

# Brachial Flow-Mediated Dilation and Incident Atrial Fibrillation

## The Multi-Ethnic Study of Atherosclerosis

Wesley T. O'Neal, Jimmy T. Efirid, Joseph Yeboah, Saman Nazarian, Alvaro Alonso, Susan R. Heckbert, Elsayed Z. Soliman

**Objective**—It is unknown whether endothelial dysfunction precedes atrial fibrillation (AF) development. The objective of this study was to examine the association of brachial flow-mediated dilation (FMD) with incident AF.

**Approach and Results**—A total of 2936 participants (mean age, 61±9.9 years; 50% women; 66% nonwhites) from the Multi-Ethnic Study of Atherosclerosis with available ultrasound brachial FMD measurements who were free of baseline AF were included in this analysis. Baseline (2000–2002) FMD was computed from the percentage difference (%FMD) in brachial artery diameter and maximum diameter during measured vasodilator response. AF was ascertained from hospitalization data including Medicare claims during a median follow-up of 8.5 years. Probability-weighted Cox proportional-hazards regression was used to compute hazard ratios and 95% confidence intervals for the association between FMD as a continuous variable (%FMD values per 1-SD increase) and incident AF. Incident AF was detected in 137 (4.7%) participants. Those with %FMD values below the sex-specific median value (median %FMD; men, 3.6%; women, 4.2%; incidence rate per 1000 person-years, 7.3; 95% confidence interval, 5.9–9.0) were more likely to develop AF than people whose %FMD values were above the median value (incidence rate per 1000 person-years, 4.5; 95% confidence interval, 3.4–5.8; log-rank  $P=0.0043$ ). In a multivariable Cox regression analysis, each 1-SD increase in %FMD values (SD, 2.8%) was associated with less incident AF (hazard ratio, 0.84; 95% confidence interval, 0.70–0.99). These results were consistent across subgroups stratified by age, sex, and race/ethnicity.

**Conclusions**—Smaller brachial FMD values are associated with higher rates of AF, suggesting a role for endothelial dysfunction in AF pathogenesis. (*Arterioscler Thromb Vasc Biol.* 2014;34:2717–2720.)

**Key Words:** atrial fibrillation ■ endothelium ■ epidemiology

Arterial flow-mediated dilation (FMD) is an indirect measurement of endothelial nitric oxide (NO) release.<sup>1</sup> Impaired FMD represents systemic vascular endothelial dysfunction that is commonly associated with cardiovascular disease (CVD).<sup>2,3</sup> In addition, the association of FMD with CVD is evidenced by its ability to predict future CVD events in population-based studies.<sup>4,5</sup>

Endothelial dysfunction, defined by impaired FMD, has been reported in patients with atrial fibrillation (AF).<sup>6,7</sup> Also, abnormalities in NO signaling have been implicated in atrial ectopy near the pulmonary veins.<sup>8,9</sup> These findings suggest a potential role for endothelial dysfunction in the development of AF. However, data from population-based studies to support this claim are lacking. The purpose of this study was to

examine the association of brachial FMD with incident AF in the Multi-Ethnic Study of Atherosclerosis (MESA).

### Materials and Methods

Materials and Methods are available in the online-only Data Supplement.

### Results

Of the 3026 participants from the FMD ancillary study with available FMD measurements, 28 participants had a diagnosis of AF before enrolment in MESA. These cases were detected by Centers for Medicare & Medicaid Services linkage and were not detected in the baseline study ECG. Of those that remained, 3 participants with missing follow-up data and 59

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### Nonstandard Abbreviations and Acronyms

<b>AF</b>	atrial fibrillation
<b>CVD</b>	cardiovascular disease
<b>FMD</b>	flow-mediated dilation
<b>MESA</b>	Multi-Ethnic Study of Atherosclerosis

participants missing either baseline characteristics or medication data were excluded. A total of 2936 study participants (mean age, 61±9.9 years; 50% women; 66% nonwhites) were included in the final analysis.

FMD was computed from the percentage difference (%FMD) in brachial artery diameter and maximum diameter during measured vasodilator responses. Baseline characteristics stratified by sex-specific median %FMD are shown in Table 1. Participants with %FMD values below the median were more likely to be older, diabetic, and nonwhite and to have lower educational attainment and income compared with higher %FMD values. Persons with %FMD values below the median value were more likely to have increased values for systolic blood pressure and high-density lipoprotein cholesterol, and lower values for total cholesterol than those with %FMD values above the median value. Higher rates of anti-hypertensive medications, aspirin, lipid-lowering therapies, and left ventricular hypertrophy also were observed in people with %FMD values below the median value.

A total of 137 (4.7%) participants developed AF during the study period. Median follow-up for study participants was 8.5 years (interquartile range, 7.9–8.7). Unadjusted cumulative incidence curves stratified by median %FMD are shown in the Figure. Participants with %FMD less than the median value (incidence rate per 1000 person-years, 7.3; 95% confidence interval, 5.9–9.0) were more likely to develop AF compared with participants who had %FMD values greater than the median value (incidence rate per 1000 person-years, 4.5; 95% confidence interval, 3.4–5.8; log-rank  $P=0.0043$ ).

In a multivariable Cox proportional-hazards analysis, each 1-SD increase in %FMD values (SD, 2.8%) was associated with less incident AF (Table 2). The association between FMD and AF remained significant after further adjustment of model 2 with amino-terminal pro-brain natriuretic peptide (hazard ratio, 0.83; 95% confidence interval, 0.69–0.99). These results were consistent across subgroups of MESA participants stratified by age, sex, and race/ethnicity (Table 2).

## Discussion

In this analysis from MESA, lower brachial FMD values were associated with increased rates of AF. These findings suggest that endothelial dysfunction, as measured by brachial FMD, plays a role in the pathogenesis of AF.

To our knowledge, only 2 studies have examined the association of brachial FMD with AF. A study of chronic AF participants showed that FMD measurements are significantly impaired compared with sinus rhythm controls.<sup>6</sup> Another case-control study showed that participants with persistent AF have impaired FMD and that FMD improves after the restoration of sinus rhythm.<sup>7</sup> However, both studies examined

**Table 1. Baseline Characteristics (n=2936)\***

Variable	<Median %FMD (n=1470)	≥Median %FMD (n=1466)	P Value†
Age, mean (SD), y	64 (10)	58 (9.1)	<0.0001
Male sex (%)	738 (50)	722 (49)	0.61
Race/ethnicity			
White (%)	440 (30)	561 (38)	...
Black (%)	396 (27)	205 (14)	...
Chinese-American (%)	263 (18)	326 (22)	...
Hispanic (%)	371 (25)	374 (26)	<0.0001
High school or less (%)	569 (39)	496 (34)	0.0060
Income <\$20 000 (%)	407 (28)	351 (24)	0.020
Body mass index, mean (SD), kg/m <sup>2</sup>	28 (5.1)	28 (5.4)	0.44
Current or former smoker (%)	697 (47)	679 (46)	0.55
Diabetes mellitus (%)	229 (16)	146 (10)	<0.0001
Systolic blood pressure, mean (SD), mm Hg	128 (20)	121 (19)	<0.0001
Total cholesterol, mean (SD), mg/dL	193 (36)	196 (34)	0.048
HDL cholesterol, mean (SD), mg/dL	51 (14)	50 (14)	0.014
Antihypertensive medications (%)	570 (39)	427 (29)	<0.0001
Statins (%)	222 (15)	185 (13)	0.052
Aspirin (%)	357 (24)	311 (21)	0.047
Lipid-lowering medications (%)	245 (17)	205 (14)	0.044
hs-CRP, mean (SD), mg/L	3.5 (5.3)	3.4 (5.7)	0.13
Left ventricular hypertrophy (%)	70 (4.8)	34 (2.3)	0.0003

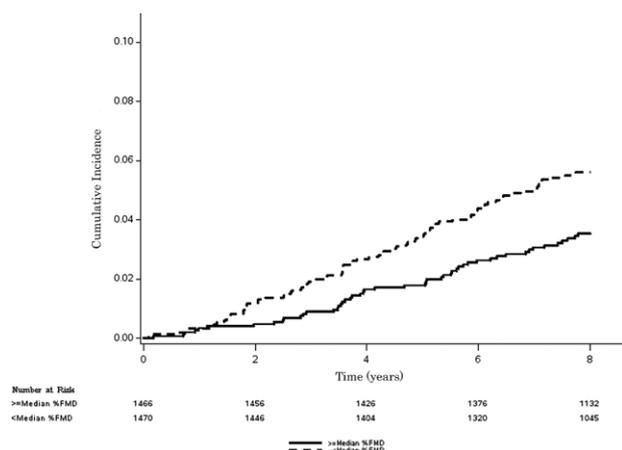
FMD indicates flow-mediated dilation; HDL, high-density lipoprotein; and hs-CRP, high-sensitivity C-reactive protein.

\*Median %FMD values for male and female participants were 3.6% and 4.2%, respectively.

†Statistical significance was tested for categorical variables using the  $\chi^2$  method and for continuous variables the Wilcoxon rank-sum method was used.

FMD among participants who already had AF. The current study examined FMD among participants without diagnosed AF and showed that lower FMD values are associated with an increased risk of AF development. In addition, participants with lower %FMD values were observed to have a higher incidence of AF than those in the general population.<sup>10</sup> Therefore, our results provide evidence that impaired FMD precedes the development of AF, suggesting a role for endothelial dysfunction in AF pathogenesis.

Endothelial cells regulate oxidative stress, vascular permeability, platelet aggregation, thrombosis, and vascular tone by controlling the release of several vasoactive substances, including NO.<sup>11</sup> The dysfunctional endothelium results in the downregulation of NO and the upregulation of adhesion



**Figure.** Cumulative incidence of atrial fibrillation by median percentage flow-mediated dilation (%FMD). Median %FMD values for male and female participants were 3.6% and 4.2%, respectively. Kaplan-Meier estimates were significantly different (log-rank  $P=0.0043$ ).

molecules that promote increased levels of inflammation and oxidative stress. Recent evidence suggests that increased oxidant generation by endothelial NADPH oxidase promotes the uncoupling of NO synthase and subsequent generation of reactive oxygen species and oxidative injury that leads to the electrophysiological remodeling observed in AF.<sup>8</sup> In addition, exogenous NO has been shown to reduce spontaneous electric activity in cardiomyocytes isolated from the pulmonary vein, implicating NO as a regulator of AF arrhythmogenesis.<sup>9</sup> Endothelial dysfunction also is associated with increased levels of inflammation that result in atrial ectopy in discharging cells near the pulmonary veins.<sup>12,13</sup> It is plausible that persons with endothelial dysfunction are more likely to have dysfunctional regulation of the aforementioned processes that increase their risk for AF. Therefore, FMD potentially is able to identify abnormal vascular biological profiles that precede AF development.

Alternatively, several shared risk factors for AF, such as increasing age, diabetes mellitus, hypertension, and smoking, have been associated with endothelial dysfunction.<sup>14-18</sup> Potentially, these conditions increase the level of vascular endothelial dysfunction and predispose individuals to AF. However, our results remained significant after adjustment for these risk factors, suggesting that the association between endothelial dysfunction and AF is not completely explained by shared risk factors.

Brachial FMD has been shown to independently predict incident CVD events among people who are free of CVD at baseline.<sup>5</sup> Abnormal FMD values possibly identify individuals with subclinical atherosclerosis who are at-risk for CVD. In addition, as evidenced by our results, FMD potentially is able to identify persons who are at-risk for AF. However, further research is needed to examine the predictive ability of FMD for AF among at-risk populations before screening programs that use FMD are introduced.

Our results should be interpreted in the context of certain limitations. Paroxysmal cases of AF possibly were missed because of its time-dependent nature. Incident AF cases were ascertained from hospitalization discharge records and inpatient Medicare claims data using *International Classification of Disease* codes, which possibly resulted in misclassification. However, this method has been reported to have adequate positive predictive value for AF case identification.<sup>19-21</sup> Brachial FMD measurements were obtained during the initial MESA visit and the association of FMD with AF may vary with repeat FMD measurements. The clinical significance of the FMD values (eg, increase per 1-SD, median) used in this study is unknown and those used were designed to demonstrate exploratory associations (eg, lower FMD values are associated with increased AF risk). Future studies are needed to define clinically relevant values. Nonsignificant interactions were observed by age, sex, and race/ethnicity but the current analysis potentially was underpowered to detect such differences. Furthermore, our results are limited regarding

**Table 2. Association of FMD With Atrial Fibrillation by Age, Sex, and Race/Ethnicity\***

	Events/No. at Risk	Model 1† HR (95% CI)	P Value	Model 2‡ HR (95% CI)	P Value	P Interaction§
All	137/2936	0.82 (0.69–0.98)	0.029	0.84 (0.70–0.99)	0.048	...
Age, y						
≤65	47/1898	0.68 (0.52–0.89)	0.0048	0.73 (0.56–0.95)	0.022	0.55
>65	90/1038	0.79 (0.63–0.99)	0.037	0.81 (0.64–1.01)	0.065	
Race						
White	70/1001	0.88 (0.70–1.1)	0.27	0.86 (0.68–1.1)	0.22	0.97
Nonwhite	67/1935	0.77 (0.59–1.0)	0.054	0.83 (0.64–1.1)	0.19	
Sex						
Male	85/1460	0.87 (0.69–1.1)	0.26	0.89 (0.70–1.1)	0.33	0.31
Female	52/1476	0.77 (0.59–0.99)	0.048	0.77 (0.59–1.01)	0.062	

CI indicates confidence interval; FMD, flow-mediated dilation; and HR, hazard ratio.

\*HRs presented are for %FMD per 1-SD increase (SD, 2.8%). Subgroups were adjusted according to models 1 and 2 excluding the covariate of interest.

†Adjusted for age, sex, race/ethnicity, income, and education.

‡Adjusted for model 1 covariates plus smoking status, systolic blood pressure, diabetes mellitus, body mass index, total cholesterol, high-density lipoprotein cholesterol, aspirin, antihypertensive and lipid-lowering medications, high-sensitivity C-reactive protein, and left ventricular hypertrophy.

§Interactions tested using model 2.

generalizability to other populations because of the older age of participants with FMD values lower than the median value for study participants.

In conclusion, we have shown that brachial FMD values are inversely associated with incident AF in MESA. Our results suggest that endothelial dysfunction precedes the development of AF and may play an important role in the pathogenesis of this common arrhythmia. Further research is needed to confirm our findings and also to explore the clinical use of FMD to identify those who are at-risk for developing AF.

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### Significance

Endothelial dysfunction has been reported in patients with atrial fibrillation. However, the temporal association between impaired endothelial function and atrial fibrillation has not been examined. In this analysis from the Multi-Ethnic Study of Atherosclerosis, we have shown that impaired brachial artery flow-mediated dilation, an indirect marker of endothelial dysfunction, precedes the development of atrial fibrillation. These findings support the hypothesis that alterations in vascular biology predispose to atrial fibrillation arrhythmogenesis.

# Arteriosclerosis, Thrombosis, and Vascular Biology



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## MATERIALS AND METHODS

### *Study Population*

Details of MESA have been reported previously.<sup>1</sup> Briefly, between July 2000 and September 2002, 6,814 participants were recruited at 6 field centers (Baltimore, Maryland; Chicago, Illinois; Forsyth County, North Carolina; Los Angeles, California; New York, New York; and St. Paul, Minnesota). Requirement for study participation included age between 45 and 84 years and no history of clinical cardiovascular disease. For the purpose of this analysis, participants were excluded if baseline characteristics, medication data, anthropometric measurements, and/or laboratory data were missing from the first examination. Participants with missing follow-up data also were excluded.

### *Brachial FMD Measurement*

A total of 6,489 participants underwent ultrasound brachial FMD measurement. For cost reasons, only a subset of participants had their brachial FMD tapes analyzed (N=3,026). A nested case-cohort involving a randomly selected sample (subcohort, n=2,844) and participants with cardiovascular events (cases, n=182) after 5 years of follow-up comprised the original FMD ancillary study.<sup>2,3</sup> Briefly, participants underwent FMD measurement after 15 minutes of rest in the supine position and after a 6-hour fast. A standard blood pressure cuff was positioned around the right arm, 2 inches below the antecubital fossa, and the artery was imaged 5 to 9 cm above the antecubital fossa. A linear-array multifrequency transducer operating at 9 MHz (GE Logiq 700 Device) was used to image the brachial artery. After baseline images were obtained, the cuff was inflated to 50 mm Hg above each participant's systolic blood pressure for 5 minutes. Digitized images of the right brachial artery were captured continuously for 30 seconds before cuff inflation and for 2 minutes beginning immediately before cuff deflation to document the vasodilator response. FMD was computed from the percent difference in baseline brachial artery diameter and maximum diameter during the vasodilator response. %FMD was defined using the following formula:  $\%FMD = [(maximum\ diameter - baseline\ diameter) / baseline\ diameter] \times 100\%$ . In MESA, the intraclass correlation coefficients for baseline diameter, maximum diameter, and %FMD were 0.90, 0.90, and 0.54, respectively.<sup>3</sup> Additionally, the percent technical error of measurement was 1.39% for baseline diameter measurement, 1.47% for maximum diameter measurement, and 28.4% for %FMD measurement.<sup>3</sup>

### *Atrial Fibrillation*

Follow-up phone calls to study participants every 9-12 months were used to identify hospitalizations. Medical records, including discharge diagnoses, were obtained for each hospitalization. Additionally, for participants 65 years or older enrolled in fee-for-service Medicare, Medicare claims data were used to identify inpatient AF cases. Incident AF was defined by International Classification of Disease Ninth Revision codes 427.31 or 427.32.

### *Baseline Characteristics*

Participant characteristics recorded during the initial MESA visit were used in this analysis. Age, sex, race/ethnicity, income, and education were self-reported. Annual

income was dichotomized at \$20,000 (<\$20,000 vs. ≥\$20,000). Similarly, education was dichotomized at high school education (“high school or less” vs. “some college or more”). Smoking was defined as “ever” (current or former) vs. “never” smoker. Blood samples were obtained after a 12-hour fast. Serum measurements of total cholesterol, high-density lipoprotein cholesterol, plasma glucose, and high-sensitivity C-reactive protein (hs-CRP) were used in this analysis. Additionally, a subgroup of study participants’ blood samples were analyzed for levels of amino-terminal-pro-brain natriuretic peptide and these values were used. Diabetes was defined as a fasting glucose value ≥126 mg/dL or a history of diabetes medication use. After the participant rested for 5 minutes in a seated position, blood pressure was measured 3 separate times and the mean of the last 2 values was recorded. The use of aspirin, statins, and antihypertensive and lipid-lowering medications were self-reported. Body mass index was computed as the weight in kilograms divided by the square of the height in meters. Left ventricular hypertrophy was defined by the Cornell criteria (R wave amplitude  $AV_L$  plus S wave amplitude  $V_3$  ≥2800 mm for men and ≥2000 mm for women) using baseline electrocardiogram data.<sup>4</sup>

### *Statistical Analysis*

Due to variation in the measurement of FMD, sex-specific median values for %FMD were computed (median %FMD; males=3.6%, females=4.2%).<sup>3</sup> Baseline data for study participants were stratified by sex-specific %FMD median values. Categorical variables were reported as frequency and percentage while continuous variables were recorded as mean ± standard deviation (SD). Statistical significance for categorical variables was tested using the chi-square method and the Wilcoxon rank-sum procedure for continuous variables. Kaplan-Meier estimates were used to compute cumulative incidence of AF by median %FMD and the difference in estimates was compared using the log-rank procedure.<sup>5</sup> Follow-up time was defined as the time between the initial study visit until the diagnosis of AF or until death, loss to follow-up, or end of follow-up which was December 31, 2010. To account for the sampling probabilities of the case-cohort design, probability-weighted Cox regression was used to compute hazard ratios (HR) and 95% confidence intervals (95%CI) for the association between FMD as a continuous variable (%FMD values per 1-SD) and AF. Multivariable models were constructed with incremental adjustments as follows: Model 1 adjusted for age, sex, race/ethnicity, income, and education; Model 2 adjusted for Model 1 covariates plus smoking status, systolic blood pressure, diabetes, body mass index, total cholesterol, high-density lipoprotein cholesterol, aspirin, antihypertensive and lipid-lowering medications, hs-CRP, and left ventricular hypertrophy. We tested for interactions between our main effect variable and age, sex, and race/ethnicity. A sensitivity analysis was performed with further adjustment for amino-terminal-pro-brain natriuretic peptide among participants who had baseline measurements (N=2,475).<sup>6</sup> The proportional hazards assumption was not violated in our analysis. Statistical significance was defined as  $p < 0.05$ . SAS Version 9.3 (Cary, NC) was used for all analyses.

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