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ORIGINAL RESEARCH

Coronary Microvascular Endothelial Dysfunction in Patients With Angina and Nonobstructive Coronary Artery Disease Is Associated With Elevated Serum Homocysteine Levels

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BACKGROUND: Elevated levels of serum homocysteine, via impaired nitric oxide production, and coronary microvascular dysfunction are associated with increased risk of major adverse cardiovascular events. However, whether serum homocysteine levels and coronary microvascular endothelial dysfunction (CMED) are linked remains unknown.

METHODS AND RESULTS: This study included 1418 patients with chest pain or an abnormal functional stress test and with nonobstructive coronary artery disease (<40% angiographic stenosis), who underwent CMED evaluation with functional angiography and had serum homocysteine levels measured. Patients were classified as having normal microvascular function versus CMED. Patients in the CMED group (n=743; 52%) had higher mean age (52.1±12.2 versus 50.0±12.4 years; $P<0.0001$), higher body mass index (29.1 [25.0–32.8] versus 27.5 [24.2–32.4]; $P=0.001$), diabetes mellitus (12.5% versus 9.4%; $P=0.03$), and fewer women (63.5% versus 68.7%; $P=0.04$) compared with patients in the normal microvascular function group. However, they had lower rates of smoking history, and mildly lower low-density lipoprotein cholesterol levels. Serum homocysteine levels were significantly higher in patients with CMED, and the highest quartile of serum homocysteine level (>9 μmol/L) was an independent predictor of CMED (odds ratio, 1.34 [95% CI, 1.03–1.75]; $P=0.03$) after adjustment for age; sex; body mass index; chronic kidney disease (CKD); diabetes mellitus; smoking exposure; low-density lipoprotein cholesterol; high-density lipoprotein cholesterol and triglycerides; and aspirin, statin, and B vitamin use.

CONCLUSIONS: Patients with CMED have significantly higher levels of serum homocysteine. Elevated serum homocysteine levels were associated with a significantly increased odds of an invasive diagnosis of CMED. The current study supports a potential role for homocysteine for diagnosis and target treatment in the patients with early coronary atherosclerosis.

Key Words: endothelial dysfunction ■ homocysteine ■ microvascular

Coronary endothelial dysfunction is the earliest clinically detectable form of coronary atherosclerosis. Sixty percent of patients presenting with angina and nonobstructive coronary artery disease (CAD) at clinically indicated coronary angiography have coronary endothelial dysfunction detected with pharmacologic provocation testing.[1–3] Coronary microvascular endothelial dysfunction (CMED) has been associated with increased mortality and a higher risk of major adverse cardiovascular events, including myocardial infarction, progressive congestive heart failure, and sudden cardiac death.[4–10] CMED was also

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CLINICAL PERSPECTIVE

What Is New?

- Coronary endothelial microvascular dysfunction, a marker of early atherosclerosis, is associated with elevated levels of serum homocysteine.
- High-normal levels of homocysteine were associated with coronary endothelial microvascular dysfunction, as diagnosed invasively by acetylcholine provocative testing, even after adjusting for cardiovascular risk covariables.
- In subpopulation analysis, there was no sex difference for this relationship; this association was also augmented in patients with elevated levels of serum homocysteine and taking B-vitamin supplementation.

What Are the Clinical Implications?

- The established link between serum homocysteine and adverse cardiovascular effects might be mediated through coronary microvascular endothelial dysfunction.
- Further investigations are needed to establish a causal link and to assess if baseline serum homocysteine levels might help in risk stratification of patients at risk for future major adverse cardiovascular events.

Nonstandard Abbreviations and Acronyms

CBF	coronary blood flow
CMED	coronary microvascular endothelial dysfunction

shown to be associated with vulnerable plaque characteristics in epicardial vessel using optical coherence tomography[11,12] and virtual-histology intravascular ultrasound studies.[13] Coronary endothelial dysfunction is a systemic disease[14–16] and is usually associated with elevated levels of systemic inflammatory markers like plasma-soluble urokinase-type plasminogen activator receptor, uric acid, and high-sensitivity C-reactive protein levels.[17–19]

Elevated serum levels of homocysteine, which could be caused by an inherited deficiency of cystathionine synthase or other enzymes, have been linked to premature arteriosclerosis and increased risk of cardiovascular diseases such as CAD, peripheral arterial disease, stroke, venous thrombosis, and gestational hypertension.[20–28] Furthermore, in a study of patients with stable CAD, hyperhomocysteinemia was also found to be an independent predictor of cardiac death.[29]

Several in vitro studies displayed a direct adverse effect of homocysteine on endothelial function, mainly by reducing nitric oxide activity and increasing local oxidative stress, leading to endothelial dysfunction.[30]

We therefore hypothesize that elevated serum homocysteine levels could be associated with CMED. The aim of this study is to investigate the association between increased levels of serum homocysteine and CMED in patients with angina and nonobstructive coronary artery disease at angiography.

METHODS

A total of 1991 consecutive patients with chest pain referred for clinically indicated coronary angiography and functional assessment, between 1992 and 2019, who were found to have angiographically normal coronary arteries or mild CAD (<40%) were enrolled in the Mayo Clinic Endothelial Database. Of these, 1418 patients with serum homocysteine levels taken up to 2 weeks before the index coronary angiogram were included in this study. Patients with acute coronary syndrome, myocardial infarction, or cerebrovascular accident within the preceding 6 months, use of radiographic contrast agents within 12 hours of the catheterization, and advanced chronic kidney disease (CKD) (glomerular filtration rate <30 mL/min per m^2) were excluded. All patients fasted for at least 8 hours and withheld all prescription medications that could affect coronary vasoreactivity for at least 48 hours before the study procedure (calcium channel blockers, beta blockers, nitrates). The study was conducted in accordance with the guidelines of the Declaration of Helsinki and was approved by the Mayo Clinic Institutional Review Board. All patients provided written informed consent for participation in the current protocol. The data that support the findings of this study are available from the corresponding authors upon reasonable request.

Functional Angiography

The study protocol has been previously described in detail.[31–38] In brief, patients underwent diagnostic coronary angiography using standard clinical protocols. Those with no CAD or nonobstructive CAD (angiographic stenosis <40%) went on to receive 5000 intravenous units of heparin, after which a Doppler guidewire (FloWire, Philips/Volcano Corp., San Diego, CA) was positioned in the mid–left anterior descending coronary artery. Intracoronary acetylcholine was infused at incremental doses for 3 minutes each with increasing concentrations of 10^{-6}, 10^{-5}, and 10^{-4} mol/L to assess endothelial function. Doppler measurements of mean peak velocity were performed after each acetylcholine infusion followed

by repeat coronary angiography. Mid–left anterior descending coronary artery diameter was measured by an independent investigator in the segment 5 mm distal to the tip of the Doppler wire using a quantitative coronary angiography program (Medis Corp, Leiden, The Netherlands). Coronary blood flow (CBF) was then calculated using the following formula, as previously described:[32,33,39–41] CBF=π×(mean peak velocity/2)×(coronary artery diameter/2)^2. The maximal percent change in CBF in response to acetylcholine compared with the CBF at baseline (%ΔCBF) was then calculated, and CMED was defined as %ΔCBF <50%. This definition was predefined before analysis and is standardized as described in previous studies.[3]

Clinical Assessment

Clinical history, laboratory data, and current medications were collected from a detailed chart review by an investigator blinded to functional angiography results. Data were collected on conventional cardiovascular risk factors including age, hypertension, diabetes mellitus, hyperlipidemia, smoking status, and body mass index; biochemical parameters including serum total cholesterol, low-density lipoprotein (LDL) cholesterol, high-density lipoprotein cholesterol, triglycerides, creatinine, and glycosylated hemoglobin. Smoking was defined as positive for exposure (current or former) or never. CKD was defined as estimated glomerular filtration rate <60 mL/min per 1.73m^2. All blood levels documented had been drawn within 6 weeks of the index procedure. Dyslipidemia was defined by a documented history of hyperlipidemia, treatment with lipid-lowering therapy, an LDL cholesterol level above the target (<130 mg/dL for low-risk patients, <100 mg/dL for moderate-high-risk patients, <70 mg/dL for very-high-risk patients, and <55 mg/dL for extreme-high-risk patients on the basis of 10-year atherosclerotic cardiovascular disease risk), high-density lipoprotein cholesterol <40 mg/dL in men or <50 mg/dL in women, or triglycerides >150 mg/dL. Type 2 diabetes mellitus was defined as a documented history of or treatment for type 2 diabetes mellitus, or a glycosylated hemoglobin of >6.5%, if available. Hypertension was defined as a documented history of or treatment of the condition, a systolic blood pressure measurement of >130 mm Hg, or diastolic blood pressure measurement >90 mm Hg.[42]

Statistical Analysis

Continuous variables distributed normally were expressed as the mean±standard deviation, and those with a skewed distribution were expressed as the median with interquartile range (median [Q25–Q75]). Categorical variables were expressed as frequency (percentage). For between-group comparisons, unpaired t test was used for normally distributed continuous variables, Mann–Whitney *U* test for nonnormally distributed variables, and χ^2 test (or Fisher's exact test) for categorical variables. Univariable logistic regression analyses were performed to study the association between CMED and serum homocysteine levels. Multivariable logistic regression analyses were performed to estimate the independent association between serum homocysteine levels in the highest quartile and CMED. The covariables included were those that showed a trend of difference (P<0.25) in baseline characteristics between the CMED and non-CMED group. We also decided a priori to include CKD[43,44] and B-vitamin (B_6, B_9, and B_{12})[45] and statin use[28,46] in all adjusted models since they were previously shown to affect serum homocysteine levels and endothelial function, thus acting as potential confounders. We also examined the frequency of B-vitamin consumption and statin use in our population and sought to compare the association of serum homocysteine and CMED in patients on these supplements/medications versus those who were not. For all tests, a P<0.05 was considered statistically significant. All statistical analyses were performed using JMP Pro software (SAS Institute, Inc., Cary, NC).

RESULTS

Baseline Characteristics

Baseline patients' characteristics, categorized as CMED versus normal microvascular endothelial function, are summarized in Table 1. Of 1418 patients, 743 (52%) had CMED (%ΔCBF, −4 [−31 to −22]), while 675 had normal coronary microvascular function (%ΔCBF, 110 [76–161]) (P<0.0001). The distribution of homocysteine in our population was skewed with all but 6 patients having levels <30 μmol/L. Patients with CMED were more likely to have cardiovascular risk factors such as age, male sex, obesity, or diabetes mellitus. However, they had lower rates of smoking history, and mildly lower LDL cholesterol levels. Serum homocysteine levels were also higher in the CMED group as compared with the normal microvascular function group (8 [6–10] versus 8 [6–9]; P=0.006) (Figure 1).

Impact of Serum Homocysteine Levels on Coronary Microvascular Endothelial Function

There was a significant association between quartiles (first quartile ≤6; second quartile >6 to ≤8; third quartile >8 to ≤9; fourth quartile >9) of serum homocysteine levels

Table 1. Baseline Characteristics of Patients With Normal Microvascular Function Versus CMED as Measured by Percent Change or Coronary Blood Flow With Successive Intracoronary Acetylcholine Infusion

	Coronary Microvascular Endothelial Function		
	Normal N=675	Abnormal N=743	*P* Value
Clinical parameters			
Age, y	50±12.4	52.1±12.2	0.0003
Female sex, n (%)	464 (68.7)	472 (63.5)	0.04
Body mass index, kg/m^2	27.5 (24.2; 32.4)	29.1 (25.0; 32.8)	0.001
Hypertension, n (%)	545 (80.5)	606 (81.6)	0.69
Systolic BP, mm Hg	124±17	124±17	0.56
Diastolic BP, mm Hg	75±10	75±10	0.88
Diabetes mellitus, n (%)	60 (8.9)	98 (13.2)	0.01
HbA_{1c} (%)	5.3 (5.0 to 5.6)	5.4 (5.1 to 5.7)	0.01
Dyslipidemia, n (%)	392 (48.0)	437 (58.8)	0.77
LDL cholesterol, mg/dL	104±37	100±35	0.02
HDL cholesterol, mg/dL	55±18	53±17	0.08
Triglycerides, mg/dL	102 [70; 153]	107 [76; 158]	0.14
Creatinine, mg/dL	0.94±0.28	0.97±0.26	0.07
CKD, n (%)	74 (11.0)	95 (12.8)	0.3
Smoking history, n (%)	332 (49.3)	323 (43.5)	0.03
ΔCBF (%)	110 (76; 161)	−4 (−31 to 22)	<0.0001
Homocysteine, μmol/L	8 (6 to 9)	8 (6 to 10)	0.006
Medications			
Aspirin, n (%)	344 (51.0)	411 (55.3)	0.1
Statin, n (%)	253 (37.5)	336 (45.2)	0.003
Antihypertensives, n (%)	431 (63.9)	494 (66.5)	0.3
Antidiabetics, n (%)	36 (5.3)	69 (9.3)	0.01
Diuretics, n (%)	108 (16.0)	129 (17.4)	0.5
B-vitamin use, n (%)	160 (23.7)	184 (24.8)	0.64

BP indicates blood pressure; CKD, chronic kidney disease; CMED, coronary endothelial microvascular dysfunction; HbA1c, glycosylated hemoglobin; HDL, high-density lipoprotein; LDL, low-density lipoprotein; and ΔCBF, blood flow change.

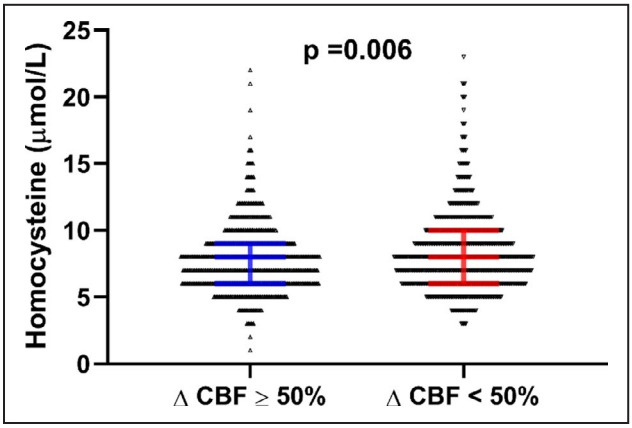

Figure 1. Difference in homocysteine levels between patients with normal coronary microvascular endothelial function and patients with CMED.
CBF indicates coronary blood flow; and CMED, coronary microvascular endothelial dysfunction.

and CMED (*P*=0.02) is shown in Figure 2. Patients in the highest quartile of serum homocysteine (>9 μmol/L) had a significantly higher prevalence of CMED as compared with those in the lowest quartile (<6 μmol/L), with an unadjusted odds ratio (OR) of 1.5 [1.2–2.1; *P*=0.0003). There were no significant differences in the frequency of CMED between the first, second, and third quartiles. Therefore, the first 3 quartiles were grouped together and were compared with the group with the highest quartile of serum homocysteine levels.

Differences between patients in the highest-quartile group versus the lower-3-quartiles group are outlined in Table 2. Patients in the highest-quartile group tended to be older, included more males, and had more hypertension, CKD, and smoking exposure. Furthermore, they had higher glycosylated hemoglobin levels and were on antidiabetics (insulin or oral hypoglycemics) more than those in other quartiles. However, they took fewer B vitamins and more diuretics than the group with serum homocysteine levels ≤9. Univariable logistic regression analysis (Table 3) showed that homocysteine levels in the highest quartile were significantly associated with CMED (OR, 1.46 [1.14–1.87]; *P*=0.003). In multivariable logistic regression analysis, elevated serum homocysteine levels remained a significant predictor of CMED (OR, 1.34 [1.03–1.75]; *P*=0.031) after adjusting for age, sex, body mass index, CKD, diabetes mellitus, smoking exposure, aspirin use, B-vitamin use, statin use, LDL cholesterol, high-density lipoprotein cholesterol, and triglycerides. Subgroup univariable regression analysis based on sex revealed that elevated serum homocysteine levels are a significant predictor of CMED in males (OR, 1.50 [1.02–2.20]; *P*=0.04) and borderline significant in females (OR, 1.36 [0.98–1.89]; *P*=0.06; $P_{interaction}$=0.71). In multivariable regression analysis, elevated levels of serum homocysteine was predictive of CMED in men (OR 1.55 [1.01–2.39]; *P*=0.047) but not in women (OR, 1.21 [0.85–1.71]; *P*=0.29; $P_{interaction}$=0.49) (Table 3). However, the $P_{interaction}$ is nonsignificant for both the unadjusted and adjusted models, which means that there is no difference in the association of homocysteine and CMED between the men and women.

Stratification by Medication/ Supplementation Use

Several medications, such as diuretics, statins, and B vitamins, affect the levels of homocysteine and thus the

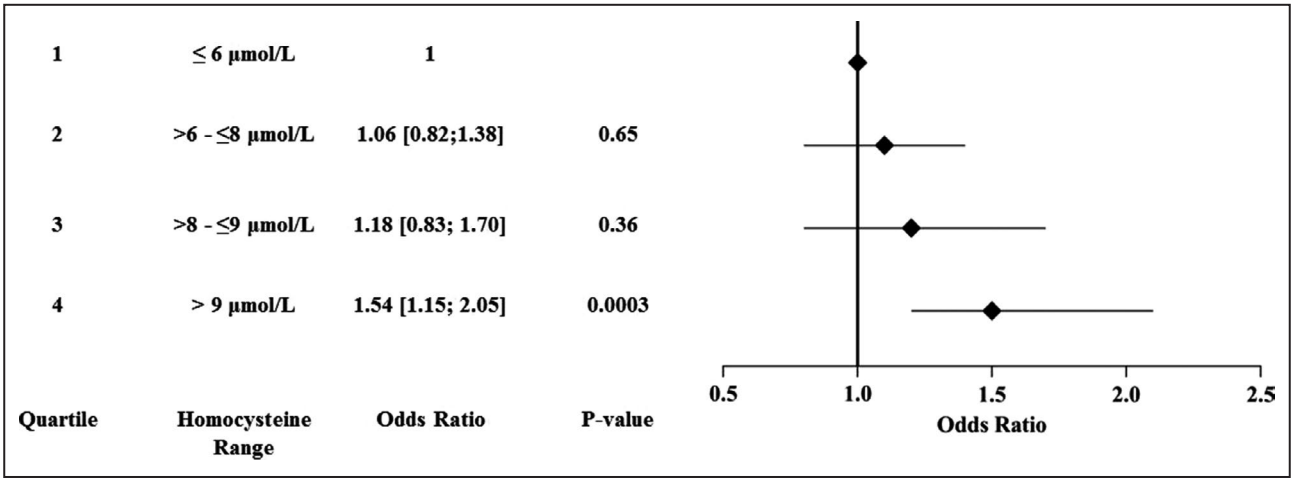

Figure 2. Association of homocysteine levels (by quartiles) and impaired blood flow reaction to acetylcholine infusion.

same relationship between serum homocysteine and CMED was investigated after stratification of medication use. The difference in baseline characteristics between patients taking B-vitamin supplementation and those who were not are summarized in Table S1. Patients on B-vitamin supplementation were generally older, included more women, and had more dyslipidemia. Patients on B-vitamin supplementation had significantly lower levels of homocysteine (7 [6–9] versus 8 [6–10] µmol/L; $P<0.0001$) than those not on a supplementation. As shown in Table 4, in univariable logistic regression analysis, patients with serum levels of homocysteine in the highest quartile who were consuming B vitamins were more likely to have CMED (OR, 3.35 [1.72–6.50]; $P=0.0004$) ($P_{interaction}=0.005$). After adjusting for age, sex, body mass index, CKD, diabetes mellitus, smoking exposure, aspirin use, statin use, LDL cholesterol, high-density lipoprotein cholesterol, and triglycerides, the association remained significant (OR, 2.72 [1.35–5.47], $P=0.005$; $P_{interaction}=0.027$). As for statin use, univariable and multivariable analyses in Table 4 showed that the association between serum homocysteine levels in the highest quartile and CMED was only present in patients not on any statin therapy at the time of testing. However, this difference is not significant as the $P_{interaction}$ was not significant between the 2 groups. The association between elevated serum homocysteine levels and CMED was not different in patients on diuretics versus those who are not, in both unadjusted (OR, 1.86 [1.09–3.18]; $P=0.02$ versus OR, 1.36 [1.02–1.80], $P=0.03$; $P_{interaction}=0.31$) or adjusted models (OR, 1.89 [1.03–3.47]; $P=0.04$ versus OR, 1.27 [0.94–1.56]; $P=0.13$; $P_{interaction}=0.44$).

DISCUSSION

In this study, we demonstrated that elevated serum homocysteine levels in patients with early coronary atherosclerosis are associated with endothelial dysfunction. The current study further supports a role for homocysteine in the mechanism, and potentially a therapeutic target, of early coronary atherosclerosis in humans. Interestingly, the association between elevated levels of serum homocysteine and CMED seemed to be augmented in patients on B-vitamin supplementation, while this association was not statistically different between patients on statin or diuretic therapy as compared with those who are not.

Homocysteine is not obtained from diet but rather biosynthesized from essential amino acid methionine via a demethylation multistep process. Homocysteine plays an important mediator role in several basic metabolic processes. It could be reused in the methionine cycle, with the help of folate (B_9) and cobalamin (B_{12}), which is important for S-adenosyl methionine production, an essential methylation agent used in several pathways. Alternatively, it could be irreversibly converted to cysteine, via transsulfuration assisted by pyridoxine (B_6), to be used in protein synthesis and other biochemical processes. Through these mechanisms, homocysteine levels are maintained at a safe range with constant turnover and little accumulation. Therefore, the lack of B vitamins as well as other genetic variations affecting these enzymatic pathways could lead to hyperhomocysteinemia. In this study, we demonstrated a clear association between the highest quartile of serum homocysteine levels and coronary microvascular endothelial function, a feature of early atherosclerosis. The chronic effects of elevated homocysteine were previously shown in a case-control study where a modest increase of homocysteine was associated with an increased risk of vascular diseases, including coronary, cerebrovascular, and peripheral artery disease.[47] This is the first study to demonstrate the association between modest elevations of

Table 2. Baseline Characteristics of Patients With Serum Homocysteine Level >9 μmol/L vs Those ≤9 μmol/L

	Homocysteine (μmol/L)		
	≤9 N=1075	>9 N=343	*P* Value
Clinical parameters			
Age, y	49.9±12.2	54.8±11.8	<0.0001
Female sex, n (%)	757 (70.2)	182 (52.9)	<0.0001
Body mass index, kg/m^2	27.9 (24.3 to 32.6)	29.1 (25.3 to 32.9)	0.02
Hypertension, n (%)	860 (80.0)	291 (84.8)	0.046
Systolic BP, mm Hg	123±17	127±17	0.0006
Diastolic BP, mm Hg	75±10	76±10	0.051
Diabetes mellitus, n (%)	110 (10.2)	48 (14.0)	0.054
HbA_{1c} (%)	5.3 [5.0; 5.6]	5.4 [5.2; 5.7]	<0.0001
Dyslipidemia, n (%)	621 (57.6)	210 (61.1)	0.26
LDL cholesterol, mg/dL	102±36	102±37	0.9
HDL cholesterol, mg/dL	55±16	53±18	0.08
Triglycerides, mg/dL	104 (68 to 154)	109 (82 to 163)	0.002
Creatinine, mg/dL	0.92±0.24	1.04±0.32	<0.0001
CKD, n (%)	102 (9.5)	67 (19.6)	<0.0001
Smoking history, n (%)	482 (44.8)	176 (51.3)	0.03
ΔCBF (%)	49 [−5 to 108)	33 (−7 to 99)	0.1
Homocysteine, μmol/L	7 (6 to 8)	11 (10 to 13)	<0.0001
Medications			
Aspirin, n (%)	560 (52.0)	196 (57.0)	0.1
Statin, n (%)	434 (40.3)	157 (45.7)	0.08
Antihypertensive, n (%)	683 (63.4)	244 (70.9)	0.01
Antidiabetic, n (%)	71 (6.6)	34 (9.9)	0.04
Diuretics, n (%)	148 (13.7)	92 (26.7)	<0.0001
B-vitamin use, n (%)	290 (26.9)	56 (16.3)	<0.0001

BP indicates blood pressure; CKD, chronic kidney disease; HbA1c, glycosylated hemoglobin; HDL, high density lipoprotein; LDL, low-density lipoprotein; and ΔCBF, blood flow change.

homocysteine on CMED, a marker of early preobstructive atherosclerosis.

Serum homocysteine accumulation might also play a role in synaptic dysfunction and cell death, through oxidative-stress generated by self-looping mechanisms.[48] Some of the pathomechanisms of homocysteine accumulation's effect on endothelial function have also been described in the literature.[49] For example, it has been hypothetically linked to increased levels of asymmetric dimethylarginine, an endogenous inhibitor of nitric oxide synthase, which is in turn a potent vasodilator vital for normal endothelial function. Another in vitro study using endothelial cells revealed that homocysteine treatment resulted in increased asymmetric dimethylarginine accumulation through suppression of dimethylarginine dimethylaminohydrolase activity.[50] In vitro studies also showed that homocysteine-induced endothelial dysfunction was mainly due to increasing superoxide production and quenching nitric oxide, a potent vasodilator, production. Furthermore, tetrahydrobiopterin attenuated homocysteine-induced damage to the endothelium by promoting nitric oxide synthase function.[51] Thus, our current study extends this previous observation and demonstrates that homocysteine is directly associated with endothelial dysfunction through dysregulation in the dilation/constriction pathways of coronary vasculature.

Serum homocysteine is usually kept below 15 μmol/L, with mild elevation defined as levels from 15 to 30 μmol/L.[27,52] We demonstrated a clear association between high-normal to mild hyperhomocysteinemia and CMED. Moreover, another study also showed that a modest increase of homocysteine (>12.1 μmol/L) was associated with cardiovascular risk.[47] This trend was also seen with another inflammatory biomarker, uric acid, where high-normal levels were associated with coronary endothelial dysfunction.[18] This suggests that currently established thresholds that define elevated serum homocysteine might not be reflective of the early pathophysiological effects of these normal-range elevations.

Studies investigating effects of hyperhomocysteinemia treatment on coronary artery disease demonstrated conflicting results. Folate supplementation was shown to improve flow-mediated dilatation of the brachial artery in human subjects with hyperhomocysteinemia.[53] In contrast, most randomized

Table 3. Association of Elevated Homocysteine Levels (>9) With Impaired CBF Reaction to Successive Acetylcholine Infusion

	All Patients			Males			Females			
	OR	95% CI	*P* Value	OR	95% CI	*P* Value	OR	CI (95%)	*P* Value	$P_{interaction}$
Unadjusted model										
Homocysteine >9 μmol/L	1.46	(1.14–1.87)	0.003	1.50	(1.02–2.20)	0.04	1.36	[0.98; 1.89]	0.06	0.71
Adjusted model										
Homocysteine >9 μmol/L	1.34	(1.03–1.75)	0.03	1.55	(1.01–2.39)	0.047	1.21	[0.85; 1.71]	0.29	0.49

Model adjusted for age, sex, body mass index, chronic kidney disease, diabetes mellitus, smoking exposure, aspirin use, statin use, B-vitamin use, LDL cholesterol, HDL cholesterol, and triglycerides. CBF indicates coronary blood flow; HDL, high density lipoprotein; LDL, low-density lipoprotein; and OR, odds ratio.

Table 4. Association of Elevated Homocysteine Levels (>9) With Impaired CBF Reaction Stratified by Taking Certain Relevant Medications (B Vitamins, Statins, or Diuretics)

	On Medication/Supplement			Not on Medication/Supplement			
	OR	95% CI	*P* Value	OR	95% CI	*P* Value	$P_{interaction}$
Statins							
Unadjusted model	1.31	(0.90–1.90)	0.16	1.55	(1.11–2.15)	0.01	0.50
Adjusted model*	1.17	(0.79–1.74)	0.44	1.52	(1.05–2.18)	0.03	0.31
B vitamins							
Unadjusted model	3.35	(1.72–6.50)	0.0004	1.26	(0.96–1.65)	0.1	0.005
Adjusted model†	2.72	(1.35–5.47)	0.005	1.20	(0.89–1.61)	0.22	0.027
Diuretics							
Unadjusted model	1.86	(1.09–3.18)	0.02	1.36	(1.02–1.80)	0.03	0.31
Adjusted model‡	1.89	(1.03–3.47)	0.04	1.27	(0.94–1.56)	0.13	0.44

CBF indicates coronary blood flow; HDL, high density lipoprotein; LDL, low-density lipoprotein; and OR, odds ratio.

*Model adjusted for age, sex, body mass index, chronic kidney disease, diabetes mellitus, smoking exposure, aspirin use, B-vitamin use, LDL cholesterol, HDL cholesterol, and triglycerides.

†Model adjusted for age, sex, body mass index, chronic kidney disease, diabetes mellitus, smoking exposure, aspirin use, statin use, LDL cholesterol, HDL cholesterol, and triglycerides.

‡Model adjusted for age, sex, body mass index, chronic kidney disease, diabetes mellitus, smoking exposure, aspirin use, statin use, B-vitamin use, LDL cholesterol, HDL cholesterol, and triglycerides.

controlled trials have failed to show a beneficial effect of B-vitamin supplementation on cardiovascular outcomes, despite reductions in serum homocysteine levels.[54–57] However, a substudy of the VISP (Vitamin Intervention for Stroke Prevention) trial demonstrated a beneficial effect of high-dose B-vitamin supplementation in patients >67 years of age by reducing risk of stroke, myocardial infarction, and death in the older cohort.[58]

B-vitamin use can decrease the levels of homocysteine in serum.[52] Yet trials have shown conflicting results regarding the effect of B-vitamin supplementation on cardiac disease.[55] Further, statin treatment in elderly patients with high serum homocysteine showed overall reduction of CVD events[59] and was also associated with improvements in coronary endothelial function.[60–62] B-vitamin supplementation was proven as a reliable method to decrease serum homocysteine level in most patients. However, the lack of efficacy of B-vitamin supplementation on cardiovascular outcomes created controversy around this topic. As shown in our analysis, patients on B-vitamin supplementation with elevated serum homocysteine levels (>9 μmol/L) are at even higher odds of having CMED, compared with those not taking supplementation. A possible explanation to this is that B vitamins may themselves play a role in atherogenesis. One study reported higher restenosis rates after angioplasty in patients taking B-vitamin supplementation.[63] Another study suggested that despite decreasing the levels of homocysteine, S-adenosyl methionine levels are increased, which alters normal methylation patterns, possibly leading to atherogenesis.[64] This effect was evident in the post-hoc analysis of one trial, which showed that patients in B-vitamin supplementation arm had more rapid progression of coronary diameter stenosis as evaluated by quantitative coronary angiography.[65] These patients may have also had higher levels of homocysteine before initiating vitamin treatment. Finally, B-vitamin supplementation might unmask patients with mild forms of genetic hyperhomocysteinemia, which is not usually affected by B-vitamin supplementation. However, no comparisons between genetic and other types of hyperhomocysteinemia effect on cardiovascular diseases were done.

In this study, statin therapy was not shown to affect the association between serum homocysteine and CMED. While some studies in the literature convey a beneficial effect of statins on endothelial function,[60–62] one study also reported that statin therapy showed the greatest benefit in individuals with increased baseline serum homocysteine levels.[59] No previous study evaluated the effect of statin on homocysteine-induced CMED. The possible protective role of statins could be attributable to the anti-inflammatory role of statins, which one in vitro study investigated and showed that statins have an attenuating effect on LDL cholesterol and homocysteine-induced oxidation.[66,67] While statins may play a role in ameliorating the association between high-normal levels of homocysteine and CMED the precise mechanism involved in this process, and its potential clinical implications will require further study.

Limitations

This study has several limitations. First, its retrospective and cross-sectional design makes it challenging to

derive causal associations, and the results should be considered as hypothesis generating. However, to our knowledge, this cohort is the largest database of patients undergoing an invasive assessment of endothelial function. Furthermore, some variables were not taken into account during clinical assessment. First, folate-fortified food intake may directly affect folate levels in the blood, so B-vitamin use is not the only factor that affects folate levels and therefore homocysteine levels.[68] We do not have the dose and frequency of B-vitamin supplementation in our population. In one randomized controlled trial in a Chinese population, only medium (400 µg/day) or high-dose (4000 µg/day) supplementation decreased homocysteine levels in patients in the quartile with the highest baseline homocysteine levels (>12 µmol/L).[69]

CONCLUSIONS

Our current study demonstrates that early coronary atherosclerosis is associated with elevated homocysteine levels and even high-normal levels of homocysteine (highest quartile, >9 µmol/L) are independently weakly yet significantly associated with CMED diagnosed invasively with pharmacologic provocation testing. Thus, the link between increased homocysteine levels and adverse cardiovascular events may potentially be mediated by coronary endothelial dysfunction; however no causal link can be established, requiring further investigation.

ARTICLE INFORMATION

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Supplementary Material

Table S1

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SUPPLEMENTAL MATERIAL

Table S1. Baseline characteristics comparing patients with versus without supplementation of vitamin B6, B12, and/or folate.

	B-vitamin Use		*P* value
	No **N = 1074**	**Yes** **N = 344**	
Clinical Parameters			
Age	49.8 ± 12.2	55.3 ± 11.8	<0.0001
Females, n (%)	682 (63.4)	257 (74.3)	0.0002
Body mass index (kg/m^2)	28.6 [24.8; 32.7]	27.4 [24.0; 32.2]	0.07
Hypertension, n (%)	873 (81.3)	278 (80.8)	0.85
Systolic BP (mmHg)	124 ± 16	125 ± 18	0.6
Diastolic BP (mmHg)	75 ± 10	75 ± 10	0.8
Diabetes mellitus, n (%)	119 (11.1)	39 (11.3)	0.9
HbA1c (%)	5.3 [5; 5.6]	5.4 [5.1; 5.6]	0.04
Dyslipidemia, n (%)	610 (56.7)	221 (63.9)	0.02
LDL Cholesterol (mg/dL)	103 ± 36	100 ± 37	0.2
HDL Cholesterol (mg/dL)	53 ± 18	57 ± 18	0.001
Triglycerides (mg/dL)	106 [74; 158]	103 [70; 145]	0.11
Creatinine (mg/dL)	0.96 ± 0.29	0.92 ± 0.19	0.002
CKD, n (%)	120 (11.2)	49 (14.3)	0.12
Smoking history, n (%)	513 (47.7)	145 (42.0)	0.07
ΔCBF (%)	44 [-7; 108]	45 [-5; 100]	0.7
Homocysteine (μmol/L)	8 [6; 10]	7 [6; 9]	<0.0001
Medications			
Aspirin, n (%)	560 (52.0)	196 (56.7)	0.14
Statin, n (%)	430 (40.0)	161 (46.5)	0.03

Anti-hypertensive, n (%)	699 (65.0)	228 (65.9)	0.8
Anti-diabetic, n (%)	83 (7.7)	22 (6.4)	0.4
Diuretics, n (%)	175 (16.3)	65 (18.8)	0.3

BP: Blood Pressure, LDL: Low-density lipoproteins, HDL: High density lipoproteins, eGFR: estimated glomerular filtration rate, CKD: Chronic Kidney Disease, ΔCBF: Blood flow change