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Remnant cholesterol, coronary atheroma progression and clinical events in statin-treated patients with coronary artery disease

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Abstract

Aim: Remnant cholesterol has been proposed to promote atherosclerotic cardiovascular disease independent of low-density lipoprotein cholesterol, yet the underlying mechanisms are not well understood. We aimed to study the association of remnant cholesterol with coronary atheroma progression and clinical events.

Methods: We analyzed data from 5754 patients with coronary artery disease undergoing serial intravascular ultrasonography who were enrolled in 10 trials examining various medical therapies. Remnant cholesterol was calculated as (non-high-density lipoprotein cholesterol – low-density lipoprotein cholesterol (estimated using the Hopkins–Martin equation)). Changes in percentage atheroma volume and 2-year major adverse cardiovascular events were compared across various levels of remnant cholesterol, and multivariable models were used to assess the independent relationship of remnant cholesterol with changes in percentage atheroma volume.

Results: The mean age was 58.1 ± 9.2 years, 28% were women and 96% received a statin. Percentage atheroma volume progression (changes in percentage atheroma volume > 0) occurred in a linear fashion at on-treatment remnant cholesterol levels of 25 mg/dL or greater. The highest on-treatment remnant cholesterol quartile demonstrated greater percentage atheroma volume progression (+0.53 ± 0.26 vs. –0.15 ± 0.25%, $P < 0.001$) and 2-year major adverse cardiovascular events (23% vs. 14%, log–rank $P < 0.001$) compared with the lowest. In multivariable analyses, changes in percentage atheroma volume significantly correlated with on-treatment remnant cholesterol ($P < 0.001$] independent of low-density lipoprotein cholesterol, apolipoprotein B, C-reactive protein, high-density lipoprotein cholesterol levels and clinical risk factors. Changes in percentage atheroma volume also significantly correlated with changes in remnant cholesterol following multivariable adjustment.

Conclusions: In statin-treated patients with atherosclerotic cardiovascular disease, remnant cholesterol was associated with coronary atheroma progression regardless of conventional lipid parameters, C-reactive protein or clinical risk factors. Higher remnant cholesterol levels also correlated with higher major adverse cardiovascular events. These data support further investigations into remnant cholesterol-lowering interventions in statin-treated patients harboring residual atherosclerotic cardiovascular disease risk.

Keywords

Remnant cholesterol, coronary artery disease, atherosclerosis, residual risk

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Introduction

Over the past 30 years, the literature has unequivocally revealed that reducing low-density lipoprotein (LDL) cholesterol levels with both statin and non-statin-based therapies leads to a significant reduction in atherosclerotic cardiovascular disease (ASCVD).[1–3] However, recurrent ASCVD continues to be a major problem despite achieving optimal LDL-cholesterol levels,[4–6] and part of this residual risk could be explained by triglyceride-rich lipoproteins (TGRLs) and their cholesterol content, known as remnant cholesterol (RC).[7,8] RC has gained increasing recognition as a biomarker driving residual ASCVD risk in this contemporary era of greater obesity, diabetes and metabolic syndrome rates.[9,10] Several studies in primary and secondary prevention cohorts have shown an association between RC levels and ASCVD,[10–12] yet the mechanisms of this underlying association and its therapeutic implications are not well understood. While non-high-density lipoprotein (HDL) cholesterol and apolipoprotein B (apoB) provide a comprehensive assessment of atherogenic lipoprotein-related risk, it is important to determine the relative independent contribution of individual fractions including LDL-cholesterol, RC and lipoprotein (a), as therapeutic strategies may differ with the advent of novel pathway-specific lipid-lowering agents.[13]

Serial measurement of coronary atheroma volume by intravascular ultrasound (IVUS) has been used to examine the effects of novel therapies on coronary artery disease (CAD) progression, and correlates closely with incident ASCVD.[14,15] In this study, we hypothesized that on-treatment RC levels would associate with changes in coronary atheroma volume and 2-year major adverse cardiovascular events (MACE) in a well-treated secondary prevention ASCVD patient cohort.

Methods

Study population

This analysis included pooled raw patient data from 10 clinical trials ($N = 5754$) studying the effect of various medical therapies and changes in coronary atheroma volume quantified using serial IVUS. The trials included assessed statin therapy (REVERSAL, ASTEROID and SATURN),[16–18] anti-hypertensive therapies (AQUARIUS and CAMELOT),[19,20] the anti-atherosclerotic efficacy of acyl-coenzyme A:cholesteryl ester transfer protein inhibition (ACTIVATE),[21] cholesteryl ester transfer protein inhibition (ILLUSTRATE),[22] endocannibanoid receptor antagonism (STRADIVARIUS),[23] peroxisome proliferator-activated receptor-gamma agonism (PERISCOPE)[24] and proprotein convertase subtilisin kexin type 9 (PCSK9) inhibition (GLAGOV).[25]

Acquisition and analysis of IVUS images

The acquisition and analysis of serial IVUS images was standardized across all trials and the details have previously been reported.[16–25] Target vessels were chosen if they had no luminal stenosis greater than 50% by angiography within a segment of at least 30 mm length. Following anticoagulation and administration of intracoronary nitroglycerin, a high frequency (40–45 MHz) ultrasound transducer was placed as distally as possible within the target coronary artery. Images were acquired as the catheter continuously withdrew through the artery and back to the aorta at a constant rate of 0.5 mm/s by a motorized pullback. Imaging was performed within the same coronary artery at baseline and at study completion, ranging from 18 to 24 months. All imaging was screened by the Atherosclerosis Imaging Core Laboratory of the Cleveland Clinic Coordinating Center for Clinical Research. Images were digitized and the analysis segments were selected using proximal and distal side branches as fiduciary points to allow for analysis of the same segment at follow-up. Images spaced 1 mm apart were selected for analysis. Leading edges of the lumen and external elastic membrane (EEM) were traced by manual planimetry.

The accuracy and reproducibility of this method have been reported previously.[14] Percentage atheroma volume (PAV) was determined by calculating the proportion of the entire vessel wall occupied by atherosclerotic plaque, throughout the segment of interest as follows:

$$PAV = \frac{\sum (EEM_{\text{area}} - lumen_{\text{area}})}{\sum EEM_{area}} \times 100 \quad (1)$$

$$\text{Change}(\Delta) \text{ in PAV was calculated as } \text{PAV}_{\text{completion of study}} - \text{PAV}_{\text{baseline}} \quad (2)$$

Determination of RC

Blood samples for the standard lipid profile were collected in the fasting state. RC was calculated from the standard lipid profile as (RC = total cholesterol (TC) – HDL-cholesterol – LDL-cholesterol), where LDL-cholesterol was either estimated using the Hopkins–Martin (HM) equation (RC_{HM}), instead of Friedewald's (RC_F), when triglycerides were less than 400 mg/dL or directly measured if triglycerides were 400 mg/dL or greater.[26] The HM equation, supported

by the recent 2018 American Heart Association (AHA)/American College of Cardiology (ACC) multisociety cholesterol guidelines,[27,28] uses an adjustable very low-density lipoprotein (VLDL):triglyceride ratio based on each subject's triglyceride and non-HDL-cholesterol level rather than Friedewald's constant of 5. Compared to prior landmark studies, we are the first to use HM LDL-cholesterol to calculate RC rather than Friedewald's LDL-cholesterol. It is important to note that this definition of RC (non-HDL-cholesterol – LDL-cholesterol) includes atherogenic TGRLs (such as VLDL remnants, chylomicron remnants and intermediate-density lipoprotein (IDL)) and larger non-atherogenic fractions (such as nascent VLDL and chylomicrons).[29] Measuring the levels of atherogenic TGRL fractions specifically is only available through advanced lipoprotein testing at an extra cost.[9,29]

Statistical analysis

Continuous variables were reported as mean ± standard deviation if normally distributed and as mean (interquartile range) if non-normally distributed. Categorical variables were expressed as frequency (percentage). RC was calculated as described above. LDL-cholesterol was calculated for subjects with triglycerides less than 400 mg/dL using the HM equation and was directly measured when triglycerides were 400 mg/dL or greater. Demographics, baseline clinical characteristics and medication use were compared across quartiles of RC. Laboratory data and IVUS parameters were compared at baseline and on-treatment (calculated as average on-treatment levels). LOWESS (locally weighted scatter plot smooth) plots were used to assess visually the overall relationship between on-treatment RC with ΔPAV.

To assess for a potential independent relationship between on-treatment and change in RC (ΔRC) levels with ΔPAV, given that the calculation of RC inherently includes LDL-cholesterol, comparative multivariable mixed effects regression models were constructed. These models were adjusted for baseline PAV, LDL-cholesterol, apoB, race (white vs. other), sex, body mass index, history of myocardial infarction, history of percutaneous coronary intervention, hypertension, diabetes mellitus, current smoking, baseline use of angiotensin-converting enzyme inhibitors or angiotensin receptor blockers, baseline HDL-cholesterol and baseline CRP. As RC is calculated using estimated HM LDL-cholesterol rather than direct LDL-cholesterol in most patients (direct LDL-cholesterol only used when triglycerides were > 400 mg/dL), RC is actually a derivative of triglycerides (RC = triglycerides/adjustable factor)[29] and they are highly correlated (Pearson correlation coefficient 0.97, $P < 0.001$). Therefore, we did not include triglycerides in the regression models to avoid collinearity and overestimation of the effect.

The association between on-treatment RC with ΔPAV was also stratified according to quartiles of RC as well as previously described clinical cut points (≤20, 21–39, 40–59 and ≥ 60 mg/dL).[30] ΔPAV in each quartile was analyzed by analysis of covariance, adjusting for clinical trial and baseline measures and reported as least squares mean ± SE.

An analysis was performed to evaluate the time to first MACE (cardiovascular death, non-fatal myocardial infarction, stroke, coronary revascularization, hospitalization for unstable angina). Log rank tests with Kaplan–Meier curves were performed on MACE rates by quartiles of on-treatment RC. A 24-month cut-off was used for the survival analysis and the time to first occurrence of MACE was determined. Those without MACE at 24 months were censored at this time point or at the last known contact. The trials included in this post hoc analysis had different durations (either 18 or 24 months). Therefore, we performed a sensitivity analysis censored at one year to account for the difference in trial duration. All analyses were based on the patient population that had baseline and achieved IVUS measurements with non-missing baseline and on-treatment lipoproteins. All analyses were performed using SAS software version 9.4 (SAS Institute, Cary, NC, USA).

Results

Table 1 shows baseline demographics, clinical characteristics and medications in the pooled cohort ($n = 5754$). The mean age was 58.1 ± 9.2 years and 28% were women. A high frequency of risk factors (hypertension 78%, hyperlipidemia 75%, smoking 25% and diabetes mellitus 28%) were encountered. The majority of patients was treated with statins (96%) and aspirin (93%).

Table 1 shows baseline, follow-up and changes in laboratory and ultrasonic parameters in the overall cohort. Reductions in LDL-cholesterol (–23.3 ± 38.3 mg/dL, $P < 0.001$), triglycerides (–7.5 (95% confidence interval (CI) 39.5, 19) mg/dL, $P < 0.001$) and RC (–2.8 (95% CI 7.7, 1.6) mg/dL, $P < 0.001$) and an increase in HDL-cholesterol (+4.6 ± 9.8 mg/dL, $P < 0.001$) were observed. Overall, PAV did not significantly change (least square mean (95% CI) of –0.04 (–0.13, 0.04)%; $P = 0.33$).

Figure 1 shows the relationship between on-treatment RC vs. ΔPAV as a LOWESS plot, which appears to be linear above RC levels of 20 mg/dL. Net atheroma progression (ΔPAV > 0) occurs at an RC level above 25 mg/dL. Figure 2 shows ΔPAV across quartiles and

Table 1. Baseline clinical characteristics and concomitant medications and baseline, follow-up and changes in laboratory and intravascular ultrasound parameters.

	Cohort ($N = 5754$)
Baseline clinical characteristics and concomitant medications	
Baseline RC range (mg/dL)	
Age (years), mean ± SD	58.1 ± 9.2
Women, *n* (%)	1609 (28)
Caucasian, *n* (%)	5357(93)
Current smoker, *n* (%)	1344/5404 (25)
Hypertension, n(%)	4502 (78)
Diabetes mellitus, *n* (%)	1613 (28)
Hyperlipidemia, *n* (%)	4293 (75)
BMI (kg/m^2), mean ± SD	30.6 ± 5.7
Prior MI, *n* (%)	1720 (30)
Prior revascularization, *n* (%)	2134/5407 (40)
Acute coronary syndrome, *n* (%)	1200/4629 (26)
Prior PVD, *n* (%)	259 (5)
Medications	
Statin, prior, *n* (%)	4482 (78)
Statin, concomitant, *n* (%)	5590 (96)
Any ACE-I or ARB, prior, *n* (%)	3646 (63)
Any ACE-I or ARB, concomitant, *n* (%)	3965 (69)
Aspirin, prior, n (%)	5270 (92)
Aspirin, concomitant, n (%)	5324 (93)
Beta-blockers, concomitant, *n* (%)	4413 (77)

Baseline, follow-up and changes in laboratory and intravascular ultrasound parameters

Parameter	Baseline	Follow-up	Change	*P* value*
Laboratory†				
RC (mg/dL), median (IQR)	23.8 (19.1, 30.8)	21.7 (17.9, 27.0)	–2.8 (–7.7, 1.6)	<0.001
Triglycerides (mg/dL), median (IQR)	134.0 (97.3, 190.0)	125.9 (93.5, 170.6)	–7.5 (–39.5, 19)	<0.001
LDL-cholesterol (mg/dL)	103.5 ± 34.6	80.2 ± 30.1	–23.3 ± 38.3	<0.001
Non-HDL-cholesterol (mg/dL)	133.6 ± 40.1	107.7 ± 35.5	–25.9 ± 42.3	<0.001
HDL-cholesterol (mg/dL)	43.7 ± 11.9	48.3 ± 14.4	4.6 ± 9.8	<0.001
Apolipoprotein B (mg/dL)	96.6 ± 33.07	77.1 ± 26.22	–19.8 ± 29.50	<0.001
TC/HDL ratio	4.3 ± 1.50	3.5 ± 1.20	–0.8 ± 1.31	<0.001
CRP (mg/L), median (IQR)	2.2 (1.0, 4.8)	1.5 (0.7, 3.7)	–0.3 (–1.8, 0.4)	<0.001
IVUS‡	LS mean (95% CI)			
PAV (%)	37.82 ± 8.90	37.78 ± 8.90	–0.04 (–0.13, 0.04)	0.33

ACE-I: angiotensin-converting enzyme inhibitor; ARB: angiotensin receptor blocker; BMI: body mass index; MI: myocardial infarction; RC: remnant lipoprotein cholesterol; LDL: low-density lipoprotein; TC: total cholesterol; HDL: high-density lipoprotein; ApoB: apolipoprotein B; CRP: C-reactive protein; IQR: interquartile range; IVUS: intravascular ultrasound; PAV: percentage atheroma volume; LS mean: least squares mean adjusted for trial and respective baseline PAV.

Data are presented as mean ± SD unless otherwise indicated.

*Tests whether change is significantly different than zero.

†Reflects % changes from baseline with mean change (95% CI) reported, unless noted.

‡Reflects absolute changes from baseline with mean change and LS mean (95% CI) reported.

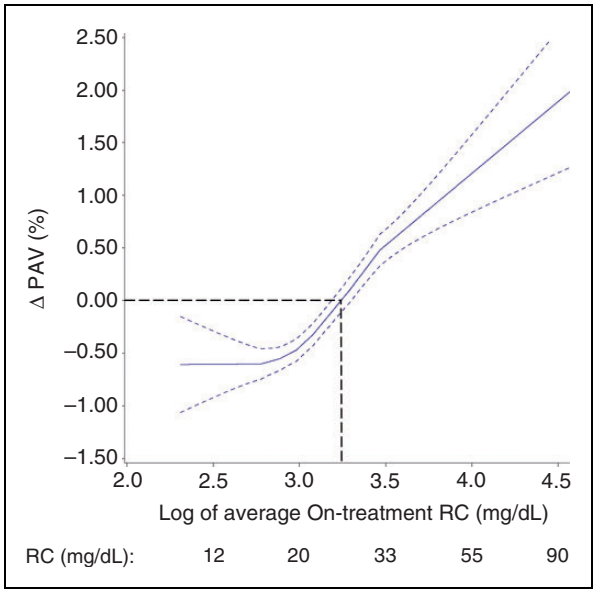

Figure 1. LOWESS (locally weighted scatter plot smooth) regression plot that shows the overall associations between average on-treatment RC versus ΔPAV (%). *There is a plateau in ΔPAV% followed by a linear increase once RC levels increase beyond 20 mg/dL. Atheroma progression (ΔPAV% > 0) occurs at an RC level of 25 mg/dL. RC: remnant cholesterol; ΔPAV: change in percentage atheroma volume.

predefined strata of on-treatment RC levels. There was a stepwise increase in ΔPAV across increasing quartiles and categories of RC ($P < 0.001$).

Two separate multivariable models were constructed to evaluate the independent association of on-treatment RC or ΔRC with plaque progression (Table 2). In the first model (Table 2), on-treatment RC levels were significantly correlated with PAV progression (β-coefficient (95% CI) 0.18 (0.08, 0.28), $P < 0.001$) after adjusting for clinical trial duration, baseline PAV, on-treatment LDL-cholesterol, apoB, HDL-cholesterol, CRP and other clinical risk factors. In the second model (Table 2), ΔRC was significantly associated with PAV progression ($\beta = 0.28$ (0.13, 0.43), $P < 0.001$] after adjusting for the above-mentioned variables in addition to baseline RC levels as well as baseline and changes in LDL-cholesterol and apoB levels. In these multivariable models, on-treatment LDL-cholesterol levels were associated with PAV progression ($\beta = 0.33$ (0.23, 0.43), $P < 0.001$) but ΔLDL-cholesterol was not ($\beta = 0.08$ (–0.12, 0.27), $P = 0.45$) (Table 2).

Figure 3 shows cumulative incidence of MACE at 24 months by quartiles of on-treatment RC. There was a progressive increase in the incidence of first MACE across increasing quartiles of on-treatment RC, with early curve separation (log rank $P < 0.001$). A sensitivity analysis censoring MACE at one year due to differences in trial durations showed similar associations (Supplementary Figure 1).

Supplementary Figure 2 demonstrates that the RC_F, estimated using the Friedewald formula, had a similar linear relationship to ΔPAV; however, atheroma progression started at higher RC_F levels of ≈ 30 mg/dL (compared with ≈ 25 mg/dL when assessing RC_{HM}).

Discussion

This post hoc analysis of 5754 mostly statin-treated patients with CAD demonstrates that higher on-treatment RC levels are significantly associated with greater coronary atheroma progression and higher 2-year MACE rates. After adjusting for conventional lipid parameters, CRP and a range of clinical risk factors, plaque progression remained independently associated with on-treatment RC levels. Furthermore, in this analysis, plaque progression was more strongly associated with changes in RC compared with changes in LDL-cholesterol or apoB levels. Our findings support further investigations into RC-lowering interventions in statin-treated patients harboring residual ASCVD risk.

It is well established that LDL-cholesterol reduction attenuates atheroma progression, induces regression and plaque stabilization, thus lowering ASCVD event rates.[15] Nevertheless, the role of other atherogenic lipoproteins (such as TGRLs and lipoprotein (a)) in atherogenesis and its clinical sequelae has received increasing attention.[31] TGRL particles are regulated by apolipoprotein C-III (apoC-III)[28–30] and their atherogenic fractions (such as IDL, VLDL remnants and chylomicron remnants) have been shown to migrate across the endothelial wall where they are engulfed by macrophages, forming foam cells, inducing low-grade inflammation and instigating atheromatous plaque growth.[32] Numerous genetic and clinical studies have shown that RC levels, or their highly correlated triglyceride levels, are predictive of ASCVD and all-cause mortality.[10–12,33–35] A Mendelian randomization study of 60,000 participants demonstrated that a 39 mg/dL (1 mmol/L) greater level of non-fasting RC was associated with a three-fold increase in CRP levels and higher rates of ischemic heart disease.[10] Similar results were seen in patients with CAD from the TNT trial suggesting that elevated on-treatment levels of RC may contribute to residual ASCVD risk.[36] Moreover, in CAD patients with LDL-cholesterol less than 70 mg/dL, elevated RC was predictive of significant coronary plaque burden as seen on computed tomography coronary angiography.[37] However, the mechanism by which RC levels associate with ASCVD events has thus far remained elusive.

Our group has previously demonstrated that higher on-treatment levels of non-HDL-cholesterol were

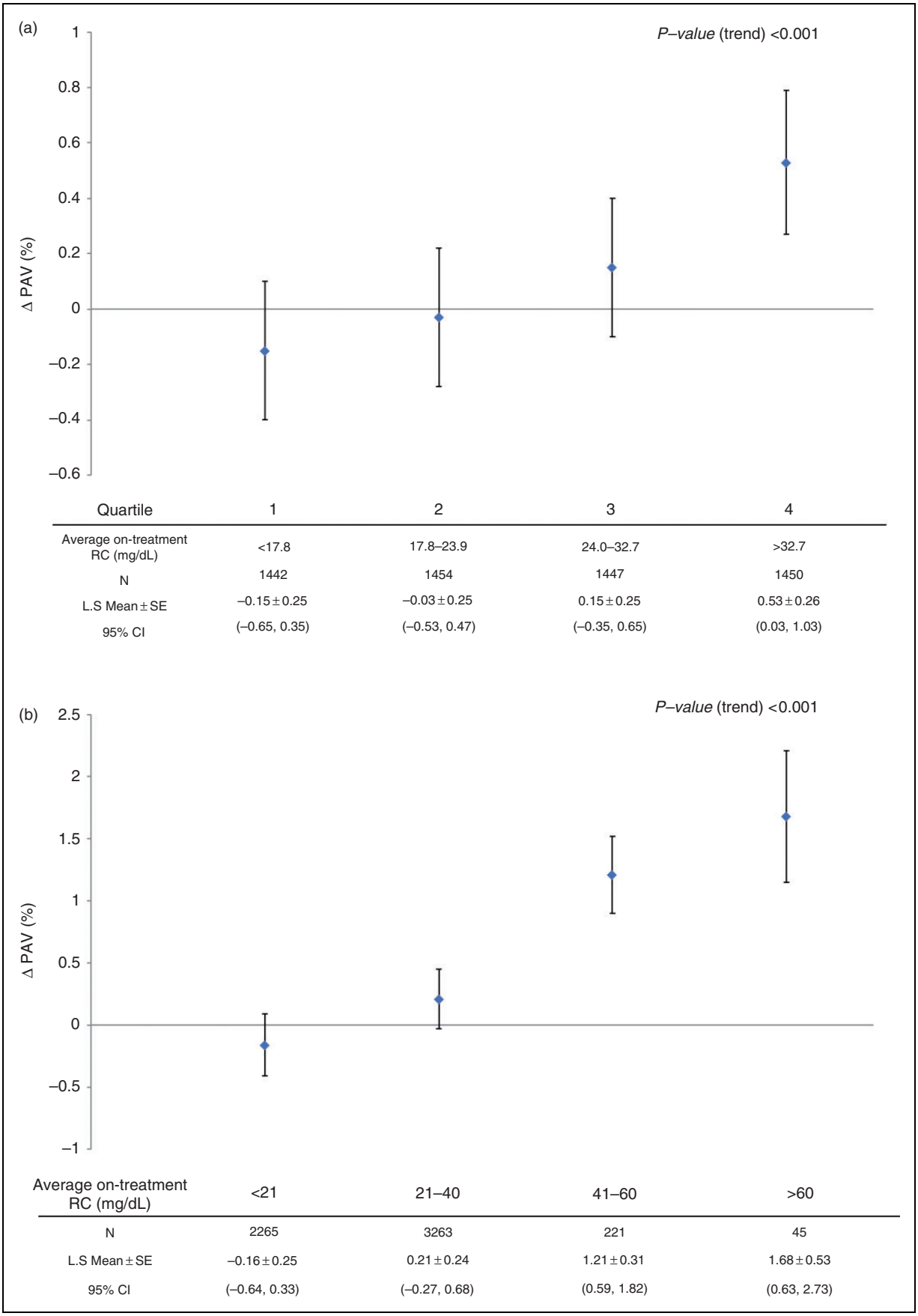

Figure 2. Change in percentage atheroma volume (ΔPAV%) across (a) quartiles of average on-treatment RC levels and (b) categorical cut points of average on-treatment RC levels. *ΔPAV% shown represents least square (LS) mean of each quartile. RC: remnant cholesterol.

Table 2. Multivariable linear regression model for change in percent atheroma volume adjusted for on-treatment RC levels and changes in RC levels.

Covariate	β-coefficient	95% CI		*P* value
		Lower	Upper	
On-treatment RC levels				
Baseline PAV	–0.08	–0.09	–0.07	<0.0001
Average on-treatment RC	0.18	0.07	0.29	0.0014
Average on-treatment LDL-cholesterol	0.31	0.13	0.50	0.00019
Average on-treatment apoB	0.001	–0.007	0.009	0.78
Hypertension	0.26	0.04	0.48	0.02
Baseline ACE-I or ARB	–0.24	–0.42	–0.06	0.008
Baseline HDL-cholesterol	0.009	0.0008	0.02	0.03
Diabetes mellitus	0.63	0.41	0.84	<0.0001
Women	–0.31	–0.51	–0.10	0.004
Changes in RC levels				
Baseline PAV	–0.09	–0.11	–0.07	<0.0001
ΔRC	0.25	0.09	0.42	0.003
ΔLDL-cholesterol	–0.05	–0.36	0.25	0.72
ΔApoB	0.007	–0.004	0.02	0.21
Baseline RC	0.04	–0.20	0.28	0.74
Baseline LDL-cholesterol	0.11	–0.26	0.48	0.55
Baseline apoB	0.01	–0.005	0.02	0.18
Diabetes mellitus	0.42	0.02	0.82	0.04

β-coefficient estimates are standardized; RC is natural log-transformed; model controls for differences in duration of IVUS trial.
*Covariates with $P < 0.05$ are shown in the table in addition to baseline RC and baseline LDL-cholesterol. Other factors considered in the multivariable analysis include race (white vs. other), sex, body mass index, history of myocardial infarction, history of percutaneous coronary intervention, hypertension, current smoking, baseline use of angiotensin-cconverting enzyme inhibitors or angiotensin receptor blockers, baseline high-density lipoprotein cholesterol and baseline C-reactive protein (natural log transformed).
RC: remnant lipoprotein cholesterol; LDL: low-density lipoprotein; ApoB: apolipoprotein B 100; PAV: percentage atheroma volume; IVUS: intravascular ultrasound.

associated with coronary atheroma progression regardless of LDL-cholesterol, but we did not directly examine RC.[38] The current analysis, which additionally includes patients from the GLAGOV trial who achieved very-low LDL-cholesterol levels with a PCSK9 inhibitor, demonstrated that on-treatment RC levels were significantly associated with ΔPAV independent of LDL-cholesterol, apoB, HDL-cholesterol and CRP levels, as well as clinical risk factors. For each standard deviation increase (9 mg/dL) in on-treatment RC, the increment in PAV progression was 0.18% (95% CI 0.07%, 0.29%). Consistent with prior studies evaluating clinical events, atheroma progression ($\Delta PAV > 0$) occurred when on-treatment RC levels were more than 25 mg/dL.[36,39] The present analysis demonstrates higher on-treatment RC levels to correlate independently with coronary atheroma progression, as a potential mechanism underscoring residual MACE rates in statin-treated CAD patients. Furthermore, reductions in RC are independently associated with atheroma regression, beyond the effects of established interventions focused on lowering LDL-cholesterol.

In the present analysis, we also found that coronary atheroma progression rates were significantly linked with changes in RC, but not with changes in LDL-cholesterol or apoB. However, our findings do not detract from previous reports that changes in LDL-cholesrerol or apoB are independently associated with atheroma progression,[15] yet highlight the simultaneous relative importance of changes in RC in modulating atheroma progression rates. Given the current availability of extremely potent LDL-cholesterol-lowering therapies,[40] targeting alternative pathways, such as triglycerides or RC lowering, may be incrementally beneficial in curbing residual ASCVD burden.[40] The recent REDUCE-IT trial (Reduction of Cardiovascular Events with Icosapent Ethyl–Intervention Trial) revealed that high-risk patients with fasting triglycerides of 135–499 mg/dL who received icosapent ethyl had a 25% relative risk reduction in ASCVD at 5 years compared

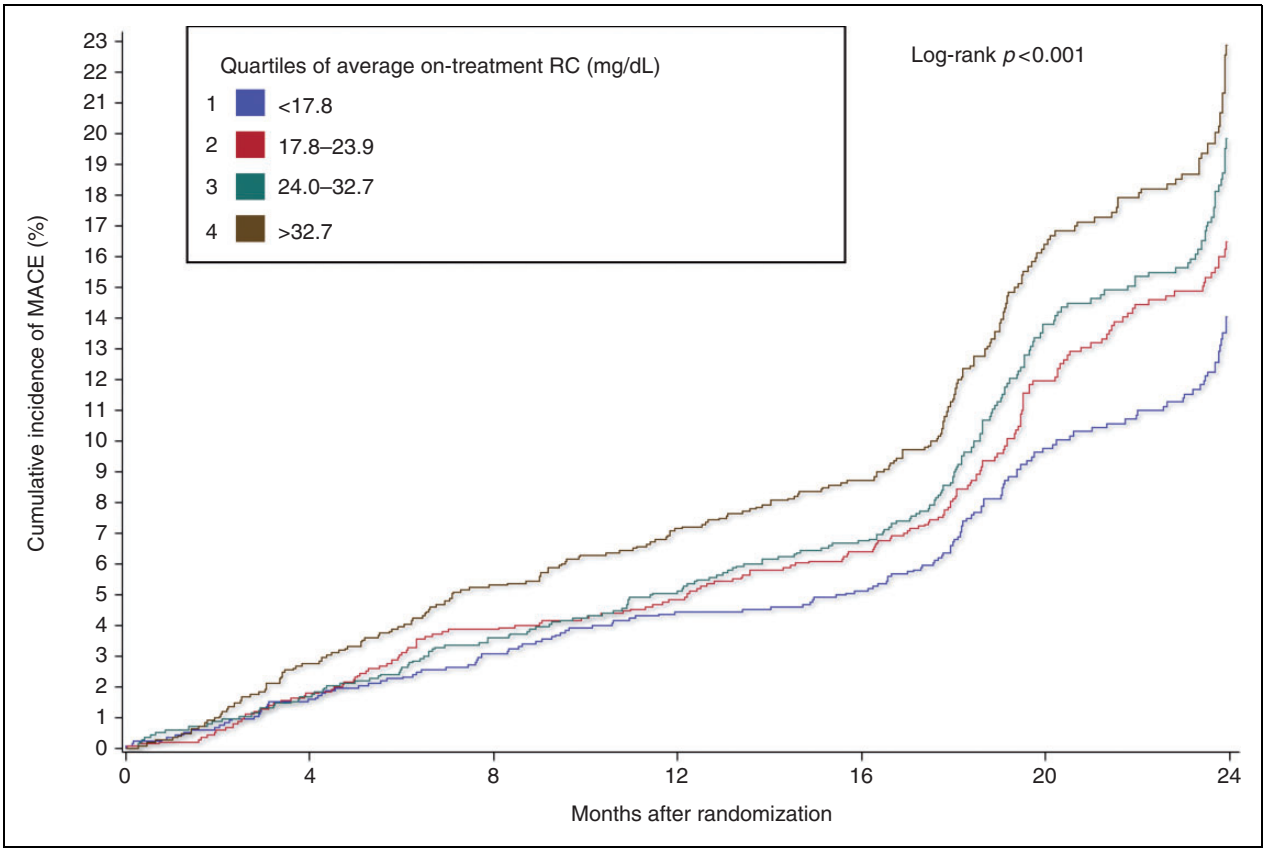

Figure 3. Log rank test with Kaplan–Meier curves of major adverse cardiovascular events (MACE) across quartiles of average on-treatment RC levels. MACE rates from quartile 1 to quartile 4 were: 14%, 17%, 20% and 23%. The log rank *P* value was less than 0.001 for detecting any difference between quartiles. *MACE defined as cardiovascular death, non-fatal myocardial infarction, stroke, coronary revascularization, hospitalization for unstable angina. RC: remnant cholesterol.

to placebo.[41] The median change across follow-up was –38 mg/dL for triglyceride levels, –5 mg/dL for non-HDL-cholesterol levels and + 2 mg/dL for LDL-cholesterol levels. While it is uncertain whether triglyceride lowering directly resulted in the benefits achieved in this trial, it highlights the importance of targeting patients with elevated residual RC or triglyceride levels despite optimal LDL-lowering therapy. In concordance with REDUCE-IT, our findings support exploring therapies that favorably reduce RC levels, through apoC-III inhibition or alternative therapeutic pathways,[35,42,43] in order to unravel novel means of incrementally reducing ASCVD risk in statin-treated patients. With the emergence of novel lipid-lowering drugs with highly specific targets,[13] comprehensive assessment of total atherogenic cholesterol or lipoproteins by measuring non-HDL-cholesterol or apoB levels may not be sufficient. In fact, accurate measurement of the relative contribution of each atherogenic cholesterol fraction (LDL vs. RC vs. lipoprotein (a)) may be essential to personalize lipid-lowering treatment strategies.

A number of caveats should be noted with regard to the present analysis. The study population included patients with CAD with an indication for coronary catheterization, and 96% were receiving statins. It is therefore uncertain whether they will extrapolate to asymptomatic patients and those not treated with lipid-modifying agents. The clinical trials incorporated in this analysis primarily focused on a surrogate efficacy endpoint and were not individually powered to detect differences in MACE between treated groups. However, the previous report that baseline and changes in coronary atheroma volume and MACE rates would suggest that our observations are clinically relevant.[14] Despite adjusting for clinical trial, variation in follow up times and a range of clinical, biochemical and ultrasonic variables, we cannot exclude the presence of unidentified potential confounders which may have impacted our results. As coronary IVUS imaging focuses on the evaluation of plaque burden, the association between RC and plaque composition remains poorly characterized. RC was estimated from the standard lipid profile using the HM equation for LDL-cholesterol estimation and not

directly measured by ultracentrifugation. Finally, lifestyle data, known to affect RC levels, were not routinely collected in all patients.

Conclusion

In conclusion, in high-risk CAD patients, RC was significantly associated with coronary atheroma progression, regardless of biochemical and clinical risk factors. This suggests that accelerated progression of atherosclerosis is an important factor underlying the observation of a greater incidence of clinical cardiovascular events. Measuring RC is likely to play an important role in identifying patients requiring more intense or personalized medical therapy for secondary prevention. These data also highlight RC and triglyceride targeted therapies as areas of interest for the clinical development of novel anti-atherosclerotic agents.

Author contribution

MBE and PM have contributed equally to the manuscript. They are the lead authors and are responsible for study design, data analysis, figure preparation, and manuscript preparation and submission for publication. DMB is responsible for data analysis, figure preparation and manuscript review. RP is the senior author and is responsible for supervising the study design, data analysis and manuscript preparation. SN, DC, SM, SRJ, RQ, ED and SJN participated in reviewing the manuscript and provided guidance to study design and data analysis.

Declaration of conflicting interests

The author(s) declared the following potential conflicts of interest with respect to the research, authorship, and/or publication of this article: SM and SRJ are listed as co-inventors on a pending patent filed by Johns Hopkins University for LDL-cholesterol estimation. SM has served as a consultant to Quest Diagnostics, Sanofi/Regeneron and the Pew Research Center. SM has been the recipient of research support from the PJ Schafer Cardiovascular Research Fund, Aetna Foundation, American Heart Association, Google, Apple and Withings, outside of the scope of this article. SRJ has received charitable gift for support for the VLDL project from the David and June Trone Family Foundation. The other authors report no conflicts of interest.

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