Published in final edited form as:

Atherosclerosis. 2019 March ; 282: 91–99. doi:10.1016/j.atherosclerosis.2019.01.011.

The novel inflammatory marker GlycA and the prevalence and progression of valvular and thoracic aortic calcification: The Multi-Ethnic Study of Atherosclerosis

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Abstract

Background and aims: GlycA is a novel composite biomarker of systemic inflammation reflecting posttranslational glycosylation of acute phase reactants. GlycA has been associated with

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Author contributions

Ms. Ezeigwe and Drs. Fashanu and Michos designed the study. Dr. Fashanu did the statistical analysis under the supervision of Dr. Zhao. Dr. Ezeigwe wrote the first draft of manuscript under guidance of Dr. Michos. Dr. Budoff was involved in the CT Core lab and supervised the measurements of the extra-cardiac calcification measures. Dr. Otvos's lab provided the GlycA measurements. Drs. Fashanu, Zhao, Budoff, Otvos, Thomas, Mora, Tibuakuu provided critical input to manuscript draft. Ms Ezeigwe and Drs. Fashanu and Michos take full responsibility for study results.

Trial registration

The MESA cohort design is registered at clinicaltrials.gov as follows: <https://clinicaltrials.gov/ct2/show/NCT00005487>

Conflicts of interest

Dr. Otvos is employed by LabCorp (formerly LipoScience). Dr. Mora received an institutional research grant from Atherotech for work unrelated to this study, and is listed as co-inventor on a patent on the use of GlycA for predicting risk of colorectal cancer. Dr. Budoff receives grant support from NIH and General Electric. The other authors do not report any disclosures.

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coronary artery calcium, cardiovascular disease (CVD) events and mortality. Vascular calcifications outside of the coronary arteries are risk markers of CVD and mortality. Whether GlycA is linked to extra-coronary calcifications (ECC) is not well established.

Methods: We studied 6,462 MESA participants free of clinical CVD who had plasma GlycA measured at baseline. ECCs [calcification in aortic valve (AVC), mitral annulus (MAC), ascending and descending thoracic aorta (ATAC, DTAC)] were ascertained at baseline and follow-up visit (median 2.3-yrs later) by cardiac CT. Poisson regression models with robust variance estimation assessed associations of GlycA with prevalent and incident ECC. Linear mixed models assessed the cross-sectional and 2-year change in ECC. Models were adjusted for demographic and lifestyle factors.

Results: In cross-sectional analysis, GlycA (per SD increment) was positively associated with prevalent AVC, ATAC and DTAC with adjusted prevalence ratios (95% CI) of 1.08 (1.01-1.14), 1.18 (1.03-1.34) and 1.10 (1.06-1.14), respectively. There was also a significant association between GlycA and baseline extent of both ATAC and DTAC. Longitudinally, GlycA was positively associated with incident MAC and DTAC, with adjusted incidence ratios of 1.18 (1.03-1.37) and 1.17 (1.07-1.28), respectively. GlycA was also associated with 2-year change in MAC and DTAC extent.

Conclusions: In this diverse cohort free from clinical CVD, we found GlycA was positively associated with prevalent and incident ECC measures, in particular for progression of MAC and DTAC.

Graphical Abstract.

Forest plot showing the associations between one standard deviation increment in GlycA and extra coronary calcification.

*Coefficients are natural log transformed. Exponentiate value to derive Prevalence & Incidence ratios.

†Models were adjusted for age, sex, race/ethnicity, study site, CT scanner type, education, BMI, smoking status, pack-years of smoking, and physical activity

‡Findings for prevalent DTAC, DTAC extent, incident DTAC, DTAC progression, and MAC progression remained statistically significantly associated with GlycA after further adjustment for

potential mediating ASCVD risk factors (systolic blood pressure, use of antihypertensive medications, total cholesterol, HDL-C, use of lipid-lowering medications, diabetes, and eGFR)

Extracoronary calcification=ECC; AVC= Aortic Valve Calcification; MAC= Mitral Annular Calcification; ATAC= Ascending Thoracic Aortic Calcification; DTAC= Descending Thoracic Aortic Calcification

Keywords

GlycA; inflammation; Thoracic aortic calcification; aortic valve calcification; Mitral annular calcification

INTRODUCTION

Atherosclerotic cardiovascular disease (ASCVD) still remains a leading cause of morbidity and mortality around the world despite advances in preventive interventions such as the use of statins.¹ Thus, there is a need to identify novel predictors of ASCVD, which may be modifiable and better risk stratify individuals.² Numerous research studies have corroborated the key role that inflammation plays in the development of atherosclerosis.^{3,4,5} GlycA is a novel composite biomarker of systemic inflammation measured by nuclear magnetic resonance (NMR) spectroscopy.^{6,7} GlycA might prove to be a superior way of assessing systemic inflammation due to its composite nature, lower analytic imprecision and lower intra-individual variability when compared to high sensitivity C-reactive protein (hsCRP) measurements.⁶ Plasma GlycA levels have been shown to be associated with major ASCVD events and all-cause mortality even after adjustment for other inflammatory markers such as hsCRP and interleukin-6 (IL-6).⁴

GlycA has also been shown to be associated with a greater prevalence of coronary artery calcium (CAC),⁸ a surrogate marker of total coronary atherosclerosis burden and predictor of incident ASCVD events.⁹ Other markers of atherosclerosis, including Extra-Coronary Calcifications (ECC), are also predictive of incident ASCVD and mortality.¹⁰ Components of ECC include thoracic aortic calcification (TAC) and left-sided valvular calcification [mitral annular calcification (MAC) and aortic valve calcification (AVC)]. Valvular calcification has been linked to increased risk for myocardial infarction, stroke, atrial fibrillation and vascular death, independent of traditional ASCVD risk factors.^{11,12} TAC is also associated with all-cause mortality,¹³ coronary events,¹⁴ non-coronary events,¹⁵ and even non-CVD morbidity/mortality.¹⁶ CAC is associated with both TAC¹⁵ and left-sided valvular calcification¹⁷ and share many similar traditional ASCVD risk factors.¹⁸⁻²⁰

However, despite similarities of cardiovascular risk factors across the various vascular beds, risk factors for vascular calcification are not necessarily overlapping.²¹ Even though GlycA is associated with CAC,²² whether GlycA is also linked to ECC (TAC, AVC, and MAC) and their progression has not been well established. Further understanding of this relationship may have clinical implications in the utilization of GlycA as a comprehensive risk marker for CVD.

Our study sought to assess the temporal relationship between GlycA and ECC in a diverse multi-ethnic cohort without known clinical CVD at baseline. We hypothesized that individuals with higher composite systemic inflammation, as measured by GlycA, would be at increased risk for progression of calcification (as a marker of atherosclerosis) in non-coronary vascular beds.

MATERIALS AND METHODS

Study population

The Multi-Ethnic Study of Atherosclerosis (MESA) consists of a diverse cohort of participants recruited from six centers across the United States and followed longitudinally to assess for progression of subclinical CVD. The baseline examination of this ongoing study took place between 2000 and 2002 and there were five subsequent follow-up visits (Exams 2 to 6). Demographically, the study population at enrollment included a total of 6,814 men and women between the ages of 45 and 84, 38% of whom were White, 28% African American, 22% Hispanic and 12% Asian. All were asymptomatic from known clinical CVD at the time of enrollment. Further details about the conduct of the MESA study have been previously published.²³

For this present analysis, of the 6,814 participants enrolled at baseline, we excluded those with missing GlycA measurements (n=30), missing baseline ECC measures (n=4), or missing covariates (n=318). This left 6,462 participants for our analysis (Supplemental Fig. 1). Institutional Review Boards at each research center approved the study and informed consent was obtained from each participant.

Exposure assessment

Plasma levels of GlycA were obtained from EDTA plasma samples stored from MESA baseline exam (2000-2002) using *NMR LipoProfile*[®] analysis. Detailed description of the protocol has been previously described.⁶ The intra-assay and inter-assay coefficients of variability for GlycA measurement were 1.9% and 2.6%, respectively.⁴ Previous work by Otvos have confirmed stability of GlycA levels when measured after long-term storage.⁶ GlycA levels have also been shown to be similar when measured in plasma vs. serum samples or in non-fasting vs. fasting samples.⁶ A prior analysis found that GlycA levels are modestly positively skewed in the MESA cohort, and women in MESA have higher GlycA levels [mean (SD) 394 (62) $\mu\text{mol/L}$] than men [mean (SD) 365 (57) $\mu\text{mol/L}$],⁶ similar to the finding of higher hsCRP levels in women than in men.

Covariates

Covariates used in these analyses were ascertained at the baseline exam. Demographic information on age, sex, race/ethnicity, education level, and smoking status was obtained from interview and questionnaire data. Height and weight were measured following standard protocol, and Body Mass Index (BMI) was calculated as weight per squared height (kg/m^2). Total amounts of moderate + vigorous physical activity (MET-minutes/week) were ascertained through survey. Baseline systolic and diastolic blood pressures were measured using the Dinamap automated blood pressure device. The CKD-Epi equation was used to

calculate the estimated glomerular filtration rate (eGFR).²⁴ Total and HDL cholesterol levels were measured from fasting blood samples. A positive diabetes status was established as taking oral hypoglycemic medications or insulin, self-report of diabetes, or a fasting glucose level at or above 126 mg/dL. Medication use was determined through a medication inventory approach. Markers of inflammation (hsCRP, IL-6 and fibrinogen) were measured from stored blood obtained at baseline exam, as previously described.²⁵

Outcome assessment

The 4 ECC measures investigated in this analyses were AVC, MAC, ascending TAC (ATAC), and descending TAC (DTAC) ascertained by cardiac computed tomography (CT), using either an electron-beam CT (EBCT) or a four slice multidetector row helical CT (MDCT). Prior work in MESA has established equivalence across scanner types.^{26, 27} There was also very good reproducibility when using duplicate scans performed on the same patient using the same scanner.^{26, 27} This pilot work suggests the accuracy of calcification measurement is reasonably valid for the assessment of ECC progression over time.

All participants underwent an ECG-gated non-contrast CT scan at baseline (2000-2002). Participants then underwent a follow-up CT scan at either Exam 2 (2002-2004) or Exam 3 (2004-2005), randomly assigned. Two scans were taken in succession and the results were averaged to improve the accuracy of the calcium score. The CT scans were initially obtained for CAC measurement but were retrospectively reviewed for the ECC measures. The Agatston scoring method²⁸ was used to quantify the respective ECC scores. Further details on the procedures for assessing the calcification measures have previously been published.^{26, 29, 30}

Statistical analyses

We modeled GlycA levels in quartiles and per one standard deviation (SD) increment. We present baseline characteristics by GlycA quartiles. Continuous variables are presented as means (SD) or median (interquartile interval) and categorical variables as frequency (percent).

Prevalent ECC was defined as a greater than zero Agatston score present at baseline, and incident ECC was defined as detectable calcium (Agatston score >0) at follow-up in a participant without any detectable calcium at baseline (for each of the 4 ECC measures, respectively). Given the skew of the calcification data, for the analyses of the extent and progression of ECC measures, the ECC scores were natural log transformed as $\log(\text{ECC}+1)$ in the models.

We used multivariable-adjusted Poisson regression with robust variance estimation to determine prevalence and incidence ratios with their respective 95% confidence intervals (CI) for the 4 ECC measures (i.e. calcium score >0). We also used linear mixed effects models with random slopes and intercepts to determine the cross-sectional and longitudinal associations of GlycA with $\log(\text{ECC}+1)$ extent and progression. The time variable was scaled to derive the 2-year change.

We adjusted for demographics and field center in our limited Model 1 (age, sex, race/ethnicity, study site, CT scanner type). Model 2, our main analytic model, additionally adjusted for lifestyle factors (education, BMI, smoking status, pack-years smoking [log(pack-years+1)], alcohol consumption, and physical activity [log(physical activity+1)]). We then performed two additional supplemental models as follows: Model 3 further adjusted for ASCVD risk factors which may be intermediate variables between inflammation and atherosclerosis (systolic blood pressure, use of antihypertensive medication, total cholesterol, HDL-cholesterol, use of lipid-lowering medications, diabetes, and eGFR). In Model 4, we further adjusted for other traditional inflammatory markers, specifically log(hsCRP), log(IL-6), and log(fibrinogen). In our incident ECC analysis, we excluded participants with prevalent ECC and additionally adjusted for the time between CT scans.

In a sensitivity analysis, we explored the cross-sectional and longitudinal associations between all the inflammatory markers (e.g., hsCRP, fibrinogen, IL-6, and GlycA) separately on a log-transformed scale per 1 SD with the various ECC measures using our main analytic Model 2. This was done in order to compare the relative magnitudes of associations across the inflammatory markers.

Statistical significance was established at a p -value less than 0.05. Analyses were performed using STATA Version 15.

RESULTS

Baseline characteristics

The baseline characteristics of the study population are shown in Table 1. Of the 6,462 participants in this study, the mean (SD) age was 62 (10) years with 53% being women, 39% White, 27% Black, 22% Hispanic and 12% Chinese. The mean (SD) GlycA level was 381 (61) $\mu\text{mol/L}$. Study participants in the higher GlycA quartiles tended to be women and have a higher average BMI, median pack-years of smoking, systolic blood pressure and total cholesterol than those in the lowest quartile of GlycA. Participants with higher GlycA levels also had lower average physical activity levels, eGFR and HDL cholesterol. Those in the higher GlycA quartiles also had higher average levels of the other markers of inflammation (hsCRP, IL-6, and fibrinogen).

Cross-sectional analysis

Among our study participants, the presence of ECC for AVC, MAC, ATAC, and DTAC (respective Agatston scores >0) at baseline was 13.2%, 9.4%, 3.4%, and 27.1%, respectively (Supplemental Fig. 1). The prevalence of these ECC measures by GlycA quartiles are shown in Table 2.

AVC—After adjustment for demographics and lifestyle factors in our primary model (model 2), participants with higher GlycA levels (per SD increment) had a greater prevalence of AVC (>0), with adjusted prevalence ratio (PR) of 1.08 (95% CI 1.01, 1.14). (Table 2). This association became attenuated and was no longer statistically significant in models that further adjusted for potential intermediary ASCVD risk factors (model 3) or additional inflammatory markers (model 4). When examining the association of GlycA with baseline

extent of AVC, GlycA was only associated with $\log(\text{AVC}+1)$ in the limited demographic model 1, with β coefficients of 0.05 (95% CI 0.02, 0.09) (Table 3), but not in our primary or additionally adjusted models.

MAC—There was no statistically significant association found for GlycA with the presence and extent of MAC at baseline (Table 2 and 3).

ATAC—After adjustment for demographics and lifestyle factors in our primary model (model 2), those with higher GlycA levels (per SD increment) had a greater prevalence of ATAC (>0) [PR 1.18 (1.03, 1.34)] (Table 2). However, similar to AVC, this was also attenuated in subsequent models adjusted for ASCVD risk factors and inflammatory markers. In our primary model (model 2), higher GlycA levels per SD were also associated with baseline ATAC extent [$\log(\text{ATAC}+1)$] with beta-coefficients of 0.03 (0.01, 0.05) (Table 3).

DTAC—After adjustment for demographics and lifestyle factors in our primary (model 2), those with higher GlycA levels (per SD increment) also had increased prevalence of DTAC (>0) [PR 1.10 (1.06, 1.14)] (Table 2). Unlike the other atherosclerosis markers, the association of GlycA with DTAC presence remained statistically significant after further adjustment for ASCVD risk factors (model 3) but not after further adjustment for inflammatory markers. Higher GlycA levels per SD were also associated with DTAC extent [$\log(\text{DTAC}+1)$] with beta-coefficients of 0.14 (0.08, 0.20) (Table 3) in our primary model (model 2), as well in the ASCVD risk factor adjusted model.

Longitudinal analysis

There was a median of 2.3 years between CT scans. After excluding those with respective ECC scores >0 at baseline, the percent of study participants who newly developed incident measures of ECC >0 on the follow-up CT scans for AVC, MAC, ATAC, and DTAC were 4.1%, 4.4%, 1.9%, and 11.4%, respectively (Supplemental Fig. 1).

AVC—In all of our adjusted models, we found no association between levels of GlycA and incident AVC as well as with 2-year change in AVC score (Tables 4 and 5).

MAC—On the other hand, higher GlycA levels, per SD, were found to have a statistically significant positive association with incident MAC for our main model with incidence ratio of 1.18 (1.03, 1.37) (Table 4). As for the association of GlycA with 2-year change in MAC score, higher GlycA (per SD) was also associated with $\log(\text{MAC}+1)$ progression in our main model with beta-coefficients of 0.04 (0.02, 0.06) (Table 5). This association with MAC progression remained statistically significant after further adjustments for ASCVD risk factors and inflammatory markers (models 3 and 4).

ATAC—Higher GlycA, per SD, was found to be positively associated with incident ATAC in model 1 alone with incidence ratio of 1.23 (1.02, 1.48); however it was not statistically significantly associated in our main model or after additional covariate adjustment (Table 4). There was also no significant association of GlycA with 2-year ATAC progression (Table 5)

DTAC—Of all the ECC markers, the association of GlycA appeared to be the most robust for incidence and progression of DTAC. Among those without baseline DTAC, higher GlycA levels (per SD) were associated with incident DTAC with incident ratio of 1.17 (1.07, 1.28) (Table 4) in our main model, which remained statistically significant after further adjustment for both ASCVD risk factors (model 3) and additional inflammatory markers (model 4). Furthermore, GlycA was associated with 2-year progression of DTAC [$\log(\text{DTAC} + 1)$] with beta-coefficients of 0.04 (0.01, 0.07) in demographic and lifestyle adjusted models (Table 5), a finding which remained unchanged and significant after further adjustments in models 3 and 4.

Comparison with other inflammatory markers

For comparison of the magnitude of associations between the other inflammatory markers, GlycA, fibrinogen, IL-6, and hsCRP were all natural log-transformed and compared per SD for the associations with the prevalence and incidence of the 4 ECC markers, as well as their baseline extent and 2-year progression (Supplemental Table 1), in our primary model 2. The confidence intervals were overlapping between markers, but GlycA appeared to be slightly stronger of a marker for incident DTAC and 2-year DTAC progression than hsCRP (which was not statistically significant).

DISCUSSION

In this diverse cohort of individuals free from clinical CVD at baseline, we found that higher levels of a novel composite marker of inflammation, GlycA, was positively and independently associated with greater prevalence and incidence of DTAC, as well as baseline DTAC extent and 2-year progression. However, we found that higher GlycA levels were not associated with incident ATAC in our main model, of which incident ATAC was much less common an occurrence in our study population than incident DTAC (1.9% vs. 11.4%), but GlycA was associated with higher prevalence of ATAC at baseline. Furthermore, we found no associations between levels of GlycA and prevalent MAC despite a positive association with incident MAC. Additionally, while an association with GlycA levels and incident AVC was not established in our study, a positive association with GlycA and prevalent AVC was found. In sum, we found GlycA was positively associated with several prevalent and incident ECC measures, in particular for progression of MAC and DTAC, associations that persisted even after adjusting for potential mediating ASCVD risk factors and other inflammatory markers.

Recently, the CANTOS randomized clinical trial showed that a pharmacologic intervention (canakinumab) targeting the inflammatory pathway reduced ASCVD events, without modifying lipid levels.⁵ This study opened the door for future inflammation-modifying therapies and a need to better understand mechanisms linking inflammation to atherosclerosis pathogenesis. GlycA, a novel composite biomarker of systemic inflammation, reflects serum concentration and glycosylation state of main acute-phase reactants such as α 1-acid glycoprotein, haptoglobin, α 1-antitrypsin, α 1-antichymotrypsin and transferrin.⁶ To our knowledge, this is the first study examining the association of GlycA with vascular calcification outside of the coronary arteries. Prior studies have examined the

relationship between GlycA and subclinical coronary artery disease (CAD) as measured by CAC score on non-contrast CT or coronary plaque by coronary CT angiography.^{8, 31} In a case-control study involving patients with psoriasis, GlycA was found to be associated with subclinical CAD beyond ASCVD risk factors and hsCRP.³¹ In another cross-sectional study conducted in patients with rheumatoid arthritis, GlycA was correlated with the presence of CAC and prevalent CAD.³²

GlycA has also been shown to be associated with clinical CVD events independent of ASCVD risk factors in the WHS (Women's Health Study)³³, CATHGEN (CATHeterization GENetics)³⁴, PREVEND (Prevention of Renal and Vascular ENd-Stage Disease)³⁵, MESA⁴, and JUPITER (Justification for the Use of Statins in Prevention: an Intervention Trial Evaluating Rosuvastatin)³⁶, and mortality in WHS³⁷. Subclinical atherosclerosis is likely an intermediary process linking inflammation to clinical CVD events and identification of ECC may represent a window of opportunity for preclinical identification of at-risk patients who would likely benefit from more intensive preventive therapies including improved lifestyle. Notably, GlycA levels have been inversely associated with a more favorable cardiovascular health profile as assessed by the American Heart Association Life Simple 7 criteria, including an inverse relationship with physical activity levels.³⁸ In contrast, statin therapy has not been shown to reduce GlycA levels.³⁶

While GlycA has previously been linked to subclinical coronary disease as mentioned above, we believe the new findings presented here, of its association with vascular calcification outside of the coronary beds, also have clinical relevance. Prior studies have linked the presence of ECC with future risk of CAD and all-cause mortality such that increasing multi-site ECC was associated with higher CAD and mortality risk.¹⁰ Notably TAC is associated with non-CVD morbidity and mortality even independent of CAC.¹⁶ ECC and CAC are correlated, but imperfectly so; thus the addition of ECC to CAC may complement ASCVD risk assessment with the potential clinical application of using ECC as an adjunct measurement to CAC.²¹ ATAC, while uncommon, is associated with ASCVD events including stroke, independently of CAC.³⁹ TAC is strongly associated with all-cause and non-CVD mortality¹⁶, whereas CAC is a better predictor of CVD mortality.⁴⁰ In sum, the location of calcification across the various vascular beds provides unique information about mortality risk,^{40, 41} and understanding the association of this new potent inflammatory marker, GlycA, with each specific type of ECC may further our understanding of inflammatory mechanisms in CVD pathogenesis.

It is unclear why the association of GlycA appeared more robust for DTAC in particular – where it was associated with both incident DTAC and 2-year progression DTAC across all 4 progressively adjusted models tested. However, both the prevalence and incidence of DTAC (at 27% and 11%) was greater than the other 3 ECC markers, so there may be less statistical power to detect associations for the other ECC markers. Of note, DTAC has been shown to be associated with CAC⁴², and CAC has a positive association with GlycA⁸. Alternatively, there may be distinct biological mechanisms that lead to preferential calcification across the various vascular beds.⁴³ Vascular smooth muscle cells (VSMC) are increasingly recognized as the central cell type responsible for arterial calcification.⁴⁴ The distribution of VSMC varies by the embryological origin of each vascular bed, with unique VSMC subpopulations

present in the coronary artery, ascending aorta, descending thoracic aorta, and abdominal aorta.⁴⁵ As VSMC calcification appears to differ based on embryological origin,⁴⁶ differences in VSMC lineage type may explain variations in ECC prevalence and incidence. Calcification in the aorta tends to involve both medial and intimal vessel layers, while CAC primarily involves the intimal layer, which may explain why cardiovascular risk factors associated with various vascular calcifications tend to be similar but not necessarily overlapping.²¹ For example, smoking (another pro-inflammatory condition) is more strongly associated with abdominal aortic calcification than coronary or carotid calcified plaque.⁴⁷

There is a need to identify and study novel predictors of ASCVD, which may better risk stratify an individual. Understanding the relation between GlycA and ECC is one additional step toward elucidating pathways linking inflammation to clinical ASCVD risk. The key role that inflammation plays in the development and progression of ASCVD has been established.^{5, 48-50} Further understanding of the role of GlycA contributes in particular and the mechanisms that lead to the development of ASCVD could have potential clinical implications for primary and secondary prevention of ASCVD. The composite nature of GlycA suggest that it may actually be superior in certain ways to hsCRP,⁶ although hsCRP is the more commonly used marker of inflammation in clinical practice. GlycA has also been shown to predict CVD risk beyond hsCRP⁴ and could potentially be utilized similarly or complementary to hsCRP in clinical settings. Some potential therapies, such as those targeting tumor necrosis factor (anti-TNF) therapy, have been shown to reduce GlycA with a demonstrated improvement of vascular inflammation in patients with psoriasis³¹ and could be of potential clinical use. However, future interventional studies are needed to understand how to best incorporate measures of inflammation such as GlycA for ASCVD screening and treatment in clinical practice.

Limitations and strengths

Our study should be interpreted in the context of several limitations. First, our study is observational and therefore, we cannot assert causality. It should also be noted that our reporting of cross-sectional associations is subjected to survival and temporal biases. Our result could still be prone to residual confounding even after adjustments for other CVD risk factors. Many associations were attenuated after further adjustment for ASCVD risk factors (in model 3) that might be intermediary factors in the pathway between inflammation and atherosclerosis. Second, we were unable to adjust for use of anti-inflammatory drugs. Third, GlycA levels were measured only once at baseline, and thus we were unable to assess change in GlycA levels with change in the various ECC measures. Fourth, we only had data over a median of 2.3 years available for ECC progression. Since the incidence was relatively low for some of the ECC measures in this study population (incident AVC, MAC, and ATAC all occurred in less than 5% of study population), longer follow-up may be needed to examine progression in those vascular beds. Another limitation is that the aortic arch is not visualized on these cardiac CT scans, yet is known to be rich with calcification. Finally, we performed multiple analyses and findings may be due to chance; however our findings were internally consistent and the purpose of our study was meant to be exploratory to stimulate further research in this area.

Of note, our study had a number of important strengths. We used data from a racially and gender-diverse study population free of CVD at baseline who were followed longitudinally to assess the natural history of subclinical atherosclerosis, including atherosclerosis outside of the coronary beds. We were able to examine the cross-sectional and prospective relations of GlycA with 4 distinct measures of ECC (AVC, MAC, ATAC, and DTAC), each of which has prognostic value for clinical CVD events. We were able to adjust for potential confounders and/or mediators in our assessment of GlycA levels with incident and prevalent ECC. Our study adds to the emerging body of literature on the potential uses of GlycA as an additional tool for the assessment of clinical or subclinical ASCVD disease.

Conclusion

In conclusion, higher levels of GlycA are positively associated with prevalent AVC and ATAC, incident MAC, and both prevalent and incident DTAC in this diverse cohort free from clinical CVD. While GlycA was associated with several measures of ECC, there were differences in both the associations and the strengths of these relationships across the 4 ECC markers. In particular, the association of GlycA with progression of MAC and DTAC remained statistically significant even after accounting for potential mediating ASCVD risk factors and other inflammatory markers. This work may contribute to better understanding of inflammatory pathways in various subclinical ASCVD manifestations. Potential directions for future studies include assessing whether the association of GlycA with ECC has longitudinal implications for risk prediction of ASCVD events. Secondly, further evaluating the entirety of the aorta including the aortic arch, another important site of subclinical atherosclerosis. Lastly, future studies are needed to see if therapeutic lowering of GlycA can slow or prevent atherosclerosis progression.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

The authors thank the investigators, staff, and participants of the MESA study for their valuable contributions. A full list of participating MESA investigators and institutions can be found at <http://www.mesa-nhlbi.org>.

Financial support

Drs. Michos and Zhao were supported by the Blumenthal Scholars Fund for Preventive Cardiology at Johns Hopkins University. This research was supported by grant R01 HL071739 from the National Heart, Lung, and Blood Institute (NHLBI) to Dr. Budoff. This MESA study was funded by contracts HHSN268201500003I, N01-HC-95159, N01-HC-95160, N01-HC-95161, N01-HC-95162, N01-HC-95163, N01-HC-95164, N01-HC-95165, N01-HC-95166, N01-HC-95167, N01-HC-95168 and N01-HC-95169 from the NHLBI, and by grants UL1-TR-000040, UL1-TR-001079, and UL1-TR-001420 from NCATS. Dr. Mora was supported by grants K24 HL136852 and R01HL134811 from the NHLBI.

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Highlights

- GlycA is a novel composite marker of inflammation
- We found GlycA was associated with prevalent and incident extra-coronary calcium
- Associations for progression of MAC and DTAC were independent of CVD risk factors
- This work adds to the understanding of the role of inflammation in atherosclerosis
- Whether GlycA lowering prevents atherosclerosis progression warrants further study

Table 1.

Baseline characteristics (MESA, 2000-2002)^a

Characteristics	GlycA (μmol/L)				p-value
	Q1 204.7 - 337.0	Q2 338.0 - 374.9	Q3 375.0 - 418.6	Q4 419.8 - 787.8	
N	1,637	1,598	1,626	1,601	-
Age, years	61.4 ± 10.5	62.4 ± 10.2	62.6 ± 10.2	62.2 ± 10.1	0.005
Female	629 (38.4%)	753 (47.1%)	909 (55.9%)	1133 (70.8%)	<0.001
Race/ethnicity					
White	605 (37.0%)	655 (41.0%)	609 (37.5%)	642 (40.1%)	<0.001
Black	416 (25.4%)	384 (24.0%)	469 (28.8%)	477 (29.8%)	
Hispanic	292 (17.8%)	339 (21.2%)	378 (23.3%)	407 (25.4%)	
Chinese	324 (19.8%)	220 (13.8%)	170 (10.5%)	75 (4.7%)	
Education					
Less than high school	244 (14.9%)	280 (17.5%)	319 (19.6%)	332 (20.7%)	<0.001
High school or vocational school	581 (35.5%)	630 (39.4%)	700 (43.1%)	749 (46.8%)	
College, graduate or professional school	812 (49.6%)	688 (43.1%)	607 (37.3%)	520 (32.5%)	
BMI, kg/m ²	26.4 ± 4.7	27.6 ± 4.9	28.8 ± 5.3	30.4 ± 6	<0.001
Current smoker	137 (8.4%)	174 (10.9%)	223 (13.7%)	281 (17.6%)	<0.001
Pack-years of smoking ^{b,c}	14 (5 - 26)	16 (6 - 32)	17 (7 - 34)	20 (8 - 38)	<0.001
Physical activity, MET-minutes/week ^b	4268 (2228 - 8190)	4155 (1995 - 7650)	4073 (1980 - 7320)	3645 (1703 - 6893)	<0.001
Systolic blood pressure, mmHg	122.8 ± 20.8	125.7 ± 21.3	128.2 ± 21.6	129.3 ± 21.9	<0.001
eGFR, ml/min per 1.73 m ²	79.0 ± 15.0	78.0 ± 14.8	77.1 ± 16.5	76.7 ± 18.1	<0.001
Total cholesterol, mg/dL ^d	186.2 ± 32.1	194.0 ± 33.7	196.4 ± 34.8	200.6 ± 39.2	<0.001
HDL-cholesterol, mg/dL ^d	52.6 ± 15.5	51.1 ± 15.1	50.3 ± 14.6	50.1 ± 13.8	<0.001
Diabetes status	136 (8.3%)	164 (10.3%)	213 (13.1%)	294 (18.4%)	<0.001
Antihypertensive medications	375 (22.9%)	480 (30.0%)	583 (35.9%)	688 (43.0%)	<0.001
Lipid-lowering medications	228 (13.9%)	227 (14.2%)	290 (17.8%)	315 (19.7%)	<0.001

Characteristics	GlycA (µmol/L)				p-value
	Q1	Q2	Q3	Q4	
	204.7 - 337.0	338.0 - 374.9	375.0 - 418.6	419.8 - 787.8	
hsCRP, mg/L ^b	0.9 (0.5 - 1.7)	1.4 (0.7 - 2.8)	2.4 (1.1 - 4.6)	4.6 (2.4 - 9.3)	<0.001
IL-6, pg/mL ^b	0.9 (0.6 - 1.4)	1.1 (0.7 - 1.6)	1.3 (0.9 - 1.9)	1.6 (1.1 - 2.5)	<0.001
Fibrinogen, mg/dL ^b	300 (268 - 337)	327 (291 - 369)	351 (308 - 394)	385 (337 - 440)	<0.001

BMI = body mass index; eGFR = estimated glomerular filtration rate; HDL= high density lipoprotein; hsCRP = high-sensitivity C-reactive protein; IL-6 = interleukin 6; meds=medication.

^aData are presented as mean ± standard deviation for continuous variables and frequency (percentage) for categorical variables unless otherwise specified.

^bData presented as median (interquartile interval)

^cEstimates are for participants with pack-years of smoking >0

^dTo convert total and HDL-C cholesterol from mg/dL to mmol/L, divide by 38.67.

Table 2.

Prevalence ratios (95% confidence interval)^a of extra-coronary calcification by GlycA at MESA baseline exam (2000-2002)

GlycA	Q1	Q2	Q3	Q4	p-for-trend	Per 1SD increment
Aortic valve, n (%)	205 (12.52%)	198 (12.39%)	219 (13.47%)	232 (14.49%)	-	854 (13.22%)
Model 1 ^b	1 (reference)	0.94 (0.80 - 1.12)	1.07 (0.91 - 1.27)	1.25 (1.06 - 1.48)	0.004	1.11 (1.04 - 1.17)
Model 2 ^c	1 (reference)	0.92 (0.78 - 1.09)	1.01 (0.85 - 1.20)	1.15 (0.97 - 1.37)	0.08	1.08 (1.01 - 1.14)
Model 3 ^d	1 (reference)	0.84 (0.71 - 1.00)	0.90 (0.76 - 1.06)	0.96 (0.80 - 1.14)	0.85	1.00 (0.94 - 1.07)
Model 4 ^e	1 (reference)	0.83 (0.70 - 0.99)	0.87 (0.73 - 1.04)	0.89 (0.73 - 1.09)	0.41	0.98 (0.91 - 1.05)
Mitral valve, n (%)	125 (7.64%)	152 (9.51%)	161 (9.9%)	168 (10.49%)	-	606 (9.38%)
Model 1 ^b	1 (reference)	1.05 (0.85 - 1.31)	1.04 (0.84 - 1.29)	1.06 (0.86 - 1.31)	0.64	1.03 (0.96 - 1.11)
Model 2 ^c	1 (reference)	1.02 (0.82 - 1.27)	0.96 (0.77 - 1.19)	0.93 (0.75 - 1.16)	0.42	0.98 (0.91 - 1.06)
Model 3 ^d	1 (reference)	0.99 (0.80 - 1.24)	0.92 (0.74 - 1.15)	0.87 (0.69 - 1.09)	0.15	0.96 (0.88 - 1.03)
Model 4 ^e	1 (reference)	0.99 (0.80 - 1.23)	0.92 (0.74 - 1.16)	0.85 (0.67 - 1.10)	0.17	0.95 (0.87 - 1.04)
Ascending aorta, n (%)	43 (2.63%)	46 (2.88%)	51 (3.14%)	77 (4.81%)	-	217 (3.36%)
Model 1 ^b	1 (reference)	1.04 (0.69 - 1.56)	1.13 (0.76 - 1.69)	1.84 (1.26 - 2.69)	0.001	1.25 (1.10 - 1.41)
Model 2 ^c	1 (reference)	0.99 (0.66 - 1.48)	0.99 (0.67 - 1.47)	1.54 (1.05 - 2.25)	0.03	1.18 (1.03 - 1.34)
Model 3 ^d	1 (reference)	0.89 (0.59 - 1.34)	0.89 (0.59 - 1.32)	1.34 (0.89 - 2.00)	0.12	1.12 (0.97 - 1.30)
Model 4 ^e	1 (reference)	0.88 (0.59 - 1.32)	0.88 (0.59 - 1.33)	1.28 (0.83 - 1.96)	0.24	1.10 (0.94 - 1.28)
Descending aorta, n (%)	364 (22.24%)	426 (26.66%)	460 (28.29%)	502 (31.36%)	-	1752 (27.11%)
Model 1 ^b	1 (reference)	1.14 (1.03 - 1.27)	1.21 (1.09 - 1.35)	1.39 (1.25 - 1.54)	<0.001	1.13 (1.09 - 1.16)
Model 2 ^c	1 (reference)	1.12 (1.01 - 1.24)	1.16 (1.04 - 1.29)	1.29 (1.16 - 1.43)	<0.001	1.10 (1.06 - 1.14)
Model 3 ^d	1 (reference)	1.06 (0.95 - 1.18)	1.07 (0.96 - 1.19)	1.15 (1.03 - 1.28)	0.01	1.05 (1.02 - 1.09)

GlycA	Q1	Q2	Q3	Q4	p-for-trend	Per 1SD increment
Model 4 ^e	1 (reference)	1.04 (0.93 - 1.16)	1.04 (0.93 - 1.16)	1.08 (0.96 - 1.21)	0.26	1.03 (0.98 - 1.07)

^a Adjusted prevalence ratios (95% CI) for the presence of an ECC score >0 were derived from Poisson regression models.

^b Model 1: adjusted for age, sex, race/ethnicity, study site, and CT scanner type

^c Model 2: model 1 plus education, BMI, smoking status, pack-years of smoking, and physical activity

^d Model 3: model 2 plus systolic blood pressure, use of antihypertensive medication, total cholesterol, HDL-cholesterol, use of lipid-lowering medications, diabetes, and eGFR

^e Model 4: model 3 plus log transformed hsCRP, log transformed IL-6, and log transformed fibrinogen

Table 3.

Cross-sectional associations^a between GlycA Levels and extra-coronary calcification (log transformed): MESA (2000-2002)

GlycA	Q1	Q2	Q3	Q4	p-for-trend	Per 1SD increment
Aortic valve						
Model 1 ^b	0 (reference)	-0.01 (-0.11, 0.09)	0.05 (-0.05, 0.15)	0.12 (0.02, 0.22)	0.01	0.05 (0.02, 0.09)
Model 2 ^c	0 (reference)	-0.03 (-0.13, 0.07)	0.01 (-0.09, 0.11)	0.06 (-0.05, 0.16)	0.21	0.03 (-0.01, 0.07)
Model 3 ^d	0 (reference)	-0.06 (-0.16, 0.04)	-0.04 (-0.14, 0.06)	-0.03 (-0.14, 0.08)	0.68	-0.002 (-0.04, 0.04)
Model 4 ^e	0 (reference)	-0.07 (-0.17, 0.03)	-0.06 (-0.16, 0.05)	-0.06 (-0.18, 0.06)	0.36	-0.02 (-0.06, 0.03)
Mitral valve						
Model 1 ^b	0 (reference)	0.01 (-0.08, 0.11)	0.02 (-0.08, 0.12)	0.03 (-0.08, 0.13)	0.61	0.01 (-0.02, 0.05)
Model 2 ^c	0 (reference)	-0.01 (-0.11, 0.09)	-0.03 (-0.13, 0.07)	-0.06 (-0.16, 0.05)	0.26	-0.02 (-0.06, 0.02)
Model 3 ^d	0 (reference)	-0.02 (-0.12, 0.08)	-0.05 (-0.15, 0.05)	-0.09 (-0.20, 0.01)	0.07	-0.03 (-0.07, 0.01)
Model 4 ^e	0 (reference)	-0.01 (-0.10, 0.09)	-0.02 (-0.12, 0.09)	-0.05 (-0.17, 0.07)	0.41	-0.01 (-0.06, 0.03)
Ascending aorta						
Model 1 ^b	0 (reference)	-0.01 (-0.07, 0.05)	0.01 (-0.04, 0.07)	0.09 (0.03, 0.15)	0.002	0.04 (0.02, 0.06)
Model 2 ^c	0 (reference)	-0.02 (-0.07, 0.04)	-0.003 (-0.06, 0.05)	0.07 (0.01, 0.13)	0.03	0.03 (0.01, 0.05)
Model 3 ^d	0 (reference)	-0.03 (-0.09, 0.03)	-0.03 (-0.08, 0.03)	0.04 (-0.02, 0.10)	0.25	0.02 (-0.01, 0.04)
Model 4 ^e	0 (reference)	-0.03 (-0.09, 0.03)	-0.03 (-0.09, 0.03)	0.03 (-0.03, 0.10)	0.37	0.01 (-0.01, 0.04)
Descending aorta						
Model 1 ^b	0 (reference)	0.12 (-0.03, 0.27)	0.22 (0.07, 0.37)	0.45 (0.30, 0.61)	<0.001	0.18 (0.12, 0.23)
Model 2 ^c	0 (reference)	0.09 (-0.06, 0.24)	0.15 (0.002, 0.31)	0.35 (0.19, 0.51)	<0.001	0.14 (0.08, 0.20)
Model 3 ^d	0 (reference)	0.02 (-0.13, 0.17)	0.03 (-0.13, 0.18)	0.16 (-0.002, 0.32)	0.07	0.07 (0.01, 0.13)

GlycA	Q1	Q2	Q3	Q4	p-for-trend	Per 1SD increment
Model 4 ^e	0 (reference)	0.01 (−0.14, 0.16)	0.02 (−0.14, 0.18)	0.14 (−0.04, 0.32)	0.16	0.06 (−0.003, 0.13)

^aEstimates are β coefficients (95% CI) derived from linear mixed effect models and outcomes are natural log transformed (ECC+1)

^bModel 1: adjusted for age, sex, race/ethnicity, study site, and CT scanner type

^cModel 2: model 1 plus education, BMI, smoking status, pack-years of smoking, and physical activity

^dModel 3: model 2 plus systolic blood pressure, use of antihypertensive medication, total cholesterol, HDL-cholesterol, use of lipidlowering medications, diabetes, and eGFR

^eModel 4: model 3 plus log transformed hsCRP, log transformed IL-6, and log transformed fibrinogen

Table 4.

Incidence ratios (95% confidence interval)^a of extra-coronary calcification by GlycA levels: MESA (2000 to 2005)

GlycA	Q1	Q2	Q3	Q4	p-for-trend	Per 1SD increment
N	1,248	1,232	1,183	1,134	-	4,797
Aortic valve	45 (3.61%)	56 (4.55%)	40 (3.38%)	55 (4.85%)	-	196 (4.09%)
Model 1 ^b	1 (reference)	1.18 (0.80 - 1.73)	0.89 (0.59 - 1.34)	1.39 (0.94 - 2.06)	0.25	1.11 (0.96 - 1.28)
Model 2 ^c	1 (reference)	1.13 (0.77 - 1.65)	0.82 (0.54 - 1.24)	1.22 (0.81 - 1.84)	0.66	1.05 (0.90 - 1.24)
Model 3 ^d	1 (reference)	1.07 (0.72 - 1.57)	0.74 (0.48 - 1.14)	1.05 (0.69 - 1.60)	0.77	0.99 (0.84 - 1.18)
Model 4 ^e	1 (reference)	1.08 (0.73 - 1.60)	0.76 (0.49 - 1.20)	1.08 (0.67 - 1.75)	0.86	1.00 (0.82 - 1.23)
N	1,315	1,266	1,217	1,179	-	4,977
Mitral valve	40 (3.04%)	55 (4.34%)	57 (4.68%)	68 (5.77%)	-	220 (4.42%)
Model 1 ^b	1 (reference)	1.26 (0.84 - 1.89)	1.41 (0.94 - 2.12)	1.83 (1.24 - 2.70)	0.002	1.28 (1.12 - 1.45)
Model 2 ^c	1 (reference)	1.19 (0.80 - 1.79)	1.24 (0.82 - 1.87)	1.49 (1.00 - 2.24)	0.06	1.18 (1.03 - 1.37)
Model 3 ^d	1 (reference)	1.09 (0.73 - 1.62)	1.10 (0.73 - 1.65)	1.24 (0.82 - 1.86)	0.32	1.10 (0.95 - 1.27)
Model 4 ^e	1 (reference)	1.03 (0.69 - 1.54)	1.01 (0.67 - 1.54)	1.00 (0.65 - 1.56)	0.98	1.01 (0.85 - 1.20)
N	1,389	1,352	1,310	1,250	-	5,301
Ascending aorta	19 (1.37%)	25 (1.85%)	31 (2.37%)	26 (2.08%)	-	101 (1.91%)
Model 1 ^b	1 (reference)	1.32 (0.72 - 2.41)	1.64 (0.90 - 2.99)	1.49 (0.79 - 2.78)	0.15	1.23 (1.02 - 1.48)
Model 2 ^c	1 (reference)	1.20 (0.66 - 2.20)	1.33 (0.72 - 2.49)	1.10 (0.58 - 2.08)	0.74	1.12 (0.90 - 1.38)
Model 3 ^d	1 (reference)	1.04 (0.57 - 1.90)	1.09 (0.59 - 2.02)	0.79 (0.41 - 1.52)	0.49	1.00 (0.79 - 1.26)
Model 4 ^e	1 (reference)	0.94 (0.51 - 1.72)	0.90 (0.47 - 1.73)	0.53 (0.26 - 1.11)	0.10	0.87 (0.65 - 1.16)
N	1,117	1,039	980	921	-	4,057
Descending aorta	111 (9.94%)	111 (10.68%)	121 (12.35%)	120 (13.03%)	-	463 (11.41%)

GlycA	Q1	Q2	Q3	Q4	<i>p</i> -for-trend	Per 1SD increment
Model 1 ^{<i>b</i>}	1 (reference)	1.06 (0.83 - 1.34)	1.28 (1.01 - 1.62)	1.52 (1.19 - 1.94)	<0.001	1.22 (1.12 - 1.33)
Model 2 ^{<i>c</i>}	1 (reference)	1.02 (0.80 - 1.29)	1.18 (0.93 - 1.50)	1.35 (1.04 - 1.74)	0.01	1.17 (1.07 - 1.28)
Model 3 ^{<i>d</i>}	1 (reference)	0.94 (0.74 - 1.20)	1.06 (0.83 - 1.35)	1.17 (0.90 - 1.53)	0.16	1.11 (1.01 - 1.23)
Model 4 ^{<i>e</i>}	1 (reference)	0.94 (0.74 - 1.21)	1.08 (0.84 - 1.39)	1.18 (0.88 - 1.59)	0.19	1.14 (1.02 - 1.27)

^{*a*}Incidence ratios (95% CI) for incident ECC score >0 were derived from Poisson regression among those with respective scores of 0 at baseline.

^{*b*}Model 1: adjusted for age, sex, race/ethnicity, study site, CT scanner type, and time between CT scans

^{*c*}Model 2: model 1 plus education, BMI, smoking status, pack-years of smoking, and physical activity

^{*d*}Model 3: model 2 plus systolic blood pressure, use of antihypertensive medication, total cholesterol, HDL-cholesterol, use of lipid-lowering medications, diabetes, and eGFR

^{*e*}Model 4: model 3 plus log transformed hsCRP, log transformed IL-6, and log transformed fibrinogen

Table 5.

Associations^a between GlycA and 2-year change in extra-coronary calcification (log transformed): MESA

	Q1	Q2	Q3	Q4	p-for-trend	Per 1SD increment
N	1,421	1,388	1,350	1,310	-	5,469
Aortic valve						
Model 1	0 (reference)	-0.01 (-0.06, 0.04)	-0.02 (-0.07, 0.03)	0.01 (-0.04, 0.06)	0.91	0.004 (-0.01, 0.02)
Model 2	0 (reference)	-0.01 (-0.06, 0.04)	-0.02 (-0.07, 0.03)	0.01 (-0.04, 0.06)	0.92	0.004 (-0.01, 0.02)
Model 3	0 (reference)	-0.01 (-0.06, 0.04)	-0.02 (-0.07, 0.03)	0.01 (-0.04, 0.06)	0.90	0.004 (-0.01, 0.02)
Model 4	0 (reference)	-0.01 (-0.06, 0.04)	-0.02 (-0.07, 0.03)	0.01 (-0.04, 0.06)	0.90	0.004 (-0.01, 0.02)
Mitral valve						
Model 1	0 (reference)	0.05 (-0.0002, 0.10)	0.06 (0.01, 0.11)	0.10 (0.05, 0.15)	<0.001	0.04 (0.02, 0.06)
Model 2	0 (reference)	0.05 (-0.0002, 0.10)	0.06 (0.01, 0.11)	0.10 (0.05, 0.15)	<0.001	0.04 (0.02, 0.06)
Model 3	0 (reference)	0.05 (-0.0002, 0.10)	0.06 (0.01, 0.11)	0.10 (0.05, 0.15)	<0.001	0.04 (0.02, 0.06)
Model 4	0 (reference)	0.05 (-0.0003, 0.10)	0.06 (0.01, 0.11)	0.10 (0.05, 0.15)	<0.001	0.04 (0.02, 0.06)
Ascending aorta						
Model 1	0 (reference)	0.02 (-0.02, 0.06)	0.03 (-0.01, 0.07)	-0.01 (-0.06, 0.03)	0.72	0.003 (-0.01, 0.02)
Model 2	0 (reference)	0.02 (-0.02, 0.06)	0.03 (-0.01, 0.07)	-0.01 (-0.06, 0.03)	0.73	0.003 (-0.01, 0.02)
Model 3	0 (reference)	0.02 (-0.02, 0.06)	0.03 (-0.01, 0.07)	-0.01 (-0.05, 0.03)	0.75	0.003 (-0.01, 0.02)
Model 4	0 (reference)	0.02 (-0.02, 0.06)	0.03 (-0.01, 0.07)	-0.01 (-0.05, 0.03)	0.75	0.003 (-0.01, 0.02)
Descending aorta						
Model 1	0 (reference)	0.03 (-0.04, 0.10)	0.06 (-0.01, 0.14)	0.08 (0.01, 0.16)	0.02	0.04 (0.01, 0.07)
Model 2	0 (reference)	0.03 (-0.04, 0.11)	0.07 (-0.01, 0.14)	0.09 (0.01, 0.16)	0.02	0.04 (0.01, 0.07)
Model 3	0 (reference)	0.03 (-0.04, 0.10)	0.07 (-0.01, 0.14)	0.09 (0.01, 0.16)	0.02	0.04 (0.01, 0.07)
Model 4	0 (reference)	0.03 (-0.04, 0.10)	0.07 (-0.01, 0.14)	0.09 (0.01, 0.16)	0.02	0.04 (0.01, 0.07)

^aEstimates are β coefficients from linear mixed effect models and outcomes are natural log transformed (extra-coronary calcification +1). Time has been scaled for 2-year change.

^bModel 1: adjusted for age, sex, race/ethnicity, study site, and CT scanner type

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c, Model 2: model 1 plus education, BMI, smoking status, pack-years of smoking, and physical activity
d, Model 3: model 2 plus systolic blood pressure, use of antihypertensive medication, total cholesterol, HDL-cholesterol, use of lipidlowering medications, diabetes, and eGFR
e, Model 4: model 3 plus log transformed hsCRP, log transformed IL-6, and log transformed fibrinogen