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**FULL RESEARCH PAPER**
*Cardiovascular disease*

# The role of systemic inflammation in remnant cholesterol-associated cardiovascular risk: insights from the EPIC-Norfolk study

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**Aims** Both plasma levels of remnant cholesterol and low-density lipoprotein (LDL) cholesterol levels are independent risk factors for atherosclerotic cardiovascular disease. However, only remnant cholesterol has consistently been associated with systemic inflammation. In this study, we aimed to assess the extent to which inflammation mediates the effect of remnant and LDL cholesterol on (non)fatal major adverse cardiovascular events (MACE), comprising of coronary artery disease and ischaemic stroke.

**Methods and results** This prospective study included 16,445 participants without prior atherosclerotic cardiovascular disease from the EPIC-Norfolk study, with a mean age of 58.8 ± 9.1 years, of which 9,357 (56.9%) were women. Every 1 mmol/L higher remnant cholesterol was associated with 29.5% higher high-sensitivity C-reactive protein (hsCRP) levels [95% Confidence Interval (CI): 22.1, 37.4, $P < 0.001$], whereas LDL cholesterol was not significantly associated with hsCRP levels in the fully adjusted model. Additionally, each 1 mmol/L higher remnant cholesterol was associated with a hazard ratio (HR) of 1.31 (95% CI: 1.14, 1.50, $P < 0.001$) for MACE, compared with an HR of 1.21 (95% CI: 1.13, 1.31, $P < 0.001$) for LDL cholesterol. Mediation analysis showed that hsCRP mediated 5.9% (95% CI: 1.2, 10.6%, $P < 0.001$) of the effect of remnant cholesterol on MACE, whereas hsCRP did not mediate the effect of LDL cholesterol.

**Conclusion** Plasma remnant cholesterol levels are independently associated with systemic inflammation and cardiovascular events. Inflammation, as measured with hsCRP, contributed minorly to the association between remnant cholesterol and MACE. This underscores the need to address both remnant cholesterol and systemic inflammation separately in the clinical management of cardiovascular disease.

**Lay summary** This study finds that systemic inflammation does not influence the effect remnant cholesterol has on cardiovascular disease risk, suggesting the importance of addressing both remnant cholesterol and inflammation to manage cardiovascular health.

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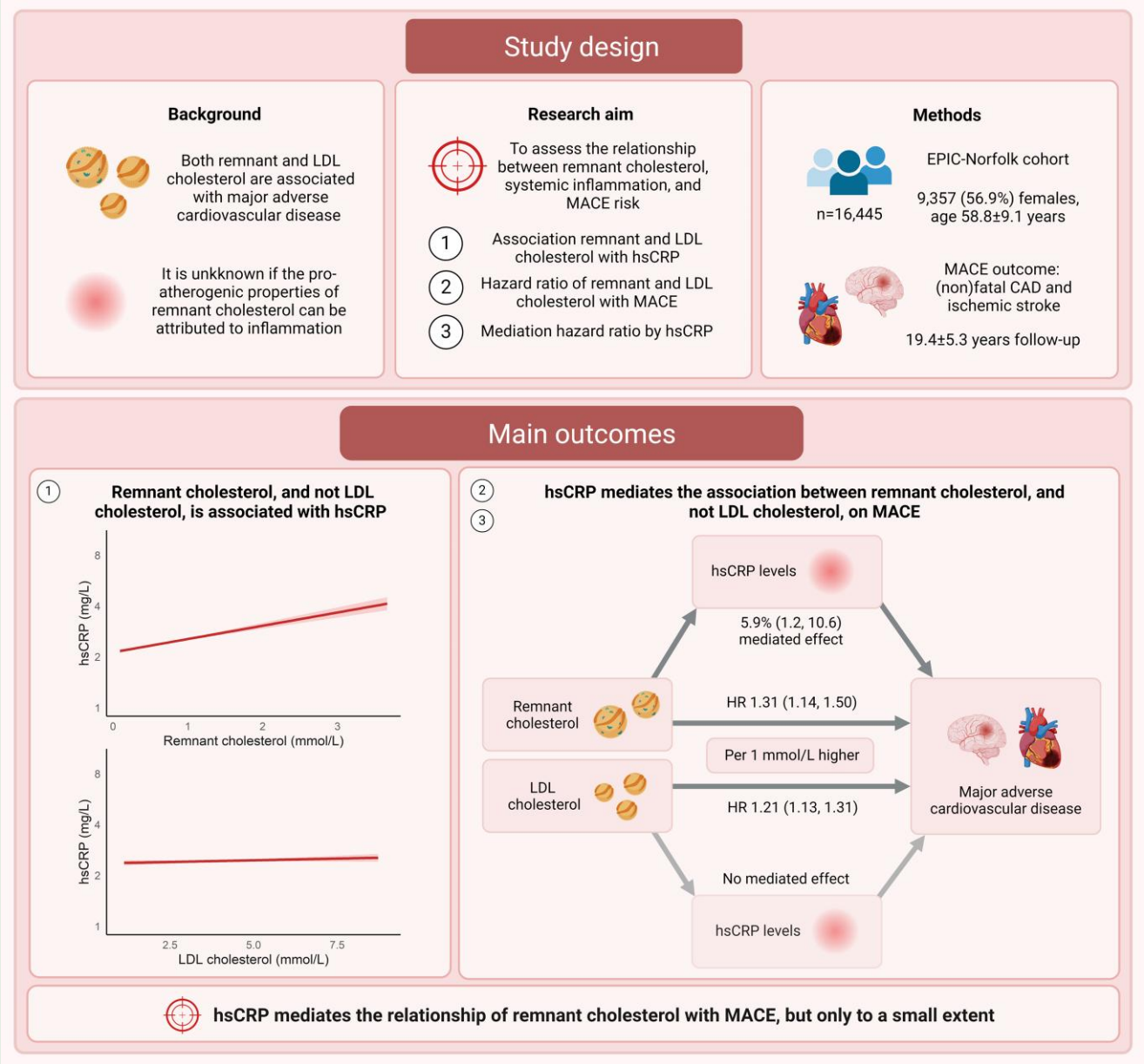
## Graphical Abstract




The study assessed the relationship between remnant cholesterol, systemic inflammation, and MACE risk in 16,445 participants free from atherosclerotic cardiovascular disease from the EPIC-Norfolk study. Every 1 mmol/L higher remnant cholesterol was associated with 29.5% higher hsCRP levels (95% CI: 22.1, 37.4, $P < 0.001$), while LDL cholesterol was not significantly associated with hsCRP levels. Additionally, each 1 mmol/L higher remnant cholesterol was associated with an HR of 1.31 (95% CI: 1.14, 1.50, $P < 0.001$) for MACE, compared with an HR of 1.21 (95% CI: 1.13, 1.31, $P < 0.001$) for LDL cholesterol. hsCRP mediated 5.9% (95% CI: 1.2, 10.6%, $P < 0.001$) of the effect of remnant cholesterol on MACE, while it did not mediate the effect of LDL cholesterol. LDL, low-density lipoprotein cholesterol; HR, hazard ratio; CI, confidence interval; MACE, major adverse cardiovascular events.

**Keywords** Atherosclerotic cardiovascular disease • Primary prevention • LDL cholesterol • Remnant cholesterol • Inflammation • hsCRP

## Key findings

- Higher levels of remnant cholesterol are associated with more inflammation and a higher risk of cardiovascular disease.
- Inflammation plays a small role in how remnant cholesterol affects cardiovascular disease risk, indicating other factors are also important.

# Introduction

Atherosclerotic cardiovascular disease (ASCVD) is the leading cause of mortality worldwide.[1] The development of atherosclerosis begins early in life and is accelerated by the presence of modifiable risk factors over time.[2] Entrapment of apolipoprotein B (apoB)-containing particles in the arterial wall is the causal process leading to initiation and progression of atherosclerotic plaques. Hence, apoB concentration is a major modifiable risk factor for ASCVD.[3–5] Triglyceride-rich apoB-containing particles are produced in the liver as very-low-density-lipoproteins (VLDL) which are subsequently metabolized to VLDL-remnant particles and low-density lipoproteins (LDL) through lipoprotein-lipase mediated lipolysis.[6] Several metabolic disorders such as obesity and insulin resistance increase VLDL production and impair lipolysis, resulting in accumulation of remnant particles in the circulation.[7] The cholesterol content of remnant particles, commonly referred to as remnant cholesterol, have been independently associated with major adverse cardiovascular disease (MACE).[8–12] For example, in the Copenhagen studies, 1 mmol/L higher remnant cholesterol was associated with a hazard ratio of 1.4 (95% CI: 1.3–1.5) for cardiovascular events,[9] while similar observations were reported in secondary prevention patients.[13,14]

Recent genetic studies have implied that remnant cholesterol may have an even bigger impact on cardiovascular risk compared with cholesterol in LDL particles (LDL cholesterol), indicating a pro-atherogenic effect beyond apoB particle concentration.[15] It was suggested that this observation could be attributed to the impact of remnant particles on inflammation.[16] Inflammation plays a critical role in atherogenesis, and involves various mediators that contribute to accelerated plaque formation, rupture and subsequent clinical events.[17,18] A variety of lipid and metabolic factors, chronic inflammatory diseases as well as other risk factors can activate the endothelium, facilitating the influx of immune cells and apoB-containing particles. These immune cells can take up lipid components, thereby triggering a pro-inflammatory response and amplifying plaque development.[16] Clinical studies showed a correlation between remnant particles and arterial wall inflammation assessed by 18F-fluorodeoxyglucose positron emission tomography ($^{18F}$FDG-PET) as well as systemic inflammation measured by high-sensitivity C-reactive protein (hsCRP) levels in healthy individuals.[19–25] However, it remains to be established whether remnant cholesterol's atherogenic risk is mediated by this pro-inflammatory effect. Both inflammation and apoB-containing lipoproteins are key-modifiable risk factors as was shown by numerous lipid-lowering[26–29] and anti-inflammatory (i.e. canakinumab, colchicine) studies.[30–32] However, their interplay is only partly understood and further investigation into this relationship could inform us which patient groups might benefit most from what strategy. The current study's primary objective was to assess the effect of remnant cholesterol on MACE in the prospective EPIC-Norfolk study. We specifically investigated the mediation effect of inflammation on this relationship. The secondary objective was to examine whether this relationship is influenced by the presence of metabolic syndrome factors, which are independently associated with systemic inflammation and elevated remnant cholesterol levels. Finally, to gain more mechanistic insights, we explored the association of remnant particle size and apoC-III levels with systemic inflammation in a nested case-control sub study within the EPIC-Norfolk study.

# Methods

## Study population

The study included participants from the European Prospective Investigation into Cancer and Nutrition (EPIC)-Norfolk prospective cohort study, involving 25,639 men and women between the ages of 40 and 79, all from general practices in Norfolk, UK.[33] Participants were recruited between 1993 and 1997 and completed a detailed health and lifestyle questionnaire, with additional data collected by trained nurses during clinic visits. The study cohort demonstrated robust response rates[34] and was broadly representative of the UK population in terms of characteristics such as anthropometry, blood pressure, and lipid levels, though it had a lower proportion of smokers compared with national averages.[33] Study participants were followed for over 25 years with check-up visits and questionnaires, with follow-up data available up to 31 March 2016.

For our secondary objective, we examined the association between remnant cholesterol, remnant particle-related measures, including concentrations of small, medium, and large VLDL particles and apoC-III levels, and $\log_2$-transformed hsCRP levels. This study was performed in a subset of participants, which were included in a nested case-control sub study within the EPIC-Norfolk study. This sub study was initiated in 2004 to explore the relationship between plasma biomarkers and cardiovascular disease, and included participants with available plasma samples.[35] Cases were study participants who did not have prevalent cardiovascular disease at baseline, but did develop coronary artery disease (CAD) (details provided below) during follow-up through the end of 2003.[35,36] Controls, matched on sex, age (±5 years), and enrollment date (±3 months) in a 1:2 ratio, were selected from participants who remained free of cardiovascular disease during follow-up. In this sub study, VLDL particle size and apoC-III levels measurements were performed (details provided below). Ethical approval was granted by the Norwich District Health Authority Ethics Committee, with all participants having provided written informed consent.

## Cardiovascular disease and other disease definitions

In the current study, the endpoint was a composite of MACE, defined as (non)fatal CAD, including myocardial infarction and angina pectoris (ICD-10: I20–I25), and (non)fatal ischaemic stroke (ICD-10: I63), consistent with prior definitions used in the EPIC-Norfolk study.[36,37] MACE was recorded during follow-up if participants were hospitalized or died with CAD or ischaemic stroke as the primary cause. Hospital admissions were tracked through the ENCORE (East Norfolk Health Authority) system using participants' NHS numbers, capturing all hospital interactions for Norfolk residents across England and Wales to identify relevant events during the study.

Metabolic syndrome was defined as the presence of 3 out of 5 characteristics: increased waist circumference (≥102 cm for men and ≥88 cm for women), elevated triglyceride levels (≥1.7 mmol/L), high systolic blood pressure (≥130 mmHg) or high diastolic blood pressure (≥85 mmHg), hyperglycaemia defined as elevated haemoglobin A1C (HbA1c) of 42 mmol/mol (6%) and above, and low high-density lipoprotein (HDL) cholesterol levels (<1.03 mmol/L for men and <1.30 mmol/L for women).[38] Diabetes mellitus was defined as either use of diabetic mediation or an HbA1c of 48 mmol/mol (6.5%) and above.

## Laboratory measurements

Non-fasting blood samples were collected in both plain and citrate tubes. These samples were either immediately processed at the Department of Clinical Biochemistry, University of Cambridge, or preserved at −80°C

for future analysis. Plasma levels of total cholesterol, HDL cholesterol, triglycerides and apoB were measured in fresh samples using a RA 1000 auto-analyzer (Bayer Diagnostics, Basingstoke, UK). LDL cholesterol levels were estimated with the NIH Sampson equation[39] in order to achieve more precision in estimating LDL cholesterol levels at elevated triglyceride levels and enhanced accuracy in calculating VLDL cholesterol levels compared with the Friedewald formula.[40] Remnant cholesterol was estimated by subtracting HDL cholesterol and LDL cholesterol from total cholesterol levels.[41] hsCRP levels were measured in 2010 in all participants with available frozen baseline serum samples using a high-sensitivity Olympus AU640 Chemistry Immuno Analyzer (Olympus Diagnostics, Watford, UK).[37] Participants with hsCRP levels above 10 mg/L were excluded from the analysis to eliminate individuals with acute infectious or manifest systemic inflammatory diseases. In the nested case-control sub study, the size and particle concentrations of VLDL, comprising of small, medium, and large particles, were measured using an automated 400-megahertz proton nuclear magnetic resonance spectroscopic assay.[42] VLDL particle size was quantified in nmol/L and categorized based on the diameter: small particles 27–35 nm, medium particles 35–60 nm, and large particles >60 nm.[42] Plasma concentrations of apoC-III were measured using a chemiluminescent enzyme-linked immunoassay (Abgent, San Diego, USA).[43]

## Study outcomes

The primary outcome was to assess the mediation effect of hsCRP on the relationship between remnant cholesterol or LDL cholesterol and future MACE within the EPIC-Norfolk study. The secondary outcomes included evaluating the influence of metabolic syndrome factors on the association between remnant and LDL cholesterol and MACE, as well as examining the relationship between hsCRP levels and the concentrations of small, medium, and large VLDL particles, and apoC-III levels in the nested case-control sub study.

## Statistical analysis

Normally distributed data and non-normally distributed data are presented as mean [standard deviation (SD)] and as median ± interquartile range [IQR], respectively. Categorical data are reported as absolute numbers and percentages. Demographic, clinical, and biochemical characteristics are provided for the complete cohort and stratified by remnant cholesterol quartiles. An ANOVA test was performed for normally distributed continuous variables, while a Kruskal–Wallis test was used for non-normally distributed variables. Correlations were examined with the Pearson's rank correlation test for non-normally distributed data.

To explore the associations between remnant cholesterol, LDL cholesterol, and $\log_2$-transformed hsCRP levels, linear regression analysis was used with additional adjustment for age and sex. Next, we corrected the association for the presence of metabolic syndrome factors diabetes mellitus (DM), body mass index (BMI), and systolic blood pressure (SBP). Next, we corrected these models for current smoking. In a final model, we corrected for LDL cholesterol in the remnant cholesterol model and vice versa.

Cox proportional hazards models were used to calculate hazard ratios (HRs) per 1 mmol/L or 1 standard deviation increment in remnant and LDL cholesterol in relation to MACE.[44] The initial model was adjusted for age, sex, DM, BMI, systolic blood pressure, and current smoking. An additional model was then constructed with further adjustment for apoB levels and LDL cholesterol in the remnant cholesterol model and vice versa. Proportionality of the models was tested using the method described by Grambsch and colleagues.[45] Regression-based mediation analysis was conducted to investigate the role of hsCRP in mediating the relationship between remnant or LDL cholesterol levels and cardiovascular disease events using the CMAverse package.[46] This method utilizes Cox proportional hazard modelling with nonparametric bootstrapping with 1000 runs to estimate the total, direct, and indirect effects. The total effect represents the overall association between the exposure and the outcome, the direct effect quantifies the association that is not mediated by the mediator, and the indirect effect reflects the portion of the association that operates through the mediator. Estimates and confidence intervals (CI) below 0% were truncated to 0%. Sensitivity analyses were performed in subgroups stratified for sex (men and women) and metabolic syndrome (0–2 MS features vs. 3 or more). Restricted cubic splines with three knots were used to visualize the relationship between remnant and LDL cholesterol with MACE.

To ascertain the independent effects of remnant cholesterol or LDL cholesterol on cardiovascular events and their mediation by hsCRP levels, we conducted a discordance analysis using Cox proportional hazards modelling with four groups based on the median values of remnant (RC) and LDL cholesterol (LDL-C) levels: low RC/low LDL-C as the reference group (remnant and LDL cholesterol < median cohort levels), low RC/high LDL-C, high RC/low LDL-C, and high RC/high LDL-C. These analyses were fully adjusted for age, sex, BMI, DM, SBP, and smoking status.

As part of the second study design, in the nested case-control sub study, we examined the association between remnant cholesterol, remnant particle-related measures, including concentrations of small, medium, and large VLDL particles and apoC-III levels, and $\log_2$-transformed hsCRP levels. We used a linear regression model, adjusting for age, sex, DM, BMI, SBP, smoking status, and LDL cholesterol. To validate the role of systemic inflammation in these remnant-related measures, we applied logistic regression models to assess the odds ratio for incident CAD. These models were adjusted for age, sex, DM, BMI, SBP, smoking status, and an additional model including LDL cholesterol.

Statistical significance was set at a $P$-value of <0.05 and all statistical analyses were performed using RStudio version 4.3.2. (R Foundation, Vienna, Austria).

# Results

In the EPIC-Norfolk study, plasma lipid and hsCRP levels were available in 18,445 individuals. After excluding patients with atherosclerotic cardiovascular disease at baseline and participants with hsCRP levels >10 mg/L, this prospective study included 16,445 participants with a mean age of 58.8 ± 9.1 years, of which 9357 (56.9%) were women (*Table 1*). The median levels of remnant and LDL cholesterol were 0.61 [0.42, 0.88] mmol/L and 4.0 (1.0) mmol/L, respectively. A comparison of the included participants with those excluded is provided in Supplementary material online, *Table S1*.

At baseline, 1,805 (11.1%) participants were current smokers, 450 (2.7%) had DM, the mean BMI was 25.6 [23.5, 28.1] kg/m$^2$, the mean waist circumference of 87.1 (12.1) cm, and the mean systolic blood pressure was 134.6 (18.2) mmHg. Additionally, 174 (1.1%) were using lipid-lowering medication, and 2,581 (15.7%) were on antihypertensive medication. The prevalence of obesity, increased waist circumference and hypertension were higher in those with higher remnant cholesterol levels (*Table 1*). Moreover, compared with those in the lowest remnant cholesterol quartile, study participants in the highest quartile had higher LDL cholesterol and lower HDL cholesterol levels (4.4 ± 1.0 vs. 3.7 ± 1.0 mmol/L, $P < 0.001$, and 1.2 ± 0.3 vs. 1.7 ± 0.4 mmol/L, $P < 0.001$, respectively). A total of 1,876 participants (13.3%) had obesity (BMI of 30 kg/m$^2$ and above). These individuals were older (58.7 ± 9.2 vs. 59.6 ± 8.9 years, $P < 0.001$) and were less likely to be female (56.1% vs. 62.0%, $P < 0.001$) compared with those without obesity. Additionally, participants with obesity had higher remnant cholesterol levels (0.6 ± 0.4 vs. 0.8 ± 0.6 mmol/L, $P < 0.001$) and higher hsCRP levels (1.3 ± 0.6 vs. 2.7 ± 1.5 mg/L, $P < 0.001$). The baseline characteristics according to the metabolic syndrome scoring are provided in Supplementary material online, *Table S2*.

## The association between remnant cholesterol, LDL cholesterol, and plasma hsCRP levels

Every 1 mmol/L higher remnant cholesterol was associated with 72.7% (95% CI: 66.0, 80.0, $P < 0.001$) higher hsCRP levels after adjustment for

**Table 1 Baseline characteristics**

| Characteristics | Overall cohort | Q1 remnant cholesterol <0.42 mmol/L | Q2 remnant cholesterol 0.42–0.61 mmol/L | Q3 remnant cholesterol 0.61–0.88 mmol/L | Q4 remnant cholesterol >0.88 mmol/L | *P*-value |
|---|---|---|---|---|---|---|
| Number of patients | 16,445 | 4,112 | 4,113 | 4,110 | 4,110 | |
| Age (years) | 58.8 (9.1) | 56.0 (8.9) | 58.7 (9.2) | 60.0 (9.1) | 60.5 (8.7) | **<0.001** |
| Male sex | 9,357 (56.9%) | 2,937 (71.4%) | 2,492 (60.6%) | 2,110 (51.3%) | 1,818 (44.2%) | **<0.001** |
| Current smoker | 1,805 (11.1%) | 410 (10.0%) | 439 (10.8%) | 457 (11.2%) | 499 (12.2%) | **0.013** |
| Diabetes mellitus | 450 (2.7%) | 66 (1.6%) | 86 (2.1%) | 123 (3.0%) | 175 (4.3%) | **<0.001** |
| Lipid-lowering medication | 174 (1.1%) | 28 (0.7%) | 38 (0.9%) | 55 (1.3%) | 53 (1.3%) | **0.009** |
| Antihypertensive medication | 2,581 (15.7%) | 408 (9.9%) | 573 (13.9%) | 716 (17.4%) | 884 (21.5%) | **<0.001** |
| BMI (kg/m$^2$) | 25.6 [23.5, 28.1] | 24.1 [22.3, 26.3] | 25.1 [23.1, 27.3] | 26.1 [24.1, 28.5] | 27.1 [25.1, 29.5] | **<0.001** |
| Waist circumference (cm) | 87.1 (12.1) | 80.6 (10.5) | 85.3 (11.3) | 89.4 (11.4) | 93.5 (11.1) | **<0.001** |
| Systolic blood pressure (mmHg) | 134.6 (18.2) | 129.0 (17.6) | 133.2 (18.0) | 136.5 (17.8) | 139.7 (17.7) | **<0.001** |
| Diastolic blood pressure (mmHg) | 82.0 (11.1) | 78.8 (10.7) | 81.2 (10.9) | 83.0 (11.0) | 85.2 (10.9) | **<0.001** |
| Apolipoprotein B (g/L) | 1.0 (0.2) | 0.9 (0.2) | 0.9 (0.2) | 1.0 (0.2) | 1.1 (0.3) | **<0.001** |
| Total cholesterol (mmol/L) | 6.2 (1.1) | 5.7 (1.0) | 5.9 (1.0) | 6.3 (1.1) | 6.8 (1.1) | **<0.001** |
| LDL cholesterol (mmol/L) | 4.0 (1.0) | 3.7 (1.0) | 3.9 (1.0) | 4.2 (1.0) | 4.4 (1.0) | **<0.001** |
| HDL cholesterol (mmol/L) | 1.4 (0.4) | 1.7 (0.4) | 1.5 (0.4) | 1.3 (0.4) | 1.2 (0.3) | **<0.001** |
| Triglycerides (mmol/L) | 1.5 [1.1, 2.1] | 0.8 [0.7, 0.9] | 1.3 [1.1, 1.4] | 1.8 [1.6, 1.9] | 2.6 [2.3, 3.1] | **<0.001** |
| VLDL cholesterol (mmol/L) | 0.7 [0.5, 1.0] | 0.4 [0.3, 0.4] | 0.5 [0.5, 0.6] | 0.8 [0.7, 0.9] | 1.2 [1.1, 1.5] | **<0.001** |
| Remnant cholesterol (mmol/L) | 0.6 [0.4, 0.9] | 0.3 [0.3, 0.4] | 0.5 [0.5, 0.6] | 0.7 [0.7, 0.8] | 1.1 [1.0, 1.4] | **<0.001** |
| High-sensitivity C-reactive protein (mg/L) | 1.4 [0.7, 2.8] | 0.9 [0.5, 1.9] | 1.3 [0.7, 2.6] | 1.6 [0.8, 3.1] | 1.9 [1.0, 3.5] | **<0.001** |

The *P*-values in bold are statistical significant.
Baseline characteristics for the complete cohort and per remnant cholesterol quartile. Normally distributed variables are reported as mean (SD), non-normally distributed variables as median ± interquartile range [IQR], and categorical variables as number (%). These were analysed using a one-way ANOVA, a Kruskal–Wallis test, or a $\chi$2-test, respectively. BMI, body mass index; LDL, low-density lipoprotein; HDL, high-density lipoprotein; VLDL, very-low-density lipoprotein.

age and sex. This association was attenuated after adjustment for MS components, with 1 mmol/L higher remnant cholesterol levels corresponding to 33.1% (95% CI: 25.6, 41.1, $P < 0.001$) higher hsCRP levels. After further adjustment for current smoking, hsCRP levels were 30.4% (95% CI: 23.0, 38.2, $P < 0.001$) higher per 1 mmol/L increase in remnant cholesterol. Lastly, after LDL cholesterol adjustment, a 1 mmol/L higher remnant cholesterol corresponded to 29.5% (95% CI: 22.1, 37.4, $P < 0.001$) higher hsCRP levels (see Supplementary material online, *Table S3* and *Figure 1A*).

LDL cholesterol showed a markedly weaker association with hsCRP levels. For every 1 mmol/L increment in LDL cholesterol levels, hsCRP levels were 7.5% (95% CI: 6.0, 9.1, $P < 0.001$) higher in the age and sex-adjusted model, 3.8% (95% CI: 1.5, 6.1, $P < 0.001$) higher in the MS component adjusted model and 3.3% (95% CI: 1.1, 5.6, $P = 0.004$) higher in the model additionally adjusted for smoking. After adjustment for remnant cholesterol, no significantly higher hsCRP per 1 mmol/L higher LDL cholesterol was observed (see Supplementary material online, *Table S3* and *Figure 1B*). Results per one SD higher remnant and LDL cholesterol are provided in Supplementary material online, *Table S3*.

## The impact of remnant and LDL cholesterol on the risk for cardiovascular disease

During a median follow-up period of 19.4 ± 5.3 years, 3,466 MACE events occurred. One mmol/L higher remnant cholesterol was associated with a HR of 1.70 (95% CI: 1.56, 1.84, $P < 0.001$) for MACE in the age and sex-adjusted model (*Table 2*). In the fully adjusted model following adjustment for MS components, current smoking, LDL cholesterol and apoB, the hazard ratio was 1.31 (95% CI: 1.14, 1.50, $P < 0.001$) per 1 mmol/L higher remnant cholesterol (*Figure 2*). Mediation analysis showed that 5.9% (95% CI: 1.2, 10.6%, $P < 0.001$) of the relationship between remnant cholesterol levels and MACE was mediated by hsCRP (*Figure 3*). One mmol/L higher LDL cholesterol was associated with a hazard ratio of 1.19 (95% CI: 1.16, 1.23, $P < 0.001$) for MACE in the age and sex-adjusted model. In the fully adjusted model, one mmol/L higher LDL cholesterol resulted in a hazard ratio of 1.21 (1.13, 1.31, $P < 0.001$) (*Figure 2*). In contrast to remnant cholesterol, no significant mediation by hsCRP was observed (*Figure 3*). Because the metabolic syndrome is associated with systemic inflammation, we investigated if these results were different for those with or without 3 or more metabolic syndrome features. For the 1,434 participants with MS, the hazard ratio was not significant, at 1.03 (95% CI: 0.82, 1.31, $P = 0.776$), with no significant mediation (see Supplementary material online, *Table S4*). Conversely, the 5,506 individuals with no MS, the hazard ratio for MACE was 1.40 (95% CI: 1.13, 1.75, $P < 0.001$) per 1 mmol/L higher remnant cholesterol of which 11.0% (95% CI: 1.3–23.4, $P < 0.001$) was mediated by hsCRP.

Lastly, we found no sex difference in the magnitude of the effect of hsCRP on the association between remnant cholesterol and MACE. In men, the hazard ratio of 1 mmol/L higher remnant cholesterol for MACE was 1.29 (95% CI: 1.09, 1.53, $P = 0.004$; Supplementary material

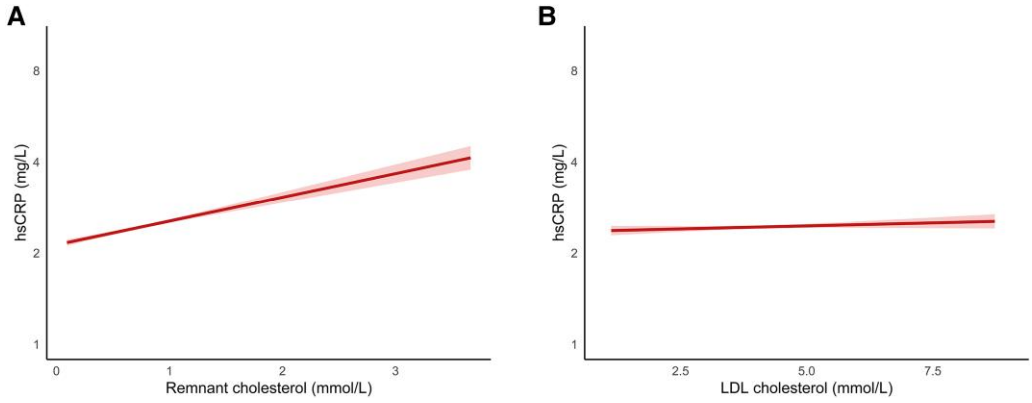



**Figure 1** Relationship between remnant cholesterol, LDL cholesterol and hsCRP levels. Visualization of linear regression analyses for (*A*) remnant cholesterol and (*B*) LDL cholesterol plasma levels on hsCRP levels (on a $\log_2$ scale) in a multivariable adjusted model, corrected for age, sex, DM, BMI, SBP, current smoking and LDL cholesterol for (*A*) and remnant cholesterol for (*B*). Shown are the 95% confidence interval of the fitted regression line. LDL, low-density lipoprotein; hsCRP, high-sensitivity C-reactive protein; DM, diabetes mellitus; BMI, body mass index; SBP, systolic blood pressure.

**Table 2 MACE hazard ratios for remnant and LDL cholesterol**

| | Hazard ratio (95% CI) Per 1 mmol remnant cholesterol | Hazard ratio (95% CI) Per 1 mmol LDL cholesterol |
|---|---|---|
| Model 1—Age and sex | 1.70 (1.56, 1.84) | 1.19 (1.16, 1.23) |
| Model 2—Model 1 + MS components | 1.38 (1.22, 1.57) | 1.19 (1.13, 1.23) |
| Model 3—Model 2 + current smoking | 1.36 (1.20, 1.55) | 1.19 (1.13, 1.26) |
| Model 4—Model 3 + apoB, RC/LDL-C | 1.31 (1.14, 1.50) | 1.21 (1.13, 1.31) |

Cox proportional modelling was used to determine hazard ratios for remnant and LDL cholesterol on future MACE. MACE was defined as (non)fatal coronary artery disease and (non)fatal ischaemic stroke. Model 1 was age and sex adjusted, model 2 was additionally adjusted for MS factors BMI, DM and SBP, model 3 additionally for current smoking and model 4 for apoB and LDL cholesterol in model A and remnant cholesterol in model B. RC, remnant cholesterol; LDL, low-density lipoprotein; MACE, major adverse cardiovascular disease; BMI, body mass index; DM, diabetes mellitus; SBP, systolic blood pressure; apoB, apolipoprotein B.

online, *Table S4*), with the mediated effect of hsCRP being 8.8% (95% CI: 3.4, 18.2, $P < 0.001$). For women, the hazard ratio was 1.28 (95% CI: 1.02, 1.60, $P = 0.031$), and the mediated effect was non-significant.

## Analysis of discordance: for the impact of remnant and LDL cholesterol on MACE and the mediation by hsCRP levels

Four groups, based on the median of remnant and LDL cholesterol levels, were constructed to investigate discordancy in MACE-associated risk. The low RC/low LDL-C served as reference group (remnant cholesterol and LDL cholesterol below median cohort levels).

Compared with the reference group, the HR for MACE was 1.22 (95% CI: 1.02, 1.45, $P = 0.031$, Supplementary material online, *Table S5*) in the low RC/high LDL-C group and 1.20 (95% CI: 1.01, 1.42, $P = 0.036$) in the high RC/low LDL-C group (*Figure 4* and Supplementary material online, *Table S5*) in the fully adjusted model. For the high RC/high LDL-C group, the HR was 1.46 (95% CI: 1.26, 1.70, $P < 0.001$). Mediation by hsCRP was observed in the high RC/low LDL-C group with 2.8% (1.5, 4.1, $P < 0.001$) and in the high RC/high LDL-C group with 2.0% (1.0, 3.0, $P < 0.001$) (*Figure 4* and Supplementary material online, *Table S5*). No mediation was observed in the low RC/high LDL-C group.

## The impact of remnant cholesterol and particle properties on hsCRP in the nested case-control sub study

To further detail the effect of remnant particle characteristics on the relationship between remnant cholesterol and hsCRP, we utilized the nested case-control sub study within the EPIC-Norfolk study as a second study design. This sub study included 2,984 participants, consisting of 961 cases and 2,063 controls, all of whom had available hsCRP levels below 10 mg/L (see Supplementary material online, *Table S1*). A comparison of baseline characteristics between the cohort study and case-control sub study is presented in Supplementary material online, *Table S1* and a flow chart is provided in Supplementary material online, *Figure S1*. Remnant cholesterol levels showed the following correlation with triglycerides (Pearson correlation coefficient ($\rho$) = 0.99, $P < 0.001$), small VLDL particles ($\rho = 0.20$, $P < 0.001$), medium VLDL particles ($\rho = 0.58$, $P < 0.001$, large VLDL particles ($\rho = 0.85$, $P < 0.001$) and with apoC-III ($\rho = 0.34$, $P < 0.001$; Supplementary material online, *Figure S2*).

In accordance with the longitudinal cohort analysis, remnant cholesterol was associated with systemic inflammation. One SD higher remnant cholesterol was associated with 11.4% (95% CI: 7.4, 15.5, $P < 0.001$) higher hsCRP levels in a model adjusted for age, sex, DM, BMI, SBP, current smoking, and LDL cholesterol (*Table 3*). One

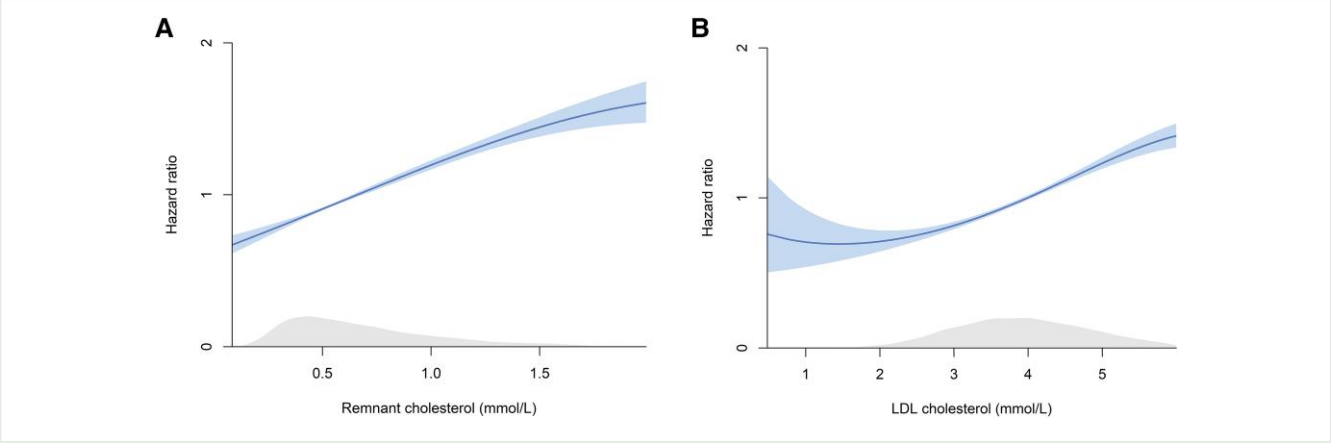



**Figure 2** Association of remnant and LDL cholesterol with MACE. Cox proportional hazard ratios for remnant (*A*) and LDL cholesterol (*B*) on future MACE. MACE was defined as (non)fatal coronary artery disease and (non)fatal ischaemic stroke. Results are shown for the restricted cubic splines in the multivariable adjusted model, corrected for age, sex, DM, BMI, SBP, current smoking, apoB, and LDL cholesterol in panel (*A*) and remnant cholesterol in panel (*B*). Grey area indicates the density of measurements of remnant (*A*) and LDL cholesterol (*B*). LDL, low-density lipoprotein; MACE, major adverse cardiovascular disease; DM, diabetes mellitus; BMI, body mass index; SBP, systolic blood pressure; apoB, apolipoprotein B.

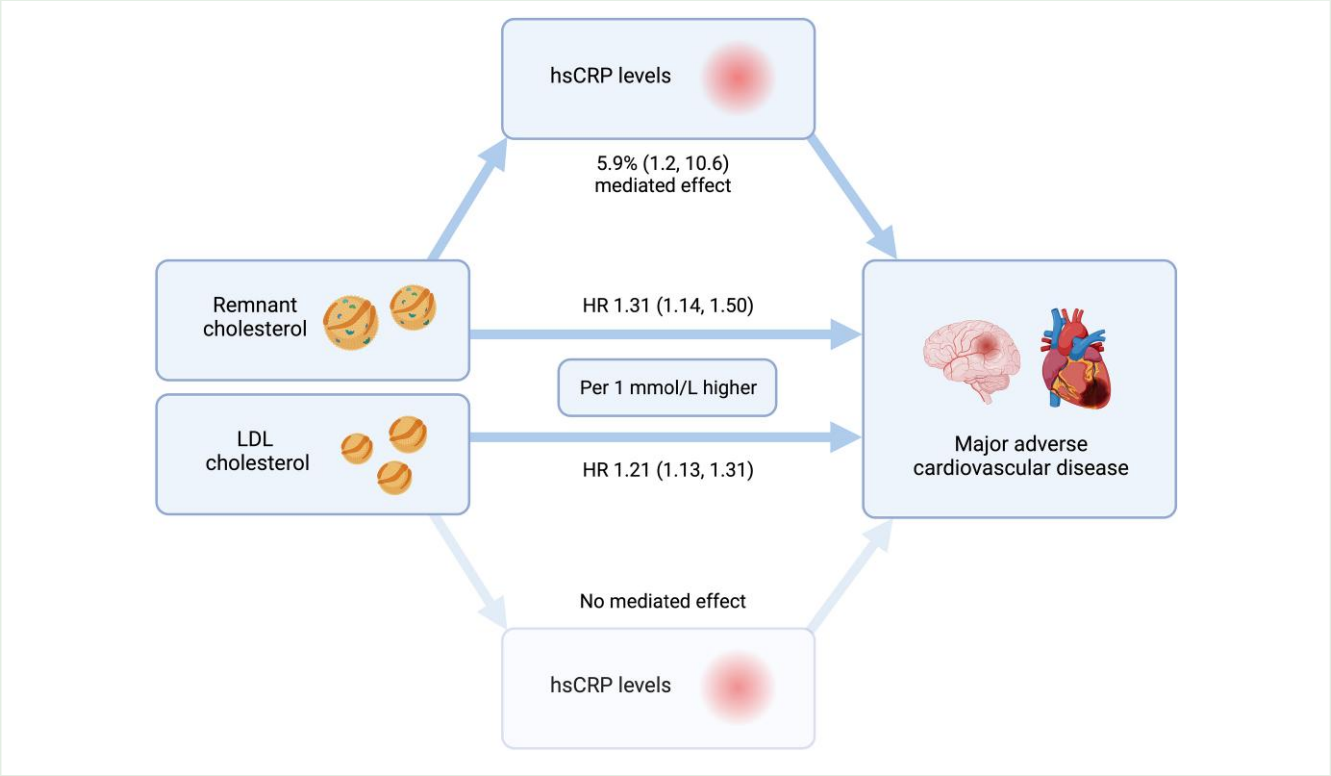



**Figure 3** Mediation analysis of hsCRP levels of remnant and LDL cholesterol on MACE. Mediation analysis between the proportion effect of hsCRP in the association between remnant and LDL cholesterol with major adverse cardiovascular disease. hsCRP, high-sensitivity C-reactive protein; LDL, low-density lipoprotein; HR, hazard ratio. Created with Biorender.com.

standard deviation higher concentrations of medium and large VLDL particle were significantly associated with 4.9% (95% CI: 1.0, 9.0, $P = 0.014$) and 13.9% (95% CI: 9.5, 18.4, $P < 0.001$) higher hsCRP levels respectively. Small VLDL size concentrations were not associated with hsCRP levels. One standard deviation higher plasma apoC-III levels was associated with 8.9% (95% CI: 4.7, 13.3, $P < 0.001$) higher hsCRP levels.

One SD higher remnant cholesterol was associated with an odds ratio of 1.19 (95% CI: 1.09, 1.30, $P < 0.001$) for CAD, adjusted for age, sex, DM, BMI, SBP, current smoking, and LDL cholesterol with 7.6%

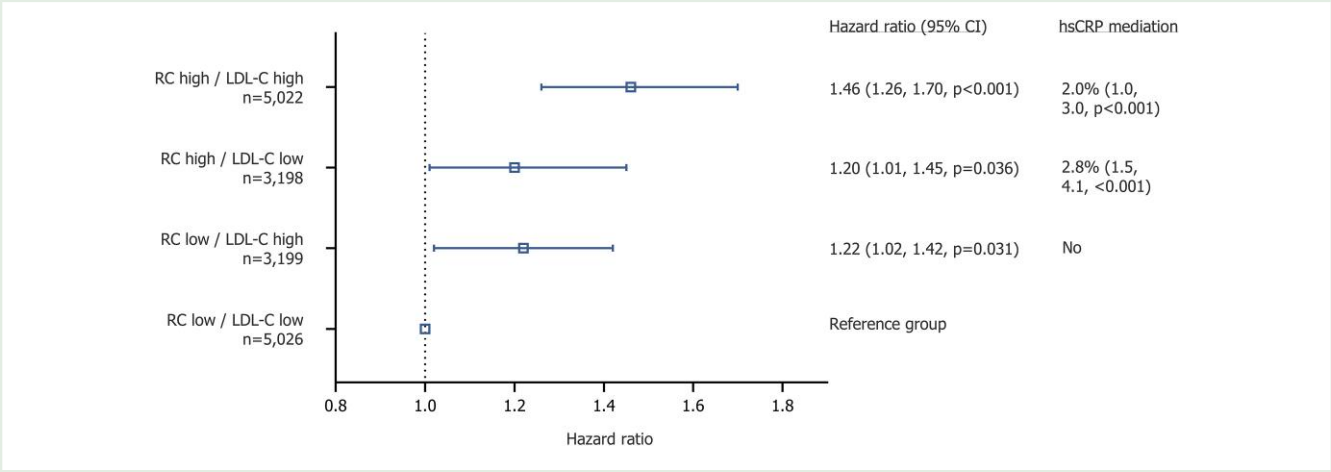



**Figure 4** Hazard ratio and mediation analysis in discordant analysis. Discordance analyses based on the median values of remnant cholesterol (0.6 mmol/L) and LDL cholesterol (4.0 mmol/L) levels; the low RC/low LDL-C (reference group), high RC/low LDL-C, low RC/high LDL-C, and high RC/high LDL-C group. Cox proportional hazard ratios calculated for future MACE, defined as (non)fatal coronary artery disease and (non)fatal ischaemic stroke and its mediation by hsCRP levels. Results are shown for the multivariable adjusted model, corrected for age, sex, DM, BMI, SBP, and current smoking. RC, remnant cholesterol; LDL, low-density lipoprotein; MACE, major adverse cardiovascular disease; hsCRP, high-sensitivity C-reactive protein; DM, diabetes mellitus; BMI, body mass index; SBP, systolic blood pressure.

(95% CI: 3.0, 15.0, $P < 0.001$) mediation by hsCRP levels (see Supplementary material online, *Table S6*). Each standard deviation higher medium and large VLDL particle concentration was associated with an odds ratio of 1.11 (95% CI: 1.01, 1.21, $P = 0.002$) and 1.08 (95% CI: 0.98, 1.19, $P = 0.104$) for CAD, respectively, with no significant mediation by hsCRP (see Supplementary material online, *Table S6*). Each standard deviation increase in apoC-III levels was associated with an odds ratio of 1.13 (95% CI: 1.03, 1.25, $P = 0.012$) for CAD, with no significant mediation by hsCRP.

## Discussion

In the present study, we confirm that plasma levels of remnant cholesterol are associated with hsCRP levels, whereas hsCRP only modestly mediated the association between remnant cholesterol and cardiovascular events Graphical abstract. The association between remnant cholesterol and hsCRP is markedly attenuated by metabolic syndrome features and could potentially be attributed to large, triglyceride loaded, particles carrying the apolipoprotein apoC-III. In contrast to remnant cholesterol, LDL cholesterol was only weakly correlated with hsCRP, and hsCRP did not significantly mediate LDL-associated cardiovascular disease risk.

The results of our study align with previous studies showing that remnant cholesterol is associated with cardiovascular disease beyond apoB and LDL cholesterol. In a study by Quispe and coworkers, remnant cholesterol levels were associated with the risk of cardiovascular events in 17,532 primary prevention individuals, independent of traditional cardiovascular risk factors, LDL cholesterol, and apoB.[47] This is corroborated by a pooled cohort study of intravascular ultrasound trials in patients with known coronary artery disease, which showed that on-treatment remnant cholesterol levels were associated with coronary atheroma progression after adjusting for apoB.[8] Conversely, these results contrast with other studies suggesting that cardiovascular risk associated with remnant cholesterol can be completely attributed to the fact that it is just cholesterol carried in apoB containing lipoproteins that can become trapped in the arterial wall.[48–52]

One explanation for our observation that remnant cholesterol is associated with ASCVD beyond apoB could lie in its inflammatory effects. Multiple large-scale studies indicated that both observational and genetically determined plasma levels of remnant cholesterol are associated with higher hsCRP levels indicative of an increased systemic inflammatory state.[22,24,25] Our study adds to this knowledge by showing a strong correlation between remnant cholesterol levels and hsCRP levels and remnant cholesterol levels and ASCVD in another large cohort. In a study by Varbo and colleagues, a 1 mmol/L increase in remnant cholesterol was associated with a 37% rise in hsCRP levels, a result that closely aligns with the findings of the current study.[24] Additionally, the effect of 1 mmol/L higher remnant cholesterol on cardiovascular events in the same cohort was similarly consistent with our study.[9] Through our mediation analyses, we aimed to assess which proportion of the observed effect of remnant and LDL cholesterol on cardiovascular event risk could be attributable to hsCRP. Only 5% of the MACE risk associated with remnant cholesterol was found to be mediated through hsCRP. This is consistent with the discordance analyses suggesting that, although there is a strong association between remnant cholesterol and systemic inflammation, the pro-inflammatory effects of remnant cholesterol as quantified by hsCRP are unlikely major drivers of the increased cardiovascular event risk. The weak mediation effect of hsCRP in the association of large VLDL particles and apoC-III with coronary artery disease further substantiates this finding and suggests that the independent association of remnant cholesterol (related markers) with systemic inflammation might be of limited relevance. Clinically, the minimal mediation effect of hsCRP on the relationship between remnant cholesterol and cardiovascular risk suggests that targeting remnant cholesterol may not have a significant impact on reducing cardiovascular events associated with inflammation. This highlights the need for therapeutic strategies that independently address both remnant cholesterol and inflammation, as their contributions to cardiovascular risk appear to operate through distinct mechanisms.

The current findings raise the question whether other factors contribute to remnant cholesterol atherogenicity. One plausible

**Table 3 Relationship between remnant cholesterol (related markers) and hsCRP**

| | Higher hsCRP (95% CI) Per 1 mmol increase | *P*-value |
|---|---|---|
| Remnant cholesterol | 11.4% (7.4, 15.5) | **<0.001** |
| Small VLDL size | −0.1% (−4.6, 4.6) | 0.95 |
| Medium VLDL size | 4.9% (1.0, 9.0) | **0.014** |
| Large VLDL size | 13.9% (9.5, 18.4) | **<0.001** |
| Apolipoprotein C-III | 8.9% (4.7, 13.3) | **<0.001** |

The *P*-values in bold are statistical significant.
Linear regression analysis for remnant cholesterol and standardized higher small, medium, and large VLDL concentration and apoC-III levels on hsCRP levels. All models are multivariable adjusted for age, sex, DM, BMI, SBP, smoking status, and LDL cholesterol. hsCRP, high-sensitivity C-reactive protein; VLDL, very-low-density lipoprotein; DM, diabetes mellitus; BMI, body mass index; SBP, systolic blood pressure; LDL, low-density lipoprotein.

explanation is that the pro-inflammatory effects of remnant particles may be primarily occurring at the cellular level, localized within the arterial wall, which might not be fully captured by plasma hsCRP levels. Experimental studies have shown that the triglyceride and free fatty acid load in these particles are susceptible to lipolysis, which can exert pro-inflammatory effects on endothelial cells and subendothelial macrophages.[49,53] Clinical studies have found that remnant cholesterol levels are independently associated with vascular inflammation using $^{18F}$FDG-PET/CT imaging, which was not correlated with hsCRP levels.[23,54] Furthermore, the observation that LDL cholesterol lowering with PCSK9 inhibitors significantly reduces vascular inflammation without affecting hsCRP levels suggests that atherosclerotic inflammation can change independently of hsCRP.[55] Beyond inflammation, another factor to consider is that remnant particles, on a per particle basis, contain more cholesterol and have a longer residence time in the vasculature compared with LDL particles.[6] ApoC-III could play a particular role in this process, as *in vitro* studies have shown that apoC-III on triglycerice-rich lipoproteins enhance the binding to biglycans; negatively charged glycosaminoglycans present in lesions, potentially increasing vascular retention of atherogenic lipoproteins.[56] Additionally, remnant particles are more potent in inducing macrophage foam cell formation than LDL particles.[57] Unlike LDL particles, they can initiate receptor-independent uptake by macrophages without requiring oxidative modification, a process necessary for the uptake of LDL particles.[57] Lastly, it is important to recognize that remnant cholesterol may be a marker of an underlying risk factor for ASCVD. One factor could be insulin resistance, as one recent study reported that approximately 80% of the MACE risk associated with remnant cholesterol was in fact mediated by insulin resistance,[58] highlighting its potential role as a key driver in the relationship between remnant cholesterol and cardiovascular disease. While we did adjust for components of metabolic syndrome in our analysis, we did not measure insulin resistance directly.

## Limitations

Our study has several limitations that warrant further discussion. First, the low number of participants with diabetes and those receiving lipid-lowering treatments restricts the applicability of our findings to more contemporary primary prevention populations.[1,59] Second, blood was withdrawn in the non-fasted state, leading to elevation of remnant cholesterol levels which may have impacted our observations and limit the generalizability to the fasting state. Third, remnant cholesterol levels have not been measured directly, but were calculated using the NIH Sampson formula. Yet, it has demonstrated a very good correlation with remnant cholesterol levels measured via ultracentrifugation.[40,60] Fourth, although the demographic characteristics of the Norfolk cohort were largely comparable to national samples, the lower percentage of current smokers may suggest a degree of selection bias. Additionally, while we adjusted for key measures of metabolic syndrome, underlying factors such as insulin resistance could still have caused residual confounding in our study. Last, the genetic and ethnic homogeneity of the European cohort may limit the applicability of our findings to other regions and patient groups. Future studies should address the underlying biological mechanisms by which remnant cholesterol cause atherosclerosis and the exact role of inflammation in this regard. Especially, they should establish if systemic inflammation measured by hsCRP does or does not reflect local atherosclerotic inflammatory processes in these patients.

# Conclusions

Remnant cholesterol is independently associated with systemic inflammation, even after correction for presence of metabolic syndrome features. This could be partly attributed to the presence of large VLDL particles and apoC-III concentrations. Importantly, remnant cholesterol is associated with cardiovascular events, but mediation analysis revealed only a minor role for inflammation in this association. These findings underscore the necessity of addressing both remnant cholesterol and systemic inflammation as distinct targets in the clinical management of cardiovascular disease.

# Supplementary material

Supplementary material is available at *European Journal of Preventive Cardiology*.

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# Authors' contributions

J.M.K. performed the analysis and wrote the manuscript. E.S.G., S.M.B., and L.F.R. provided supervision. All authors discussed the results, provided critical feedback, and contributed to develop the final manuscript.

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## Data availability

All data are available from the corresponding author upon reasonable request.

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