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Authors

Karger, Amy B
Steffen, Brian T
Nomura, Sarah O
et al.

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ORIGINAL RESEARCH

Association Between Homocysteine and Vascular Calcification Incidence, Prevalence, and Progression in the MESA Cohort

Amy B. Karger, MD, PhD; Brian T. Steffen, PhD; Sarah O. Nomura, PhD; Weihua Guan, PhD; Parveen K. Garg, MD, MPH; Moyses Szklo, MD, MPH; Matthew J. Budoff, MD; Michael Y. Tsai, PhD

Background—While elevated homocysteine has been associated with calcification in several studies, its importance as a cardiovascular risk factor remains unclear. This study examines the relationship between homocysteine and vascular and valve calcification in the MESA (Multi-ethnic Study of Atherosclerosis) cohort.

Methods and Results—MESA participants with baseline homocysteine measurements and cardiac computed tomography scans were included (N=6789). Baseline and follow-up assessment of vascular (coronary artery [CAC], descending thoracic aorta [DTAC]) and valve (aortic valve [AVC], mitral annular [MAC]) calcification was performed. Prevalence ratio/relative risk regression was used to assess the relationship of homocysteine with prevalent and incident calcification, and multivariable logistic regression was used to assess associations between homocysteine and calcification progression. Elevated homocysteine was associated with greater relative risk of prevalent and incident CAC and incident DTAC. We also identified a strong association between elevated homocysteine and CAC and DTAC progression. Elevated homocysteine was found to confer a >2-fold increased risk of severe CAC progression (defined as ΔCAC $\geq$100/year) and an $\approx$1.5-fold increased risk for severe DTAC progression (defined as ΔDTAC $\geq$100/year).

Conclusions—To our knowledge, this is the first study demonstrating an association between elevated homocysteine and both incidence and progression of coronary and extra-coronary vascular calcification. Our findings suggest a potential role for elevated homocysteine as a risk factor for severe vascular calcification progression. Future studies are warranted to further assess the utility of homocysteine as a biomarker for vascular calcification incidence and progression.

Clinical Trial Registration—https://www.clinicaltrials.gov/. Unique identifier: NCT00005487. **(*J Am Heart Assoc*. 2020;9: e013934. DOI: 10.1161/JAHA.119.013934.)**

Key Words: calcification progression • cardiovascular disease • homocysteine • vascular calcification

Elevated plasma homocysteine (Hcy) was first proposed as a cause of vascular pathology in patients with inherited disorders of homocysteine metabolism,[1] leading to the hypothesis that individuals with mild to moderately elevated homocysteine levels may have an increased risk for vascular disease. As an amino acid with a reactive sulfhydryl group, homocysteine has been proposed to mediate vascular inflammation and damage by promoting oxidative stress secondary to reactive oxygen species accumulation,[2] which in turn leads to an increase in cardiac and vascular disease risk by promoting endothelial dysfunction, smooth muscle cell proliferation, and vascular calcification.[2,3] Consistent with this hypothesis, hyperhomocysteinemia has been associated with an increased risk for coronary heart disease (CHD), heart failure, atrial fibrillation, stroke, and mortality.[4–9]

However, the importance of homocysteine as a risk factor for heart disease remains uncertain. A recent meta-analysis concluded that methylene tetrahydrofolate reductase gene (*MTHFR*) variants associated with moderate hyperhomocysteinemia have little to no effect on CHD when unpublished data sets are analyzed, suggesting publication bias.[10] An additional meta-analysis found that folic acid supplementation, which lowers homocysteine levels, had little to no effect on CHD risk.[11] Despite this evidence, studies have demonstrated clear associations between elevated homocysteine

From the Department of Laboratory Medicine & Pathology (A.B.K., B.T.S., S.O.N., M.Y.T.), and Division of Biostatistics, School of Public Health (W.G.), University of Minnesota, Minneapolis, MN; Division of Cardiology, University of Southern California, Los Angeles, CA (P.K.G.); Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD (M.S.); Los Angeles Biomedical Research Center at Harbor-UCLA, Torrance, CA (M.J.B.).

Correspondence to: Michael Y. Tsai, PhD, 420 Delaware St. SE, Mayo Mail Code 609, Minneapolis MN 55455-0392. E-mail: tsaix001@umn.edu

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Clinical Perspective

What Is New?

- This is the first study showing an association between elevated homocysteine and both incidence and progression of coronary and extra-coronary vascular calcification.
- This study challenges prior research which has questioned homocysteine's role as a cardiovascular disease risk factor because of lack of impact of homocysteine-lowering therapies, which were administered for short time frames that may have been inadequate to halt or reverse effects of long-term homocysteine elevation.

What Are the Clinical Implications?

- Homocysteine may have a role in predicting risk for incidence and progression of vascular calcification.
- Further studies are needed to ascertain the role of homocysteine as a marker and risk factor for atherosclerosis and cardiovascular disease.

and calcification,[3,12,13] and vascular and valvular calcification are well-established risk factors for cardiovascular morbidity and mortality.[14–16]

Given the controversy about the role of homocysteine as a risk factor for development of CHD, we were interested in assessing the potential role of homocysteine in vascular disease. The objective of this study was to determine whether elevated homocysteine is associated with valvular and/or vascular calcification among participants in the MESA cohort.

Methods

Study Population

The objectives and design of MESA (Multi-Ethnic Study of Atherosclerosis) have been previously described.[17] Briefly, MESA is a prospective study involving 6814 men and women aged 45 to 85 years at baseline (2000–2002), from 4 racial/ethnic groups (white, black, Hispanic, and Chinese). Subjects were recruited from 6 field centers in the United States and were free of known cardiovascular disease at the time of recruitment. The primary purpose of MESA is to study the development and progression of subclinical cardiovascular disease. Our study population included all MESA participants with baseline homocysteine measurements and baseline cardiac computerized tomography (CT) scans (n=6789). Study participants gave informed consent, and institutional review board approval was given at all MESA sites. The data, analytic methods, and study materials will not be made available to other researchers for purposes of reproducing the results or replicating the procedure.

Laboratory Measurement of Plasma Homocysteine and Other Blood Biomarkers

Plasma homocysteine was measured with high performance liquid chromatography, using fluorometric detection. The assay's coefficient of variation (CV) was 3.8%. Total cholesterol was measured in EDTA plasma with the cholesterol oxidase method on the Roche COBAS analyzer (Roche Diagnostics) at the University of Minnesota Advanced Research and Diagnostic Laboratory, with an assay CV of 1.6%. High-density lipoprotein (HDL) cholesterol was measured by the same method after precipitation of non-HDL cholesterol with magnesium/dextran, with an assay CV of 2.9%. CRP (C-reactive protein) was measured with a BNII nephelometer (Dade Behring Inc) at the Laboratory for Clinical Biochemistry Research at the University of Vermont with an assay CV of 3.6%.

CT Imaging for Assessment of Arterial and Valvular Calcification

Quantitation of coronary artery calcification (CAC), descending thoracic aorta calcification (DTAC), aortic valve calcification (AVC) and mitral annular calcification (MAC) have been described previously, and all were assessed using cardiac computed tomography (CT) with the Agatston scoring method.[18,19] Ascending thoracic aorta calcification (ATAC) was not included in the analysis because of the low prevalence in the MESA cohort.[20] All cardiac CT scans were read at the MESA central reading center (Los Angeles Biomedical Research Institute, at Harbor-UCLA, Torrance, California).

Cardiac CT scans were performed at baseline and follow-up visits on participants. Specifically, the first follow-up scans were performed at visit 2 (n=2914) or visit 3 (n=2925),[21] occurring an average of 2.5 years after baseline scans, with CAC, DTAC, AVC, and MAC assessed. A prior study assessing risk factors for CAC in MESA characterized the individuals with missing follow-up cardiac CT scans, and found that they were slightly older, more likely to have CAC at baseline, had higher systolic blood pressure, increased prevalence of diabetes mellitus, and more likely to be a current smoker.[22] Visit 4 scans for CAC were assessed in a subset of participants (n=1349), preferentially targeting those that had missed a visit 3 scan.[21] At visit 5, an average of 10 years after baseline scans, CAC was measured an additional time in participants (n=3304).[21]

Measurement of Other Covariates

Standardized questionnaires were used to collect demographic and medication use history, including age, sex, education, hypertension medication use, lipid lowering medication use, smoking status, and alcohol use.[17] Body mass index parameters (weight and height) and blood pressure were measured by trained clinical site staff, and cholesterol

and HDL-C measurements were performed on blood samples collected from participants.[17] Diabetes mellitus status was defined as a fasting glucose of ≥126 mg/dL, self-reported physician diagnosis, or use of insulin and/or diabetes mellitus medications, as previously described.[23]

Statistical Analysis

Statistical analysis was conducted using STATA 15.0 (College Station, TX). To obtain a robust association, homocysteine was examined as either tertiles or a dichotomous variable using a current clinical reference value (Hcy>12 μmol/L), which is approximately the 87th percentile in the MESA data at baseline. Calcification outcomes (CAC, AVC, MAC, DTAC) were treated as binary variables (0=Agatston score of 0 or 1=Agatston score>0) in prevalence and incidence models. Prevalence and incidence ratios were estimated using relative risk regression (log-link) for associations between homocysteine and calcification outcomes with adjustments for age, race/ethnicity, sex, education, clinic site, body mass index, hypertension, diabetes mellitus, cigarette smoking, total cholesterol, HDL-C, creatinine-based estimated glomerular filtration rate ($eGFR_{cr}$), statin use, and CRP. CAC or DTAC progression was evaluated as the change from baseline measure to the last examination with CAC or DTAC scans, normalized with the follow-up time (years). Progression status for CAC was categorized into 3 groups: no progression (defined as ΔCAC ≤0/year), moderate progression (defined as 100>ΔCAC>0/year) and severe progression (defined as ΔCAC ≥100/year). Progression of DTAC was categorized in the same manner as CAC. Association between CAC/DTAC progression and homocysteine was evaluated using multinomial logistic regression adjusted for the same covariates previously described.

Results

Demographic, lifestyle, and clinical characteristics of 6789 MESA participants were evaluated by the clinical homocysteine cutoff of Hcy>12 μmol/L (Table 1) and by homocysteine tertiles (Table 2). Individuals with higher homocysteine levels were more likely to be older, men, less educated, taking hypertension or lipid-lowering medications, to have elevated systolic blood pressure, lower estimated glomerular filtration rate, diabetes mellitus, elevated total cholesterol and HDL, and increased prevalence of CAC, AVC, MAC, and DTAC.

Homocysteine-related prevalence and incidence ratios for prevalent and incident calcification, respectively, are reported in Table 3, adjusted for age, race, sex, education, clinic site, body mass index, hypertension, diabetes mellitus, cigarette smoking, total cholesterol, HDL-C, $eGFR_{cr}$, statin use, and CRP. Homocysteine levels above the clinical cut-off of 12 μmol/L were associated with prevalent CAC (prevalence ratio= 1.07, 95% CI 1.02–1.12), as were homocysteine levels in the second and third tertiles (prevalence ratio=1.08, 95% CI 1.02–1.15 and prevalence ratio=1.11, 95% CI 1.05–1.17 respectively). Elevated

Table 1. Demographic, Lifestyle, and Clinical Characteristics of 6789 MESA Participants Stratified by a Clinical Homocysteine Cutoff Level of 12.0 μmol/L

Variable	Hcy≤12 (n=5889)	Hcy>12 (n=900)
Age, mean (SD)	61.4 (10.0)	67.0 (10.2)
Sex, n (% women)	3260 (55.4)	326 (36.2)
Education: highest level, n (%)		
<High school	1004 (17.1)	218 (24.2)
Completed high school/GED	1048 (17.9)	182 (20.2)
Some college, <4 y degree	1710 (29.1)	217 (24.1)
Bachelor's degree	1023 (17.4)	146 (16.2)
Graduate or professional school	1082 (18.4)	136 (15.1)
Race, n (%)		
White	2287 (38.8)	326 (36.2)
Chinese	704 (12.0)	98 (10.9)
Black	1591 (27.0)	289 (32.1)
Hispanic	1307 (22.2)	187 (20.8)
Cigarette smoking status, n (%)		
Former	2148 (36.6)	332 (36.9)
Current	741 (12.6)	140 (15.6)
Alcohol intake, n (%)		
Former	1361 (23.3)	256 (28.6)
Current	3272 (56.0)	465 (51.9)
BMI (kg/m^2), mean (SD)	28.3 (5.5)	28.4 (5.4)
Systolic blood pressure (mm Hg), mean (SD)	125.7 (21.0)	132.4 (23.3)
Hypertension medication, n (%)	2033 (34.5)	489 (54.3)
GFR (mL/min), mean (SD)	82.8 (16.7)	70.8 (25.0)
CRP (mg/L), median (Q1, Q3)	1.9 (0.8, 4.3)	2.0 (0.8, 4.2)
Diabetes mellitus, n (%)	703 (11.9)	161 (17.9)
Lipid-lowering medication, n (%)	905 (15.4)	189 (21.0)
Total cholesterol (mg/dL), mean (SD)	194.8 (35.3)	189.6 (38.0)
HDL-C (mg/dL), mean (SD)	51.3 (14.8)	49.1 (14.7)
Prevalent CAC, n (%)	2779 (47.2)	607 (67.4)
Prevalent AVC, n (%)	701 (11.9)	209 (23.2)
Prevalent MAC, n (%)	491 (8.3)	149 (16.6)
Prevalent DTAC, n (%)	1481 (25.1)	364 (40.5)

AVC indicates aortic valve calcification; BMI, body mass index; CAC, coronary artery calcification; CRP, C-reactive protein; DTAC, descending thoracic aorta calcification.; GED, General Education Development test; GFR, glomerular filtration rate; Hcy, Homocysteine; HDL-C, high-density lipoprotein cholesterol; MAC, mitral annular calcification; MESA, Multi-Ethnic Study of Atherosclerosis.

Table 2. Demographic, Lifestyle, and Clinical Characteristics of 6789 MESA Participants Stratified by Tertiles of Homocysteine

Variable	Homocysteine Tertile		
	1	2	3
Hcy, μmol/L (range)	3.2 to 7.7	7.8 to 9.8	9.9 to 118
Age, mean (SD)	58.5 (9.4)	62.3 (9.9)	65.7 (10.1)
Sex, n (% women)	1590 (69.9)	1122 (48.8)	874 (39.5)
Education: highest level, n (%)			
<High school	401 (17.7)	366 (16.0)	455 (20.6)
Completed high school/GED	398 (17.5)	422 (18.4)	410 (18.6)
Some college, <4-y degree	661 (29.1)	638 (27.9)	628 (28.5)
Bachelor's degree	400 (17.6)	403 (17.6)	366 (16.6)
Graduate or professional school	410 (18.1)	462 (20.2)	346 (15.7)
Race, n (%)			
White	835 (36.7)	938 (40.8)	840 (38.0)
Chinese	323 (14.2)	252 (11.0)	227 (10.3)
Black	560 (24.6)	619 (26.9)	701 (31.7)
Hispanic	558 (24.5)	492 (21.4)	444 (20.1)
Cigarette smoking status, n (%)			
Former	745 (32.8)	855 (37.3)	880 (39.9)
Current	269 (11.9)	298 (13.0)	314 (14.2)
Alcohol intake, n (%)			
Former	512 (22.7)	527 (23.1)	578 (26.3)
Current	1225 (54.3)	1329 (58.2)	1183 (53.8)
BMI (kg/m^2), mean (SD)	28.0 (5.7)	28.5 (5.3)	28.5 (5.4)
Systolic blood pressure (mm Hg), mean (SD)	122.5 (20.6)	126.7 (21.1)	130.7 (22.0)
Hypertension medication, n (%)	616 (27.1)	839 (36.5)	1067 (48.3)
GFR (mL/min), mean (SD)	88.0 (16.5)	81.4 (15.5)	74.0 (20.5)
CRP (mg/L), median (Q1, Q3)	1.9 (0.8, 4.5)	1.9 (0.8, 4.3)	1.9 (0.8, 4.1)
Diabetes mellitus, n (%)	254 (11.2)	264 (11.5)	346 (15.6)
Lipid-lowering medication, n (%)	321 (14.1)	358 (15.6)	415 (18.8)
Total cholesterol (mg/dL), mean (SD)	195.5 (34.5)	195.2 (35.9)	191.6 (36.7)
HDL-C (mg/dL), mean (SD)	52.9 (15.1)	50.7 (14.7)	49.3 (14.4)
Prevalent CAC, n (%)	837 (36.8)	1172 (50.9)	1377 (62.3)
Prevalent AVC, n (%)	184 (8.1)	267 (11.6)	459 (20.8)
Prevalent MAC, n (%)	128 (5.6)	202 (8.8)	310 (14.0)
Prevalent DTAC, n (%)	424 (18.6)	634 (27.6)	787 (35.6)

AVC indicates aortic valve calcification; BMI, body mass index; CAC, coronary artery calcification; CRP, C-reactive protein; DTAC, descending thoracic aorta calcification; GED, General Education Development test; GFR, glomerular filtration rate; Hcy, Homocysteine; HDL-C, high-density lipoprotein cholesterol; MAC, mitral annular calcification; MESA, Multi-Ethnic Study of Atherosclerosis.

homocysteine above the clinical cut-off was also associated with incident CAC (RR=1.21, 95% CI 1.04–1.40) and incident DTAC (RR=1.29, 95% CI 1.01–1.64), but no associations were observed across tertiles. Additionally, homocysteine levels in the third tertile was significantly associated with prevalent mitral annular calcification (RR=1.43, 95% CI 1.12–1.82). Homocysteine-related odds ratios are reported in Table 4 for moderate (100>ΔCAC or ΔDTAC >0/year) and severe (ΔCAC or ΔDTAC ≥100/year) progression of calcification for CAC and DTAC, compared with no progression (ΔCAC or ΔDTAC ≤0/year). Elevated homocysteine was found to be significantly associated with moderate progression of CAC when evaluated using the

Table 3. Homocysteine-Related Prevalence and Incidence Ratios for Calcification Outcomes Among Participants of the MESA*

	Hcy Tertile			Hcy
Calcification Outcome	1	2	3	>12 μmol/L
Prevalent†				
Coronary artery calcium	ref	1.08 (1.02–1.15) 0.007‡	1.11 (1.05–1.17) <0.001‡	1.07 (1.02–1.12) 0.003‡
Aortic valve calcification	ref	0.92 (0.77–1.11) 0.39	1.15 (0.96–1.38) 0.14	1.13 (0.97–1.32) 0.12
Mitral annular calcification	ref	1.14 (0.89–1.47) 0.31	1.43 (1.12–1.82) 0.004‡	1.18 (0.97–1.43) 0.09
Descending thoracic aortic calcification	ref	1.09 (1.00–1.19) 0.04‡	1.07 (0.98–1.17) 0.16	1.02 (0.95–1.10) 0.57
Incident§				
Coronary artery calcium	ref	1.00 (0.89–1.12) 0.98	1.05 (0.93–1.19) 0.44	1.21 (1.04–1.40) 0.01‡
Aortic valve calcification	ref	1.15 (0.66–1.99) 0.62	1.03 (0.56–1.88) 0.94	1.07 (0.62–1.85) 0.80
Mitral annular calcification	ref	0.94 (0.56–1.56) 0.81	0.90 (0.51–1.61) 0.73	1.21 (0.60–2.43) 0.60
Descending thoracic aortic calcification	ref	0.93 (0.71–1.21) 0.57	1.00 (0.75–1.32) 0.98	1.29 (1.01–1.64) 0.04‡

Hcy indicates homocysteine; MESA, Multi-Ethnic Study of Atherosclerosis; ref, reference group.
*Relative risk regression (log-link) adjusted for age, race, sex, education, clinic site, body mass index, hypertension, diabetes mellitus, cigarette smoking, total cholesterol, high-density lipoprotein cholesterol, creatinine-based estimated glomerular filtration rate ($eGFR_{cr}$), statin use, and C-reactive protein. Individuals with missing covariate data were excluded (n=70 for prevalent-case analysis, and n=32 for incident-case analysis).
†Data shown are prevalence ratios, 95% CIs and *P* values.
‡Data with significant *P* values.
§Data shown are incidence ratios, 95% CIs and *P* values.

clinical cut-off (odds ratio [OR]=1.43, 95% CI 1.16–1.75) and for the third tertile of homocysteine levels (OR=1.26, 95% CI 1.07–1.48). Similarly, for severe progression of CAC, there was a greater than 2-fold increased odds associated with elevated homocysteine above the clinical cut-off (OR=2.21, 95% CI 1.60–3.04) or within the third tertile (OR=2.18, 95% CI 1.53–3.10). For DTAC, the association between homocysteine and moderate DTAC progression was not statistically significant, but there was a significant association between severe DTAC progression and elevated homocysteine above the clinical cut-off (OR=1.42, 95% CI 1.06–1.92), as well as for the second (OR=1.64, 95% CI 1.20–2.24) and third tertiles (OR=1.63, 95% CI 1.16–2.27).

Discussion

In this multi-ethnic cohort of black, white, Chinese, and Hispanic participants, homocysteine was significantly associated with prevalence, incidence, and progression of CAC and DTAC. To our knowledge, this is the first study to demonstrate an association between elevated homocysteine and incidence and progression of coronary and extra-coronary calcification. Additionally, null findings were largely observed in analyses of valvular calcification, suggesting that the influence of homocysteine on calcification is restricted to vessels rather than valves.

It has been previously established that CAC and DTAC are tightly linked and share similar risk factor profiles for progression in the MESA cohort.[20,22,24] Thus, our finding of significant associations between elevated homocysteine and both types of vascular calcification is consistent with prior studies on MESA participants, indicating that the pathophysiological processes driving CAC and DTAC are similar. These findings have been reproduced in other cohorts as well; a recent analysis of CAC and TAC in the Heinz Nixdorf Recall study found that the main

Table 4. Homocysteine-Related Odds Ratios for Moderate-to-Severe Progression of Calcification in Coronary Arteries and the Descending Thoracic Aorta*†

	Homocysteine Tertile			Hcy
Calcification Progression	1	2	3	>12 μmol/L
CAC progression rate‡§				
100>ΔCAC >0/y	ref	1.13 (0.98–1.30) 0.10	1.26 (1.07–1.48) 0.007‖	1.43 (1.16–1.75) <0.001‖
ΔCAC ≥100/y	ref	1.33 (0.94–1.89) 0.11	2.18 (1.53–3.10) <0.001‖	2.21 (1.60–3.04) <0.001‖
DTAC progression rate§¶				
100>ΔDTAC >0/y	ref	1.04 (0.87–1.26) 0.66	1.05 (0.86–1.29) 0.63	1.19 (0.96–1.47) 0.12
ΔDTAC ≥100/y	ref	1.64 (1.20–2.24) 0.002‖	1.63 (1.16–2.27) 0.004‖	1.42 (1.06–1.92) 0.02‖

CAC indicates coronary artery calcification; DTAC, descending thoracic aorta calcification; Hcy, homocysteine; ref, reference group.
*Multinomial logistic regression adjusted for age, race, sex, education, clinic site, body mass index, hypertension, diabetes mellitus, cigarette smoking, total cholesterol, high-density lipoprotein cholesterol, creatinine-based estimated glomerular filtration rate ($eGFR_{cr}$), statin use, and C-reactive protein. Individuals with missing covariate data were excluded (n=61).
†Respective analyses include participants with baseline measurements and at least 1 follow-up measurement of CAC (n=5992) and DTAC (n=5811).
‡CAC progression rate, cases: 100>ΔCAC >0/year (3366 cases) or ΔCAC >100/year (408 cases).
§Data shown are odds ratios, 95% CIs and *P* values.
‖Data with significant *P* values.
¶DTAC progression rate, cases: 100>ΔDTAC >0/year (1171 cases) or ΔDTAC >100/year (444 cases).

determinants for CAC and TAC incidence and progression were predominantly the same.[25]

In contrast to vascular calcification, the associations between homocysteine and valvular calcification were largely non-significant, indicating that the pathophysiologic mechanism for valvular calcification differs from that of vascular calcification as had been reported previously.[26,27] Specifically, a recent study using positron emission tomography and CT to measure active calcification and inflammation in the aortic valve, aorta and coronary arteries found that active inflammation was more pronounced in areas of vascular calcification than in calcific valves.[26]

While elevated homocysteine has been previously associated with prevalent coronary calcification, no study has demonstrated an association between incident coronary calcification and elevated homocysteine. Additionally, there has been only 1 small study (n=133) demonstrating a significant association between elevated homocysteine and coronary artery calcification progression.[28] However, this prior study did not show an association between elevated homocysteine and CAC prevalence or incidence, nor did it assess extra-coronary calcification. Therefore, to our knowledge, our study is the first to demonstrate significant associations between homocysteine and prevalence, incidence and progression of both coronary and extra-coronary calcification. Prior in vitro studies provide mechanistic support for the associations observed in the present study, with several studies demonstrating an association between homocysteine, inflammation, and calcification.[2,29,30] Specifically, in vitro studies have demonstrated that elevated homocysteine increases reactive oxygen species production in microvascular endothelial cells, leading to induction of inflammatory responses mediated by nuclear factor-κB.[2,31] Additionally, homocysteine has been shown to promote osteogenic differentiation and calcium deposition in vitro, and homocysteine was found at higher levels in calcified human atheroma, when compared with non-calcified biopsies.[29] Therefore, we hypothesize that elevated homocysteine is a contributor to this pathophysiologic process of vascular inflammation and damage that leads to vascular calcification but does not play a significant role in valvular calcification.

There is currently considerable debate in the literature as to whether homocysteine is a causal risk factor for CHD, a risk marker for CHD, or neither. A recent Cochrane systematic review assessed 15 randomized controlled trials and concluded that they demonstrate no effects of homocysteine-lowering interventions on myocardial infarction, adverse events, or mortality.[32] Additionally, a 2012 meta-analysis of 19 unpublished data sets found that genetic variants in the methylene tetrahydrofolate reductase gene (*MTHFR*) associated with moderate hyperhomocysteinemia were not associated with an increased risk for CHD.[10] The investigators

concluded that the discrepancy between their null findings and prior studies demonstrating an association between homocysteine and CHD should be attributed to publication bias of prior studies because of positivity of results and/or methodologic issues with those earlier studies.[10] Given these recent publications, interest in homocysteine as a cardiovascular biomarker has diminished. However, the randomized controlled trials and meta-analysis have some important limitations that may have influenced their findings. First, the majority of randomized controlled trials using homocysteine-lowering interventions had short treatment and follow-up periods (≤2 years).[32,33] The longest randomized controlled trial, WAFACS (Women's Antioxidant and Folic Acid Cardiovascular Study), had a follow-up period of 7.3 years and only measured homocysteine directly in 5% of participants, and therefore could not directly identify participants with high homocysteine levels to determine whether this subgroup had a significant reduction in cardiovascular events from homocysteine-lowering vitamin regimens.[33] It is additionally important to note that WAFACS enrolled only high-risk women with known cardiovascular disease or at least 3 cardiovascular risk factors.[33] Therefore, this study likely had a high percentage of subjects with established coronary artery and/or thoracic aorta calcification. Calcification is not reversible, and therefore if homocysteine contributes to risk for vascular calcification, reduction of homocysteine would not be expected to impact outcomes in a high-risk cohort likely to already have established vascular calcification. Additionally, prior studies have demonstrated that, while homocysteine levels are influenced by both genetic and environmental factors, levels in an individual are relatively constant over time.[34,35] Therefore, it is possible that short-term reduction in homocysteine levels through B vitamin supplementation is not sufficient to reverse or impact the cardiovascular effects of lifelong, chronic elevation of homocysteine. Relatedly, the 2012 meta-analysis that found no association between hyperhomocysteinemia-associated *MTHFR* variants and CHD also did not have directly measured homocysteine levels for participants. Therefore the meta-analysis data support the conclusion that *MTHFR* variants are not associated with CHD, but are not sufficient to discount the impact of elevated homocysteine on cardiovascular outcomes.[10] In fact, a 2009 study assessed the association of *MTHFR* variants with homocysteine levels in the Coronary Artery Risk Development in Young Adults cohort, both before and after folate fortification was implemented in the United States in 1998, and found that *MTHFR* variants in the post-fortification era were neither required nor sufficient to cause hyperhomocysteinemia.[36] Relatedly, it is important to note that the MESA study occurred in the era of folate fortification, and yet 900 of 6789 MESA subjects had homocysteine levels above the clinical cut-off, with those elevated levels associated with vascular calcification in our study.

Our study does have some limitations. While CAC was assessed at multiple follow-up visits, the remaining calcification measures (DTAC, AVC, MAC) were assessed at only a single follow-up visit. Therefore, the weaker associations of elevated homocysteine with prevalent and incident DTAC and DTAC progression may be attributable to the longer follow-up period for CAC. Assessing visit 4 and 5 scans for DTAC progression would determine whether a longer follow-up period strengthens associations between elevated homocysteine and DTAC incidence and progression. An additional limitation of the study is that homocysteine was measured at the baseline visit only, and not measured at follow-up visits in parallel with calcification assessments. However, as stated previously, prior studies have demonstrated relative stability of homocysteine over time and therefore lack of repeat homocysteine measurements is unlikely to impact our results.[34,35]

Conclusions

This study confirms prior research demonstrating an association between homocysteine and vascular calcification prevalence and demonstrates a new potential role for homocysteine as a biomarker for both coronary and extra-coronary vascular calcification incidence and progression. CAC and DTAC progression have been shown to be associated with increased risk for all-cause mortality, hard and total CHD events,[37–39] and therefore a biomarker that identifies risk for vascular calcification progression would be a valuable tool for providers to more accurately risk stratify their patients. While recent literature has concluded that homocysteine is not associated with increased risk for cardiovascular disease, these conclusions may be premature, as the majority of these studies failed to directly measure homocysteine in participants and instead correlated *MTHFR* variants or B vitamin supplementation with cardiovascular end points. Therefore, future research is needed in large cohorts with direct homocysteine measurement, not only to confirm our novel findings, but to further clarify the role of homocysteine in cardiovascular disease.

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Disclosures

None.

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