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Ambar Kulshreshtha
Emory University

Yan Zheng
Emory University

Arshed A. Quyyumi
Emory University

Emir Veledar
Emory University; Department of Biostatistics, Florida International University, eveledar@fiu.edu

John Votaw
Emory University

See next page for additional authors

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Authors
Ambar Kulshreshtha, Yan Zheng, Arshed A. Quyyumi, Emir Veledar, John Votaw, Irina Uphoff, J. Douglas Bremmer, Jack Goldberg, and Viola Vaccarino

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Endothelial dysfunction is associated with occult coronary artery disease detected by positron emission tomography

Ambar Kulshreshtha [a,b], Yan Zheng [a], Arshed A. Quyyumi [b], Emir Veledar [a,b], John Votaw [c], Irina Uphoff [c], J. Douglas Bremner [d], Jack Goldberg [e,f], Viola Vaccarino [a,b,*]

[a] Department of Epidemiology, Emory University School of Public Health, Atlanta, GA, United States
[b] Division of Cardiology, Emory University School of Medicine, Atlanta, GA, United States
[c] Department of Radiology, Emory University School of Medicine, Atlanta, GA, United States
[d] Department of Psychiatry, Emory University School of Medicine, Atlanta, GA, United States
[e] Vietnam Era Twin Registry, Seattle, WA, United States
[f] University of Washington School of Public Health, Seattle, WA, United States

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ABSTRACT

Objective: Silent myocardial ischemia is common in asymptomatic subjects without a prior history of coronary artery disease (CAD) and is associated with increased morbidity and mortality. Our objective was to determine whether endothelial dysfunction is associated with silent myocardial ischemia and whether the association is independent of genetic and familial factors.

Material and methods: We examined 416 male monozygotic and dizygotic twins aged 47 to 63 years, free of symptomatic CAD. Subclinical ischemia was diagnosed by [13N] ammonia positron emission tomography at rest and after adenosine stress. Endothelial function was measured by flow-mediated dilation (FMD) of the brachial artery. Generalized estimating equations were used for analysis.

Results: Fixed perfusion defects were found in 24 (6%) twins and reversible perfusion defects in 90 (22%) twins, indicating subclinical ischemia. There was an inverse correlation between FMD and the reversible perfusion defect score ($r = -0.14$, $p = 0.01$) but not the fixed defect score ($r = -0.017$, $p = 0.73$). From the lowest to the highest quartiles of FMD, the prevalence of reversible defects decreased from 28% to 14%, $p = 0.008$. In multivariable analysis, reversible defects were significantly associated with each quartile of decreasing FMD (OR = 1.3; 95% 1.1, 2.5). In 54 twin pairs discordant for endothelial dysfunction (FMD ≤ 7% dilation from baseline), twins with endothelial dysfunction had 9% higher likelihood of having perfusion defects than their co-twins without endothelial dysfunction ($p = 0.041$).

Conclusions: Endothelial dysfunction is independently associated with silent ischemia and this association is not confounded by genetic or other shared familial factors.

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1. Introduction

Normal vascular endothelium, by secreting several mediators including nitric oxide, promotes arterial vasodilation, prevents thrombosis, and has anti-proliferative and anti-inflammatory actions. Dysfunction of the endothelium is characterized by impaired vasodilation in response to endothelial-specific agonists that reflects abnormalities in the integrity and function of the vascular endothelium.[1,2] This dysfunction plays a critical role in the pathogenesis of atherosclerotic coronary artery disease (CAD) and often precedes development of structural atherosclerosis [3–7]. Endothelial dysfunction can be measured by intra-arterial infusion of agonists that promote release of nitric oxide, such as acetylcholine, but these techniques are invasive and thus have limited applicability [8]. Flow-mediated dilation (FMD) of the brachial artery is an ultrasound-based method that allows non-invasive assessment of vascular nitric oxide release in response to increased shear stress [9]. FMD correlates with traditional vascular risk factors and is an independent measure of long term outcomes in both patients with CAD and in the general population [10–18].

Based on myocardial perfusion imaging, asymptomatic subjects frequently (20–50%) have perfusion abnormalities suggestive of silent ischemia [19]. These perfusion abnormalities may be due to either hemodynamically significant coronary stenosis, or occur in the absence

Abbreviations: CAD, coronary artery disease; FMD, flow-mediated dilation; PET, positron emission tomography.

* Corresponding author at: Dept. of Epidemiology, 1518 Clifton Rd NE, Room 3011, RSPH, Emory University, Atlanta, GA 30322, United States. Tel.: +1 404 727 8710; fax: +1 404 727 8737.
E-mail address: viola.vaccarino@emory.edu (V. Vaccarino).

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of significantly obstructive CAD, and in this case have been attributed to coronary micro vascular endothelial dysfunction [20]. However, the relationship between silent myocardial ischemia and peripheral vascular endothelial dysfunction remains unknown. Such an association may provide mechanistic explanation for the worse long term prognosis in subjects with endothelial dysfunction, and potentially provide a way to identify a high risk group within an asymptomatic population. In this study, we investigated the relationship between peripheral vascular endothelial dysfunction and silent myocardial ischemia in asymptomatic middle-aged, male twins without a prior history of CAD, with the hypothesis that endothelial dysfunction, measured as FMD, will identify a population at risk of silent myocardial ischemia diagnosed by positron emission tomography (PET). Twin studies provide a unique opportunity to examine the association between risk factors and disease because twins are matched on shared early environment and genetic factors, since twin siblings share genes (50% on average if dizygotic (DZ) and 100% if monozygotic (MZ)), maternal, and early familial environmental factors [21].

2. Material and methods

2.1. Study population

The Emory Twin Studies includes samples recruited in two companion studies: the Twins Heart Study (THS) and the Stress and Vascular Evaluation in Twins (SAVEIT). The purpose of these studies was to elucidate the role of psychological, behavioral, and biologic risk factors for subclinical cardiovascular disease in twins. Both studies recruited randomly selected samples of middle-aged male MZ and DZ twin pairs (who were raised in the same household) from the Vietnam Era Twin (VET) Registry, which includes 7369 male–male twin pairs both of whom served in the US military during the time of the Vietnam War [22]. Both studies followed identical procedures, measurements, and protocols. THS enrolled 180 twin pairs between 2002 and 2006 and SAVEIT included 127 twin pairs enrolled between 2005 and 2010 as previously described [20,23,24]. After excluding the second visit of a few pairs who participated in both studies, the combined sample included 281 pairs. Pairs of twins were examined at the same time at the Emory University General Clinical Research Center, and all data collection occurred during a 24-hour admission under controlled conditions. Both studies were approved by the Emory Institutional Review Board, and all twins signed an informed consent. Zygosity information by means of DNA typing was available for all twin pairs.

2.2. Cardiovascular risk assessment

A medical history and a physical examination were obtained on all twins. Systolic and diastolic blood pressure was measured by mercury sphygmomanometer on the right arm with the subject in sitting position after 10 min of rest. The average of two measurements 5 min apart was used in the statistical analyses. Venous blood samples were drawn for the measurement of glucose and lipid profile after an overnight fast. Direct low-density lipoprotein (LDL) cholesterol was obtained using homogeneous assays (Equal Diagnostics, Exton, Pennsylvania). Cigarette smoking was classified into current smoker (any number of cigarettes) versus never or past smoker. Diabetes mellitus was defined as having a fasting glucose level of >126 mg/dl or being treated with anti-diabetic medications.

2.3. Flow-mediated dilation (FMD)

Endothelium-dependent brachial artery FMD was determined using bi-mode ultrasound according to standardized procedures as described previously [25,26]. Images were obtained with an Acuson 10-mHz linear array transducer and ultrasound system (Mountain View, CA, USA). We performed imaging with the subject resting supine for at least 10 min on a hospital bed in a quiet setting. Optimal brachial artery images were obtained between 2 and 10 cm above the antecubital crease. After baseline measurements, a blood pressure cuff was inflated to 200 mm Hg over the proximal portion of the right forearm for 5 min. Endothelium-dependent function was determined during the first 2 min of release of the cuff [27]. After a 15 min period to re-establish baseline conditions, endothelium-independent dilation was assessed with similar procedures before and 3 min after administration of 0.4 mg of sublingual nitroglycerin. Images were digitized online, and arterial diameters were measured with edge-detection software (Medical Imaging Applications, Coralville, IA, USA) by an individual blinded to subject data. Arterial diameter was measured in millimeters from the leading edge of the intima–lumen interface of the near wall (echo zone 3) to the leading edge of the lumen–intima interface of the far wall (echo zone 5), coincident with the R-wave on the electrocardiogram (i.e. end-diastole). The brachial artery vasodilator response was quantified as percentage change in vessel diameter from baseline. In our laboratory, the mean difference in FMD (%) between two consecutive assessments performed in 11 subjects an average of 8 days apart was 1.26% (±0.76%), with a Pearson's correlation of 0.75. The mean difference in the FMD (%) between two readings of the same 11 measurements was 0.82% (±0.48%), with a Pearson's correlation of 0.97.

2.4. Myocardial perfusion

Twins underwent imaging of myocardial blood flow with PET [13N] ammonia at rest and following pharmacologic (adenosine) stress. On the day prior to the PET scan, they abstained from smoking and drinking alcoholic or caffeinated beverages. All medications were held the morning of the PET scan.

Initially, a 2–3 mCi dose of [13N] ammonia was injected and a 4-minute static scan was collected and reconstructed without any corrections to verify subject position. Then, rest and pharmacological stress (adenosine) ammonia imaging was performed on each subject. The rest and stress imaging protocols were identical except that a 4-minute infusion of adenosine (0.14 mg/kg/min) was started 2 min prior to the ammonia injection for the stress imaging session. 20 mCi of [13N] ammonia was injected and a 5-minute, 31 frame dynamic acquisition was started (12 frames × 5 s, 3 frames × 20 s, 1 frame × 300 s). Data were collected in 47 planes 3.375 mm thick covering a range of 16 cm for the CTI ECAT 921 camera or in 35 planes, 4.25 mm thick, covering a range of 15 cm for the GE PET-CT Discovery LS scanner. Immediately after the conclusion of the dynamic sequence, a 15-min gated (8 equally spaced phased gates) acquisition was started. The injections of ammonia were separated by at least 50 min to allow [13N] ammonia from the first injection to decay to a level where it would not interfere with the second study. Images were reconstructed with filtered back projection using a Hann filter cutoff at 1 cycle/cm and included attenuation correction.

We constructed a summary score describing the number and the severity of visible perfusion defects across 20 myocardial segments. In each segment, the defect severity was quantified on a 4-point scale (0: normal; 4: absent perfusion) and subsequently summed across the 20 segments to yield a total score. Separate scores were obtained for the rest (summed rest score) and stress (summed stress score) scans. The difference between these scores, the summed difference score, was computed to provide an overall indication of reversible ischemia. In addition we computed dichotomous indicators of perfusion abnormalities, defined as a summed stress score ≥4 across all 20 segments [28].

2.5. Statistical analysis

Continuous variables were described as mean ± SD and categorical variables as frequencies (percent). We compared baseline demographic

characteristics, cardiovascular risk factors, and perfusion defect scores (both reversible and fixed) across quartiles of FMD treating the twins as individuals, while accounting for correlated data using mixed models or generalized estimating equation (GEE) models. In additional analyses, we examined the Spearman correlation between perfusion scores and FMD both treated as continuous variables. Next, we analyzed the relationship between FMD and perfusion defects using mixed model linear regression analysis adapted for twin studies [29]. We treated perfusion defects as an outcome for these models using dichotomous categorization (normal or abnormal based on a score ≥ 4). We then adjusted for cardiovascular risk factors that include age, body mass index (BMI), high, total, and low density lipoprotein cholesterol, current smoking, systolic blood pressure, fasting plasma glucose and history of major depression. In a separate model we accounted for cardiovascular risk factors, use of aspirin and statins, as well as anti-hypertensive medications, including beta blockers, ace-inhibitors, diuretics, and angiotensin receptor blockers. Potential multicollinearity was investigated using condition indexes and variance decomposition proportions by means of a SAS macro including both a condition index of >20 and at least 2 non-intercept variables with variable decomposition proportion values of >0.05.

We next analyzed twin pairs discordant for endothelial dysfunction where one member had endothelial dysfunction and his twin brother did not. The highest quartile of hyperemia dilation (>7%) compared to lower quartiles was used to categorize endothelial dysfunction as normal vs abnormal. This within-pair analysis automatically takes into account shared familial and many early environmental factors. Within-pair analysis was further stratified by zygosity to determine whether the relationship between reversible defects and FMD was different between monozygotic and dizygotic twins. The analysis of within-pair differences in monozygotic twins is fully controlled for genetic factors shared between FMD and myocardial ischemia. Dizygotic twins share on average 50% of genes and differences among the twins are only partially controlled for shared genetic factors. Shared genetic factors would be indicated if the within-pair difference in reversible defects in endothelial dysfunction-discordant pairs is smaller in monozygotic than in dizygotic pairs. All statistical analyses were conducted using SAS software, version 9.2 (SAS Institute Inc., NC). Significance level was set at 0.05, two-sided.

3. Results

From the initial sample of 281 twin pairs (562 subjects), we excluded 65 twins because of previous history of CAD. Furthermore, 81 twins were excluded because of missing data on PET or FMD due to scheduling conflicts or technical problems with the imaging equipment or processing. The final sample of 416 twins (mean age 55 years, range 47 to 63 years) was 94% white and 3% black, 64% MZ and 36% DZ, a distribution that is similar to that of the Vietnam Era Twin Registry. There were no significant differences in demographic variables between MZ and DZ twins. Twins in the higher FMD quartiles had lower systolic blood pressure and alcohol consumption; no other significant differences were noted (Table 1).

Fixed perfusion defects were observed in 24 (6%), and reversible perfusion defects in 90 (22%) twins. The summed stress score, but not the rest score, tended to be lower in twins with higher FMD, and the summed difference score was negatively correlated with FMD quartiles (Table 2). When stress perfusion was dichotomized as normal or abnormal based on a score ≥ 4, twins in the highest quartile of FMD had lower prevalence of stress defects compared with twins in the lower FMD quartiles. Thus, from the lowest to the highest quartile of FMD, the prevalence of reversible defects decreased from 28% to 14% (p for trend = 0.008). In contrast, there was little difference in fixed defects based on endothelial function. Similarly, when FMD was treated as a continuous variable, it was negatively correlated with the reversible perfusion defect score (Spearman $r = -0.14$, $p = 0.01$,) but not the fixed defect score (Spearman $r = -0.017$, $p = 0.73$).

In the unadjusted model, subjects in the lowest FMD quartile exhibited twice the odds of myocardial ischemia (presence of reversible perfusion defects) (OR = 2.0, 95% CI 1.6–2.7) compared with the highest quartile (Table 3) This association was only mildly diminished after adjusting for age, smoking, BMI, systolic blood pressure, plasma lipids and fasting glucose. There was no association between FMD and fixed defects.

Our final analyses focused on twin pairs who were discordant for endothelial dysfunction (defined as FMD ≤ 7% dilation from baseline diameter), i.e. where one member had endothelial dysfunction and his twin brother did not. In 54 discordant twin pairs, twins with endothelial dysfunction had 9% higher likelihood to have perfusion defects than their co-twins without endothelial dysfunction ($p = .041$). Results for MZ and DZ twin pairs were similar.

4. Discussion

In asymptomatic subjects without a prior history of CAD, endothelial dysfunction, as demonstrated by reduced FMD, is independently associated with inducible myocardial ischemia. This relationship remained significant even after adjustment for traditional CAD risk factors. These findings were confirmed in twin pairs discordant for endothelial dysfunction, where those with endothelial dysfunction were more likely to have reversible perfusion defects than their co-twins (both monozygotic and dizygotic) without endothelial dysfunction.

Table 1
Cardiovascular risk factors according to FMD quartiles.

Cardiovascular risk factors	FMD quartiles 1st (<2.7%)	2nd (2.7–4.6%)	3rd (4.6–7.0%)	4th (>7%)	p-Value for trend
N	110	97	107	102	
Age (mean ± s.e)	55.6 ± 2.9	55.3 ± 3.16	55.5 ± 3.16	54.8 ± 3.6	0.12
Systolic BP, mm Hg (mean ± s.e)	132.8 ± 14.4	133.6 ± 16.5	127.5 ± 15.1	127.7 ± 15.0	0.02
BMI, kg/m^2 (mean ± s.e)	29.4 ± 4.9	29.6 ± 4.5	29.4 ± 4.3	29.7 ± 5.8	0.71
Current smoking (%)	29.0	22.0	27.0	22.0	0.34
Alcohol (no. drinks/week)	5.6 ± 9.6	6.6 ± 12.6	3.5 ± 5.2	3.5 ± 5.7	0.03
LDL cholesterol, mg/dl (mean ± s.e)	122.3 ± 32.7	131.0 ± 35.5	122.2 ± 31.2	119.8 ± 31.2	0.29
History of hypertension (%)	23.6	25.0	26.4	25.0	0.78
History of diabetes (%)	37.1	28.9	34.3	25.7	0.95
Taking beta-blockers (%)	28.6	28.5	28.6	14.2	0.20
Taking diuretics (%)	18.5	37.0	14.8	29.6	0.83
Taking statins (%)	24.1	20.4	27.7	27.7	0.47
Taking ACE-inhibitors (%)	22.2	20.3	33.3	24.1	0.57
Taking aspirin (%)	26.9	25.4	29.4	18.3	0.13

FMD = flow mediated dilation; BMI = body mass index; LDL = low density lipoprotein.

Table 2
Myocardial perfusion imaging data by FMD quartile.

Myocardial perfusion scores	FMD Quartiles 1st (<2.7%)	2nd (2.7–4.6%)	3rd (4.6–7.0%)	4th (>7%)	p-Value for trend
N	110	97	107	102	
Summed stress score, mean ± SD	2.26 ± 5.2	2.12 ± 6.2	1.85 ± 3.6	1.22 ± 3.2	0.06
Summed rest score, mean ± SD	0.51 ± 2.1	0.62 ± 2.6	0.45 ± 2.0	0.45 ± 1.5	0.64
Summed difference score, mean ± SD	1.7 ± 4.9	1.5 ± 4.7	1.4 ± 2.8	0.7 ± 2.1	0.04
Abnormal stress perfusion[a], N (%)	39 (27)	41 (29)	36 (25)	24 (17)	0.007
Fixed defects[b], N (%)	10 (24)	12 (27)	10 (24)	10 (24)	0.82
Reversible defects[c], N (%)	29 (28)	28 (26)	26 (25)	14 (14)	0.008

[a] Summed stress score ≥4.
[b] Summed rest score ≥4.
[c] Summed difference score ≥4.

Therefore, it appears that the relationship between FMD and myocardial ischemia is not confounded by CVD risk factors, shared genetic or other familial factors. Thus, in asymptomatic individuals without a history of CAD, endothelial dysfunction is associated with a higher risk of reversible myocardial ischemia. Our results suggest that endothelial dysfunction may be implicated in asymptomatic myocardial ischemia.

Endothelial function is an excellent barometer of vascular health [2,30]. Abnormal endothelial function is associated with greater risk factor burden and coronary atherosclerosis, and improves in response to anti-atherosclerotic therapies [31–33]. Coronary endothelial dysfunction correlates with peripheral endothelial dysfunction and both are markers of subsequent increased risk of adverse cardiovascular events in patients with and without established CAD [14,16,34]. Although, prior studies in CAD patients have linked endothelial dysfunction to myocardial perfusion defects [35], no previous studies have examined if this is also true in asymptomatic individuals without prior history of CAD among whom the etiology of perfusion abnormalities is not known [36–38]. In our study of asymptomatic, community individuals without a clinical history of CAD, a lower FMD was associated with a doubling in risk of silent myocardial ischemia, suggesting that endothelial dysfunction is an important underlying mechanism.

Silent ischemia, defined as myocardial ischemia occurring in the absence of angina or angina equivalents, is a common yet frequently unrecognized manifestation of CAD, accounting for more than 75% of ischemic episodes during daily life [39,40]. Silent ischemia is an independent predictor of future cardiac events even in patients without a history of CAD, whether it is detected by exercise testing, ambulatory electrocardiography, or imaging modalities [41,42]. Importantly, up to 48% of subjects with known CAD and silent ischemia detected by electrocardiography have an adverse cardiac event within 4 to 6 years [43,44]. Whether community screening with FMD is of benefit in reducing the risk of coronary events and cardiac death, however, needs to be further evaluated.

Table 3
Unadjusted and adjusted odds ratios for the relationship between FMD quartiles and presence of myocardial perfusion defects.

Myocardial perfusion defects	FMD Quartiles 1st OR (95% CI)	2nd OR (95% CI)	3rd OR (95% CI)	4th	p-Value for trend
Reversible defects					
Unadjusted	2.0 (1.6, 2.7)	1.6 (1.2, 2.7)	1.4 (1.1, 2.7)	1.0	0.04
Adjusted[a]	1.9 (1.6, 2.6)	1.5 (1.2, 2.1)	1.3 (1.1, 2.5)	1.0	0.03
Fixed defects					
Unadjusted	1.5 (0.7, 2.9)	1.1 (0.4, 2.6)	1.3 (0.6, 3.1)	1.0	0.51
Ajusted[a]	1.5 (0.7, 1.1)	1.1 (0.4, 2.6)	1.3 (0.6, 3.1)	1.0	0.50

[a] Adjusted for age, smoking, body mass index (BMI), systolic blood pressure, plasma lipids, and fasting plasma glucose.

Despite being asymptomatic and without a clinical history of CAD, 22% of our sample had reversible perfusion defects on cardiac PET perfusion imaging. Consistent with our data, the prevalence of myocardial perfusion abnormalities in asymptomatic subjects has been reported to be between 20 and 50% and is attributed to the presence of CAD risk factors and/or family history [19,45,46]. A number of underlying mechanisms have been suggested for ischemia being silent, including subclinical epicardial disease, microvascular dysfunction [47], blunted pain perception or insufficient degree of ischemia to stimulate pain receptors. Our findings suggest that endothelial dysfunction can play an important pathophysiologic role, independent of traditional CAD risk factors and family background and provides mechanistic insight for the link between FMD and coronary heart disease events [14,34,48,49].

Our study has several strengths. We have the advantage of a relatively large sample size and a twin study design. Twins provide naturally matched pairs where the potential confounding effects of a large number of factors such as sociodemographic, genetic and other familial influences are removed by comparisons within twin pairs. Our study also had the advantage of detailed measures of CVD risk factors and lifestyle behaviors. There are also a few limitations. Our analyses are cross-sectional and thus we are unable to address the temporal relationship between FMD and silent ischemia. Further, the sample is restricted to predominantly healthy middle-aged male Vietnam era veterans, and therefore, our results may not be generalizable to women and may not extend to younger subjects or populations with clinically manifest cardiovascular disease. Also we have no coronary angiography data on these participants to confirm or exclude the presence of obstructive CAD.

In conclusion, we showed that endothelial dysfunction, as assessed by means of brachial artery FMD, is independently associated with a 2-fold increased risk of silent ischemia in predominantly healthy asymptomatic men, independent of CVD risk factors, and shared genetic or environmental factors. Our results support growing evidence linking endothelial dysfunction to the early phases of CAD and point to a key role of the endothelium in the pathophysiology of myocardial ischemia.

Conflict of interest

The authors report no relationships that could be construed as a conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.ijcme.2014.07.004.

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