CORRELATES OF FEMORAL ARTERY FLOW MEDIATED DILATION IN A MULTI-ETHNIC SAMPLE OF 12- TO 26-YEAR-OLDS

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Objective: Endothelial dysfunction is one of the earliest events in the pathogenesis of cardiovascular disease (CVD). Studies involving healthy adults have found endothelial function, measured via flow mediated dilation (FMD), to be impaired in African Americans (AAs) compared to European Americans (EAs). The purpose of this study was to determine whether ethnic differences exist in FMD in a group of healthy teenagers and young adults, and to examine separately, by ethnic group, the relationships between FMD and several measures of adverse cardiovascular prognoses.

Design: Subjects underwent measurement of various anthropometric, hemodynamic, and echocardiographic variables.

Setting: Measurements were made in a laboratory setting.

Participants: Subjects were 159 12- to 26-year-old AAs and EAs.

Main Outcome Measures: FMD, endothelin-1 (ET-1) levels, at rest and in response to stress, and measures of cardiac structure and function.

Results: Ethnic differences were not observed in FMD. Only pre-occlusion arterial diameter was significantly related to FMD in EAs ($P<.001$); whereas, among AAs, FMD was negatively correlated (all $Ps<.03$) with gender, pre-occlusion arterial diameter, relative wall thickness (RWT), resting plasma ET-1, and ET-1 increases, related to a behavioral stressor. Multiple linear regressions for AAs revealed that gender and RWT each explained a unique variation in FMD (Total model $R^2=.36$, $P<.0001$).

Conclusion: Ethnic differences in FMD are not in evidence for subjects in this age range. The inverse relationships observed in AAs between FMD and measures of altered endothelial system function, and ventricular structure and function, provide additional evidence for early clustering of measures of adverse cardiovascular prognoses in this group. (*Ethn Dis*. 2004; 14:227–232.)

INTRODUCTION

African Americans (AAs) experience greater morbidity and mortality from cardiovascular disease (CVD), compared to European Americans (EAs).[1] This disparity is partially reflected in AAs' higher prevalence, and earlier onset, of essential hypertension, as well as in associated target organ changes ranging from changes in vascular endothelin system function, to alterations in left ventricular (LV) structure and function.[2]

Vascular endothelial dysfunction is one of the earliest events in the pathogenesis of CVD.[3,4] High resolution ultrasound can be utilized to non-invasively assess endothelial function by measuring the dilation of systemic arteries in response to reactive hyperemia. This flow mediated dilation (FMD) has been shown to be an endothelium-dependent phenomenon, reflecting shear stress-induced release of nitric oxide (NO) from the vascular endothelium.[5,6] An endothelium-produced counter-regulatory hormone that serves to balance NO is the potent vasoconstrictor endothelin-1 (ET-1),[7] which plays a significant role in the regulation of vascular tone, as well as in the development of CVD.[8] Plasma ET-1 levels have been demonstrated to be greater in AAs than EAs, both at rest, and in response to behavioral stress.[9–11] It should be noted that ET-1 is released by the endothelium abluminally[12]; therefore, the circulating levels of ET-1 likely do not reflect its full physiologic impact.[13] Whether measures of altered endothelin system function, such as increased plasma ET-1 at rest and/or in response to acute stress, are associated with decreased FMD, is unknown.

Two recent studies involving normotensive adults (with mean ages of 37 and 30) found FMD to be significantly lower in AAs, compared to EAs.[14,15] Reasons for these ethnic differences are poorly understood. Impaired FMD has been linked with numerous measures of adverse cardiovascular (CV) prognoses, including increased blood pressure (BP) at rest and in response to acute stress, increased LV mass, cigarette smoking, and adiposity, among others.[16–20] These, and other CV risk factors, have been shown to cluster by ethnicity, with AAs exhibiting higher levels compared to EAs.[21,22] Whether these measures are related to FMD among young normotensives, particularly AAs, is unknown.

The objectives of this exploratory study involving normotensive 12- to 26-year-old subjects were two-fold: to determine whether AAs have decreased

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An endothelium-produced counter-regulatory hormone that serves to balance NO is the potent vasoconstrictor endothelin-1 (ET-1),[7] which plays a significant role in the regulation of vascular tone, as well as in the development of CVD.[8]

FMD compared to EAs; and to examine relationships separately by ethnic group between FMD and several measures of adverse CV prognoses, including altered endothelin system function, and left ventricular structure and function.

METHODS

Study Population

One hundred fifty-nine individuals (66 AAs [35 males], 93 EAS [51 males]) participated in the study. They were recruited from subjects of a longitudinal study of the development of CVD risk factors, based upon a verified family history of essential hypertension and/or premature myocardial infarction.[23] The subjects had acceptable ultrasound measurements of the femoral artery and the left ventricle, as well as ET-1 plasma levels at rest and in response to a brief behavioral stressor. They had a mean age of 17.9 years (range 12.4–26.6), and were apparently healthy, normotensive, taking no vaso-active medications, and undergoing no intensive training regimens. Subjects who were smokers (N=33) were asked to refrain from smoking for at least 4 hours prior to the study visit. All were compliant, based upon self-report.

Subjects were classified as African-American if: 1) one or both parents reported being of African heritage; 2) they and the child were born and raised in the United States; 3) parents considered themselves and their child to be African-American, Black, or Negro. Subjects were classified as European Americans if: 1) both parents reported being of European ancestry; 2) they and the child were born and raised in the United States; 3) they considered themselves and their child to be White, Caucasian, or European-American, and not of Hispanic, native African, or Asian descent. As recommended by Anderson et al,[24] ethnicity classification was used as a sociological index representing differential exposure to chronic social and environmental stressors (eg, poverty, racism, etc) rather than as an index of possible genetic differences between groups of individuals.

Protocol

The process for obtaining informed consent and the experimental protocol were approved by the Institutional Review Committee of the Medical College of Georgia. After obtaining informed consent, anthropometric measurements, including height (cm), weight (kg), and waist and hip circumferences (cm), were obtained using established protocols.[23] Body mass index (BMI, kg/m^2) and body surface area (BSA, m^2) were derived from these data.

Once anthropometric measurements were obtained, an appropriately sized blood pressure (BP) cuff was placed on each subject's right arm for measurement of systolic BP (SBP), diastolic BP (DBP), and heart rate (HR), using a Dinamap Vital Signs Monitor (Model 1846SX, Criticon Incorporated, Tampa, Fla.). The subject was then placed in a supine position, and the left elbow was stabilized with an arm board. A 21-gauge needle was inserted into the antecubital vein, and a 3-way plastic stopcock was attached. Immediately following needle placement, a 5 mL blood sample was drawn, transferred to a 10 mL pre-chilled EDTA tube vacutainer, and maintained on ice.

A 20-minute supine rest period followed, in which CV measurements were obtained at 11, 13, 15, 17, and 19 minutes; the average of the 15-, 17-, and 19-minute measurements represented CV function. A 5 mL blood draw was conducted at minute 19 of the rest period.

A 10-minute video game challenge ("Break Out," Atari, Inc.) immediately followed, based on a protocol initially developed by Murphy et al.[25] Cardiovascular (CV) measurements were recorded every other minute during the stressor. Another 5 mL blood sample was acquired upon conclusion of the behavioral stressor. A cardiovascular (CV) reactivity change scores (ie, peak CV response—pre-stressor CV level), and an ET-1 change score (ie, post-video game stressor ET-1 level − pre-stressor ET-1 level), were derived.

Plasma ET-1 levels were determined with an ELISA (QuantiGlo, R&D Systems, Minneapolis, Minn.) according to the manufacturer's instructions, except that the standard curve was limited to a maximum of 6 pg/mL. All samples and standards were processed in duplicate. The intra-assay variability was 4.2%.

Two-dimensional directed M-mode echocardiographic evaluations were conducted using a Hewlett-Packard (HP) Sonos echocardiograph (Andover, Mass.). Left posterior wall thickness, interventricular septal thickness, and left ventricular internal dimension, were measured according to the American Society of Echocardiography convention.[26] Left ventricular mass (LVM) was derived using a validated formula of Devereux and colleagues.[27] Left ventricular mass/Ht$^{2.7}$, relative wall thickness (RWT), and mid-wall fractional shortening (MFS), were calculated, using established formulas.[28] Intra- and inter-rater coefficients of variation for all cardiac structures were less than 10%.

Following the echocardiogram, FMD of the left superficial femoral artery (FA) was assessed as previously described.[29] The sonographer was unaware

of any of the subjects' laboratory evaluation results. A 7.5 MHz vascular transducer with an HP Sonos echocardiograph was used to identify the left superficial FA in a longitudinal section 2 to 3 cm distal to its origin from the common FA. After a 10-minute supine rest, 5 to 7 images of arterial diameter in end-diastole were measured and averaged. Arterial flow velocity was also measured at baseline. With the transducer held in place, a BP cuff was inflated on the subject's left thigh, 1–2 cm above the knee. To provoke hyperemia, the BP cuff was inflated to 240 mm Hg for 4 minutes. Flow measurements were obtained during the first 30 seconds following cuff deflation. The diameter of the artery was measured from the anterior to the posterior "m" line in end-diastole, during 5 to 7 cardiac cycles beginning 60 seconds after cuff release, and averaged to obtain the maximum arterial diameter. Flow-mediated dilation was calculated as the percent change in FA diameter from pre- to post-occlusion. The intra- and inter-rater coefficients of variation were less than 10%.

Table 1. Clinical characteristics of sample by ethnicity*

	AAs	EAs	
Sociodemographic	*N* = 66	*N* = 93	
Gender (% male)	52	54	.78
Age (years)	18.2 ± 2.2	17.7 ± 2.4	.25
Socioeconomic status†	42.0 ± 12.9	47.5 ± 13.0	.02
Anthropometric			
Height (cm)	170.5 ± 9.1	169.5 ± 10.2	.51
Weight (kg)	67.1 ± 12.3	64.3 ± 14.0	.21
Waist (cm)	29.8 ± 3.1	30.1 ± 3.6	.61
BMI (kg/m^2)	23.0 ± 3.1	22.2 ± 3.4	.15
BSA (m^2)	1.78 ± 0.19	1.74 ± 0.22	.23
Echocardiographic			
FA pre-occlusion diameter (mm)	4.8 ± 0.80	4.8 ± 0.65	.65
FMD (% change)	4.52 ± 4.85	4.10 ± 4.25	.55
RWT (%)	0.36 ± 0.06	0.32 ± 0.05	.0001
MFS (%)	18.8 ± 2.4	19.6 ± 2.1	.02
LV mass/$height^{2.7}$ ($g/m^{2.7}$)	33.2 ± 9.1	29.9 ± 7.8	.01
Hemodynamic			
Resting SBP (mm Hg)	113.7 ± 9.3	109.9 ± 8.7	.01
Resting DBP (mm Hg)	65.0 ± 7.7	59.1 ± 5.4	.001
SBP video game reactivity (mm Hg)‡	7.9 ± 8.8	8.2 ± 7.1	.82
DBP video game reactivity (mm Hg)‡	5.5 ± 6.4	5.1 ± 5.6	.69
Biochemical			
ET-1 (pg/ml)	1.68 ± 0.67	1.39 ± 0.54	.003
ET-1 video game reactivity (pg/ml)‡	0.16 ± 0.23	0.07 ± 0.20	.008

* Means ± standard deviations are presented.

† Socioeconomic status as measured by the Hollingshead Four Factor Social Status Index.

‡ Change score derived from post-video game stressor value − pre-video game stressor value. BMI=body mass index; BSA=body surface area; DBP=diastolic blood pressure; ET-1=endothelin-1; FA=femoral artery; FMD=flow mediated dilation; LV=left ventricular; MFS=mid-wall fractional shortening; RWT=relative wall thickness; SBP=systolic blood pressure.

Statistical Methods

The data were initially evaluated for distributional characteristics. All variables were approximately normally distributed, with approximately equal variance across ethnic classifications. Univariate analyses of variance were conducted, examining possible ethnic differences in FA pre-diameter, FMD, and the comparison variables of interest (Table 1). Because many of these comparison variables have been found to differ by ethnicity, comparisons with FMD were conducted separately by ethnicity, using Pearson product moment correlations. A multiple regression model was then constructed to determine the unique (ie, non-redundant) contribution of the statistically significant CVD risk factors to FMD. Results of statistical tests are given as exact *P* values, and reflect the probability of obtaining the given effect, assuming a chance (ie, error) only model.

RESULTS

Table 1 gives descriptive statistics for all variables used in the analysis by ethnicity (means and standard deviations). Compared to EAs, AAs exhibited statistically higher resting SBP and DBP, RWT, $LVM/Ht^{2.7}$, basal ET-1, and ET-1 reactivity, as well as lower MFS and socioeconomic status.

Table 2 provides the univariate correlations of FMD with the comparison variables, by ethnic group. For EAs, only FA pre-occlusion diameter was significantly associated with FMD (R^2=.14). For AAs, sex and FA pre-occlusion diameter demonstrated moderately high correlations with FMD (R^2=.31 and .41, respectively). Females exhibited a 7.27% change in FMD, compared to a 1.94% change in males. Various other factors were significantly correlated with FMD among AAs, including ET-1 resting levels, ET-1 reactivity change scores, RWT, MFS, height, BSA, and weight (R^2 range=.04 to .22).

Multiple linear regression analysis was used to determine the unique contributions of the comparison variables significantly associated with FMD among AAs. The analysis did not include height, due to the high correlation and possible multicolinearity problems with BSA. Gender accounted for the majority of the variance in the regression model (R^2=.31, P<.001). The only other variable to statistically account for the variance in FMD, after

Table 2. Correlation coefficients of FMD with comparison variables by ethnicity*

	AAs	EAs
Sociodemographic		
Gender†	0.55 (.001)	0.13
Age (yrs)	−0.15	−0.04
Smoking status‡	−0.17	−0.10
Anthropometric		
Height (cm)	−0.47 (.001)	−0.17
Weight (kg)	−0.21 (.09)	−0.10
Waist (cm)	−0.07	−0.11
BMI (kg/m^2)	−0.08	−0.03
BSA (m^2)	−0.32 (.01)	−0.13
Echocardiographic		
FA pre-occlusion diameter (mm)	−0.64 (.001)	−0.38 (.001)
RWT (%)	−0.30 (.02)	−0.05
MFS (%)	0.25 (.05)	−0.05
LV mass/height$^{2.7}$ (g/m$^{2.7}$)	−0.13	0.01
Hemodynamic		
SBP (mm Hg)	−0.06	0.01
DBP (mm Hg)	0.12	0.15
SBP video game reactivity (mm Hg)§	−0.15	−0.16
Biochemical		
ET-1 (pg/ml)	−0.28 (.03)	−0.08
ET-1 video game reactivity (pg/ml)§	−0.29 (.03)	−0.08

* Significant $P<$values in parentheses.
† Gender coded as males=1, females=2 in analyses.
‡ Smoking status coded as non-smokers=0, smokers=1 in analyses.
§ Change score derived from post-video game stressor value − pre-video game stressor value. BMI=body mass index; BSA=body surface area; DBP=diastolic blood pressure; ET-1=endothelin-1; FA=femoral artery; LV=left ventricular; MFS=mid-wall fractional shortening; RWT=relative wall thickness; SBP=systolic blood pressure.

accounting for gender, was RWT (R^2 increase .05, $P=.02$; Total Model $R^2=.36$, $P<.0001$). The multiple regression coefficient associated with RWT was −0.19, which indicated that, after controlling for gender, there was a 0.2% decrease in FMD for every 1% increase in RWT.

A second statistical model for AAs was conducted that excluded gender, but included FA pre-occlusion diameter. This was done since pre-occlusion arterial diameter was highly correlated with FMD in this study ($r=-.64$, $P<.0001$), and in others,[14] and because gender differences were observed in pre-occlusion arterial diameter in our study (males=5.40 mm; females=4.22 mm, $P<.02$) and in others.[30] Similar to gender, FA pre-diameter accounted for a large amount of the variance ($R^2=.40$, $P<.001$). Relative wall thickness (RWT) represented an additional variance of approximately 4%, after accounting for pre-diameter (total model $R^2=.44$, $P<.001$). Although this was a 12% increase in full model R^2, compared to the model that included gender, the R^2 attributable to RWT was approximately the same in both models.

DISCUSSION

The primary objectives of this study were 2-fold: 1) to evaluate whether ethnic differences in FMD are manifest in normotensive adolescents and young adults; and 2) to determine patterns of relationships between FMD and indices of adverse CV prognoses, including measures of altered endothelin system function, and left ventricular structure and function.

Contrary to our hypothesis, AAs did not exhibit compromised FMD compared to EAs. This contrasts with recent studies among older samples of normotensive adults (mean ages of 30 and 37) which found significantly lower FMD in AAs compared to EAs.[14,15] However, consistent with other adult and pediatric studies, ethnic differences were observed in which AAs exhibited higher resting SBP and DBP, increased plasma ET-1 at rest, and in response to a brief behavioral stressor, greater LVM/Ht$^{2.7}$ and RWT, and decreased MFS.[31–34] There were no ethnic differences in gender ratio, age, anthropometric measures, or FA baseline diameter, that might have accounted for these observations. Collectively, these findings suggest that in asymptomatic 12- to 26-year-old AAs (mean age=17.9 years), although resting BP and LV geometry and function are already altered, endothelial function based upon FMD is still comparable to that of EAs. Given these findings, and those of Campia et al[14] and Perregaux et al,[15] ethnic differences in FMD do not appear to manifest until later in adulthood.

None of the examined variables, with the exception of FA pre-occlusion diameter, demonstrated a significant relationship with FMD in the EA group. Reasons for this are unclear. It should be noted that the majority of FMD studies have involved predominantly Europeans or EAs. These studies involved youth and adults, and inconsistent findings were observed regarding correlates of FMD. Some studies have found inverse relationships between FMD and age, cholesterol, increased BP, smoking, and obesity (eg, body mass index), while others have not found significant associations.[14,16,29,31,35] These inconsistencies can be partly attributed to differences across studies in subject characteristics (eg, age range, dietary patterns, vaso-active medication and tobacco usage, socioeconomic status, etc.), and variations in methodology (eg, site of occlusion, specific artery examined, artery image acquisition, FMD image analysis, etc.).[3]

To our knowledge, our findings among AAs are the first to demonstrate inverse relationships between FMD and adverse changes in cardiac remodeling (ie, increased RWT) and function (ie, decreased MFS), in otherwise asymptomatic, healthy individuals.

A number of variables were significantly negatively correlated to FMD in the AA group. Consistent with previous adult studies, measures of body habitus and male gender were inversely related to FMD.[18,36] To our knowledge, our findings among AAs are the first to demonstrate inverse relationships between FMD and adverse changes in cardiac remodeling (ie, increased RWT) and function (ie, decreased MFS), in otherwise asymptomatic, healthy individuals. The multivariate analysis revealed RWT as the only independent predictor of FMD, after accounting for the contributions of gender.

Reasons why gender accounted for such a large amount of the variance in FMD are not completely clear. Although, statistically, gender suppressed the inclusion of other explanatory variables into the statistical model, other than RWT, this does not mean these variables are not associated with the gender effect upon FMD; that is, females were significantly different from males on several parameters associated with increased FMD (eg, lower ET-1 in response to stress, greater MFS, and lower pre-occlusion diameter). Therefore, although ET-1 increases did not explain unique variance in FMD after gender was considered, they still may help to explain why women exhibited greater FMD, compared to men. Similarly, women showed greater FMD, compared to men, in part, because of smaller pre-occlusion arterial diameter. This is true despite the fact that gender and pre-occlusion diameter essentially cancel each other out of a statistical model that attempts to explain FMD. The detailed partitioning of these complex interrelationships, based solely on statistical manipulation, is not possible with observational data of this type. Only through experimental manipulation and control can the physiologic mechanisms responsible for gender differences in FMD be determined.

A second important novel finding was the inverse relationship between FMD and plasma ET-1 levels at rest, and in response to acute stress, in the AA group. Interestingly, both youth and adult AAs, compared to EAs, have been shown to exhibit greater vasoconstrictive-mediated responsivity to a variety of stressors.[37–39] The present findings, as well as others,[40] suggest that AAs vasoconstrictive tone-related blood pressure control problems are, in part, related to greater levels of, and/or increases in, plasma ET-1. A plausible endothelin system-related factor that may play a role in the increased impact of ET-1 on FMD in AAs involves ethnic differences in ET_B receptor density. This receptor produces vasoconstriction when present on vascular smooth muscle (VSM) cells.[41,42] However, it can also induce vasodilation when located on the endothelium, via release of endothelial-derived relaxing factors (EDRFs).[43] Ergul et al observed a lower density of ET_B receptors on endothelial cells, and a greater density of ET_B receptors on VSM cells in AAs. The altered ratio of endothelial to VSM ET_B receptors implies a shift in favor of vasoconstriction-promoting receptors among AAs.[44]

Although these findings are intriguing, this study had several limitations that should be noted. First, endothelium-independent vasodilation was not assessed in this sample. However, numerous FMD studies involving youth and young adults have found endothelium-independent dilation to be intact, with changes in endothelium-independent vascular-wall smooth-muscle relaxation typically becoming evident much later in the atherosclerotic process.[45] Second, other biochemical measures, including nitric oxide metabolites, angiotensin II, insulin, norepinephrine, cholesterol, and homocysteine, were not evaluated, due to lack of available plasma. Therefore, their potential impact upon FMD could not be determined. Third, the cross-sectional design of this study prevents our drawing any specific causative inferences with respect to any potential roles played by the various CVD risk factors in endothelial function changes, and vice-versa. Finally, the degree of generalizability of these findings to other adolescents and young adults is unclear, since all subjects had a verified family history of essential hypertension, and/or premature myocardial infarction.

In conclusion, ethnic differences in FMD are not apparent in this age group. However, our results indicate that in healthy African-American adolescents and young adults, FMD is negatively related to measures of altered left ventricular structure and function, and altered endothelin system function. This pattern is not seen in similarly aged European Americans. These findings provide additional evidence for early clustering of indices of adverse cardiovascular outcomes in African Americans.

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Data analysis and interpretation: Treiber, Kapuku, Hartley, Ludwig
Manuscript draft: Treiber, Hartley, Ludwig
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