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Association of Circulating Sclerostin with Vascular Calcification in Afro-Caribbean Men

Allison L Kuipers, PhD[1], **Iva Miljkovic, MD, PhD**[1], **J Jeffery Carr, MD**[2], **James G Terry, MS**[2], **Cara S Nestlerode, MS**[1], **Yaorong Ge, PhD**[3], **Clareann H Bunker, PhD**[1], **Alan L Patrick, FRCP**[4], and **Joseph M Zmuda, PhD**[1,5]

[1]Department of Epidemiology, University of Pittsburgh, Pittsburgh, PA, USA

[2]Department of Radiology, Vanderbilt University Medical Center, Nashville, TN, USA

[3]Department of Software and Information Sciences, University of North Carolina, Charlotte, NC, USA

[4]Tobago Health Studies Office, Scarborough, Tobago, Trinidad and Tobago

[5]Department of Human Genetics, University of Pittsburgh, Pittsburgh, PA, USA

Abstract

Objective—Sclerostin, a Wingless (Wnt) pathway antagonist, is an established regulator of bone mineralization in humans but its potential importance in the regulation of vascular calcification is less clear. Therefore, our objective was to assess the relationship of serum sclerostin levels with coronary and aortic artery calcification (CAC and AAC, respectively) in Afro-Caribbean men on the island of Tobago.

Methods—Serum sclerostin levels and computed tomography of CAC and AAC were measured in 191 men (age mean(SD): 62.9(8.0)years) recruited without regard to health status. Multivariable logistic regression models were used to assess the cross-sectional association of sclerostin with prevalent arterial calcification.

Results—Mean(SD) sclerostin was 45.2 pmol/L (15.6 pmol/L). After adjusting for risk factors including age, physical and lifestyle characteristics, comorbidities, lipoproteins and kidney function, 1 SD greater sclerostin level was associated with a 1.61-times (95%CI 1.02–2.53) greater odds of having CAC. Sclerostin was not associated with AAC in any model.

Conclusions—This is the first study to show that, among Afro-Caribbean men, greater serum sclerostin concentrations were associated with prevalence and extent of CAC. Further studies are needed to better define the role of the Wnt signaling pathway in arterial calcification in humans.

Please address requests for reprints to the corresponding author: Allison L Kuipers, PhD, 130 DeSoto St, A543 Crabtree Hall, Pittsburgh PA 15261, kuipers@pitt.edu, Phone: +01 (412) 624-2781, Fax: +01 (412) 624-7397.

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Keywords

sclerostin; coronary artery calcification; aortic artery calcification; African ancestry

INTRODUCTION

With aging, calcifications develop within the vasculature and in the presence of vascular disease progression, calcification arise within the medial vessel wall and/or as calcified plaques(1). The presence and amount of arterial calcification predicts cardiovascular disease (CVD) events and mortality(2–4). Emerging evidence indicates that the Wingless (Wnt) plays a role in vascular biology including vascular calcification(5–7), angiogenesis(8–10), and atherosclerosis(11–13). Wnt signaling occurs when the Wnt ligand binds to co-receptors, Frizzled and Low-density Lipoprotein Receptor related Protein, which induces β-catenin translocation to the nucleus to regulate the transcription of Wnt target genes. The Wnt pathway is involved in many aspects of biology including cell survival, stem cell development and cell differentiation, including bone and vascular lineages(14).

Sclerostin, one of the most studied circulating Wnt inhibitors in humans, is a secreted glycoprotein that acts as a Wnt antagonist(15, 16). Although sclerostin is an established regulator of bone mineralization (17), its potential role in vascular biology and arterial health is less clear. Sclerostin has been detected in the human aorta(18) and is up-regulated in calcifying vascular smooth muscle cells(19, 20) and calcified valvular plaques(21). The relationship of human serum sclerostin and vascular calcification, to our knowledge, has been investigated in four previous studies with two showing a direct correlation with calcification(22, 23) and two reporting an inverse correlation(24, 25). These inconsistent results may be due, in part, to the measurement of calcification since three of the studies(22–24) only used vertebral x-ray scans to measure aortic artery calcification and did not assess the more clinically relevant(26) coronary arteries. The previous studies also included vastly different population samples such as only diabetic patients(23, 25), chronic kidney disease patients(24) or post-menopausal women(22). Therefore, in the current study we assessed the association of serum sclerostin with vascular calcification measures from computed tomography imaging of the clinically relevant coronary and aortic arteries in a community-dwelling sample of 191 adult Afro-Caribbean men.

MATERIALS AND METHODS

Study Sample

The computed tomography (CT) scans were obtained as an ancillary study of the Tobago Bone Health Study (TBHS), a population-based prospective study of 2,652 community-dwelling men aged 40 years and older, residing on the Caribbean island of Tobago(27). Participants for the TBHS were recruited without regard to health status and men were eligible if they were ambulatory, not terminally ill and without a bilateral hip replacement. Men from Tobago are of homogeneous African ancestry with low European admixture (<6%)(28). The CT sample consisted of 304 men who were recruited consecutively during a follow-up visit of the TBHS from 2011–2012. During this ancillary visit, participants

underwent a chest and abdominal CT, an extensive clinical exam and health history assessment, and collection and biobanking of specimens including fasting serum samples. Serum measures of sclerostin (SOST) were obtained in a random sample of 191 men who serve as the basis of the current analysis. The men with sclerostin measured were more likely to be diabetic but less likely to be hypertensive than men from the larger cohort who did not have sclerostin measured (both P-values<0.05; data not shown). Other factors, such as age, demographics, physical and lifestyle characteristics, biochemical markers, and vascular calcification measures, did not differ between groups (data not shown). Written informed consent was obtained from each participant using forms and procedures approved by the University of Pittsburgh Institutional Review Board, the U.S. Surgeon General's Human Use Review Board, and the Tobago Division of Health and Social Services Institutional Review Board.

Serum Sclerostin and Other Biochemical Assays

Blood samples were collected from participants in the morning after an overnight fast. Serum was separated and stored at −80°C until time of assay. Serum sclerostin levels were measured according to the manufacturer's protocol using a validated sandwich enzyme-linked immunosorbent assay (ELISA) (Biomedica Gruppe, Vienna, Austria) and standardized across plates. Intra- and inter-assay coefficients of variation were 5% and 3%, respectively.

Fasting serum glucose was measured using a coupled enzymatic reaction similar to the procedure described by Bondar and Mead(29). Low-density lipoprotein cholesterol (LDL-c) was calculated by the Friedewald equation. High-density lipoprotein cholesterol (HDL-c) was determined using the selective heparin/manganese chloride precipitation method. Triglycerides were determined enzymatically using the procedure of Bucolo and David(30). Serum creatinine was quantitatively determined by the VITROS CREA Slide method. The Modification of Diet in Renal Disease Study formula was used to estimate glomerular filtration rate (eGFR) as: eGFR [mL $\cdot$ min^{-1} $\cdot$ (1.73 m^2) $^{-1}$] = 175 × (serum creatinine [mg/dL] $^{-1.154}$× age [years] $^{-0.203}$[× 0.742, if female] [× 1.212, if African American] (31).

Arterial Calcification

Arterial calcification was assessed by central computed tomography using a dual slice, high-speed NX/I scanner, 120 KVp, 290 mA and gantry speed 0.7 seconds (GE Medical Systems, Waukesha, WI). The scans were obtained using the axial, two-slice scan mode (2i) and a segmented (a.k.a "half-scan") reconstruction to provide an effective temporal resolution of approximately 350 msec for each 3 mm thick slice without cardiac gating. Coronary artery calcification (CAC) values were obtained from cross-sectional slices through the chest from the carina through the entire inferior aspect of the heart and measurements made by vessel for each of the major epicardial coronary arteries. For the abdominal scan series, a helical scan mode (120 KVp, 250 mA, 3 mm slice thickness and pitch of 1.5:1 was utilized since the higher temporal resolution for the coronary arteries was not required. For participants with body weight greater than 200 lbs, the mA was increased to 300. Aortic artery calcification (AAC) values were obtained from cross-sectional slices through the abdomen from L3 to S1 and included the summation of calcification in the abdominal aorta and

common iliac arteries. Measurements were performed by experienced analysts using an FDA approved computer workstation and software (Calcium, Aquarius workstation, TeraRecon San Mateo, CA) that accounts for slice thickness and scan field of view. The Agatston method(32) method was used to report scores of calcified plaque using a 130 HU threshold, minimum lesion size of >1 mm2 and a display field of view of 350 mm designed to be comparable to other population based studies that have measured CAC. In this report, presence of CAC was defined by an Agatston score of >10 to further reduce false positive classification. The lead reader at the Wake Forest University CT Reading Center read all CT scans for the present study. This reader also led the Coronary Artery Risk Development In young Adults (CARDIA) Study CT analyses, and a careful blinded re-read of 153 CARDIA scans found intra-reader technical error of 6.6%(32).

Other Characteristics

Demographic, health history and anthropomorphic characteristics were assessed by trained staff using interview and clinical exams. Body weight was measured to the nearest 0.1 kg on a balance beam scale and standing height was measured to the nearest 0.1 cm using a wall-mounted stadiometer, both without participants wearing shoes. Body mass index (BMI) was calculated as weight in kilograms divided by standing height in meters squared. Total body fat was measured using dual X-ray absorptiometry. Smoking status was classified both as either current or not, or ever or not wherein participants reporting smoking <100 cigarettes in their lifetime were considered never-smokers. Alcohol consumption is very limited in this cohort sample and was, therefore, coded as consuming >3 drinks per week (yes/no) to identify individuals with greater than average cohort alcohol intake. As walking is the predominate form of physical activity on the island, physical activity was dichotomized into participants reporting >60 min of walking for exercise per week vs. not. Grip strength was calculated as the average grip strength of four trials (two left handed and two right handed) as measured using a dynamometer (Preston Grip Dynamometer, JA Preston Corp.).

Diabetes was defined as a fasting serum glucose level ≥126 mg/dl, current self-reported use of diabetes medication or an affirmative response to the question “has a doctor ever told you that you have diabetes?”. Blood pressure was measured three times while seated and the average of the 2nd and 3rd reading were used in this analysis. Hypertension was defined as a systolic blood pressure (SBP) ≥140 mmHg, diastolic blood pressure (DBP) ≥90 mmHg, current self-reported use of antihypertensive medication or an affirmative to the question “has a doctor ever told you that you have hypertension or high blood pressure?” Use of statin medications was self-reported.

Statistical Analysis

The distribution of sclerostin was assessed prior to analysis and did not violate the assumption of normality. One outlier, defined as ≥4 SD from the mean, was removed prior to analyses. Differences in means or frequencies by CAC or AAC presence were tested by chi-squared test or T-test, as appropriate. Multivariable logistic regression was used to evaluate the association of serum sclerostin (independent variable) with prevalent CAC or AAC (dependent variables) after adjustment for potential confounding factors including age, total body fat, ever smoking, alcohol consumption, walking, grip strength, diabetes,

hypertension, LDL-c, HDL-c, triglycerides, statin use and eGFR. We expressed odds ratios from these models per a 1 standard deviation increase in serum sclerostin. Partial spearman correlation, a non-parametric test, was used to determine the association of serum sclerostin with CAC and AAC score due to the skewness and high prevalence of men without any calcification. Models were adjusted for the same potential confounding factors as used in the logistic regression models of calcification presence.

RESULTS

Sample Characteristics

Of the 191 Afro-Caribbean men, 30.0% had prevalent CAC and 68.6% had prevalent AAC (Table 1). Median CAC score, overall, was 0 with interquartile range of 0 to 21.5. In men with any CAC, the median CAC score was 85.5 (IQR: 26.6–283.2). Median AAC score, overall, was 92.3 with interquartile range of 0 to 686.0. In men with any AAC, the median AAC score was 302.3 (IQR: 78.5–1085.8). Mean serum SOST levels were 45.2 pmol/L (median: 44.0; range: 15.3–88.6 pmol/L). The Afro-Caribbean men were aged 63 on average and had 24% body fat. While only 11% were current smokers, 35% reported smoking >100 cigarettes in their lifetime. Only 16% drank more than 3 alcoholic beverages per week and 54% reported walking more than 60 minutes per week. Average grip strength was 39.5 kg. Diabetes was present in 30.7% of the sample with nearly 76% of these men taking diabetes medication. Hypertension was present in 56% of the sample but only 61% of these men were on antihypertensive medication. The mean eGFR was 84.1 ml/min/1.73m^2, only 6% of men had an eGFR <60 ml/min/1.73m^2.

In unadjusted analysis (Table 1), serum sclerostin was greater among men with than without prevalent CAC (P=0.04). In contrast, serum sclerostin was not associated with AAC. Greater age and prevalent hypertension were associated with both CAC and AAC (P<0.01 for all). eGFR was greater in men with CAC than in those without CAC (P=0.02), but was not associated with AAC. Total body fat and statin use were greater in men with AAC than in those without AAC (P=0.03 and P=0.01, respectively), but were not associated with CAC.

Association of Serum Sclerostin with Arterial Calcification Presence and Extent

Consistent with unadjusted analyses, greater serum sclerostin concentrations were associated with greater odds of prevalent CAC (Table 2). In unadjusted models, 1 SD greater serum SOST was associated with 1.95-fold (95% CI: 1.30–2.91; P-value=0.001) increased odds of prevalent CAC. After adjustment for age, physical and lifestyle characteristics, comorbidities and cholesterol, a 1 SD greater serum SOST remained significantly associated with prevalent CAC (OR: 1.76; 95%CI: 1.13–2.76; P-value=0.013). Additional adjustment for kidney function attenuated the association slightly, though it was still significant (Model 4; OR: 1.61; 95%CI: 1.02–2.53; P-value=0.042). Consistent with the prevalence analysis, SOST was also significantly and directly correlated with the extent of CAC as assessed by Agatston score (Table 2). The Spearman correlation coefficient between SOST and CAC extent was 0.2854 (P-value <0.001) in an unadjusted model and the correlation remained strong after adjustment for age, physical and lifestyle characteristics, comorbidities and cholesterol (Spearman r=0.2090, P-value=0.007). Additional adjustment for kidney function

did attenuate the correlation, although it remained significant (Spearman r=0.1701, P-value=0.035). Serum SOST was not significantly associated with prevalence or extent of AAC in any model.

DISCUSSION

In these Afro-Caribbean men, greater serum sclerostin was associated with greater coronary artery calcification. We found that serum sclerostin concentrations were associated with both increased odds of prevalent CAC and the extent of CAC measured by Agatston score, independent of potential confounding factors. On the other hand, serum sclerostin levels were not associated with abdominal aortic calcification in our study sample.

This is the first study to report a significant association between serum sclerostin and coronary artery calcifications. Previous studies have found associations with aortic or carotid plaques (22–25), although the direction of association was inconsistent. Only the study by Register *et al* used a non-contrast CT protocol that was specifically designed to assess vascular calcification in the coronary and abdominal aortic regions. Other previous studies used a secondary imaging analysis of vertebral fracture assessment (VFA) scans to identify any abdominal aortic calcification(22–24). While this validated method is widely used by studies initially intended for osteoporosis research(33), it is a semi-quantitative scale that has much lower reproducibility (ICC: 0.71–0.85 in (33)) than CT-derived calcification measures (ICC: 0.94 in the current study). In addition, since the aorta is not an intended included landmark for VFA scans, there may be greater missing calcification data in participants with greater central adiposity and, therefore, may lead to biased results. Therefore, only the present study and the previous report by Register *et al* include precise measurement of vascular calcification in both the abdominal aortic and coronary arteries.

Register *et al* found that, in contrast to our study, greater sclerostin was weakly associated with less calcified carotid plaque in diabetic African American men, and was not associated with aortic or coronary calcification. However, they only assess calcification score not prevalence. While our study participants were recruited without regard to health status, in a sensitivity analysis of only the 58 diabetic men in our study, results were similar to the whole group (data not shown). Thus, our results suggest that diabetes status alone cannot explain these conflicting results. Also of note, Register *et al* used a different assay to measure sclerostin in plasma samples, which may also explain at least some of the conflicting results. Nevertheless, our results are in agreement with two previous studies in humans which found a positive association between serum sclerostin concentrations and vascular calcification (22, 23) and with the cellular and animal data showing an up-regulation of sclerostin in calcifying vascular cells and plaques(19–21). Additionally, while we had similar power to detect associations with AAC and CAC prevalence, the findings for AAC were not statistically significant in the current study. These findings might imply that sclerostin may differentially influence vascular calcification in different vascular beds. Thus, differences in the location of vascular calcification assessment, type of measure used (visualizing plaques vs. scoring calcium deposition), and/or imaging methodology may explain why studies of sclerostin and calcification have been inconsistent.

The positive association of sclerostin, a Wnt pathway inhibitor, with vascular calcification may initially seem paradoxical. One might hypothesize that if Wnt signaling increases skeletal and ectopic mineralization, then a Wnt inhibitor would be associated with decreased mineralization. Indeed, sclerostin antibody treatment increases bone mineralization in humans (34) and individuals with deleterious mutations in the *SOST* gene display increased bone overgrowth and mineralization(35–38). Nonetheless, our findings of greater serum sclerostin being associated with greater arterial calcification is consistent with previous epidemiologic studies that have found higher concentrations of serum sclerostin are associated with greater bone mineral density(17). One hypothesis to explain this apparent paradox is that increased overproduction of sclerostin may be a physiological adaptation to increased calcification. It is also possible that serum sclerostin levels may be a marker of some other mineralization pathway with which the Wnt pathway interacts with, such as the nuclear factor κB (RANK)/ RANK ligand/ osteoprotegerin (OPG) pathway(39). The OPG pathway is also associated with vascular disease in humans(40–44). Further studies will be needed to determine which factors may play a direct role in vascular calcification.

Our study was conducted in generally healthy, Afro-Caribbean men and, therefore, cannot be generalized to women, other race/ethnicities or patient groups. Previous studies have assessed the relationship between circulating sclerostin levels and vascular calcification in specific disease states, such as diabetes(23, 25) or chronic kidney disease(24, 45, 46), which have particularly high rates of coronary artery calcification. While 31% of our study sample had diabetes, only 10 (5%) men had any impaired kidney function (eGFR<60). Thus, our study sample was fairly healthy and unlikely to have high levels of CAC compared with other high-risk population segments. Sclerostin may act differently in individuals with disorders related to mineral metabolism than in healthy individuals. Therefore, it is possible that underlying disease state, along with different methods and locations for CT assessment and different types of sclerostin assays may explain some of the different findings across studies. Additionally, our study did not include measures of mineral metabolism, such as fibroblast growth factor 23, calcium, phosphate or parathyroid hormone and, thus, we were unable to assess their potential role in the association of sclerostin with coronary artery calcification. It will be important to investigate these other biochemical measures in future studies.

We have shown a positive association of serum sclerostin levels with coronary, but not aortic, artery calcification in Afro-Caribbean men. This finding is independent of confounders, including age, physical characteristics, lifestyle factors, comorbidities, cholesterol levels and kidney function, and adds to the scant and inconclusive literature on this topic. Our findings implicate the Wnt pathway in the atherogenic calcification process that occurs within the coronary arteries but suggest that this pathway may not be as important to aging-related calcification that occurs within the abdominal aorta. While involvement of the Wnt pathway in cardiovascular disease has been studied in *in vitro* cellular and animal models, the current findings highlight the need for further research on the Wnt pathway in CVD disease humans.

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Highlights

- Serum sclerostin, a Wingless pathway antagonist, levels and computed tomography of coronary and aortic artery calcification (CAC and AAC, respectively) were measured in 191 Afro-Caribbean men.
- Multivariable logistic regression models were used to assess the cross-sectional association of sclerostin with prevalent vascular calcification.
- After adjusting for risk factors, greater sclerostin level was associated with greater odds of having CAC.
- Sclerostin was not associated with AAC in any model.

Table 1

Characteristics of the Afro-Caribbean Men

Trait	Overall (N=191)	CAC Present		AAC Present	
		No (N=134)	Yes (N=57)	No (N=60)	Yes (N=131)
CAC Agatston Score*	0 (0–21.5)	0 (0–0)	85.5 (26.6–283.2)	0 (0–0)	0 (0–52.8)
AAC Agatston Score*	92.3 (0–686.0)	43.9 (0–272.0)	728.5 (55.1–1794.0)	0 (0–0)	302.3 (78.5–1085.8)
Serum SOST (pmol/L)	45.2 (15.6)	43.7 (16.5)	48.7 (12.8)	46.5 (17.4)	44.6 (14.8)
Age (years)	62.9 (8.0)	**61.2 (7.3)**	**66.9 (8.4)**	**58.0 (5.8)**	**65.2 (7.9)**
Body Mass Index (kg/m^2)	27.9 (4.2)	27.6 (4.0)	28.7 (4.7)	27.2 (3.9)	28.3 (4.4)
Total Body Fat (%)	23.8 (6.3)	23.4 (6.2)	24.9 (6.4)	22.3 (5.9)	24.5 (6.4)
Current smoking (%)	11.0	10.5	12.5	6.7	13.0
Ever Smoked (%)	35.1	31.3	44.6	30.0	37.4
>3 Drinks per week (%)	15.7	14.9	17.9	18.3	14.5
Walking >1hr per week (%)	53.9	54.5	53.6	60.0	51.1
Grip Strength (kg)	39.5 (9.0)	40.3 (8.3)	37.6 (10.3)	41.4 (9.8)	38.6 (8.5)
Diabetes (%)	30.7	27.6	36.8	21.7	34.9
Diabetes Medication (%)	23.4	20.2	29.8	15.0	27.3
Hypertension (%)	56.0	**49.3**	**71.4**	**40.0**	**63.4**
Antihypertensive Medication (%)	34.0	**25.4**	**53.6**	21.7	39.7
LDL-c (mg/dL)	131.4 (39.7)	129.9 (40.8)	135.3 (37.3)	128.2 (39.3)	132.8 (40.0)
HDL-c (mg/dL)	44.2 (12.0)	44.5 (10.7)	43.5 (15.0)	46.5 (11.0)	43.2 (12.4)
Triglycerides (mg/dL)*	94.0 (73.0–123.5)	94.0 (71.0–120.0)	96.0 (79.0–140.0)	102.5 (76.5–121.5)	93.0 (70.5–126.0)
Statin Use (%)	11.6	9.9	14.5	3.3	15.5
eGFR (ml/min/1.73m^2)	84.1 (15.7)	85.8 (15.5)	79.9 (15.5)	86.6 (14.5)	82.9 (16.2)

Characteristics shown as Mean(SD) or Frequency, as appropriate. Significant differences by CAC or AAC status are underlined ($P<0.05$) and/or **bolded** ($P<0.01$)

* Due to skewed trait median(IQR) shown

CAC: coronary artery calcification; AAC: aortic artery calcification; SOST: sclerostin; LDL-c: low-density lipoprotein cholesterol; HDL-c: high density lipoprotein cholesterol

Table 2

Association of Serum Sclerostin with Coronary and Aortic Artery Calcification in Afro-Caribbean Men

Model	Odd Ratio (95% CI) for Calcification Prevalence[#]		Spearman Correlation Coefficient for Calcification Score	
	CAC	AAC	CAC	AAC
1	**1.95 (1.30–2.91)**	0.96 (0.65–1.40)	**0.2854**	0.0816
2	**1.74 (1.15–2.63)**	0.73 (0.47–1.14)	**0.2301**	0.0018
3	**1.76 (1.13–2.76)**	0.64 (0.39–1.05)	**0.2090**	-0.0125
4	**1.61 (1.02–2.53)**	0.65 (0.39–1.10)	**0.1701**	0.0173

[#]Odds ratios are shown as the effect per 1 SD greater serum sclerostin (15.6 pmol/L) Significant differences by CAC or AAC status are underlined ($P<0.05$) and **bolded**($P<0.01$)

Model 1, Unadjusted
Model 2, Age adjusted
Model 3, Fully adjusted: Age + physical characteristics (total body fat (%), grip strength), lifestyle factors (ever smoked, alcohol consumption, walking), comorbidities (diabetes, hypertension), and cholesterol measures (LDL-c, HDL-c, triglycerides, statin use)
Model 4, Fully adjusted plus Kidney function: Model 3 + eGFR CAC: coronary artery calcification; AAC: aortic artery calcification; SOST: sclerostin