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ORIGINAL INVESTIGATIONS

Impact of Statins on Serial Coronary Calcification During Atheroma Progression and Regression

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ABSTRACT

BACKGROUND Statins can regress coronary atheroma and lower clinical events. Although pre-clinical studies suggest procalcific effects of statins in vitro, it remains unclear if statins can modulate coronary atheroma calcification in vivo.

OBJECTIVES This study compared changes in coronary atheroma volume and calcium indices (CaI) in patients receiving high-intensity statin therapy (HIST), low-intensity statin therapy (LIST), and no-statin therapy.

METHODS In a post-hoc patient-level analysis of 8 prospective randomized trials using serial coronary intravascular ultrasound, serial changes in coronary percent atheroma volume (PAV) and CaI were measured across matched coronary segments in patients with coronary artery disease.

RESULTS Following propensity-weighted adjustment for differences in baseline and changes in clinical, laboratory, and ultrasonic characteristics, HIST ($n = 1,545$) associated with PAV regression from baseline ($-0.6 \pm 0.1\%$; $p < 0.001$), whereas both LIST ($n = 1,726$) and no-statin therapy ($n = 224$) associated with PAV progression ($+0.8 \pm 0.1\%$ and $+1.0 \pm 0.1\%$; $p < 0.001$, respectively; $p < 0.001$ for both HIST vs. LIST and HIST vs. no-statin; $p = 0.35$ for LIST vs. no-statin). Significant increases in CaI from baseline were noted across all groups (median [interquartile range] HIST, +0.044 [0.0–0.12]; LIST, +0.038 [0.0–0.11]; no-statin, +0.020 [0.0–0.10]; $p < 0.001$ for all), which could relate to statin intensity ($p = 0.03$ for LIST vs. no-statin; $p = 0.007$ for HIST vs. no-statin; $p = 0.18$ for HIST vs. LIST). No correlations were found between changes in CaI and on-treatment levels of atherogenic and antiatherogenic lipoproteins, and C-reactive protein, in either of the HIST groups or the no-statin group.

CONCLUSIONS Independent of their plaque-regressive effects, statins promote coronary atheroma calcification. These findings provide insight as to how statins may stabilize plaque beyond their effects on plaque regression. (J Am Coll Cardiol 2015;65:1273–82) © 2015 by the American College of Cardiology Foundation.

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ABBREVIATIONS AND ACRONYMS

Cal = calcium index

CRP = C-reactive protein

CT = computed tomography

HIST = high-intensity statin therapy

IVUS = intravascular ultrasound

LDL-C = low-density lipoprotein cholesterol

LIST = low-intensity statin therapy

PAV = percent atheroma volume

TAV = total atheroma volume

Statins are the cornerstone for treating atherosclerotic cardiovascular disease and can regress atherosclerosis (1,2) and lower cardiovascular event rates (3). The most recent U.S. guidelines now advocate high-intensity statin therapy (HIST) in all individuals with known atherosclerosis, regardless of baseline lipoprotein levels (4).

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Coronary arterial calcification has been extensively evaluated, and the baseline extent of coronary calcium measured noninvasively strongly associates with incident cardiovascular events (5). Underlying this imaging approach is the presumption that coronary calcium scoring using computed tomography (CT) represents a reliable surrogate measure of coronary atheroma volume. Given the direct relationship between achieved low-density lipoprotein cholesterol (LDL-C) levels, serial measures of plaque burden, and cardiovascular events, it is therefore logical to deduce that the effects on both plaque and its calcific component following statin therapy might be concordant. However, prior serial CT evaluations of the effect of statins on coronary calcification yielded conflicting results (6–11).

Mechanistic studies have demonstrated the potential procalcific effects of statins in vitro (12). Coronary intravascular ultrasound (IVUS) has high imaging resolution for measuring atheroma volume, and techniques to measure plaque calcification on IVUS are well described (13). Moreover, serial coronary IVUS has been pivotal in elucidating factors promoting the progression and regression of coronary atheroma (14). Using serial coronary IVUS in patients with coronary artery disease, we tested the hypothesis that statin therapy would associate with concordant changes of both coronary atheroma volume and plaque calcification. We specifically compared these changes in patients receiving HIST, low-intensity statin therapy (LIST), and no-statin therapy.

METHODS

STUDY POPULATION. The present analysis included patients participating in 8 clinical trials assessing the impact of medical therapies on serial changes in coronary atheroma burden using IVUS. Included in this analysis were trials assessing intensive lipid lowering with statins (REVERSAL [Reversal of Atherosclerosis With Aggressive Lipid Lowering] and SATURN [The Study of Coronary Atheroma by Intravascular Ultrasound: Effect of Rosuvastatin Versus Atorvastatin]) (2,15), antihypertensive therapies (AQUARIUS [Aliskiren Quantitative Atherosclerosis Regression Intravascular Ultrasound Study] and NORMALIZE [Norvasc for Regression of Manifest Atherosclerotic Lesions by Intravascular Sonographic Evaluation]) (16,17), the antiatherosclerotic efficacy of acyl-coenzyme A:cholesteryl ester transfer protein inhibition (ACTIVATE [ACAT Intravascular Atherosclerosis Treatment Evaluation]) (18), cholesteryl ester transfer protein inhibition (ILLUSTRATE [Investigation of Lipid Level Management Using Coronary Ultrasound to Assess Reduction of Atherosclerosis by CETP Inhibition and HDL Elevation]) (19), endocannibanoid receptor antagonism (STRADIVARIUS [Strategy to Reduce Atherosclerosis Development Involving Administration of Rimonabont–The Intravascular Ultrasound Study]) (20), and the peroxisome proliferator-activated receptor-gamma agonism (PERISCOPE [Pioglitazone Effect on Regression of Intravascular Sonographic Coronary Obstruction Prospective Evaluation]) (21). The ASTERIOD (A Study to Evaluate the Effect of Rouvastatin on Intravascular-Ultrasound Derived Indices of Coronary Atheroma Burden) study was not included in this analysis because smoking status and C-reactive protein (CRP) levels were not collected (1). From each of these trials, patients receiving HIST (n = 1,545), LIST (n = 1,726), or no-statin therapy (n = 224) were included in the present analysis. In the present analysis, HIST was defined as atorvastatin 80 mg or rosuvastatin 40 mg, whereas LIST was defined as atorvastatin dosing <40 mg, rosuvastatin <20 mg, simvastatin <40 mg, pravastatin <80 mg, lovastatin <20 mg, and fluvastatin dosing <40 mg. Hence, the present analysis comprises a patient-level analysis of 8 randomized trials in which patients were stratified on the basis of statin treatment (or no-statin treatment).

ACQUISITION AND ANALYSIS OF SERIAL IVUS IMAGES. The acquisition and serial analysis of IVUS images in each of these trials has been previously described in detail (1,2,15,17–22). Briefly, target vessels for imaging were selected if they contained no luminal stenosis >50% angiographic severity within a segment of at least 30 mm length. Imaging was performed within the same coronary artery at baseline and at study completion, which ranged from 18 to 24 months. Imaging in all trials was screened by the Atherosclerosis Imaging Core Laboratory of the Cleveland Clinic Coordinating Center for Clinical Research. Patients meeting pre-specified requirements for image quality were eligible for randomization. An anatomically matched segment was defined at the 2 time points on the basis of proximal and distal side branches (fiduciary points). Cross-sectional images spaced precisely 1 mm apart were selected for measurement. Leading

edges of the lumen and external elastic membrane were traced by manual planimetry. Plaque area was defined as the area occupied between these leading edges. The accuracy and reproducibility of this method have been reported previously (23). The percent 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:

$$PAV = \frac{\sum(EEM_{area} - Lumen_{area})}{\sum EEM_{area}} \times 100$$

The total atheroma volume (TAV) was calculated by summating the plaque areas in all measured images. To account for heterogeneity of segment length in individual subjects, the TAV was normalized by multiplying the mean atheroma area in each pullback by the median segment length for the entire study cohort as follows:

$$TAV_{Normalized} = \frac{\sum(EEM_{\text{area}} - Lumen_{\text{area}})}{\text{Number of Images in Pullback}} \times Median\ number\ of\ images\ in\ cohort$$

Calcium was identified by an echogenic signal brighter than the adventitia with corresponding acoustic shadowing. A calcium grade was assigned for each analyzed image, reflecting the degree of acoustic shadowing (0 = no calcium; 1 = calcium with acoustic shadowing <90°; 2 = calcium with shadowing ≥90° but <180°; 3 = calcium with shadowing ≥180° but <270°; 4 = calcium ≥270°) (13,24). For images containing multiple calcium deposits, the grade represented the summation of all angles of acoustic shadowing. For each pullback, a calcium index (CaI) was thus calculated as follows (25):

$$CaI = \frac{Total\ no.\ of\ analyzed\ frames\ with\ any\ Calcium}{Total\ no.\ of\ analyzed\ frames} \times \frac{Maximal\ arc\ of\ Calcium}{4}$$

Change in CaI was defined as follow-up CaI minus baseline CaI.

STATISTICAL ANALYSIS. Continuous variables were reported as mean ± SD if normally distributed and as median (interquartile range) if non-normally distributed. Demographics, baseline clinical characteristics, baseline medications, laboratory biochemical data, and baseline IVUS parameters were compared. Two-sample Student *t* tests were used for normally distributed continuous variables, Wilcoxon rank sum tests for non-normally distributed continuous variables, and chi-square tests (or exact tests) for categorical variables.

Because of differences in various baseline characteristics across the treatment groups, a propensity score weighting method was applied. The multiple treatment propensity scores and corresponding inverse probability of treatment weight (the reciprocal of the propensity scores) were estimated by generalized boosted models using an iterative estimation procedure (26), using all the related baseline characteristics and medications as covariates. The balance of the pre-treatment covariates was assessed, and significant improvement in baseline balance was achieved following weighting.

All subsequent analyses were weighted by inverse probability of treatment weight, except the analysis of baseline CaI. Serial changes in IVUS measurements were analyzed by analysis of covariance, adjusting for their baseline counterparts, and are reported as least squares mean ± SE, and the causal effects of each therapy were examined using inverse probability of treatment weight weighted generalized linear regression models in the context of survey design controlling for baseline IVUS values. Such survey-weighted generalized linear models have robust design-based standard errors. Because the CaI (both baseline and change) had many zero values, a rank-transformation was performed, and the same strategy of survey-design generalized linear models was created using the rank-transformed CaI changes as

TABLE 1 Baseline Demographics, Clinical Characteristics, and Medications

	High-Intensity Statin (n = 1,545)	Low-Intensity Statin (n = 1,726)	No-Statin (n = 224)
Age, yrs	57.1 ± 8.8	58.4 ± 9.3	59.5 ± 9.9
Female	413 (26.7)	514 (29.8)	69 (30.8)
Body mass index, kg/m^2	29.7 ± 5.6	31.2 ± 5.9	32.7 ± 6.8
Diabetes	327 (21.2)	549 (31.8)	101 (45.1)
Hypertension	1,101 (71.3)	1,357 (78.6)	182 (81.3)
Current smoker	468 (30.3)	361 (20.9)	40 (18.0)
History of MI	432 (28.0)	479 (27.8)	52 (23.2)
History of PCI	539 (34.9)	734 (42.5)	64 (28.6)
History of CABG	19 (1.2)	49 (2.8)	4 (1.8)
History of PAD	58 (3.8)	86 (5.0)	20 (8.9)
History of CVA	38 (2.5)	60 (3.5)	9 (4.0)
Prior statin use	1,045 (67.6)	1,473 (85.3)	15 (6.7)
Baseline aspirin	1,451 (93.9)	1,594 (92.4)	185 (82.6)
Baseline beta blockers	1,170 (75.7)	1,252 (72.5)	145 (64.7)
Baseline ACE inhibitor/ARB	942 (61.0)	1,121 (64.9)	128 (57.1)
Baseline nitrates	324 (21.0)	454 (26.3)	78 (34.8)

Values are mean ± SD or n (%). Prior statin use was defined as statin use on any occasion prior to study enrolment.

ACE = angiotensin-converting enzyme; ARB = angiotensin receptor blocker; CABG = coronary artery bypass graft; CVA = cerebrovascular accident; MI = myocardial infarction; PAD = peripheral arterial disease; PCI = percutaneous coronary intervention.

TABLE 2 Laboratory Findings*

	High-Intensity Statin (n = 1,545)	Low-Intensity Statin (n = 1,726)	No-Statin (n = 224)
Baseline			
LDL-C	119.5 ± 33.8	96.3 ± 33.9	110.0 ± 36.2
HDL-C	43.9 ± 11.0	44.0 ± 12.0	41.7 ± 14.2
Non-HDL-C	149.8 ± 39.5	126.6 ± 40.1	141.9 ± 39.2
Triglycerides	137.5 (97–190)	135 (97–193)	157.7 (106.2–228)
apoB	110.3 ± 30.8	91.3 ± 35.7	96.2 ± 28.3
apoA-1	126.0 ± 24.5	127.9 ± 28.0	133.3 ± 33.6
apoB:apoA-1	0.83 ± 0.24	0.64 ± 0.22	0.78 ± 0.45
CRP	1.8 (0.9–4.3)	2.4 (1.1–5.4)	3.1 (1.4–6.4)
Follow-up			
LDL-C	70.8 ± 25.5	89.1 ± 25.0	107.2 ± 30.9
HDL-C	48.0 ± 12.2	50.7 ± 17.1	43.5 ± 14.9
Non-HDL-C	96.6 ± 29.0	117.2 ± 30.8	138.8 ± 34.3
Triglycerides	117.7 (90.4–158.5)	129.6 (94.0–177.4)	150.7 (105.2–216.3)
apoB	76.8 ± 21.5	82.9 ± 27.6	93.5 ± 27.2
apoA-1	140.5 ± 24.6	138.9 ± 29.9	135.3 ± 28.1
apoB:apoA-1	0.55 ± 0.17	0.57 ± 0.20	0.72 ± 0.25
CRP	1.1 (0.6–2.8)	2.0 (0.9–4.4)	2.6 (1.1–5.1)
Change from baseline			
LDL-C			
% change	-36.2 ± 29.5	-2.2 ± 28.5	2.8 ± 24.6
p value†	<0.001	0.002	0.10
HDL-C			
% change	11.0 ± 20.1	16.4 ± 27.9	10.3 ± 73.5
p value†	<0.001	<0.001	0.04
Non-HDL-C			
% change	-31.9 ± 25.5	-3.7 ± 23.5	-0.6 ± 18.8
p value†	<0.001	<0.001	0.66
Triglycerides			
Median of % change	-12.8	-5.2	-3.6
p value‡	<0.001	<0.001	0.72
apoB			
% change	-27.6 ± 21.7	-4.8 ± 28.7	-0.02 ± 26.3
p value†	<0.001	<0.001	0.99
apoA-1			
% change	13.2 ± 18.6	11.8 ± 37.0	7.4 ± 45.8
p value†	<0.001	<0.001	0.052
apoB:apoA-1			
% change	-32.3 ± 19.6	-8.7 ± 27.5	-2.0 ± 24.0
p value†	<0.001	<0.001	0.30
CRP			
Median of % change	-33.3	-17.6	-19.6
p value‡	<0.001	0.001	0.52

Values are mean ± SD or median (95% confidence interval). *Unless otherwise noted, laboratory values obtained during treatment are the time-weighted averages of all post-baseline values. †P value for test of % change = 0. ‡p value for signed rank test. All lipoprotein measurements are in mg/dl. CRP measurements are mg/l.

apoA-1 = apolipoprotein A-1; apoB = apolipoprotein B; CRP = C-reactive protein; HDL-C = high-density lipoprotein cholesterol; LDL-C = low-density lipoprotein cholesterol.

the outcome. Because calcium is a component of plaque, atheroma volume (PAV or TAV) was adjusted within the model for CaI. Clinical trial and baseline CaI were controlled for in the CaI model as well. Average treatment effects on IVUS and on CaI were compared in a pairwise fashion among the statin therapy groups. Given that each trial's duration varied between 18 and 24 months, changes in PAV, TAV, and CaI were also interpolated at 1 year and thus reported as annualized changes. Because of the intrinsic relationships between plaque progression and calcification, changes in coronary atheroma volume and CaIs were also compared according to plaque progression/nonprogression. A 2-sided probability value of 0.05 was considered statistically significant. Analyses were performed using SAS software version 9.2 (SAS Institute, Cary, North Carolina) and the twang package and survey package in (open-source) R software.

RESULTS

CLINICAL CHARACTERISTICS OF THE STUDY POPULATION. Table 1 describes baseline demographics, clinical characteristics, and medication use in each of the treatment groups. Significant trends for between-group differences were noted across certain baseline variables. The no-statin group was of older age, more likely female, had a higher body mass index, and had a higher incidence of diabetes mellitus, hypertension, peripheral arterial disease, and nitrate use compared with the HIST and LIST groups.

BASELINE AND CHANGES IN LABORATORY MEASURES. Table 2 describes baseline, follow-up, and changes in laboratory biochemical measures within each treatment group. Significant trends for between-group differences were noted across various baseline laboratory variables. Patients receiving HIST had the highest baseline LDL-C levels (119.5 ± 34 mg/dl) but the lowest CRP levels (1.8 mg/l). The no-statin group had the lowest baseline high-density lipoprotein cholesterol levels (41.7 ± 14 mg/dl) but the highest triglyceride (158 [106 to 228] mg/dl) and CRP levels (3.1 [1.4 to 6.4] mg/l). At follow-up, patients receiving HIST had the lowest levels of LDL-C, non–high-density lipoprotein cholesterol, triglycerides, and CRP compared with the LIST and no-statin groups (LDL-C, 70.8 ± 26 mg/dl vs. 89.1 ± 25 mg/dl vs. 107.2 ± 31 mg/dl, respectively; non–high-density lipoprotein cholesterol, 96.6 ± 29 mg/dl vs. 117.2 ± 31 mg/dl vs. 138.8 ± 34 mg/dl, respectively; triglycerides, 118 [90 to 159] mg/dl vs. 130 [94 to 177] mg/dl vs. 151 [105 to 216] mg/dl, respectively; CRP, 1.8 [0.6 to 2.8] mg/l vs. 2.0 [0.9 to 4.4] mg/l vs. 2.6 [1.1 to 5.1] mg/l, respectively).

BASELINE AND CHANGES IN CORONARY ATHEROMA VOLUME ACCORDING TO THERAPY. Table 3 describes baseline and changes in PAV and TAV of each treatment group, and pairwise comparisons for changes in atheroma volume following propensity-weighting. Baseline PAV was 36.9 ± 8.9%, 38.0 ± 9.0%, and 37.2 ± 9.0% in the HIST, LIST, and no-statin groups, respectively. The HIST group had significantly lower

TABLE 3 Baseline and Change in Coronary Atheroma Volume According to Treatment Allocation

IVUS Parameter	Therapies: High-Intensity Statin (n = 1,545)	Low-Intensity Statin (n = 1,726)	No-Statin (n = 224)	Predicted Mean Difference (95% CI), p Value: High vs. Low	No vs. Low	High vs. No
Percent atheroma volume, %						
Baseline	36.9 ± 8.9	38.0 ± 9.0	37.2 ± 9.0	-1.1 (-1.8 to -0.3), p = 0.002	-0.8 (-2.3 to 0.7), p = 0.40	-0.3 (-1.8 to 1.2), p = 0.91
Change from baseline	-0.6 ± 0.1	0.8 ± 0.1	1.0 ± 0.1	-1.4 (-1.7 to -1.1), p < 0.001	0.2 (-0.3 to 0.7), p = 0.35	-1.6 (-2.1 to -1.1), p < 0.001
Annualized changes	-0.3 ± 0.1	0.5 ± 0.1	0.6 ± 0.1	-0.8 (-0.9 to -0.6), p < 0.001	0.1 (-0.1 to 0.4), p = 0.32	-0.9 (-1.2 to -0.6), p < 0.001
p value for test of change = 0*	<0.001	<0.001	<0.001			
Total atheroma volume, mm^3						
Baseline	183.9 ± 81	188.2 ± 84	195.3 ± 87	-4.4 (-11 to 2.4), p = 0.29	7.0 (-6.7 to 21), p = 0.46	-11.4 (-25.3 to 2.5), p = 0.13
Change from baseline	-6.6 ± 0.6	-2.1 ± 0.6	3.0 ± 0.7	-4.4 (-6.1 to -2.8), p < 0.001	5.1 (1.5 to 8.8), p = 0.006	-9.6 (-13 to -5.9), p < 0.001
Annualized changes	-3.2 ± 0.4	-1.1 ± 0.3	1.8 ± 0.4	-2.1 (-3.1 to -1.2), p < 0.001	2.9 (0.9 to 5.0), p = 0.005	-4.9 (-6.9 to -2.9), p < 0.001
p value for test of change = 0*	<0.001	<0.001	<0.001			

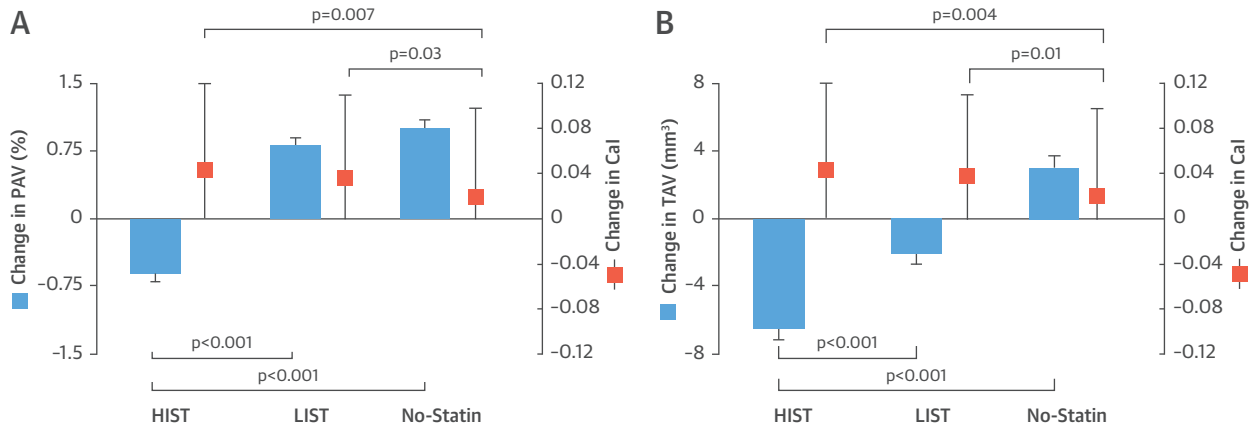
Baseline values are mean ± SD, change values are least squares mean ± SE controlling for the baseline counterpart. Pairwise comparisons for baseline IVUS were conducted using the general linear model. Pairwise comparisons for changes in IVUS parameters from baseline were conducted using survey-design inverse probability of treatment weight weighted generalized linear models. *From Wilcoxon signed rank test.
CI = confidence interval; IVUS = intravascular ultrasound.

PAV at baseline compared with the LIST group (p = 0.002). At follow-up, the HIST group demonstrated significant PAV regression from baseline (−0.6 ± 0.1%; p < 0.001), whereas both the LIST and no-statin groups each demonstrated significant PAV progression (+0.8 ± 0.1% and +1.0 ± 0.1%; p < 0.001 from baseline, respectively). These changes in PAV differed significantly for pairwise comparisons between the HIST versus LIST (p < 0.001) and the HIST versus no-statin groups (p < 0.001) (Figure 1A).

Baseline TAV was similar across all treatment groups, with no significant between-group differences. At follow-up, both the HIST and LIST groups demonstrated significant TAV regression from baseline (−6.6 ± 0.6 mm^3 and −2.1 ± 0.6 mm^3; p < 0.001 from baseline, respectively), whereas the no-statin group demonstrated significant TAV progression (+3.0 ± 0.7 mm^3; p < 0.001). Differences in the magnitude of TAV regression were significant for pairwise comparisons among the HIST versus LIST (p < 0.001), HIST versus no-statin (p < 0.001), and LIST versus no-statin (p = 0.006) groups (Figure 1B).

BASELINE AND CHANGES IN CaI ACCORDING TO THERAPY. Table 4 describes baseline and changes in CaI between treatment groups, pairwise comparisons for changes in CaI following propensity-weighting and further adjustment for clinical trial, and baseline measures of plaque burden and calcium. When adjusting for PAV in the statistical model, no significant differences in pairwise comparisons were noted

FIGURE 1 Statins and Coronary Plaque Calcification: Changes in Coronary Atheroma Volume and Calcium Indices According to Therapy



(A) Percent atheroma volume (PAV) adjusted model, depicting corresponding changes in PAV and calcium index (CaI). **(B)** Total atheroma volume (TAV) adjusted model depicting corresponding changes in TAV and CaI. Changes in PAV and TAV **(blue boxes)** are reported as least squares mean ± standard error of the mean, whereas the changes in CaI **(salmon boxes)** are reported as median (interquartile range). CI = confidence interval; HIST = high-intensity statin therapy; LIST = low-intensity statin therapy.

for baseline CaI. However, the TAV-adjusted model yielded significantly greater baseline CaI for the LIST versus no-statin ($p = 0.002$) and HIST versus no-statin ($p = 0.001$) pairwise comparisons.

All treatment groups demonstrated significant progression of coronary calcium from baseline, measured as a change in CaI (HIST, +0.044 [0.0 to 0.12]; LIST, +0.038 [0.0 to 0.11]; no-statin, +0.02 [0.0 to 0.10]; $p < 0.001$ for all treatment groups). In a PAV-adjusted model, pairwise comparisons demonstrated that the change in CaI was significantly greater in the LIST versus no-statin groups ($p = 0.03$) and the HIST versus no-statin groups ($p = 0.007$), but not for the HIST versus LIST comparison ($p = 0.18$) (Figure 1A). In a TAV-adjusted model, similar results were found, with pairwise comparisons demonstrating the change in CaI to be significantly greater in the LIST versus no-statin groups ($p = 0.01$) and the HIST versus no-statin groups ($p = 0.004$), but not for the HIST versus LIST comparison ($p = 0.35$) (Figure 1B).

CHANGES IN CORONARY ATHEROMA VOLUME AND CaI ACCORDING TO PLAQUE PROGRESSION/REGRESSION. Table 5 describes changes in plaque volume and CaI stratified according to whether patients exhibited plaque progression (defined as change in PAV or TAV >0) or nonprogression/regression (change in PAV or TAV ≤0). Those with plaque progression demonstrated an overall +2.7 ± 0.05% and +7.5 ± 0.5 mm^3 change in PAV and TAV, respectively, whereas nonprogressors/regressors demonstrated an overall −2.2 ± 0.06% and −13.1 ± 0.5 mm^3 change in PAV and TAV, respectively. Changes in CaI were significantly greater in those with plaque progression compared with those with nonprogression/regression irrespective of whether adjusted for by changes in PAV (0.045 [0.00 to 0.12] vs. 0.034 [0.00 to 0.11]; $p = 0.002$) or changes in TAV (0.045 [0.00 to 0.12] vs. 0.034 [0.00 to 0.11]; $p < 0.001$).

RELATIONSHIPS BETWEEN CHANGES IN CaI AND ON-TREATMENT LIPOPROTEINS AND CRP. Table 6 describes correlations between changes in CaI and average on-treatment lipoprotein and CRP levels among patients receiving HIST and no-statin therapy. No significant correlations were found between HIST-mediated changes in lipoprotein or CRP levels and changes in CaI. Similarly, no significant associations were found between changes in lipoprotein and CRP levels and changes in CaI in those patients receiving no-statin therapy.

DISCUSSION

In this post-hoc propensity-weighted analysis of patients with coronary artery disease undergoing serial

TABLE 4 Baseline and Change in Calcium Index According to Treatment Allocation

Calcium Index	Therapies							
	High-Intensity Statin (n = 1,545)	Low-Intensity Statin (n = 1,726)	No-Statin (n = 224)	PAV-Adjusted t Statistic, p Value			TAV-Adjusted t Statistic, p Value		
				High vs. Low	No vs. Low	High vs. No	High vs. Low	No vs. Low	High vs. No
Baseline	0.27 (0.08–0.52)	0.29 (0.08–0.58)	0.26 (0.07–0.49)	0.96, p = 0.60*	−1.2, p = 0.46*	1.6, p = 0.23*	1.0, p = 0.55*	−3.4, p = 0.002*	3.7, p = 0.001*
Change from baseline	0.044 (0.0–0.12)	0.038 (0.0–0.11)	0.020 (0.0–0.10)	1.3, p = 0.18†	−2.2, p = 0.03†	2.7, p = 0.007†	0.94, p = 0.35†	−2.6, p = 0.01†	2.9, p = 0.004†
Annualized changes	0.023 (0.0–0.06)	0.019 (0.0–0.06)	0.012 (0.0–0.06)	1.3, p = 0.18†	−2.4, p = 0.02†	2.9, p = 0.004†	0.93, p = 0.35†	−2.8, p = 0.005†	3.0, p = 0.003†
p value for test of change = 0‡	<0.001	<0.001	<0.001						

Calcium index values are reported as median (interquartile range). *Values are from the general linear model for baseline calcium index ranks, controlling for baseline plaque burden and clinical trial. The t statistic has 3,484 degrees of freedom. †Values are from the generalized linear model for change in calcium index ranks, controlling for baseline calcium index ranks, baseline plaque burden, change in plaque burden, and clinical trial. The t value has 3,482 degrees of freedom. Pairwise comparisons were conducted using rank transformed calcium index and survey-design inverse probability of treatment weight weighted generalized linear models. ‡From Wilcoxon signed rank test.

PAV = percent atheroma volume; TAV = total atheroma volume.

coronary IVUS, we demonstrate the significant procalcific effects of both high- and low-intensity statins, and the calcific nature of coronary atheroma progression in statin-naive patients during follow-up. The novel finding of this analysis was the dominant influence of statins on changes in plaque calcification, irrespective of net plaque progression or regression. The greatest increases in calcium were evident in patients receiving HIST to coincide with significant plaque regression, and statin-naive patients demonstrated the smallest increase in plaque calcification over time, despite profound atheroma progression. Despite both the LIST and no-statin groups each demonstrating comparable degrees of serial plaque progression, the increases in CaI within the LIST group were double that of the no-statin group. These findings point to possible procalcific effects of statins, which are consistent with possible plaque-stabilizing effects of statins beyond simply their effects on atheroma volume.

At first glance, the significant increase in coronary calcification following HIST seems paradoxical to the demonstrated net plaque regression in these patients. Prior investigations testing the serial effects of statins on coronary calcium have largely been undertaken via calcium scoring using CT, and findings across those studies were inconsistent (6–11). Common to most of those studies was the comparatively shorter follow-up period and smaller sample sizes. Achieved LDL-C levels were often >100 mg/dl following the use of mild statin regimens, not reflective of current practice guidelines for patients with atherosclerotic cardiovascular disease (4). Moreover, the lack of plaque volume measurement in those studies limited their ability to truly ascertain statin-mediated effects on the vessel wall. It is important to note, however, that calcium-scoring via CT also has a much lower resolution compared with IVUS, with CT capable of detecting only relatively large calcium deposits (1.03 to 1.37 mm^2) (27). Conversely, the higher resolution of IVUS in the present analysis was sensitive enough to elucidate subtle, yet significant, changes in atheroma calcification, in addition to changes in plaque volume. Hence, it remains unclear how the findings of the present analysis relate to the measured effects of statins on CT scanning. Nevertheless, the current analysis is the first to simultaneously describe, in a large number of patients, the evolution of both coronary calcium and atheroma volume following mild and potent statin regimens, as well as in patients with coronary artery disease remaining statin-naive.

Findings of the present analysis are supported by several prior clinical and pre-clinical observations. In individuals with diabetes, statin use independently

TABLE 5 Changes in Coronary Atheroma Volume and Calcium Indices According to Plaque Progression/Regression

Parameter	Change Parameter: Plaque Progressors	Change Parameter: Plaque Nonprogressors or Regressors	Comparison: LS Mean Difference (95% CI)	Comparison: p Value
Change in PAV, %	2.7 ± 0.05	-2.2 ± 0.06	4.9 (4.7–5.0)	<0.001
Annualized change	1.6 ± 0.03	-1.2 ± 0.03	2.8 (2.7–2.9)	<0.001
Change in TAV, mm^3	7.5 ± 0.50	-13.1 ± 0.5	20.6 (19.3–21.9)	<0.001
Annualized change	4.5 ± 0.30	-7.2 ± 0.3	11.7 (11.0–12.5)	<0.001

	Median (IQR) for Progressors	Median (IQR) for Nonprogressors or Regressors	t Value*	p Value*
Change in CaI (PAV adjusted)	0.045 (0.00–0.012)	0.034 (0.00–0.11)	3.1	<0.002
Annualized change	0.025 (0.00–0.070)	0.018 (0.00–0.054)	3.2	0.001
Change in CaI (TAV adjusted)	0.045 (0.00–0.12)	0.034 (0.00–0.11)	4.1	<0.001
Annualized change	0.025 (0.00–0.070)	0.018 (0.00–0.054)	4.2	<0.001

Progressors defined as change in PAV or TAV >0; nonprogressors defined as change in PAV or TAV ≤0. Changes in plaque burden are reported as reported as mean ± SD. Change in PAV and TAV are obtained from linear mixed models controlling for the baseline counterpart. *Values are from the linear mixed models for rank transformed change in calcium index, controlling for baseline calcium index ranks, baseline plaque burden, change in plaque burden, and clinical trial. The t value has 3,483 degrees of freedom.

CaI = calcium index; IQR = interquartile range; LS = least squares; other abbreviations as in Tables 3 and 4.

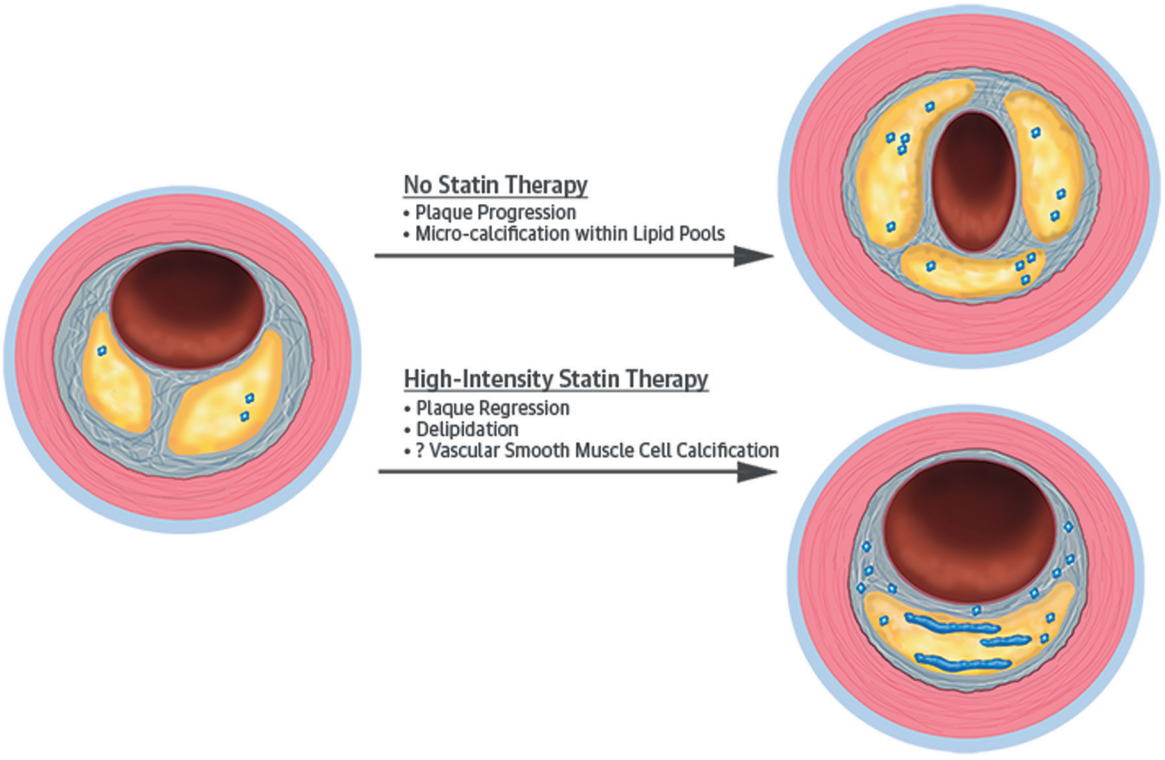
TABLE 6 Relationships of Change in CaI With Average Follow-Up Lipoprotein and CRP Levels in Patients Receiving High-Intensity Statins or No-Statins*

	LDL-C		apoB		HDL-C		apoA-1		apoB/apoA-1		CRP		Triglycerides	
	R	p Value	R	p Value	R	p Value	R	p Value	R	p Value	R	p Value	R	p Value
High-intensity statin therapy														
ΔCaI†	-0.02	0.41	-0.003	0.90	0.02	0.39	0.04	0.16	-0.04	0.21	0.02	0.43	0.01	0.59
ΔCaI‡	-0.003	0.92	0.005	0.85	0.02	0.44	0.04	0.19	-0.02	0.39	0.03	0.29	0.01	0.62
No-statin therapy														
ΔCaI†	0.04	0.54	0.10	0.21	0.10	0.12	0.11	0.19	0.03	0.68	0.04	0.62	0.005	0.94
ΔCaI‡	0.03	0.69	0.09	0.24	0.10	0.15	0.13	0.11	0.02	0.80	0.02	0.73	0.02	0.81

Baseline and change in CaI were rank transformed. Average follow-up CRP and triglycerides values were log transformed. *Pearson correlation coefficient (R) between average follow-up lipid parameters and the residuals of change in calcium index using rank analysis of variance, controlling for baseline CaI, baseline plaque burden, change in plaque burden, and clinical trial. †Plaque variable that was adjusted in the model was PAV. ‡Plaque variable that was adjusted in the model was TAV.

Abbreviations as in Tables 2 and 4.

CENTRAL ILLUSTRATION **Plaque Calcification in the Setting of No-Statin Therapy or High-Intensity Statin Therapy**



Puri, R. et al. J Am Coll Cardiol. 2015; 65(13):1273–82.

Natural plaque progression likely involves lipid-pool expansion coupled with microcalcifications within lipid pools. Following long-term high-intensity statin therapy, plaque regression manifests as delipidation and probable vascular smooth muscle cell calcification, promoting plaque stability.

associated with progressive coronary atheroma calcification (28,29), with similar observations on CT noted in nondiabetic individuals receiving statins from MESA (Multi-Ethic Study of Atherosclerosis) (30). A trend toward increasing atheroma calcification following statins was also reported by several other investigators (11,31-33). Although lacking a placebo-controlled arm, serial coronary plaque compositional analyses via interrogation of the ultrasonic radiofrequency IVUS backscatter signal were consistent in demonstrating progressive coronary calcification following aggressive statin therapy (34,35). Serial ultrasonic carotid evaluation also revealed intensive statin therapy to cause greater increases in plaque echogenicity compared with a less intensive statin regimen (36,37). Importantly, changes in plaque echogenicity correlated inversely with changes in levels of serum inhibitors of vascular calcification (osteopontin and osteoprotegerin), which were independent of alterations of lipid profile. Our analysis also failed to demonstrate associations between changes in CaI with on-treatment lipoprotein or CRP levels during statin treatment, suggesting that the procalcific effects of statins are possibly mediated by pleiotropic mechanisms unrelated to lipoprotein metabolism.

Pre-clinical studies testing the modulatory effects of statins on vascular smooth muscle cells have also yielded conflicting results; however, this may depend on the nature of calcification-induction method performed in vitro. Following an inflammation-induced calcification model, statins inhibited vascular smooth muscle cells calcification, consistent with their known anti-inflammatory pleiotropic effects (38). However, using a noninflammatory organic phosphate model of in vitro calcification, statins dose-dependently stimulated vascular smooth muscle cells apoptosis and subsequent calcification (12). Despite these paradoxical findings, such mechanistic observations are consistent with pathological observations

pointing to a central role of vascular smooth muscle cells and macrophage apoptosis driving plaque calcification in humans (39,40). The finding of progressive atheroma calcification in the no-statin group, who demonstrated marked atheroma progression, is also consistent with pathological observations of microcalcifications within plaque lipid pools (41), which can coalesce into speckles and fragments during atheroma progression (**Central Illustration**) (40).

Aside from lipid regression within plaques following long-term potent statin therapies (35), statin-mediated atheroma calcification may improve plaque stability. Microcalcifications are commonly found within an overlying fibrous cap, and were once thought to enhance the risk of plaque rupture (42). However, more recent research suggests that a very low proportion of plaques containing microcalcification actually rupture (43), and that if statins rendered plaque microcalcifications more confluent and dense, then vessel wall stresses might fall considerably, contributing to plaque stability (44). The current analysis provides supportive evidence for the possible plaque-stabilizing effects of statins via inducing microcalcification.

STUDY LIMITATIONS. Despite a rigorous statistical approach to account for the differences of baseline characteristics and trial effect, we cannot exclude the possibility of unmeasured confounding variables biasing our results. However, inclusion/exclusion criteria for all these trials were relatively uniform, and all analysis was performed within a single core laboratory using standardized analytical techniques. The exact reasons for 224 patients with demonstrable coronary disease not to be prescribed statins during an 18- to 24-month trial period are unclear. However, these patients pose as an extremely unique population exhibiting the true phenotype of untreated, progressive coronary atherosclerosis, unlikely ever to be formally prospectively investigated in a plaque imaging study again. Depth analysis of calcium is not a standard component of our core laboratory's IVUS imaging protocol, and the degree of calcium was ultimately coded semiquantitatively. Therefore, we cannot comment on the precise nature or phenotype of statin versus non-statin-induced serial coronary calcification. However, unique to the present analysis is the accurate and concomitant assessment of serial changes in coronary atheroma volume across the entire length (median length of 50 mm) of the imaged vessel. Furthermore, we sampled a single epicardial coronary artery as a broad representation of the coronary vasculature. Therefore, findings of the present analysis do not apply to patients with pre-existing extensive coronary calcification, nor are such findings directly applicable to angiographically severe or hemodynamically significant lesions. Serum osteopontin and osteoprotegerin were not measured, therefore we can only speculate on mechanisms promoting statin-induced plaque calcification. Lastly, none of these serial IVUS trials were powered for detecting differences in clinical events, and therefore no specific association to clinical event rates can be drawn from the present analysis. Nevertheless the plaque-stabilizing effects and mortality benefit of statins in patients with atherosclerosis are well described (3).

CONCLUSIONS

The present analysis provides unique insight into the procalcific effects of prolonged statin therapy on coronary atheroma in vivo, potentially underscoring the plaque-stabilizing effects of statins.

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PERSPECTIVES

COMPETENCY IN MEDICAL KNOWLEDGE: Serial analysis of coronary atheroma in vivo demonstrates that despite the association with plaque regression, statins possess procalcific effects related to the intensity of therapy.

TRANSLATIONAL OUTLOOK: Further research should be directed toward understanding the mechanisms responsible for plaque calcification that occurs during statin therapy and identifying those that concurrently stabilize coronary atheroma.

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