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

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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.

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.

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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