and sex. In Figure 8, observational odds ratios, also adjusted for age and sex but stratified for diabetes mellitus and obesity, were estimated by logistic regression in the CGPS and CCHS combined.

In Figure 7, causal risk ratios for IHD were estimated by the multiplicative generalized methods of moments estimator. $F$ statistics and $R^2$ as a percentage were estimated from the first stage of a 2-stage least-squares regression. Genotypes were included as allele or genotype scores in the same way as described above. In Figure 7 (left), analyses included participants from the CGPS and CCHS with information on all covariates and with remnant cholesterol, LDL cholesterol, and CRP.
in participants using lipid-lowering therapy multiplied by 1.33, 1.42, and 1.33, respectively, corresponding to average reductions of 25%, 30%, and 25% using common statin treatment regimens. In Figure 7 (right), analyses included all available participants from the CGPS, CCHS, and CIHDS to maximize statistical power; however, lipoprotein and CRP values were not available for all patients in the CIHDS and were imputed from age, sex, and genotypes on the basis of the known influence of genotypes on levels of lipoproteins and CRP in the CGPS and the CCHS. The same was done for participants from the CGPS and CCHS using lipid-lowering therapy. In Figure 8, causal risk ratios for IHD stratified for diabetes mellitus and obesity were estimated in the same way as in Figure 7 (left), including participants from the CGPS and CCHS with information on diabetes mellitus and body mass index.

Results

Table I in the online-only Data Supplement shows baseline characteristics of participants in the CGPS by nonfasting remnant cholesterol, LDL cholesterol, and CRP in tertiles. Genotype distributions for all studies were in Hardy-Weinberg equilibrium (P > 0.1).

Nonfasting Remnant Cholesterol, LDL Cholesterol, and CRP: Observational Associations

Associations between plasma levels of the lipoproteins and CRP are shown in Figure 2. Plasma levels of CRP were 37% (95% CI, 35–39) higher per 1-mmol/L (39 mg/dL) higher level of nonfasting remnant cholesterol and 7% (95% CI, 6–7) higher per 1-mmol/L (39 mg/dL) higher level of plasma LDL cholesterol (Figure 2, top). Associations were robust when adjusted for age, sex, and lipid-lowering therapy (Figure 2, middle) and even when additionally adjusted for smoking, hypertension, and menopausal status and hormone replacement therapy in women (Figure 2, bottom).

Figure 6. Observational and causal associations of a 1-mmol/L (39 mg/dL) higher level of remnant cholesterol with C-reactive protein (CRP) levels stratified by diabetes mellitus and obesity. Observational changes in plasma levels were by linear regression and causal estimates were by instrumental variable analyses in 48,250 participants from the Copenhagen General Population Study. Observational and causal analyses represent numbers 1 and 3 in Figure 1, respectively. BMI indicates body mass index.
Observational associations were also robust when stratified for diabetes mellitus (Figure 3, bottom) and with no sign of the association between remnant cholesterol and CRP levels being modified by diabetes mellitus (P for interaction=0.2). When stratified for obesity, the observational association was less pronounced for obese participants with body mass index >30 kg/m² compared with participants with body mass index ≤30 kg/m². Interaction test showed evidence that the association between remnant cholesterol and CRP levels was modified by obesity (P<0.001).

Genotypes and Levels of Lipoproteins and CRP

Lipoprotein and CRP levels as a function of increasing number of lipoprotein-/CRP-increasing alleles for the CGPS are shown in Figure 4 for groups of genotypes with similar effects. Corresponding effects of the individual genotypes are shown in Figure I in the online-only Data Supplement.

For nonfasting remnant cholesterol, 5 to 7 versus 0 to 2 alleles were associated with a 0.2-mmol/L (8-mg/dL) higher level of nonfasting remnant cholesterol and low-density lipoprotein cholesterol (LDL-C) and for a doubling in plasma C-reactive protein (CRP) levels for ischemic heart disease. Left, Results for 55,667 participants with information on all covariates from the Copenhagen General Population Study (CGPS) and the Copenhagen City Heart Study (CCHS) combined, with remnant cholesterol, LDL-C, and CRP in participants using lipid-lowering therapy (n=4666) multiplied by 1.33, 1.42, and 1.33, respectively, corresponding to average reductions of 25%, 30%, and 25% using common statin treatment regimens. Right, Results for 60,608 participants from the CGPS, the CCHS, and the Copenhagen Ischemic Heart Disease Study (CIHDS) combined with remnant cholesterol, LDL-C, and CRP in participants using lipid-lowering therapy (n=4666) and in participants from the CIHDS (n=4941), imputed from age, sex, and genotypes on the basis of the known association between genotypes and levels of lipoprotein and CRP (Figure 4). Observational risk estimates were by logistic regression, and causal risk estimates were by instrumental variable analyses. Observational and causal analyses represent numbers 4 and 5 in Figure 1, respectively. IL6R indicates interleukin 6 receptor.

Nonfasting Remnant Cholesterol, LDL Cholesterol, and CRP: Causal Associations

We examined potential causal associations between the lipoproteins and CRP in instrumental variable analyses (Figure 5). For nonfasting remnant cholesterol, a 1-mmol/L (39-mg/dL) higher level was observationally associated with a 37% (95% CI, 35–39) higher level of CRP and causally with a 28% (95% CI, 10–48) higher CRP level for remnant cholesterol-increasing alleles. For LDL cholesterol, a 1-mmol/L (39-mg/dL) higher level was associated observationally with a 7% (95% CI, 6–7) higher level of CRP, but there was no causal association for LDL-increasing alleles. For CRP, genotype combination 9 versus 1 was associated with a 0.071-mmol/L (3-mg/dL; 95% CI, 0.068-0.075) higher level of nonfasting remnant cholesterol; however, there was no causal association for either CRP or IL6R alleles (Figure 5, middle). In addition, a doubling in plasma CRP was associated observationally with a 0.065-mmol/L (2-mg/dL; 95% CI,
0.057-0.072) higher level of LDL cholesterol but also with no causal associations (Figure 5, bottom).

Both causal and observational associations between remnant cholesterol and CRP levels were robust when stratified by diabetes mellitus and by obesity and, importantly, were present even in those without diabetes mellitus and in those without obesity (Figure 6).

Risk of IHD: Observational and Causal Associations
We also examined the potential causal association of the lipoproteins and CRP with risk of IHD in instrumental variable analyses (Figure 7). For the CGPS, CCHS, and CIHDS combined (Figure 7, right), the causal risk estimate for IHD for a 1-mmol/L (39-mg/dL) genetically higher level of remnant cholesterol was 3.3 (95% CI, 2.1–5.2) with a corresponding observational odds ratio of 1.3 (95% CI, 1.2–1.4). For LDL cholesterol, the corresponding values were 1.8 (95% CI, 1.5–2.2) for causal and 1.2 (95% CI, 1.1–1.2) for observational. A doubling in plasma CRP levels was associated with an observational odds ratio of 1.2 (95% CI, 1.2–1.3); however, the causal association for CRP alleles with IHD was only of borderline significance and was in the opposite direction from the observational estimate, and the causal association for IL6R alleles with IHD was not significant, with a risk ratio of 1.7 (95% CI, 0.7–3.8; Figure 8, bottom).

Risk factors for IHD other than lipoproteins were generally distributed equally among genotypes, confirming that the genotypes are not confounded (Table II in the online-only Data Supplement). The only clear exceptions were the expected greater use of lipid-lowering therapy among those with the highest levels of nonfasting remnant cholesterol and LDL cholesterol and a difference in menopausal status for IL6R genotypes, which could be a chance finding.

Discussion
In this study, we find a causal association between elevated nonfasting remnant cholesterol and low-grade inflammation, marked by elevated CRP levels, together with IHD, but no causal association between elevated LDL cholesterol and
low-grade inflammation. These findings are novel. We have previously demonstrated that elevated remnant cholesterol is causally associated with increased risk of IHD and that this association is independent of low high-density lipoprotein cholesterol levels.\(^1\)\(^2\)

The causal association of nonfasting remnant cholesterol with low-grade inflammation observed in this study could be explained by remnant cholesterol causing atherosclerosis, a disease with a known inflammatory element.\(^14\) Randomized, clinical trials have found that statin therapy prevents cardiovascular disease when CRP levels are increased, even if LDL cholesterol levels are below traditional intervention levels.\(^15\) This could be explained by statins having anti-inflammatory effects that in themselves prevent cardiovascular disease; however, it could also be explained by statins lowering not only LDL cholesterol levels but also triglyceride and thus nonfasting remnant cholesterol levels, thereby reducing atherosclerosis and inflammation. Indeed, randomized, clinical intervention trials aimed at lowering LDL cholesterol levels with statins have shown that triglycerides and non–high-density lipoprotein cholesterol, the latter being LDL cholesterol plus remnant cholesterol, were also lowered during treatment.\(^16\)\(^17\) Nonfasting triglycerides and remnant cholesterol are highly correlated\(^1\) because remnant cholesterol is the cholesterol content of triglyceride-rich lipoproteins, composed of very low-density lipoproteins and intermediate-density lipoproteins in the fasting state, and of these 2 lipoproteins together with chylomicron remnants in the nonfasting state. Therefore, lowering of triglycerides is at the same time a lowering of remnant cholesterol levels.

The association between elevated levels of nonfasting remnant cholesterol and low-grade inflammation was robust even after stratifying by sex, hormone replacement therapy, diabetes mellitus, and obesity, with the highest effect size found in premenopausal women, who are likely the healthiest participants with presumably the lowest levels of other cardiovascular risk factors, including elevated CRP levels. Also in support of our conclusions, we found that the causal association between elevated remnant cholesterol and both low-grade inflammation and IHD remained significant even in participants without diabetes mellitus and obesity, indicating that the associations between elevated remnant cholesterol and both low-grade inflammation and IHD are not driven solely by diabetes mellitus or obesity. The lack of causal association between remnant cholesterol and IHD in participants with diabetes mellitus could be explained by a lack of statistical power or by the fact that the relatively small change in remnant cholesterol levels caused by the genetic variants is not large enough to show causality in the presence of other risk factors in individuals with diabetes mellitus, that lipid-lowering therapy in participants with diabetes mellitus will attenuate the causal effect of remnant cholesterol, and that remnant cholesterol may not cause atherosclerosis in individuals with diabetes mellitus. However, we are not aware of other data to support the last possibility.

A surprising finding was that we did not observe a causal association between elevated LDL cholesterol and low-grade inflammation like we did for elevated nonfasting remnant cholesterol. In accordance with this apparent paradox, in the present study, the effect size for the observational association between elevated LDL cholesterol and elevated CRP was small compared with that between elevated nonfasting remnant cholesterol and elevated CRP. Therefore, the simplest explanation is that elevated LDL cholesterol does not cause low-grade inflammation. In favor of such an interpretation, studies of patients with heterozygous familial hypercholesterolemia, which is an inherited condition with very high levels of LDL cholesterol caused by a defect in the LDL receptor, found either no difference in CRP levels between patients and control subjects\(^18\)\(^21\) or only slightly higher levels of CRP in patients compared with control subjects.\(^22\)\(^24\) Even patients with homozygous familial hypercholesterolemia, who have extremely high levels of LDL cholesterol and therefore have a very high risk of early atherosclerosis, had only slightly increased levels of CRP compared with control subjects.\(^25\) In addition, it has always been puzzling that LDL particles need to be oxidized before they can be taken up by macrophages, whereas triglyceride-rich lipoproteins or remnants can be taken up by macrophages without oxidation.\(^26\) These observations from cell culture studies may therefore help explain why elevated remnant cholesterol in humans is causally associated with low-grade inflammation but elevated LDL cholesterol is not. Taken together, this suggests that elevated LDL cholesterol causes atherosclerosis without a major inflammatory component, whereas an inflammatory component of atherosclerosis is driven by elevated nonfasting remnant cholesterol.

Strengths of the present study include the very large sample size, the >10,000 IHD events included with no loss to follow-up, and the use of a multidirectional mendelian randomization design to compare causal associations of elevated remnant cholesterol and elevated LDL cholesterol simultaneously within the same individuals. It is also a strength that our genetic instruments had \(F\) values much larger than 10, indicating sufficient strength of the instruments. Finally, it is a strength that we used whites of Danish descent only, thus eliminating population admixture as a potential problem.

The mendelian randomization approach nevertheless has potential limitations, the most important being pleiotropy of the genetic variants used; that is, the genetic variants may affect the end point (levels of nonfasting remnant cholesterol/LDL cholesterol/CRP or risk of IHD) through mechanisms other than their effects on lipoprotein/CRP levels.\(^27\) For a proper mendelian randomization study, a variant that exclusively affects the biomarker studied is needed. Because lipoproteins are the result of multiple interrelated metabolic processes involving many genes, ideal variants that affect levels of only 1 lipoprotein simply may not exist. However, choosing >1 variant in several different genes and on different chromosomes makes it unlikely that the variants have the same pleiotropic effects.\(^27\) Another limitation is that we studied whites of Danish descent only; however, we are not aware of data suggesting that our results should not apply to most races and countries.
Conclusions

In this study of 60,608 individuals, we found a causal association between elevated nonfasting remnant cholesterol and low-grade inflammation, together with increased risk of IHD; however, we found no causal association between elevated LDL cholesterol and low-grade inflammation. These findings are important because, even when LDL cholesterol is lowered to recommended levels, there is still a substantial residual risk of IHD, which could be explained by the association between nonfasting remnant cholesterol and low-grade inflammation.

Acknowledgments

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Disclosures

Dr Nordestgaard has received lecture or consultancy honoraria from Omthera, Sanofi-Aventis/Regeneron, Aegerion, AstraZeneca, and ISIS Pharmaceuticals. The other authors report no conflicts.

References

Elevated levels of both nonfasting remnant cholesterol and low-density lipoprotein cholesterol are causally associated with ischemic heart disease, but whether both lipoproteins also are associated with low-grade inflammation is unknown. This is a clinically important question because atherosclerosis is a disease with an inflammatory component and because randomized, clinical intervention trials have shown that statins prevent cardiovascular disease in individuals with low-grade inflammation with moderately elevated levels of C-reactive protein, even when low-density lipoprotein cholesterol levels are relatively low. The reason could be that statins lower not only low-density lipoprotein cholesterol levels but also triglycerides levels and thus remnant cholesterol levels. In this study, we found both elevated levels of nonfasting remnant cholesterol and elevated levels of low-density lipoprotein cholesterol to be causally associated with increased risk of ischemic heart disease, but only elevated levels of nonfasting remnant cholesterol were causally associated with low-grade inflammation. Taken together, this suggests that elevated low-density lipoprotein cholesterol levels cause atherosclerosis without a major inflammatory component, whereas an inflammatory component of atherosclerosis is driven by elevated nonfasting remnant cholesterol levels. Thus, we here point toward a new direction for reducing cardiovascular disease beyond that achieved through low-density lipoprotein cholesterol lowering.
Elevated Remnant Cholesterol Causes Both Low-Grade Inflammation and Ischemic Heart Disease, Whereas Elevated Low-Density Lipoprotein Cholesterol Causes Ischemic Heart Disease Without Inflammation

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Supplementary material

Elevated remnant cholesterol causes both low-grade inflammation and ischemic heart disease, while elevated low-density lipoprotein cholesterol causes ischemic heart disease without inflammation.

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2 Supplementary Tables and 4 Supplementary Figures
**Supplementary Table 1.** Characteristics of individuals in the Copenhagen General Population Study by tertiles of nonfasting remnant cholesterol, LDL cholesterol, and C-reactive protein.

<table>
<thead>
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<th>Remnant cholesterol tertiles (mmol/L)</th>
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<td>&lt;0.5 0.5-0.8 &gt;0.8</td>
<td>&lt;2.9 2.9-3.6 &gt;3.7</td>
<td>&lt;1.2 1.2-2.0 &gt;2.0</td>
</tr>
<tr>
<td>Number</td>
<td>16,187 16,002 16,061</td>
<td>17,220 15,831 15,199</td>
<td>16,111 16,068 16,071</td>
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<tr>
<td>Women, %</td>
<td>69 56 40</td>
<td>57 55 54</td>
<td>54 55 57</td>
</tr>
<tr>
<td>Age, years</td>
<td>53(44-63) 58(47-67) 58(49-67)</td>
<td>54(43-66) 56(47-66) 58(50-66)</td>
<td>53(45-63) 56(46-65) 60(49-69)</td>
</tr>
<tr>
<td>Lipid-lowering therapy, %</td>
<td>7 10 12</td>
<td>19 6 2</td>
<td>7 10 11</td>
</tr>
<tr>
<td>Current smokers, %</td>
<td>18 22 26</td>
<td>19 21 25</td>
<td>19 19 27</td>
</tr>
<tr>
<td>Hypertension, %</td>
<td>54 66 75</td>
<td>60 65 71</td>
<td>56 65 74</td>
</tr>
<tr>
<td>Diabetes mellitus, %</td>
<td>3 4 8</td>
<td>8 4 3</td>
<td>3 4 8</td>
</tr>
<tr>
<td>Body mass index, kg/m^2</td>
<td>24(22-26) 26(23-28) 27(25-30)</td>
<td>25(22-28) 26(23-28) 26(24-29)</td>
<td>24(22-26) 26(23-28) 27(25-31)</td>
</tr>
<tr>
<td>Menopausal status, % (women only)</td>
<td>53 69 78</td>
<td>51 65 80</td>
<td>57 65 71</td>
</tr>
<tr>
<td>Hormone replacement therapy, % (postmenopausal women only)</td>
<td>20 17 15</td>
<td>21 18 14</td>
<td>15 17 20</td>
</tr>
<tr>
<td>Ischemic Heart Disease, %</td>
<td>6 9 11</td>
<td>11 7 7</td>
<td>7 7 11</td>
</tr>
</tbody>
</table>

Continuous values are summarized as median and interquartile range and categorical values are summarized in percent. P-values for trend are by Cuzick’s extension of a Wilcoxon rank-sum test. LDL=low-density lipoprotein.
Supplementary Table 2. Characteristics of individuals in the Copenhagen General Population Study by genotypes.

<table>
<thead>
<tr>
<th>Alleles/Combinations</th>
<th>No.</th>
<th>Age years</th>
<th>Women %</th>
<th>Body mass index kg/m²</th>
<th>Diabetes mellitus %</th>
<th>Menopause, women only %</th>
<th>Hypertension %</th>
<th>Smoking %</th>
<th>Lipid-lowering therapy %</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Remnant cholesterol</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TRIB1 rs2954029</td>
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<td></td>
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<tr>
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<td>65</td>
<td>65</td>
<td>22</td>
</tr>
<tr>
<td>LPL rs1801177, rs118204057, rs268, rs328</td>
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<td>32,517</td>
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<td>26(23-28)</td>
<td>5</td>
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<td>65</td>
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<td></td>
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<td>57</td>
<td>25(23-28)</td>
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<td>61</td>
<td>63</td>
<td>21</td>
</tr>
<tr>
<td>P-trend</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td><strong>LDL cholesterol</strong></td>
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<td>26(23-28)</td>
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<td>63</td>
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<td>P-trend</td>
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<tr>
<td><strong>IL6 receptor</strong></td>
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<td>P-trend</td>
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<td>0.9</td>
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</tr>
</tbody>
</table>

Continuous values are summarized as median and interquartile range and categorical values are summarized in percent. P-values for trend are by Cuzick's extension of a Wilcoxon rank-sum test. IL6=interleukin 6, LDL=Low-density lipoprotein.
- **Remnant cholesterol**
  - *TRIB1* rs2954029: 11,059 0, 24,077 1, 13,114 2
    - p < 0.001
    - p = 0.6
  - *APOA5* rs651821: 42,461 0, 5,599 1, 190 2
    - p < 0.001
    - p = 0.003
  - *LPL* rs1801177: 46,836 0, 1,406 1, 8 2
    - p = 0.03
    - p = 0.9
  - *LPL* rs118204057: 48,210 0, 40 1
    - p = 0.006
    - p = 0.2
    - p = 0.6
  - *LPL* rs268: 45,873 0, 2,352 1, 25 2
    - p < 0.001
    - p = 0.005
    - p = 0.6
  - *LPL* rs328: 503 0, 8,860 1, 38,887 2
    - p < 0.001
    - p = 0.04
    - p = 0.03

- **LDL cholesterol**
  - *APOB* rs5742904: 48,220 0, 30 1
    - p = 0.3
    - p < 0.001
  - *LDLR* W23X: 48,245 0, 5 1
    - p = 0.2
    - p = 0.6
  - *LDLR* W66G: 48,225 0, 25 1
    - p = 0.8
    - p < 0.001
  - *LDLR* W556S: 48,248 0, 2 1
    - p = 0.03
    - p = 0.4
  - *PCSK9* rs11591147: 8 0, 1,268 1, 46,974 2
    - p = 0.4
    - p < 0.001
    - p = 0.8

Supplementary Figure 1, part 1
**Supplementary Figure 1.**
Lipoprotein and C-reactive protein levels as a function of single site genotypes in the Copenhagen General Population Study. Columns show mean lipoprotein/C-reactive protein levels with standard error bars, and P-values for trend across allele counts. LDL=Low-density lipoprotein.
Supplementary Figure 2.
Lipoprotein and C-reactive protein levels as a function of genotype in allele counts in the CGPS. Participants using lipid-lowering therapy are excluded. Genetic variants included in each group are shown in brackets. Columns show mean lipoprotein/C-reactive protein levels with standard error bars, and P-values for trend across allele counts. CGPS=Copenhagen General Population Study, LDL=Low-density lipoprotein.
Supplementary Figure 3.
Lipoprotein and C-reactive protein levels as a function of genotype in allele counts in the Copenhagen General Population Study. Participants with ischemic heart disease are excluded. Genetic variants included in each group are shown in brackets. Columns show mean lipoprotein/C-reactive protein levels with standard error bars, and P-values for trend across allele counts. CGPS=Copenhagen General Population Study, IHD=ischemic heart disease, LDL=Low-density lipoprotein.
Remnant cholesterol levels as a function of genotype in allele counts in the Copenhagen General Population Study and the Copenhagen City Heart Study combined stratified by diabetes mellitus and obesity. Remnant cholesterol levels were multiplied by 1.33 in participants using lipid-lowering therapy, corresponding to an average reduction of 25% using common statin treatment regimens. Genetic variants included are shown in brackets. Columns show mean remnant cholesterol levels with standard error bars, and P-values for trend across allele counts. Percents are percent higher level of remnant cholesterol compared to the 0-2 allele group.