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Directly measured vs. calculated remnant cholesterol identifies additional overlooked individuals in the general population at higher risk of myocardial infarction

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Aims We tested the hypothesis that high directly measured remnant cholesterol is associated with increased risk of ischaemic heart disease (IHD) and myocardial infarction (MI) in the general population. We also explored whether directly measured vs. calculated remnant cholesterol is superior in identifying individuals at increased risk.

Methods and results Overall, 16 207 individuals from the Copenhagen General Population Study with both directly measured and calculated remnant cholesterol, both representing cholesterol content in triglyceride-rich lipoproteins, were followed up for 14 years to analyse the risk for IHD and MI. For directly measured and calculated remnant cholesterol, hazard ratios for individuals with concentrations ≥95th percentile vs. <40th percentile were 1.75 (95% confidence interval 1.42–2.15) and 1.76 (1.42–2.17) for IHD and 2.05 (1.50–2.80) and 1.93 (1.40–2.66) for MI. Compared to individuals with both directly measured and calculated remnant cholesterol <80th percentile (75% of the whole population), those with only directly measured remnant cholesterol ≥80th percentile (5%) had hazard ratios of 1.42 (1.15–1.75) for IHD and 1.83 (1.35–2.47) for MI. Corresponding hazard ratios for individuals with only calculated remnant cholesterol ≥80th percentile (5%) were 1.14 (0.91–1.44) and 1.14 (0.80–1.62), respectively, and corresponding hazard ratios for individuals with both directly measured and calculated remnant cholesterol ≥80th percentiles (15%) were 1.48 (1.30–1.68) and 1.67 (1.38–2.01), respectively. In individuals with high directly measured or high calculated remnant cholesterol, the median directly measured remnant cholesterol was 1.9 and 1.5 mmol/L, the median plasma triglycerides were 2.0 and 2.7 mmol/L, and the median plasma apolipoprotein B was 132 and 142 mg/dL, respectively.

Conclusions Directly measured vs. calculated remnant cholesterol identifies 5% overlooked individuals in the general population with cholesterol-rich, triglyceride-poor remnants and 1.8-fold increased risk of MI.

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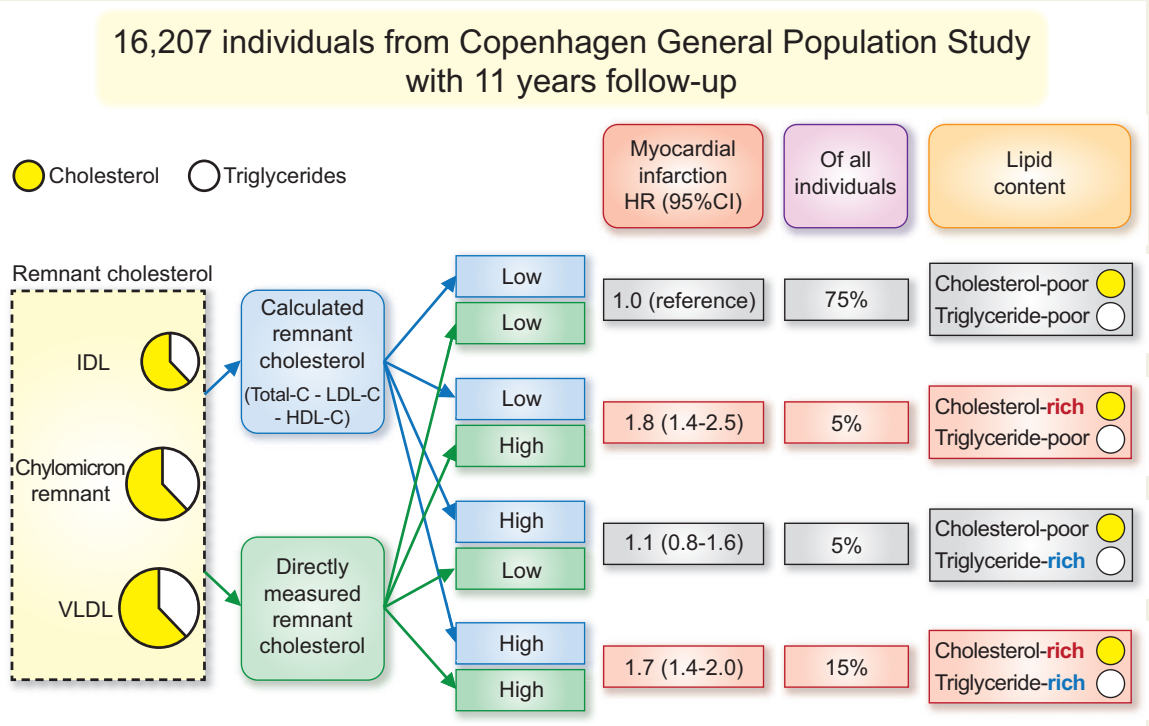
Graphical Abstract



Discordant and concordant remnant cholesterol calculated from a standard lipid profile or measured directly in relation to the risk of myocardial infarction and content of cholesterol and triglycerides in remnant particles. High indicates concentration ≥80th percentile, and low indicates <80th percentile. CI, confidence interval; HR, hazard ratio.

Keywords TRL-C • Lipoproteins • Triglyceride-rich lipoproteins • VLDL • Cardiovascular disease

Introduction

Previous studies found associations between high concentrations of calculated remnant cholesterol and increased risk of cardiovascular disease,[1] and genetic studies indicated that the association is causal.[2–4] In addition, emerging evidence from randomized clinical trials shows benefit from lowering triglycerides,[5] which are highly correlated to remnant cholesterol concentrations as they are constituents of the same triglyceride-rich lipoproteins, known as remnants.[2] A newly developed assay by Denka allows direct measurement of remnant cholesterol (i.e. cholesterol content in triglyceride-rich lipoproteins; TRL-C assay), but whether this is superior for risk prediction to calculating remnant cholesterol is unknown.

Remnant cholesterol can be calculated from a standard lipid profile as total cholesterol minus low-density lipoprotein (LDL) cholesterol minus high-density lipoprotein (HDL) cholesterol.[1,6] Remnant cholesterol is the cholesterol content of the triglyceride-rich lipoproteins called remnants, which include chylomicron remnants, very low-density lipoproteins (VLDL), and intermediate-density lipoproteins (IDL). It is an advantage that calculated remnant cholesterol can be derived from a standard lipid profile at no extra cost; however, it also has the limitation that when using the Friedewald equation to estimate LDL cholesterol and thus remnant cholesterol, a fixed ratio between triglycerides and VLDL cholesterol is assumed throughout most levels of plasma triglycerides. The ratio between triglycerides and cholesterol in remnant particles will differ somewhat between individuals, and also within the same individual at different time points. This can be mitigated partly by calculating remnant cholesterol using LDL cholesterol that is estimated without assuming a fixed ratio between triglycerides and VLDL cholesterol as done by Martin *et al.*,[7] or fully by using a direct assay that measures the cholesterol content in remnants.

Until recently, remnant cholesterol, defined as the cholesterol content in the blood stream that is not in LDL or HDL, could not be measured directly using an assay on an autoanalyzer suited for high-volume production in a hospital laboratory, but could only be measured by more laborious methods like ultracentrifugation or nuclear magnetic resonance spectroscopy.[6] If results from the present study show added benefit of measuring remnant cholesterol directly besides calculating it, this new assay opens up for broader use for risk prediction. The assay was designed to measure the cholesterol content of all lipoproteins that are not HDL or LDL, that is remnants or

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triglyceride-rich lipoproteins, and should not be mistaken with the remnant-like particle cholesterol (RLP-C) assay[8,9] that measures only a subfraction of what we define as remnant cholesterol in the present study.

In a previous study,[10] we examined the association between calculated and directly measured remnant cholesterol using this new TRL-C assay and found a high correlation between concentrations of the two, but whether directly measured remnant cholesterol concentrations are associated with risk of ischaemic heart disease (IHD) and myocardial infarction (MI) has not been explored previously by us.

In this prospective cohort study of 16 207 individuals from the Copenhagen General Population Study, we tested the hypothesis that high directly measured remnant cholesterol is associated with increased risk of IHD and MI in the general population. We also explored whether directly measured remnant cholesterol is superior to calculated remnant cholesterol in identifying individuals at increased risk of IHD and MI.

Methods

The study was approved by the Herlev and Gentofte Hospital and the Danish ethics committees (H-KF-01-144) and was conducted according to the Declaration of Helsinki. All individuals provided written informed consent and were whites of Danish descent.

The Copenhagen General Population Study

The Copenhagen General Population Study is a prospective cohort study of individuals randomly selected from the adult Danish general population, aged 20–100 years. The first examination covered the period from 2003 to 2016, and the second examination, including participants from the first examination and new participants living in the same geographical area, was initiated in 2016 and is ongoing. Individuals filled in a questionnaire, underwent a physical examination, and had blood samples drawn at study inclusion. Information on directly measured remnant cholesterol was available for 17 237 individuals recruited in 2003–2010; of these, 1030 were diagnosed with IHD and 439 with MI before baseline and were excluded from prospective analyses. Participants included were recruited consecutively ensuring that time of freezing was similar for those who did and did not develop MI and IHD during follow-up.

IHD and MI

Information on diagnoses of IHD (ICD8: 410–414; ICD10: I20–I25) and MI (ICD8: 410; ICD10: I21–I22) were obtained from the national Danish Patient Registry and the national Danish Causes of Death Registry from register start in 1977 to end of follow-up in April 2018. All Danish citizens are registered in the national registers, and follow-up is therefore without losses.

Laboratory analyses

Standard hospital assays measured nonfasting plasma triglycerides, plasma total cholesterol, and HDL cholesterol (Konelab or Roche). Calculated remnant cholesterol was total cholesterol minus HDL cholesterol and LDL cholesterol as done previously[2,8,10–12] but with LDL cholesterol calculated using the method by Martin *et al.*[7] where the ratio between triglycerides and VLDL cholesterol is dependent upon the plasma triglyceride and non-HDL cholesterol levels. For all other purposes than for calculating remnant cholesterol, LDL cholesterol was calculated using the Friedewald equation when triglycerides were ≤4 mmol/L (352 mg/dL), and otherwise measured directly (Konelab or Roche). Remnant cholesterol was measured directly using a recently developed assay by Denka (TRL-C) assay on the Cobas platform from Roche on samples from individuals who participated in 2003–2010. Samples were stored at -80°C from study inclusion to measurements performed in 2019. This automated homogeneous assay uses a two-step process where LDL and HDL are degraded and the cholesterol content of the remaining remnants (triglyceride-rich lipoproteins) is measured. The assay is traceable to cholesterol content in the ultracentrifugation density fraction <1.019 g/mL containing in the nonfasting state chylomicron remnants, VLDL, and IDL. For daily precision, average coefficient of variation was below 7%. For comparison between direct measurements of remnant cholesterol on fresh blood samples and on samples stored at -80°C, 101 samples originally measured on fresh blood samples were re-measured after ∼2 years of storage.

Other covariates

Other covariates included baseline self-reported use of antihypertensive therapy and lipid-lowering therapy (mainly statins), current or former smoking habits, self-reported diagnosed diabetes, nonfasting glucose >11 mmol/L, self-reported use of anti-diabetic medication, and/or register information on hospitalization due to diabetes before baseline (ICD8: 249–250; ICD10: E10, E11, E13, E14). Alcohol consumption was self-reported as drinks/week (1 drink ≈ 12 g of alcohol). Body mass index (BMI) was calculated by dividing body weight (in kg) by height in metre squared.

Statistical analyses

First, individuals were divided into groups of percentiles of directly measured and calculated remnant cholesterol, so that the two could be compared head-to-head as risk factors for IHD and MI. Hazard ratios for IHD and MI were estimated by Cox proportional hazards regression using left truncation and delayed entry, and with age as time-scale, which ensures the optimal adjustment for age. Individuals with IHD or MI before baseline were excluded from the analyses, and individuals were followed until first event, death, emigration, or end of follow-up in April 2018. The median follow-up time was 11 years (range 0–14 years). Besides age adjustment, hazard ratios were adjusted for possible confounders, i.e. sex, systolic and diastolic blood pressure, antihypertensive therapy, lipid-lowering therapy, and smoking. The main analyses were not adjusted for covariates in the biological pathway, including diabetes, alcohol consumption, and BMI; however, this was performed in sensitivity analyses. Also, further adjustment for LDL cholesterol and apolipoprotein B was performed in sensitivity analyses.

The association between directly measured and calculated remnant cholesterol was plotted as a scatterplot, with smoothed values from a kernel-weighted local polynomial regression.

Discordance on the risk of MI between directly measured and calculated remnant cholesterol was investigated by Cox proportional hazard regression models with individuals divided into four groups with a focus on high levels (individuals with the 20% highest levels): (i) individuals with both directly measured and calculated remnant cholesterol <80th percentile (reference), (ii) individuals with directly measured remnant cholesterol ≥80th percentile but calculated remnant cholesterol <80th percentile, (iii) individuals with calculated remnant cholesterol ≥80th percentile but directly measured remnant cholesterol <80th percentile, and (iv) individuals with both directly measured and calculated remnant cholesterol ≥80th percentile. Using the same four groups, distributions of directly measured remnant cholesterol, plasma triglycerides, and apolipoprotein B were graphed as density plots. Distributions of directly measured and calculated remnant cholesterol were also graphed as density plots in individuals with and without the metabolic syndrome.

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Discordance on the risk of MI between apolipoprotein B and calculated remnant cholesterol was investigated using a similar approach. Discordance between directly measured and calculated remnant cholesterol was also estimated by a prediction ellipse, and the risk of MI by Cox proportional hazard regression models with individuals divided into groups inside (reference) and outside the ellipse. The net reclassification index for MI using the cut point of directly measured and calculated remnant cholesterol ≥80th percentile was estimated (Supplementary material online, *Methods*), similarly to what previously done for lipoprotein(a).[13]

Comparison of directly measured remnant cholesterol in the same individuals measured on both fresh samples and after ~2 years of storage at -80°C was performed using unadjusted linear regression and was graphed as a fitted regression line with 95% confidence interval (CI).

Results

The baseline characteristics of the 16 207 individuals without previous IHD or MI are reported in Table 1, including the four groups based on <80th or ≥80th percentile levels for directly measured and calculated remnant cholesterol, both representing cholesterol content in triglyceride-rich lipoproteins. The group with only high directly measured remnant cholesterol had lower median apolipoprotein B levels but higher levels of directly measured remnant cholesterol than the group with only high calculated remnant cholesterol (132 vs. 142 mg/dL and 1.9 vs. 1.5 mmol/L, respectively). This suggests that individuals in the former group likely have fewer remnant particles but particles that are enriched in cholesterol.

Association of directly measured and calculated remnant cholesterol with risk of IHD and MI

When individuals were divided into groups with a focus on extreme high levels for directly measured and calculated remnant cholesterol, hazard ratios for individuals with concentrations ≥95th percentile vs. <40th percentile were 1.75 (95% CI 1.42–2.15) and 1.76 (1.42–2.17) for IHD and 2.05 (1.50–2.80) and 1.93 (1.40–2.66) for MI (*Figure 1*). Similar results were obtained when calculating remnant cholesterol based on Friedewald-estimated LDL cholesterol instead of using the method by Martin *et al.*[7] (Supplementary material online, *Figure S1*).

When multivariable adjusted hazard ratios were further adjusted for LDL cholesterol (Supplementary material online, *Figure S2*) or HDL cholesterol, BMI, diabetes, and alcohol intake (Supplementary material online, *Figure S3*), associations between both directly measured and calculated remnant cholesterol and risk of IHD and MI remained, although somewhat attenuated.

Association between directly measured and calculated remnant cholesterol

The association between directly measured and calculated remnant cholesterol was not perfectly linear but showed discordance especially at high concentrations (*Figure 2*).

Discordance on the risk of MI between directly measured and calculated remnant cholesterol

Because of the discordance in concentrations of directly measured and calculated remnant cholesterol, the prospective risk of IHD and MI was investigated in the four groups of individuals divided according to: (i) low concentrations of both directly measured and calculated remnant cholesterol (75% of the whole population), (ii) only high directly measured remnant cholesterol (5%), (iii) only high calculated remnant cholesterol (5%), and (iv) high concentrations of both directly measured and calculated remnant cholesterol (15%) (*Figure 3*). Compared to individuals with both directly measured and calculated remnant cholesterol <80th percentile, those with only directly measured remnant cholesterol ≥80th percentile had hazard ratios of 1.42 (95% CI 1.15–1.75) for IHD and 1.83 (1.35–2.47) for MI. Corresponding hazard ratios for individuals with only calculated remnant cholesterol ≥80th percentile were 1.14 (0.91–1.44) and 1.14 (0.80–1.62), and corresponding hazard ratios for individuals with both directly measured and calculated remnant cholesterol ≥80th percentile were 1.48 (1.30–1.68) and 1.67 (1.38–2.01), respectively.

Results were similar when remnant cholesterol was calculated using Friedewald-estimated LDL cholesterol instead of using the method by Martin *et al.*[7] (Supplementary material online, *Figure S4*). When multivariable adjusted hazard ratios were further adjusted for (i) LDL cholesterol, (ii) HDL cholesterol, BMI, diabetes, and alcohol intake, or (iii) apolipoprotein B, the associations between only high directly measured cholesterol and risk of MI remained, although somewhat attenuated (Supplementary material online, *Figure S5*).

The increased risk of MI was robust for the group with only high directly measured remnant cholesterol or with high concentrations of both directly measured and calculated remnant cholesterol, when stratified by other risk factors (*Figure 4*). The prospective risk of IHD and MI was also investigated in individuals divided based on any discordance between directly measured and calculated remnant cholesterol estimated by a prediction ellipse (Supplementary material online, *Figure S6*). Individuals with discordant vs. concordant concentrations (outside vs. within the ellipse) had hazard ratios for IHD and MI of 1.46 (95% CI 1.18–1.81) and 1.57 (1.14–2.14), respectively (Supplementary material online, *Figure S7*).

Triglycerides and apolipoprotein B

To further explore possible explanations for the discordance on the risk of IHD and MI for only high directly measured vs. only high calculated remnant cholesterol, concentration distributions of directly measured remnant cholesterol, plasma triglycerides, and apolipoprotein B for the four groups were plotted (*Figure 5*). In individuals with only high directly measured or only high calculated remnant cholesterol, the median directly measured remnant cholesterol was 1.9 and 1.5 mmol/L, the median plasma triglycerides were 2.0 and 2.7 mmol/L, and the median plasma apolipoprotein B was 132 and 142 mg/dL, respectively. This illustrates that individuals with only high directly measured remnant cholesterol have cholesterol-rich, triglyceride-poor remnant particles, while those with only high calculated remnant cholesterol have cholesterol-poor, triglyceride-rich

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Table 1 **Baseline characteristics of individuals free of ischaemic heart disease and myocardial infarction in the Copenhagen General Population Study 2003-2010**

	All	Both low directly measured and calculated remnant cholesterol	Only high directly measured remnant cholesterol	Only high calculated remnant cholesterol	Both high directly measured and calculated remnant cholesterol	*P*-value for comparison between discordant groups
Number	16 207 (100%)	12 225 (75%)	761 (5%)	772 (5%)	2449 (15%)	
Women	8824 (54%)	7291 (60%)	352 (46%)	321 (42%)	860 (35%)	<0.001
Age, years	56 (47–66)	56 (47–66)	55 (47–64)	60 (51–69)	56 (49–65)	<0.001
Hypertension	3474 (21%)	2389 (20%)	196 (26%)	200 (26%)	689 (28%)	<0.001
Lipid-lowering therapy	944 (6%)	722 (6%)	33 (4%)	67 (9%)	122 (5%)	<0.001
Ever smoking	9947 (62%)	7297 (60%)	456 (60%)	511 (67%)	1,683 (69%)	<0.001
Diabetes mellitus	620 (4%)	419 (3%)	24 (3%)	34 (4%)	143 (6%)	<0.001
High alcohol intake[a]	3,011 (20%)	2183 (19%)	111 (16%)	173 (24%)	544 (24%)	<0.001
Body mass index, kg/m^2	25.6 (23.6–28.4)	24.9 (22.8–27.7)	26.9 (24.7–29.3)	27.3 (25.1–30.1)	27.9 (25.8–30.8)	<0.001
Triglycerides, mmol/L	1.4 (1.0–2.1)	1.2 (0.9–1.6)	2.0 (1.8–2.2)	2.7 (2.4–3.0)	3.3 (2.8–4.3)	<0.001
Apolipoprotein B, mg/dL	112 (92–137)	103 (87–120)	132 (118–147)	142 (126–160)	165 (144–192)	<0.001
LDL cholesterol, mmol/L	3.3 (2.7–3.9)	3.1 (2.6–3.7)	3.7 (3.1–4.4)	3.5 (2.8–4.2)	3.8 (3.2–4.5)	<0.001
HDL cholesterol, mmol/L	1.6 (1.3–1.9)	1.7 (1.4–2.0)	1.4 (1.2–1.6)	1.3 (1.1–1.5)	1.2 (1.0–1.4)	<0.001
Directly measured remnant cholesterol, mmol/L	1.1 (0.7–1.6)	0.9 (0.6–1.2)	1.9 (1.8–2.1)	1.5 (1.3–1.6)	2.4 (2.1–3.0)	<0.001
Calculated remnant cholesterol, mmol/L	0.6 (0.5–0.8)	0.6 (0.4–0.7)	0.8 (0.8–0.9)	1.0 (0.9–1.1)	1.2 (1.0–1.5)	<0.001
Ischaemic heart disease[b]	1480 (9%)	971 (8%)	95 (12%)	80 (10%)	334 (13%)	<0.001
Myocardial infarction[b]	561 (3%)	344 (3%)	45 (6%)	28 (4%)	144 (6%)	<0.001

Both measured and calculated remnant cholesterol capture cholesterol content in triglyceride-rich lipoproteins. Data are from the Copenhagen General Population Study from study enrolment in 2003–2010 where directly measured and calculated remnant cholesterol was available. Low remnant cholesterol is <80th percentile and high remnant cholesterol is ≥80th percentile. Values are median and interquartile range or number of individuals and percentages. Numbers of individuals vary slightly according to the availability of variables.

[a]High alcohol intake was ≥14 drinks/week for women and ≥21 drinks/week for men (1 drink ≈12 g of alcohol).

[b]Ischaemic heart disease and myocardial infarction were only during follow-up (and not before baseline). *P*-values are from Pearson's chi-squared test for categorical variables and from ANOVA for continuous variables. To convert cholesterol values in mmol/L to mg/dL, multiply by 38.6.

HDL, high-density lipoprotein; LDL, low-density lipoprotein.

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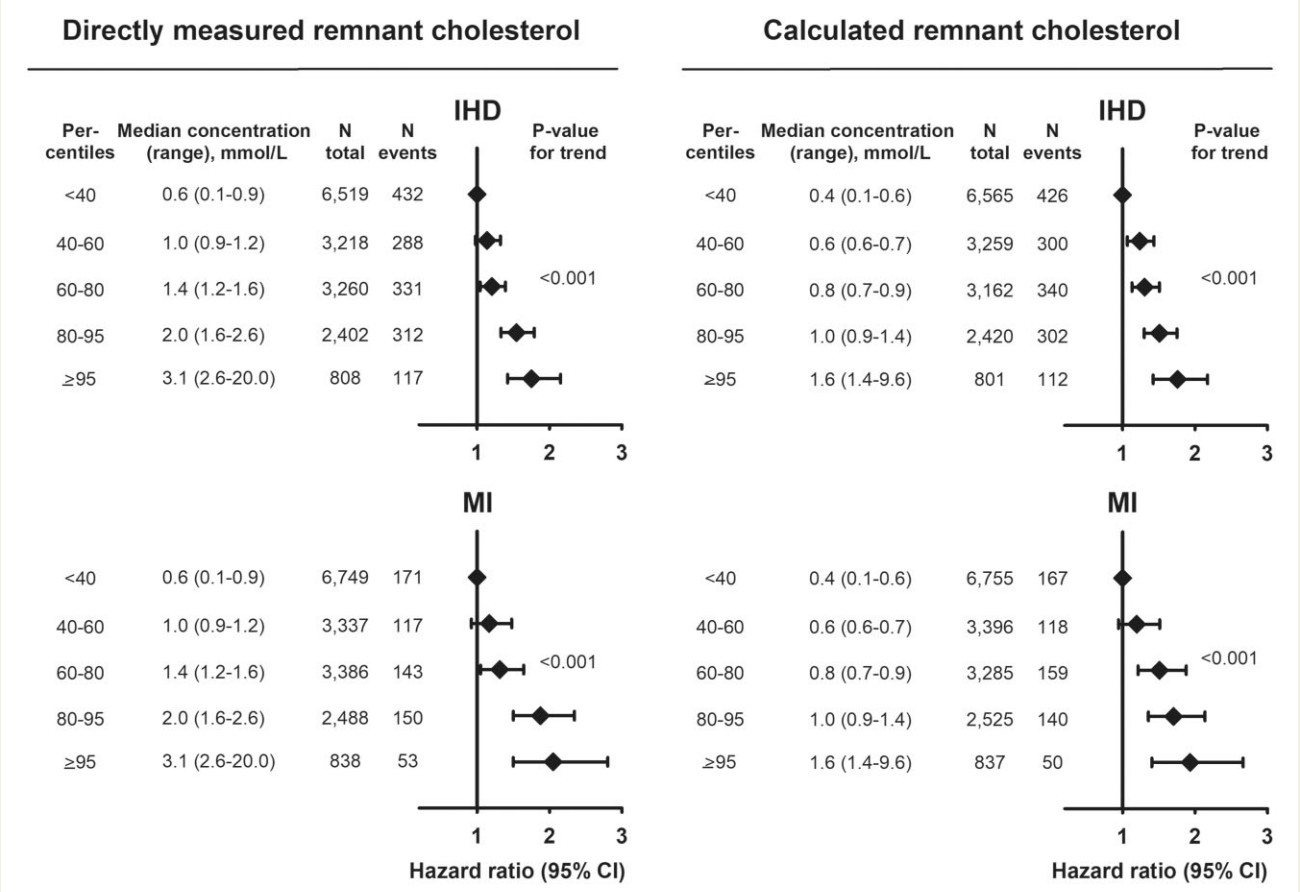

Figure 1 Association between directly measured and calculated remnant cholesterol with risk of ischaemic heart disease and myocardial infarction. Both measured and calculated remnant cholesterol capture cholesterol content in triglyceride-rich lipoproteins. Hazard ratios were from Cox proportional hazards regressions with multivariable adjustment for age (as underlying time-scale), sex, systolic and diastolic blood pressure, antihypertensive therapy, lipid-lowering therapy, and smoking. To convert cholesterol values in mmol/L to mg/dL, multiply by 38.6. CI, confidence interval; IHD, ischemic heart disease; MI, myocardial infarction.

remnant particles. Individuals with high concentrations of both directly measured and calculated remnant cholesterol had cholesterol-rich, triglyceride-rich remnant particles (*Figure 5*).

Discordance on the risk of MI between plasma apolipoprotein B and calculated remnant cholesterol was also examined (Supplementary material online, *Figure S8*). An increased risk of IHD and MI was observed for all three groups, that is (i) only apolipoprotein B ≥80th percentile, (ii) only calculated remnant cholesterol ≥80th percentile, and (iii) both apolipoprotein B and calculated remnant cholesterol ≥80th percentile, when compared to individuals with both <80th percentile.

Remnant cholesterol and the metabolic syndrome

In individuals with vs. without the metabolic syndrome, calculated remnant cholesterol was 50% higher, whereas measured remnant cholesterol was 78% higher (Supplementary material online, *Figure S9*).

Reclassification of MI risk by directly measured and calculated remnant cholesterol

Prediction of 10-year risk of MI was improved by adding directly measured or calculated remnant cholesterol to a model including the conventional risk factors, i.e. age, sex, systolic and diastolic blood pressure, antihypertensive therapy, lipid-lowering therapy, and smoking (*Figure 6*). When using a cut point ≥80th percentile for directly measured or calculated remnant cholesterol, the net reclassification index was 4.7% (1.4–8.0%; $P = 0.006$) and 7.5% (3.5–11.5%; $P < 0.001$), respectively.

Directly measured remnant cholesterol assay robustness to freezing and thawing

The Denka TRL-C assay was designed to measure cholesterol content that is not carried by LDL or HDL, and concentrations should on average be similar to calculated remnant cholesterol concentrations. However, concentrations of directly measured remnant

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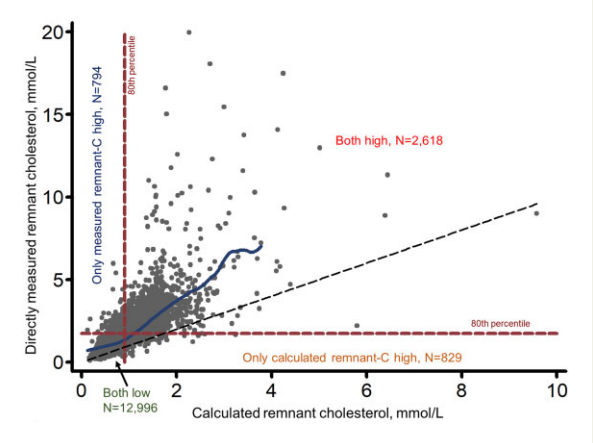

Figure 2 Association between directly measured and calculated remnant cholesterol. Each grey dot indicates concentrations for an individual, red dashed lines indicate 80th percentiles, and the dark blue line is from a local polynomial regression (unadjusted) between directly measured and calculated remnant cholesterol (both capturing cholesterol content in triglyceride-rich lipoproteins). The black dashed line is the identity line Y = X. To convert cholesterol values in mmol/L to mg/dL, multiply by 38.6.

cholesterol were found to be much higher than calculated concentrations (*Figure 2*). To explore whether this could be due to storage at -80°C for up to 14 years for the directly measured remnant cholesterol, 101 samples from the Copenhagen General Population Study second examination in 2017, where remnant cholesterol was directly measured on fresh samples, were re-measured after ∼2 years of freezer storage (Supplementary material online, *Figure S10*). This confirmed that concentrations increase with freezing; however, the association was linear, so freezing does not change, which percentile individuals are grouped into.

Discussion

In this prospective cohort study of 16 207 individuals from the Danish general population, high concentrations of directly measured remnant cholesterol are associated with higher risk of IHD and MI. Furthermore, directly measured vs. calculated remnant cholesterol identifies 5% overlooked individuals in the general population with cholesterol-rich, triglyceride-poor remnants and 1.8-fold increased risk of MI (*Graphical abstract*). This is the first study to investigate whether measuring remnant cholesterol directly adds value to calculating remnant cholesterol, which so far has been the only method applicable to high-volume testing. In the present study, both directly measured and calculated remnant cholesterol aim to capture the total cholesterol content in triglyceride-rich lipoproteins.

The aim of our study was not to search for the differences in cardiovascular risk that may exist based on different definitions of remnant cholesterol. Rather, it was to evaluate if directly measured vs. calculated remnant cholesterol could identify a group of individuals with cholesterol-rich remnants at increased risk of IHD and MI. This is important because for calculated remnant cholesterol a constant ratio of cholesterol in remnants to plasma triglycerides is assumed either throughout most levels of plasma triglycerides (Friedewald equation) or at individual levels of plasma triglycerides and non-HDL cholesterol (Martin equation[7]), which may not be the case for every individual as demonstrated in the present study. Thus, although calculated and measured remnant cholesterol may carry a similar risk of IHD and MI on a population basis, on an individual patient basis directly measured remnant cholesterol (cholesterol content in triglyceride-rich lipoproteins) appears to identify a group of high-risk individuals who are overlooked when examining only calculated remnant cholesterol.

One reason to separately consider the remnant cholesterol portion of the atherogenic cholesterol is if it presents greater risk than that of LDL cholesterol. To that end, in the Copenhagen General Population Study, directly measured VLDL cholesterol (≈remnant cholesterol) explained 50% of the risk of MI from elevated apolipoprotein B-containing lipoproteins, while LDL + IDL cholesterol only explained 29%.[14] Furthermore, in the same study, directly measured VLDL cholesterol explained 40% of the excess risk of MI associated with higher BMI, whereas LDL + IDL did not explain excess risk.[15] In support of this, in the PREDIMED trial population, elevated calculated remnant cholesterol, but not LDL cholesterol, was associated with cardiovascular outcomes in overweight and obese individuals.[16] Taken together, remnant cholesterol may be a better marker of risk for atherosclerotic cardiovascular disease than LDL cholesterol, particularly in overweight and obese individuals.

Mechanistically, the most likely explanation behind the association between high remnant cholesterol concentrations and increased risk for IHD and MI is that the cholesterol content of the remnant particles accumulates in the arterial wall and causes atherosclerosis.[1,4,17–20] Why there was a discordance on the risk of IHD and MI between directly measured and calculated remnant cholesterol could be explained by the direct assay (i) being the most accurate at higher concentrations and (ii) performing better when measuring/estimating the cholesterol content of the remnant particles, that is without being affected by plasma triglycerides for calculations. Since it is likely that triglyceride content *per se* is not atherogenic,[14] measuring cholesterol content directly seems optimal. However, this does not mean that calculating remnant cholesterol is not useful, because it did associate with high risk of IHD and MI at high concentrations, and correlated well with the directly measured concentrations, with most individuals with high directly measured remnant cholesterol also having high calculated remnant cholesterol. Also, the net reclassification index improvement was better for calculated than for directly measured remnant cholesterol, that is with the conditions we choose; however, such a finding needs to be confirmed in independent studies. This is important as calculating remnant cholesterol can be done at no extra cost from a standard lipid profile and can easily be used when direct measurement is not feasible. We found similar results on the risk of IHD and MI when using Friedewald-estimated LDL cholesterol or the method by Martin *et al.*[7] to calculate remnant cholesterol, indicating that both can be used depending on what the local laboratory offers.

The group with only high directly measured remnant cholesterol had lower levels of apolipoprotein B than the group with only high calculated remnant cholesterol. This could be seen as a strange

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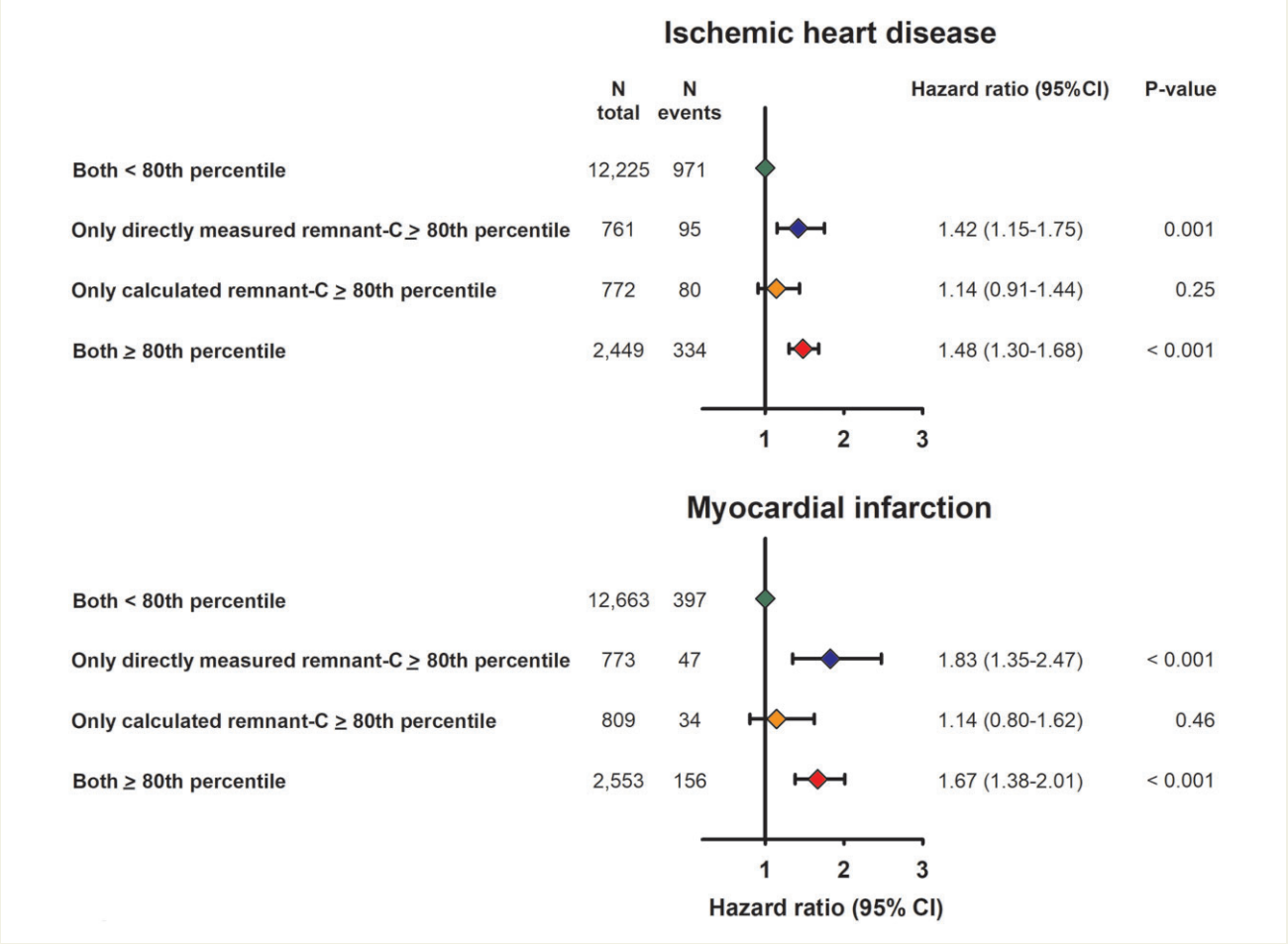

Figure 3 Discordance between directly measured and calculated remnant cholesterol on risk of ischaemic heart disease and myocardial infarction. Individuals were divided into four groups according to <80th or ≥80th percentile of directly measured and calculated remnant cholesterol (both capturing cholesterol content in triglyceride-rich lipoproteins). Hazard ratios were from Cox proportional hazards regressions with multivariable adjustment for age (as underlying time-scale), sex, systolic and diastolic blood pressure, antihypertensive therapy, lipid-lowering therapy, and smoking. CI, confidence interval.

finding; however, it seems to us that individuals in the group with only high directly measured remnant cholesterol have a lower number of remnant particles particularly enriched in cholesterol, an enrichment that most likely makes remnants to cause more atherosclerosis leading to an increased risk of IHD and MI. That cholesterol enrichment of remnant lipoproteins results in accelerated atherosclerosis, and premature IHD and MI is well known from the genetic condition of remnant hyperlipidaemia (= dysbetalipoproteinaemia or type III hyperlipidaemia). This underlines the clinical value of measuring cholesterol content directly, in addition to calculating it.

In line with our results, Duran *et al.*[21] found an increased risk of MI for higher levels of directly measured remnant cholesterol using the same assay. Also, previous studies using calculated remnant cholesterol, or other assays measuring only a fraction of what we call remnant cholesterol in this study, have found associations with cardiovascular disease.[1,2,8,10–12,22–27] The TRL-C (directly measured remnant cholesterol) assay by Denka used in this study is designed to measure the cholesterol content of triglyceride-rich lipoproteins at $d < 1.019$ g/mL, which makes it different from previous RLP-C assays measuring only a subfraction of what we define as remnant cholesterol. We therefore find higher levels of directly measured remnant cholesterol than previous studies using RLP-C assays.[9,25–27] Our study is the first to compare directly measured remnant cholesterol using the Denka TRL-C assay with calculated remnant cholesterol for estimating the risk of MI and IHD.

A strength of our study is the large number of individuals with both direct and calculated measurements of remnant cholesterol, enabling a head-to-head comparison. A limitation is that direct remnant cholesterol was not measured on fresh samples, and storage time has increased concentrations. However, since the association between concentrations in fresh and frozen samples in the same individuals was linear, dividing individuals into groups based on percentiles instead of absolute concentrations will ensure that individuals with high concentrations are identified. Also, participants were recruited consecutively ensuring that time of freezing was similar for those who did and did not develop MI and IHD during follow-up. For

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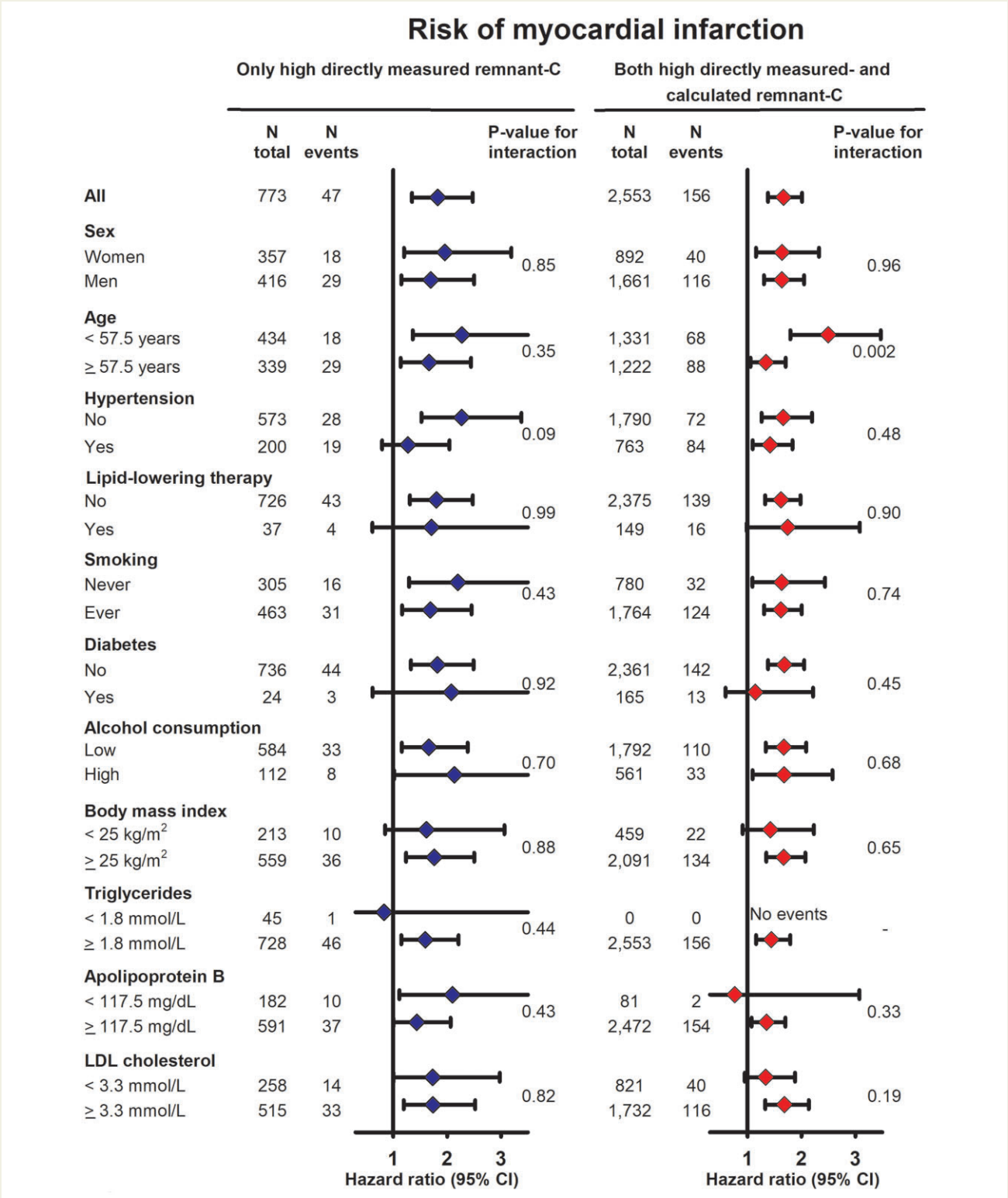

Figure 4 Risk of myocardial infarction stratified by other cardiovascular risk factors. Left section is for the group of individuals with high concentrations of directly measured remnant cholesterol, but not high calculated remnant cholesterol. Right section is for the group of individuals with high concentrations of both directly measured and calculated remnant cholesterol (both capturing cholesterol content in triglyceride-rich lipoproteins). The reference group is individuals with low concentrations of both. Numbers vary slightly according to availability of covariates. To convert cholesterol values in mmol/L to mg/dL, multiply by 38.6. C, cholesterol; CI, confidence interval.

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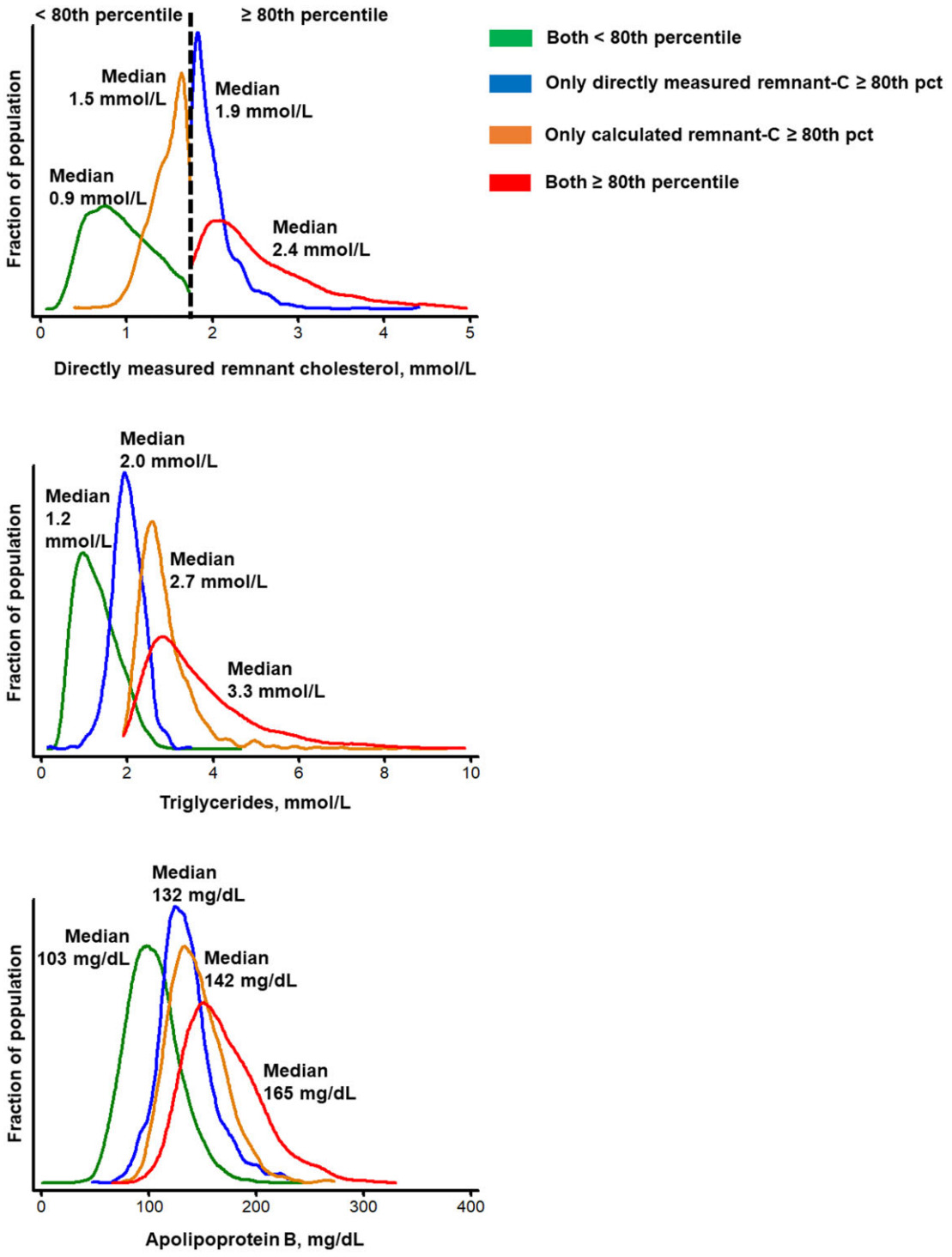

Figure 5 Distributions of directly measured remnant cholesterol, plasma triglycerides and plasma apolipoprotein B. Individuals were divided into four groups depending on whether they had below or above the 80th percentiles of directly measured and calculated remnant cholesterol (both capturing cholesterol content in triglyceride-rich lipoproteins). C, cholesterol.

future studies aiming at establishing cut-off values for clinical purposes, it will be important to use measurements on fresh samples. Furthermore, because calculated remnant cholesterol corresponds to the density <1.006 g/mL cholesterol and measured remnant cholesterol to the density <1.019 g/mL cholesterol, measured vs. calculated remnant cholesterol would on average contain slightly more

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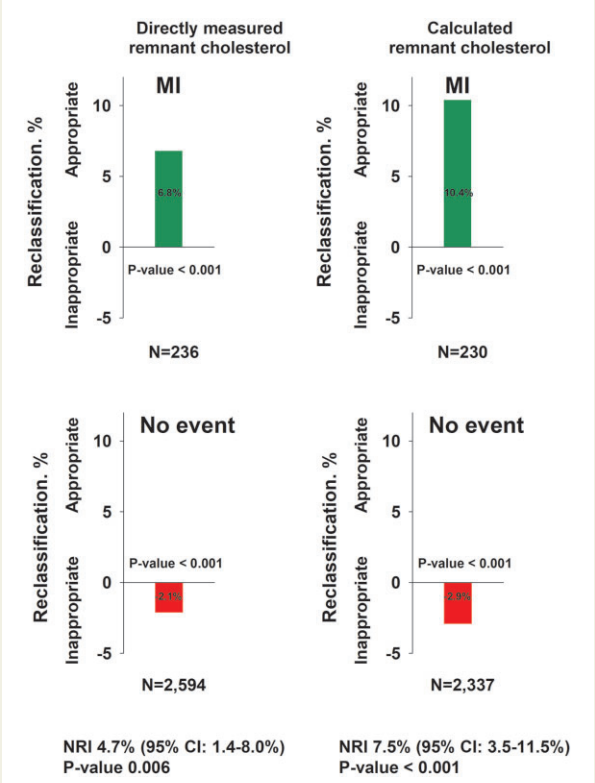

Figure 6 Net reclassification index for myocardial infarction using the cut point of ≥80th percentile of directly measured and calculated remnant cholesterol. Prediction of 10-year risk of myocardial infarction by adding directly measured or calculated remnant cholesterol (both capturing cholesterol content in triglyceride-rich lipoproteins) to a model containing the conventional risk factors of age, sex, systolic and diastolic blood pressure, antihypertensive therapy, lipid-lowering therapy, and smoking. The analyses were based on 12 456 individuals from the Copenhagen General Population Study free from myocardial infarction at baseline who had a possibility of 10 years of follow-up, i.e. excluding participants censored due to death or emigration before 10 years. CI, confidence interval; MI, myocardial infarction, NRI, net reclassification index.

cholesterol. However, this does not detract from our finding that elevated measured remnant cholesterol identifies an overlooked group of individuals in the general population at high risk of MI.

Also, a limitation is the inability of the observational study design to determine causality, partly due to the possibility of residual confounding, even though multivariable adjustment was used in the analyses. Therefore, there is a need for future randomized clinical trials to examine whether lowering directly measured remnant cholesterol in individuals with high concentrations will reduce cardiovascular risk. The recent REDUCE-IT trial[5] showed a 25% lower risk of cardiovascular disease in the group receiving 4 g of icosapent ethyl daily, when compared to placebo, both on top of statins. In that trial, individuals with moderately elevated triglycerides (1.52–5.63 mmol/L) were randomized to 4 g of icosapent ethyl daily or placebo, and were followed for a median of 4.9 years for assessing the occurrence of a composite endpoint of cardiovascular disease. When triglycerides are lowered, remnant cholesterol is also lowered since triglycerides and remnant cholesterol are components of the same lipoproteins and therefore are highly correlated. However, whether measuring remnant cholesterol directly to identify individuals for inclusion in a randomized trial would translate into even better risk reduction is unknown. This could be hypothesized from our results where a group of individuals at high risk of IHD and MI were overlooked based on calculated remnant cholesterol concentrations (and therefore would also be overlooked using plasma triglycerides) but were identified using the direct assay. Fortunately, the ongoing PROMINENT trial[28] also aimed at reducing cardiovascular disease through a reduction in triglyceride-rich lipoproteins is currently testing the direct remnant cholesterol (TRL-C) assay from Denka in all participants.

In conclusion, high concentrations of both directly measured and calculated remnant cholesterol are associated with higher risk of IHD and MI. Furthermore, directly measured vs. calculated remnant cholesterol identifies 5% overlooked individuals in the general population with cholesterol-rich, triglyceride-poor remnants and 1.8-fold increased risk of MI.

Supplementary material

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

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Conflict of interest: B.G.N. receives lecture and/or consultancy honoraria from AstraZeneca, Sanofi, Regeneron, Akcea, Amgen, Kowa, Denka, Amarin, Novartis, Novo Nordisk, Silence Therapeutics, and Esperion. A.V. is currently employed by Novo Nordisk.

Data availability

The Danish Data Protection Agency does not allow open access to our data; however, upon reasonable request to the corresponding author and the Steering Committee of the Copenhagen General Population Study, additional analyses can be performed.

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