Does Vascular Calcification Accelerate Inflammation?

A Sub-Study of the dal-PLAQUE Trial

Francis R. Joshi, MBBS, MRCP,* Nikil K. Rajani, MA MRCP,* Markus Abt, PhD,† Mark Woodward, PhD,‡ Jan Bucerius, MD, PhD,§# Venkatesh Mani, PhD,§ Ahmed Tawakol, MD,‖ David Kallend, MBBS,¶ Zahi A. Fayad, PhD, FAHA, FACC,§ James H.F. Rudd, MD, PhD*

*From the *Division of Cardiovascular Medicine, University of Cambridge, UK; † Pharma Development, F. Hoffmann-La Roche Ltd, Basel, Switzerland; ‡George Institute for Global Health, University of Sydney, Sydney, Australia and University of Oxford, UK; §Translational and Molecular Imaging Institute, Icahn School of Medicine at Mount Sinai, New York, USA; # Department of Nuclear Medicine and Cardiovascular Research Institute Maastricht (CARIM), Maastricht University Medical Center, Maastricht, the Netherlands and Department of Nuclear Medicine, University Hospital RWTH Aachen, Aachen, Germany; ‖Harvard Medical School and Massachusetts General Hospital, Boston, Massachusetts, USA; ¶Pharma Development, F. Hoffmann-La Roche Ltd, Basel, Switzerland. Present address: The Medicines Company, Zürich, Switzerland.*

Corresponding author:

James H.F. Rudd

University of Cambridge, Division of Cardiovascular Medicine, Box 110, ACCI Level

6, Addenbrooke's Hospital, Hills Road, Cambridge, CB2 0QQ, UK

Tel: +44 (0)1223 331504; Fax: +44 (0)1223 331505; Email address: jhfr2@cam.ac.uk

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Abstract

Objectives

We sought to investigate non-coronary vascular calcification and its influence on changes in vascular inflammation.

Background

Atherosclerosis is an inflammatory condition with calcification apparent late in the disease process. The extent and progression of coronary calcification predict cardiovascular events. Relatively little is known about non-coronary vascular calcification.

Methods

One hundred and thirty participants in the dal-PLAQUE study underwent fluorodeoxyglucose positron emission tomography/computed tomography at entry and at 6 months. Calcification of the ascending aorta, arch, carotid, and coronary arteries was quantified. Cardiovascular risk factors were related to arterial calcification. The influence of baseline calcification and drug therapy (dalcetrapib vs. placebo) on progression of calcification was determined. Finally, baseline calcification was related to changes in vascular inflammation.

Results

Age >65 years was consistently associated with higher baseline calcium scores. Arch calcification trended to progress more in those with calcification at baseline ($p = 0.055$). There was no significant difference in progression of vascular calcification with dalcetrapib as compared to placebo. Average carotid target-to-background ratio indices declined over 6 months if carotid calcium was absent (single hottest slice [$p =$

0.037], mean of maximum target-to-background ratio [p = 0.010], and mean most diseased segment [p = <0.001]), but did not significantly change if calcification was present at baseline.

Conclusions

Across multiple arterial regions, higher age is consistently associated with higher calcium scores. The presence of vascular calcification at baseline is associated with progressive calcification; in the carotid arteries, calcification appears to influence vascular inflammation. Dalcetrapib therapy did not affect vascular calcification.

Key words (3-5): Atherosclerosis; calcium; inflammation; dalcetrapib

Categories (1-3): 1. Vascular Disease, Hypertension and Prevention/Pharmacology/Hormones/Lipids – Clinical; 2. Vascular Disease, Hypertension and Prevention/Vascular/Pathology - Clinical

Abbreviations and Acronyms

BMI = body mass index

CHD = coronary heart disease

HDL = high-density lipoprotein

FDG = ^{18}F-fluorodeoxyglucose

LDL = low-density lipoprotein

MDS = most diseased segment

PET/CT = positron emission tomography/computed tomography

SHS = single hottest slice

SUV = standardized uptake value

TBR = target-to-background ratio

Introduction

Atherosclerosis is a chronic, systemic, multifocal inflammatory disorder, a response to the deposition of low-density lipoprotein (LDL) in the vascular wall. Thought to be an actively-inhibited but passive process of mineralization (1), there is increasing evidence that arterial calcification is an active and regulated process analogous to bone formation. That supposition is supported by histologic findings of ectopically-formed bone, the presence of osteoblast and osteoclast-like cells, and the secretion of several bone-related peptides within calcified atherosclerotic lesions. In pre-clinical models, inflammatory macrophage activity is seen to precede early osteogenesis (2).

Vascular calcification serves as a marker for the extent of atherosclerosis, and is predictive of cardiovascular events and mortality (3,4). Calcification of the coronary arteries has been extensively investigated. In clinical practice, coronary calcium scoring is used to risk-stratify patients for coronary heart disease (CHD), providing better discrimination than classical risk factors alone (4).

By comparison, relatively little is known about vascular calcification and its progression in other arterial regions. Contrary to notion that calcification represents a stable, end-stage of the disease, dynamic micro-calcification may increase the risk of plaque rupture and clinical events (5). Understanding this process is important because rapid progression of calcification, at least in the coronary arteries, is associated with an increased risk of cardiovascular events. Current medical therapies, including statins, do not alter this progression (6,7).

In this study we investigated vascular calcification detected on serial positron emission tomography/computed tomography (PET/CT) imaging in the dal-PLAQUE

study, a phase 2 randomized clinical trial that investigated the effects of the cholesteryl ester transfer protein inhibitor, dalcetrapib, on the vasculature.

Specifically, we hypothesised (1) that the presence of classical cardiovascular risk factors would increase both baseline calcification and its progression in the ascending aorta, aortic arch, and carotid and coronary arteries over 6 months. We also hypothesized (2) that arteries with the highest baseline calcium levels would undergo the greatest additional calcification over the next 6 months.

Methods

The current study is a post hoc analysis of the dal-PLAQUE study. The study design, methods and primary results have already been published (8,9). dal-PLAQUE was a phase 2b, double-blind, randomized, placebo-controlled study that investigated the effect of dalcetrapib on vessel wall inflammation assessed by ^{18}F-fluorodeoxyglucose (FDG) PET/CT. Inclusion criteria were males and females aged 18 to 75 years, with previous known CHD or at high risk thereof (diabetes or a 10-year risk of CHD events >20% by Framingham Risk scoring), triglyceride concentrations ≤400 mg/dl, LDL cholesterol (LDL-C) concentrations <100 mg/dl or on maximum tolerated doses of statins, and a target to background ratio (TBR) of 1.6 or higher in an index vessel (either right carotid, left carotid, or ascending aorta), as identified by ^{18}F-FDG uptake measured by PET/CT during the screening period.

PET/CT imaging

Details of FDG PET/CT imaging procedures, quantification of tracer uptake, and analyses have been published previously (8). FDG-PET/CT imaging of the carotid arteries and ascending aorta was performed at baseline as well as after 3 and 6 months of follow-up.

Arterial FDG uptake was quantified by manually delineating a region of interest (ROI) on co-registered transaxial PET/CT images. A circular ROI was drawn to encompass the vessel wall on each contiguous axial segment. Next, the maximum arterial standardized uptake value (SUV) was determined, being defined as the decay-corrected tissue concentration of FDG in kBq/ml, adjusted for the injected FDG dose and the body weight of the patient. We calculated TBR from the ratio of SUV of the artery compared with background venous activity and recorded values for the

individual vessels (aorta and mean right and left carotid artery) using previously reported methods (8). The vessel with the highest maximum TBR at baseline was considered the index vessel. Mean of maximum (MeanMax) TBR was the average of the maximal TBR values from each artery. The most diseased segment (MDS) was defined as the average maximum TBR of a group of 3 contiguous slices, centered on the slice with the highest maximum TBR, the single hottest slice (SHS). Active slices were defined as those with TBR >1.6. For the current analyses, we used baseline values and change in the above indices at 6 months for both the index vessel and an average of both carotid arteries.

Assessment of vascular calcification

Analysis was performed on baseline and 6-month CT scans by an experienced observer (Francis R Joshi) blinded to both clinical details and scan order. Unenhanced CT imaging acquired for localization of FDG PET uptake was loaded into the open-source DICOM viewer OsiriX (version 4.0, 64-bit, OsiriX Imaging Software, Geneva, Switzerland) and re-sampled to a 3 mm transaxial thickness.

Using the freely available ‘Calcium Scoring’ plug-in, vascular calcification (based on an attenuation threshold of 130 Hounsfield Units in 3 contiguous voxels, after the method of Agatston (10)) was analyzed on consecutive transaxial slices along the length of the arterial segment. The extent of calcification was expressed both as a score in Agatston units and as a volume in cubic millimeters. The measurement of thoracic aortic and coronary artery calcification from ungated CT studies is accurate and highly concordant with values derived from gated studies (11). Paired (baseline and follow-up) studies were visually compared to ensure that the same length of artery was analyzed on both scans.

Coronary artery calcification was scored as the sum of all 3 epicardial vessels. Ascending aortic calcification was scored on transaxial slices from the main pulmonary artery inferiorly to 1 slice below the aortic arch. Aortic arch calcification was scored on transaxial slices from the most inferior slice at which the ascending and descending aorta were contiguous to the origin of the great vessels. Calcification was scored if it appeared to be in the vascular wall. When isolated calcification of the ligamentum arteriosum was obvious it was not scored. Carotid arterial calcification was assessed bilaterally from the most inferior slice visible on the CT scan to the base of the skull, and then summed.

Coronary calcium was not recorded in those who had undergone prior percutaneous coronary intervention or coronary artery bypass surgery. If the participant had evidence of prior coronary bypass surgery, the ascending aorta was not analysed, due to artefacts from the surgical clips used in fashioning the grafts. If possible, calcification in the aortic arch was still scored in such cases, provided there were no significant artefacts from sternal wires or surgical clips. Noisy CT images of the neck, with artefacts from dental prostheses, were excluded from analyses of the carotid arteries.

Statistical analysis

Descriptive data are presented as median (Q1, Q3) as well as frequencies with proportions for nominal variables as appropriate.

The following measures of progression of vascular calcification were used:

1. The absolute difference between baseline and follow-up

2. The difference between the square root of baseline and square root of follow-up score (the ‘SQRT method’ of Budoff et al (12)). A cut-off of 2.5 was used to identify a change in calcification from baseline beyond the known interscan variability of coronary calcium scores, as per Hokanson et al. (13).

Baseline calcium scores in the presence or absence of risk factors were compared after square root transformation using analysis of variance (ANOVA). Baseline calcium scores were dichotomized into ‘zero’ and ‘non-zero’, and the effect of a non-zero score on progression of calcification (absolute change after square root transformation) was analyzed using ANOVA. The effect of a ‘non-zero’ score on absolute changes in PET indices at 3 and 6 months were assessed through linear mixed models with a visit by score interaction. The effect of dalcetrapib treatment on calcium progression (absolute change on original scale and also after square root transformation) was analyzed using ANOVA with baseline and treatment in the model. Two-sided p-values are reported throughout, with no adjustment for multiple testing. The lack of adjustment for multiple testing was pre-planned and stated in our statistical analysis plan for all Dalplaque manuscripts (8), (9). Here, we provide raw p values that should be interpreted in the light of multiple testing.

Results

dal-PLAQUE recruited 130 patients with a median age of 65 years, most (82%) of whom were male. Baseline demographics are reported in Table 1. After exclusions, both baseline and 6-month CT images yielded data for the aortic arch in 98 patients (75%), the ascending aorta in 80 patients (62%), the carotid arteries in 102 patients (78%), and the coronary arteries in 32 patients (25%). Data were only included when the whole arterial territory was visualized on both PET/CT scans.

At baseline, the median (Q1, Q3) calcium score in the aortic arch was 161 (0, 852), in the combined carotid arteries 52 (0, 267) and 13 (0, 83) in the coronary arteries. The median (Q1, Q3) calcium score in the ascending aorta was 0 (0, 0). Given the large number of zero scores (71 of 80 participants), it was not possible to analyze associations between cardiovascular risk factors and the baseline scores in the ascending aorta. Across all other arterial territories, baseline calcium scores were higher in those over the age of 65 as compared to younger participants (Table 2). Scores in the aortic arch were significantly higher in those having ever smoked (329 [43, 1073] vs. 0 [0, 551]; p = 0.003 after SQRT transformation). Calcium scores were higher in those with body mass index (BMI) ≤29 kg/m^2 than in those with BMI >29 kg/m^2 in the carotid arteries (132 (3, 411) vs. 7 [0, 158]; p = 0.007 after SQRT transformation) but not elsewhere. An LDL-C >80 mg/dl was not associated with significantly higher baseline calcium scores than those with LDL-C ≤80 mg/dl; additionally, high-density lipoprotein cholesterol (HDL-C) levels ≤40 mg/dl were not significantly associated with higher calcium scores than levels >40 mg/dl. A history of CHD, hypertension, type 2 diabetes, peripheral arterial disease, or metabolic syndrome was not associated with higher baseline calcium scores in any territory.

‘New’ calcification was seldom seen at 6 months in those with no calcium at baseline. This only occurred in 3 of 32 patients (9%) in the coronary circulation and in the carotids in 5 of 102 patients (5%). There was no incident calcification in those with zero baseline scores in either the ascending aorta or aortic arch.

Across both treatment groups, progression of SQRT volumes >2.5 was noted in 12 of 32 patients (38%) in the coronary arteries, 18 of 98 patients (18%) for the aortic arch, and 3 of 104 patients (3%) the carotid arteries.

There was a trend towards greater progression of calcium scores after SQRT transformation in the aortic arch in those with non-zero scores at baseline compared to zero at baseline ($p = 0.055$, Table 3).

Treatment assignment was not associated with a significant difference in progression of calcium scores in any arterial territory (Table 4; similar data for calcium volumes not shown).

Comparing difference in absolute change in average carotid PET indices from baseline to 6 months, TBR in active slices ($p < 0.001$), SHS ($p = 0.006$), MeanMaxTBR ($p = 0.003$) and mean MDS ($p = 0.008$) declined if baseline total carotid calcium was zero, relative to those with non-zero scores (Table 5). At 6 months, average carotid PET indices consistently declined in those with zero total carotid calcium scores (mean active segment TBR [$p < 0.001$], SHS TBR [$p = 0.037$], mean MDS TBR [$p < 0.001$], and MeanMax TBR [$p = 0.010$]; Figure 1). There was no statistically significant change ($p > 0.05$) in these indices for those with non-zero scores. The presence or absence of calcification elsewhere did not influence changes in measures of carotid PET uptake. Additionally, there was no significant

relationship between baseline calcification and change in measures of inflammation for the index vessel (ascending aorta in 75% of cases; data not shown).

Discussion

The study investigated the relationship between risk factors and vascular calcification in several arterial territories, and explored the relationship between baseline calcification and changes in inflammation on serial FDG-PET/CT imaging. Whilst prior studies have reported the relationship between arterial calcification and cardiovascular risk factors, and progression of calcification in a single arterial territory (usually coronary), the imaging undertaken in the dal-PLAQUE study afforded an opportunity to examine both these questions. In addition, the first data on progression of aortic calcification outside of renal disease are reported.

Consistently, age >65 years was significantly associated with higher baseline arterial calcification. Generally, the same cardiovascular risk factors influenced atherosclerosis in the coronary, aortic arch and carotid circulation, as also noted by others (3,14,15). Data from the Rotterdam Study found that age and current smoking were the strongest risk factors for calcification in all vessel beds, except the coronaries. Additionally, hypertension, hypercholesterolemia, and diabetes are independently related to arterial calcification, although associations are not consistent across all vessel beds and for men and women (14). Increasing thoracic aortic scores were observed with increasing age in a study by Rivera et al. (15). Most traditional cardiovascular risk factors, including age, diabetes, hypertension, smoking, and family history, were significantly associated with the presence of aortic calcification, though not LDL-C or HDL-C. Aortic calcification was also associated with incident (new) coronary calcification predicting all-cause mortality (16) and cardiovascular events, though not as strongly as coronary artery calcium (17).

An inverse association with a high BMI and carotid arterial calcification was noted in our study. Associations of BMI and vascular calcification have generally been inconsistent (18–21), describing positive, inverse, or no association. The ‘obesity paradox’ for cardiovascular risk is well-described (22). Though mechanistic insight is lacking, it has been proposed that, in females, estrogen production in adipose tissue is relatively protective for the vasculature (23).

Progression of vascular calcification has the potential to better capture the temporal exposure to risk factors as compared with a single baseline score. It has been suggested that a baseline calcium score can be thought of as a single point on an atherosclerosis-versus-time curve, whereas progression correlates with the slope of that curve (24). Similarly, although baseline scores might reflect the atherosclerotic plaque burden, progression might provide insight into ongoing current disease activity. Rapid progression of coronary calcium scores is independently predictive of mortality (12). Our study demonstrated very little newly detectable calcification at 6 months in those without it at baseline, presumably due to the relatively short interval between scans. Nevertheless, our data are consistent with those of Min et al., who demonstrated that, in subjects over 45 years of age, approximately 5% of those without detectable coronary calcium on CT would develop it over 12 months, largely dependent on the number of cardiovascular risk factors present (25).

In those with some degree of calcium at baseline, there were larger increases in calcium scores within the aortic arch and right carotid arteries compared to those with non-zero scores in the same arteries at the start of the study. To the best of our knowledge, these are the first published data on the progression of aortic

calcification outside of the context of end-stage kidney disease or renal transplantation (26).

Van Gils et al. published work describing the progression of carotid calcification in patients after transient ischemic attack or stroke, with 4.7 years between scans (27). Calcification was measured on contrast-enhanced CT scans, using a threshold of 600 Hounsfield units to define calcium. Incident calcification was seen in around 40% of those with zero calcium at baseline over the follow-up period, and was associated with advanced age and hypertension at baseline. Similarly, the most important determinant of progression of existing calcification was the baseline calcium score, although age, diabetes, hypertension, and smoking were also significantly associated. There are no published data on the prognostic implications of carotid artery calcification. Data regarding its relationship to luminal stenosis are conflicting (28,29).

There was no significant difference in progression of vascular calcification in any arterial territory with dalcetrapib as compared to placebo. *In vitro,* HDL-C inhibits osteogenic differentiation and calcification of vascular smooth muscle cells (30). Kuller et al. found an independent association of HDL-C with coronary calcification in postmenopausal females (31), though there is no evidence for an association with calcification elsewhere (14). ^{18}F-sodium fluoride has been advocated as a means of non-invasively detecting active calcification *in vivo* (32). ^{18}F-sodium fluoride uptake in the coronary arteries in that study was associated with a low HDL-C, suggesting that this might be permissive for progressive calcification. Further research is required to investigate this; to date, however, pharmacological treatment of low HDL-C has not influenced clinical outcomes (33,34).

Over the 6 month interval from first to last PET scan in the dal-PLAQUE study, carotid artery TBR indices consistently declined in patients without carotid calcification at baseline, but remained statistically unchanged when calcification was already present. Calcification in other arterial territories did not appear to influence changes in carotid inflammation, suggesting a local interaction. Inflammation and calcification are thought to be active at different phases of the disease process in atherosclerosis. Inflammation appears to be dominant early, leading to development and maturation of plaques, with calcification predominant later. Pre-clinical models have demonstrated this spatial and temporal relationship (2). Initial deposition of calcium hydroxyapatite, so-called microcalcification, may induce further inflammatory responses (35). Whilst linked, inflammation and calcification are distinct processes within atherosclerosis, and medium-term changes in the relative importance of each in vivo are not well understood. Recent work by Abdelbaky et al. suggests that local vascular inflammation as assessed by FDG PET is independently associated with progression of thoracic aortic calcification (36). In this study, arterial segments with subsequent incident calcification had the highest baseline FDG uptake, suggesting that inflammation precedes calcification. Furthermore, within individuals, arterial segments with progressive calcification from a non-zero baseline had the highest FDG uptake. This suggests that inflammation continues to be important after the development of macroscopic calcification. Segments with calcification that did not progress had the lowest FDG uptake. This is consistent with previous work showing that the final stages of calcification are associated with a reduction in inflammatory activity (37). The point at which inflammation becomes less prominent is, however, still to be defined. ^{18}F-sodium fluoride holds promise as a means of imaging active

calcification in vivo (38) and further longitudinal study of changes in the activity of both inflammation and calcification is needed to better understand these processes.

Limitations

This was a post-hoc analysis of data from the dal-PLAQUE study, with only 6 months between PET/CT imaging sessions. Consequently, despite the inclusion of a high-risk population, there was relatively little progression of vascular calcification during this short wondow. A significant number of scans were excluded from analysis of the coronary arteries because of prior coronary bypass surgery or coronary intervention. Whilst coronary calcium scores on electrocardiogram-gated and ungated CT scans are highly correlated, the interscan variability on ungated scans is not known. Comparison of ungated scans may therefore miss small increments in coronary calcium scores, but is likely to be less of a problem in the other vascular beds studied. Finally, as in any post-hoc data analysis, false positive findings are possible. We advise caution in interpretation of our results, which need to be replicated in longer, larger, prospective studies.

Conclusions

Inflammation and calcification are important in both the progression of atherosclerosis and its clinical complications.

In this study, we have described the relationship between cardiovascular risk factors and both baseline and interval calcification in several arterial beds, as well as the interactions with inflammation in those territories. We have also provided data concerning the drivers of calcification progression and the lack of a significant effect of an HDL-raising therapy on calcification. Finally, we have provided the first report of aortic calcification progression outside of the context of subjects with renal failure.

These findings are important for providing a better understanding of the links between these pathological processes, and could help to improve both patient risk-stratification and provide a platform for developing and testing new atherosclerosis treatments.

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Table 1. Baseline Demographics

	Placebo (n = 66)	Dalcetrapib (n = 64)	Overall (n = 130)
Demographics			
Age, years	64.6 (61, 71)	62.6 (60, 68)	63.6 (60, 70)
Male sex	55 (83)	51 (80)	106 (82)
Body mass index, kg/m^2	29.8 (25.5, 32.3)	29.6 (25.9, 32.1)	29.7 (25.7, 32.2)
White race	62 (94)	58 (91)	120 (92)
History of			
Coronary heart disease	54 (82)	57 (89)	111 (87)
Symptomatic coronary artery disease	5 (8)	5 (8)	10 (8)
Hypertension	48 (73)	47 (73)	95 (74)
Type 2 diabetes	20 (30)	19 (30)	39 (30)
Abdominal aortic aneurysm	2 (3)	3 (5)	5 (4)
Peripheral artery disease	10 (15)	6 (9)	16 (12)
Current smoker	8 (12)	9 (14)	17 (13)
Statin use	61 (92)	52 (81)	113 (87)
Imaging parameters at baseline			
Average carotid FDG-PET/CT			
MeanMax TBR	1.76 (1.47, 2.10)	1.79 (1.58, 2.21)	1.78 (1.50, 2.19)
Single hottest slice	1.96 (1.70, 2.40)	2.01 (1.83, 2.45)	2.00 (1.73, 2.44)
Calcium scores, AU			
Ascending aorta	0 (0, 0)	0 (0, 0)	0 (0, 0)
Aortic arch	157 (0, 797)	166 (0, 865)	161 (0, 852)
Coronary arteries	16 (0, 89)	10 (0, 58)	13 (0, 83)
Total carotid arteries	100 (0, 315)	27 (1, 234)	52 (0, 267)

Data are presented as median (Q1, Q3) for continuous variables and as n (%) otherwise.

AU = Agatston units; FDG-PET/CT = fluorodeoxyglucose positron emission tomography/computed tomography; MeanMax, mean of maximum; TBR = target-to-background ratio.

Table 2. Summary of Baseline Calcium Scores by Cardiovascular Risk Factors

		Aortic Arch			Coronary Arteries			Total Carotid Arteries		
		n	**Score (AU)**	**p Value***	**n**	**Score (AU)**	**p Value***	**n**	**Score (AU)**	**p Value***
Age (years)	≤65	53	15 (0, 280)	<0.001	17	8 (0, 21)	0.038	55	7 (0, 100)	<0.001
	>65	45	651 (165, 1181)		15	58 (0, 204)		48	175 (18, 427)	
Body mass index (kg/m^2)	≤29	48	261 (2, 941)	0.107	14	10 (0, 89)	0.750	50	132 (3, 411)	0.007
	>29	50	83 (0, 775)		18	19 (0, 76)		53	7 (0, 158)	
Coronary heart disease	Yes	86	161 (0, 797)	0.733	20	13 (0, 111)	0.617	90	45 (0, 267)	0.611
	No	12	148 (2, 860)		12	15 (0, 67)		13	68 (0, 249)	
Hypertension	Yes	74	165 (0, 852)	0.705	24	10 (0, 62)	0.078	79	54 (2, 276)	0.750
	No	24	57 (2, 930)		8	53 (2, 301)		24	13 (0, 216)	
Type 2 diabetes	Yes	31	200 (0, 1022)	0.457	15	4 (0, 76)	0.488	32	41 (4, 210)	0.565
	No	67	135 (0, 775)		17	21 (1, 93)		71	54 (0, 315)	
Peripheral arterial disease	Yes	13	280 (0, 865)	0.249	5	10 (1, 204)	0.562	13	100 (29, 423)	0.128
	No	85	135 (0, 781)		27	16 (0, 76)		90	49 (0, 249)	
Current smoker	Yes	10	510 (43, 1079)	0.127	3	4 (0, 10)	0.262	11	34 (4, 173)	0.605
	No	88	118 (0, 789)		29	21 (0, 89)		92	54 (0, 272)	
Ever smoked	Yes	57	329 (43, 1073)	0.003	20	19 (1, 109)	0.349	58	69 (4, 276)	0.334
	No	41	0 (0, 551)		12	5 (0, 67)		45	29 (0, 224)	

Metabolic syndrome	Yes	53	157 (3, 871)	0.650	20	13 (0, 62)	0.495	57	25 (0, 254)	0.185
	No	45	166 (0-651)		12	29 (1, 109)		46	106 (0, 267)	
HDL-C (mg/dl)	<=40	38	214 (3, 1011)	0.549	11	47 (0, 265)	0.279	42	27 (3, 315)	0.683
	>40	60	150 (0, 753)		21	9 (0, 48)		61	66 (0, 254)	
LDL-C (mg/dl)	<=80	65	100 (0, 775)	0.295	19	9 (0, 204)	0.391	69	54 (0, 276)	0.460
	>80	33	200 (0, 1002)		13	23 (0, 48)		34	33 (0, 249)	

Data are presented as median (Q1, Q3). No data are presented for the ascending aorta because calcium scores at baseline were zero in 71 of 80 participants. *p-values are two-sided after square root transformation.

AU = Agatston units; HDL-C = high-density lipoprotein cholesterol; LDL-C = low-density lipoprotein cholesterol.

Table 3. Progression in Vascular Calcification over 6 Months by Baseline (Zero vs. Non-Zero) Calcium Score and Volume

	Zero at Baseline		Non-Zero at Baseline		
	n (%)	Median Absolute Change	n (%)	Median Absolute Change	p Value*
Calcium scores (AU)					
Ascending aorta	71 (89)	0 (0, 0)	9 (11)	0 (-10, 29)	0.900
Aortic arch	29 (30)	0 (0, 0)	69 (70)	13 (-15, 138)	0.055
Coronary arteries	11 (34)	0 (0, 0)	21 (66)	10 (-5, 68)	0.414
Left carotid	39 (38)	0 (0, 0)	64 (62)	0 (-11, 21)	0.774
Right Carotid	43 (42)	0 (0, 0)	59 (58)	5 (-2, 23)	0.029
Total carotid arteries	28 (27)	0 (0, 0)	74 (73)	3 (-5, 33)	0.249
Calcium volumes (mm^3)					
Ascending aorta	71 (89)	0 (0, 0)	9 (11)	3 (-11, 9)	0.536
Aortic arch	29 (30)	0 (0, 0)	69 (70)	14 (-35, 121)	0.356
Coronary arteries	11 (34)	0 (0, 0)	21 (66)	6 (-17, 187)	0.648
Left carotid	39 (38)	0 (0, 0)	64 (62)	4 (-8, 23)	0.995
Right carotid	43 (42)	0 (0, 0)	59 (58)	8 (-3, 34)	0.006
Total carotid arteries	28	0 (0, 0)	74	10 (-8, 31)	0.225

Data are presented as n (%) and median (Q1, Q3). *p-values are two-sided after square root transformation.

AU = Agatston units.

Table 4. Effect of Treatment with Dalcetrapib on Progression of Calcium Scores over 6 Months

	Absolute Change from Baseline*	p Value	Change from Baseline after SQRT	p Value
Ascending aorta	-6 (-19 to 7)	0.355	0.17 (-0.73 to 0.38)	0.538
Aortic arch	83 (-4 to 171)	0.061	0.74 (-0.54 to 2.02)	0.254
Coronary arteries	-61 (-171 to 48)	0.263	-1.51 (-4.64 to 1.61)	0.330
Total carotid arteries	6 (-10 to 21)	0.459	0.28 (-0.18 to 0.74)	0.231

Data are presented as difference from placebo with (95% CI). Two-sided p values.

*Absolute change in Agatston units.

CI = confidence interval; SQRT = square root transformed values.

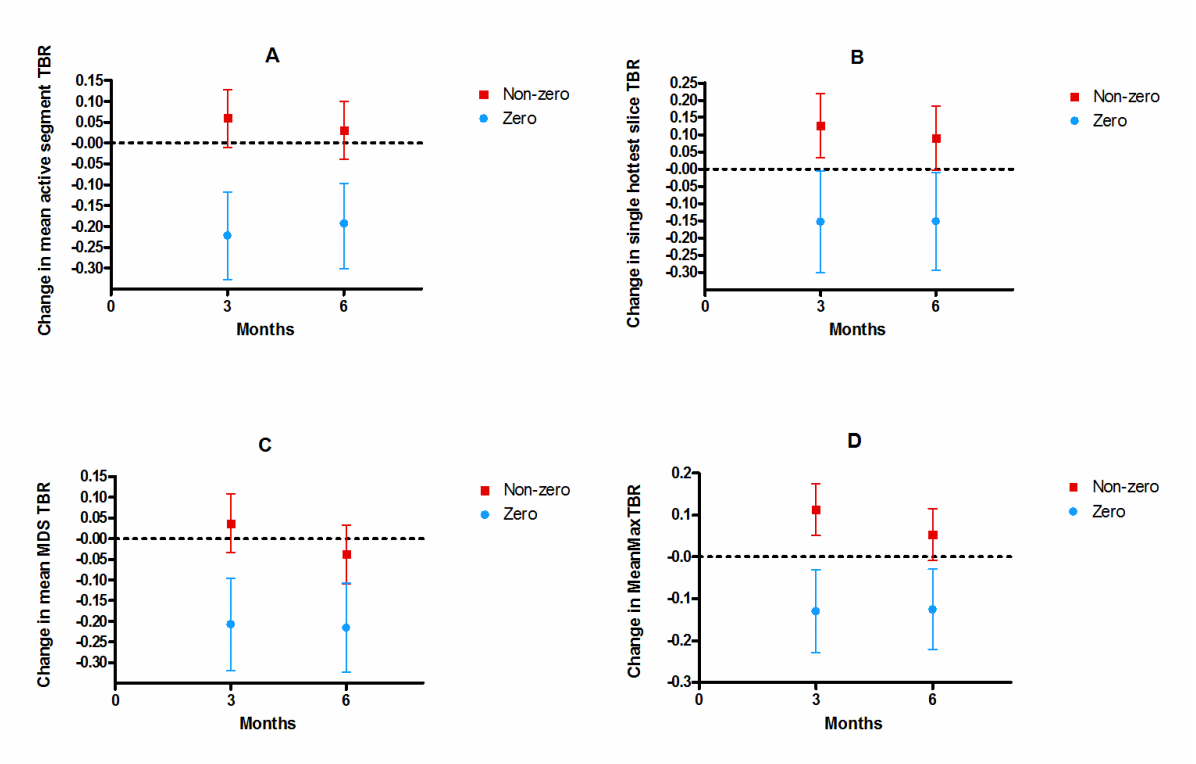
Table 5. Comparison of Change in Average Carotid ^{18}F-FDG PET Indices from Baseline to 6 Months for Patients with Zero and Non-Zero Baseline Total Carotid Calcium Scores

	Mean Difference (95% CI)	p-value
Mean active segment TBR	-0.23 (-0.35 to -0.11)	<0.001
Single hottest slice TBR	-0.24 (-0.41 to -0.07)	0.006
Mean MDS TBR	-0.18 (-0.31 to -0.05)	0.008
MeanMax TBR	-0.18 (-0.30 to -0.06)	0.003

Data are presented as mean difference (zero baseline scores minus non-zero) with (95% CI). Two-sided p-values.

FDG = fluorodeoxyglucose; TBR = target-to-background ratio; CI = confidence interval; MDS = most diseased segment; MeanMax, mean of maximum.

Figure 1. Change in Average Carotid ^{18}F-FDG PET Indices from Baseline at 3 and 6 Months by Baseline Total Carotid Calcium Score (Zero or Non-Zero).



A: Change in mean active segment (TBR >1.6) TBR. Zero calcium scores, p = <0.001; non-zero scores p = 0.399.

B: Change in single hottest slice TBR. Zero calcium scores, p = 0.037; non-zero scores p = 0.061.

C: Change in mean MDS TBR. Zero calcium scores, p < 0.001; non-zero scores p = 0.285.

D: Change in MeanMaxTBR. Zero calcium scores, p = 0.010; non-zero scores p = 0.095.

Least squares means and 95% CIs for absolute change from baseline. Two-sided p-values given for change at 6 months from baseline for total (sum left and right) carotid calcium scores dichotomized into zero (blue) and non-zero (red).

CI = confidence interval; TBR = target-to-background ratio; MDS = most diseased segment; MeanMax, mean of maximum.