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The Presence of African American Race Predicts Improvement in Coronary Endothelial Function After Supplementary L-Arginine

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OBJECTIVES The purpose of our study was to determine if the presence of African American ethnicity modulates improvement in coronary vascular endothelial function after supplementary L-arginine.

BACKGROUND Endothelial dysfunction is an early stage in the development of coronary atherosclerosis and has been implicated in the pathogenesis of hypertension and cardiomyopathy. Amelioration of endothelial dysfunction has been demonstrated in patients with established coronary atherosclerosis or with risk factors in response to infusion of L-arginine, the precursor of nitric oxide. Racial and gender patterns in L-arginine responsiveness have not, heretofore, been studied.

METHODS Invasive testing of coronary artery and microvascular reactivity in response to graded intracoronary infusions of acetylcholine (ACh) ± L-arginine was carried out in 33 matched pairs of African American and white subjects with no angiographic coronary artery disease. Pairs were matched for age, gender, indexed left ventricular mass, body mass index and low-density lipoprotein cholesterol.

RESULTS In addition to the matching parameters, there were no significant differences in peak coronary blood flow (CBF) response to intracoronary adenosine or in the peak CBF response to ACh before L-arginine infusion. However, absolute percentile improvement in CBF response to ACh infusion after L-arginine, as compared with before, was significantly greater among African Americans as a group (45 ± 10% vs. 4 ± 6%, $p = 0.0016$) and after partitioning by gender. The mechanism of this increase was mediated through further reduction in coronary microvascular resistance. L-arginine infusion also resulted in greater epicardial dilator response after ACh among African Americans.

CONCLUSIONS We conclude that intracoronary infusion of L-arginine provides significantly greater augmentation of endothelium-dependent vascular relaxation in those of African American ethnicity when compared with matched white subjects drawn from a cohort electively referred for coronary angiography. Our findings suggest that there are target populations in which supplementary L-arginine may be of therapeutic benefit in the amelioration of microvascular endothelial dysfunction. In view of the excess prevalence of cardiomyopathy among African Americans, pharmacologic correction of microcirculatory endothelial dysfunction in this group is an important area of further investigation and may ultimately prove to be clinically indicated. (J Am Coll Cardiol 2002;39:1314–22) © 2002 by the American College of Cardiology Foundation

Endothelial dysfunction represents an early pre-clinical stage in the evolution of coronary atherosclerosis and has been implicated in the pathogenesis of other cardiovascular diseases, including systemic hypertension and dilated cardiomyopathy (1–5). One indicator of endothelial dysfunction is impaired vasodilator response to intracoronary acetylcholine (ACh) (6–13). This occurs when coronary vasorelaxation after stimulated release of endothelial nitric oxide (NO) is exceeded by constriction mediated through direct stimulation of muscarinic receptors on vascular smooth muscle cells. A proposed approach for treatment of endothelial dysfunction is provision of supplementary L-arginine, the precursor of NO. Despite the fact that sufficient intracellular L-arginine exists in theory to maximally drive the reaction leading from L-arginine to NO through constitutive NO synthase, a number of studies have demonstrated improvement in systemic, forearm and coronary endothelial function after infusion or ingestion of L-arginine (14–21). Many of these studies included patients with established coronary atherosclerotic disease (CAD) or with risk factors for CAD. Furthermore, other investigations have demonstrated that systemic administra-

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Abbreviations and Acronyms

ACh = acetylcholine
ADMA = asymmetric dimethylarginine
ANOVA = analysis of variance
BMI = body mass index
CAD = coronary atherosclerotic disease
CBF = coronary blood flow
HDLC = high-density lipoprotein cholesterol
LDLC = low-density lipoprotein cholesterol
LV = left ventricle/ventricular
LVMI = left ventricular mass indexed by body surface area
NO = nitric oxide

tion of L-arginine results in transient reduction in systemic vascular resistance and blood pressure in both normotensive and hypertensive humans and in the salt-sensitive rat model of hypertension (22–26). Previous studies have not addressed racial and gender patterns in L-arginine responsiveness. Thus, the purpose of our study was to examine the possible modulating effects of race and gender on response of the coronary endothelium to intracoronary infusion of L-arginine.

METHODS

Subjects. Patients were prospectively recruited for the approved investigational study (Albany Medical College Institutional Review Board) after clinical referral for cardiac catheterization. Informed consent was obtained documenting the participants' understanding of the investigational nature of the protocol. The current investigation is part of a larger study begun in 1992 whose purpose was the examination of the effects of coronary risk factors, gender and ethnicity on coronary artery and arteriolar relaxation properties. Ethnicity was self-reported by the patient as African American (black) or Caucasian (white). No patient had CAD based on angiographic assessment. Individuals were excluded from the study because of significant valvular heart disease, prior history of myocardial infarction or coronary revascularization procedure, or other serious medical disorder. Patients were excluded for morbid obesity, defined as weight greater than twice ideal body weight.

African American and Caucasian study pairs were matched first for gender, then for the best overall fit of the parameters: age, left ventricular mass indexed by body surface area (LVMI), body mass index (BMI) and low-density lipoprotein cholesterol (LDLC). For age, differences were considered less clinically significant among those 20 to 35 years old. The parameters of age, LVMI, BMI and LDLC were selected for matching because of their previously reported adverse effects on endothelial function and because the latter three were found to differ significantly among African American and white subjects enrolled in our larger study. Hypertension was defined as reproducible blood pressure measurements ≥140/90 mm Hg or self-reported taking of antihypertensive medication. Diabetes mellitus was diagnosed by self-reported history or fasting serum glucose ≥126 mg/dl. Hypercholesterolemia was defined by LDLC ≥160 mg/dl or by current use of cholesterol-lowering medication. A risk factor score was calculated for each patient by adding one point for each of the following risk factors for CAD: male gender and age ≥45 years, female gender and age ≥55 years, hypertension, cigarette smoking, diabetes mellitus, LDLC ≥160 mg/dl, high-density lipoprotein cholesterol (HDLC) <40 mg/dl and BMI ≥30 kg/m^2. One point was subtracted for HDLC ≥60 mg/dl. Menopausal status was determined based on self-reported history. Blood was obtained in the fasting state for measurement of total cholesterol, LDLC, HDLC, lipoprotein(a) and glucose.

Left ventricular (LV) mass was calculated using M-mode echocardiographic measurements made in accordance with the PENN convention and corrected to agree with necropsy data (27,28). Analyses were performed using LV mass indexed first by body surface area in m^2 and second by height$^{2.7}$ in m$^{2.7}$. Gender specific partition values for LV hypertrophy were taken from the Framingham Heart study (29). Subjects fasted for a minimum of 8 h before the study. Current smokers were instructed to refrain from smoking for a minimum of 8 h. Coronary endothelial dysfunction was defined by one or more of the following: presence of risk factors for CAD, constriction of epicardial arteries during infusion of ACh and coronary blood flow (CBF) increase <150% above baseline during infusion of ACh (1,4,9–12).

Invasive coronary artery testing. After coronary angiography was performed, a 0.018-in. Cardiometrics Flo-Wire Doppler tipped guidewire (Cardiometrics, Mountain View, California) was advanced through a coronary guiding catheter into the proximal to mid-portion of the left anterior descending artery in 26 African American and in 25 Caucasian subjects and into the circumflex artery in 7 and 8, respectively. After stable measurements of baseline coronary flow velocity were recorded, we infused agents into the left main artery to test the capacity for vasorelaxation by endothelium-dependent and -independent mechanisms. Instantaneous coronary pressure and blood flow velocity were continuously recorded. Coronary arteriograms were obtained under baseline conditions, at the end of each graded infusion of ACh and after L-arginine and repeated peak infusion of ACh.

Intracoronary drug infusion protocols. To evaluate endothelium-independent coronary relaxation of the microcirculation, adenosine was administered by graded intracoronary bolus into the left main artery (8 μg, then 16 μg, then 20 μg), each after return to baseline values of coronary flow velocity, blood pressure and heart rate. Graded infusion of ACh was then commenced to study endothelium-dependent coronary relaxation of resistance and conduit vessels. Acetylcholine in 3 ml was infused directly into the left main artery (assumed blood flow equal to 150 ml/min) over 2 min initially at a rate of 0.15 μg/min (10^{-8} mol/l)

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using a Medfusion syringe pump (Harvard apparatus, Duluth, Georgia). Acetylcholine was similarly infused at 1.5 μg/min (10^{-7} mol/l), then 15 μg/min (10^{-6} mol/l). In 48 patients (73%), a peak infusion rate of 30 μg/min (2×10^{-6} mol/l) was additionally administered. After ACh infusions, L-arginine (3,200 μmoles) was infused over 10 min into the left main coronary artery and the peak infusion rate of ACh repeated.

Quantitative coronary angiography. Baseline arteriography of the coronary artery undergoing study was performed in an optimal right anterior oblique or anteroposterior projection so that overlapping of branches and foreshortening of the region of interest were minimized. An optimal end-diastolic cineangiographic frame was selected, and coronary artery diameter was measured at the site of Doppler velocity measurements in each of the sequential research study arteriograms. Measurements were made with electronic digital calipers (Sandhill Scientific, Colorado Springs, Colorado) by a single investigator (J. L. H.) who was blinded to the clinical, echocardiographic and Doppler velocity findings. We have previously shown by linear regression analysis that intraobserver variability for measurement of coronary artery diameter in our laboratory was minimal (30). Coronary artery area was calculated assuming a circular cross-sectional profile. Percent change in coronary artery diameter above baseline (a measure of epicardial artery vasomotor function) was calculated in response to infusions of ACh ± L-arginine. The contrast agent iohexol was used for all studies.

Coronary artery blood flow and resistance measurements. Coronary artery blood flow was calculated as the product of mean CBF velocity and corresponding coronary artery cross-sectional area. Baseline values were calculated before infusion of the predominantly endothelium-independent agent adenosine and before infusion of the endothelium-dependent agent ACh. Percent change in CBF above baseline was calculated in response to adenosine, in response to each infusion of ACh and after co-infusion of the peak dose of ACh + L-arginine. Similarly, coronary vascular resistance was calculated as the quotient of instantaneous coronary perfusion pressure (mean aortic pressure) and mean CBF. Minimum endothelium-dependent coronary vascular resistance index was calculated as the coronary vascular resistance at peak effect of ACh divided by that at baseline and expressed as a percentage. After L-arginine and repeated peak infusion of ACh, minimum coronary vascular resistance index was recalculated.

Statistical analysis. Summary clinical data and outcomes of the research studies (percentile change in CBF and coronary artery diameter measurements in response to ACh + L-arginine as compared with ACh alone) are expressed as mean ± SE. Unpaired Student *t* test (for continuous variables) and chi-square test or Fisher exact test (for categorical variables) were used for assessment of the statistical significance of group differences, where a value of $p < 0.05$ was considered significant. Patients were grouped by race and by race and gender for comparisons. The primary outcome for this study, change in the peak ACh microvascular response after intracoronary L-arginine, was defined as the absolute percentage point increase in CBF after L-arginine during peak infusion of ACh when compared with control ACh testing before L-arginine. Similarly, change in the peak ACh epicardial response after intracoronary L-arginine was defined as the absolute percentage point increase in epicardial diameter after L-arginine during peak infusion of ACh when compared with control ACh testing. Percentile change in the ACh stimulated minimum coronary vascular resistance index in response to supplementary L-arginine infusion was calculated for each subject, then compared among the groups. Two-way analysis of variance (ANOVA) was used to test for gender and racial differences in CBF and coronary artery diameter responses to graded infusions of ACh, with the Bonferroni adjustment of probability values for multiple comparisons. Linear regression analysis was performed for assessment of the association between ACh response after L-arginine and the independent variables: age, LVMI, BMI, LDLC, HDLC, lipoprotein(a), adenosine response, mean arterial pressure and risk factor score. Grubbs test was used for detection of outliers (31).

RESULTS

Subjects. Thirty-three African American subjects (20 men and 13 women) were identified as eligible for the study and were subsequently matched with 33 Caucasian subjects. Eligibility was defined as performance of coronary reserve and endothelial function testing with supplementary L-arginine infusion, presence of endothelial dysfunction as defined in the Methods section, absence of any study exclusion and presence of a matching white subject fulfilling the same criteria. African American and white subjects were matched first for gender, then for the closest overall match of age, LVMI, LDLC and BMI. Matching parameters and pertinent clinical data for African American patients are listed in Table 1. Similarly, the paired white subjects are listed sequentially using the same format in Table 2. Also listed is the percentile change in peak CBF response stimulated by ACh after L-arginine compared with before. Table 3 presents summary matching data for the two groups. There were no significant differences in matching parameters, in baseline peak ACh CBF responses (76 ± 9% vs. 86 ± 10% increase above baseline) and in peak endothelium-independent (adenosine) CBF responses (208 ± 13% vs. 174 ± 12% increase above baseline) among African American and white subjects. Using two-way ANOVA, dose response curves relating percent increase in CBF to dose of ACh were not significantly different among the African American and Caucasian groups. Similarly, there were no significant differences among groups in dose response curves relating percent increase in coronary diameter to dose of ACh. Hypertension was equally present in

Table 1. Individual Clinical Characteristics and Coronary Blood Flow Responses After L-Arginine in 33 African American Patients

Patient Number	Age (yrs) and Gender	BMI (kg/m^2)	LVMI (g/m^2)	LDLC (mg/dl)	BP (mm Hg)	HTN	DM	Tob	Percentile Change in AChP After L-Arginine
1	20 M	27.2	97	82	100/74	−	−	−	27
2	25 M	26.2	120	168	120/80	+	−	−	47
3	28 M	37	128	132	125/98	−	−	−	25
4	31 M	25	119	105	112/85	+	−	−	278
5	34 M	27.8	148	179	160/122	+	−	−	62
6	35 M	27	113	125	130/84	+	−	−	110
7	36 M	27	156	194	110/85	+	−	−	114
8	37 M	34	113	194	158/95	+	+	+	9
9	43 M	25	104	82	120/80	+	−	+	−13
10	43 F	36	130	86	115/90	−	−	+	32
11	44 M	18	81	103	140/85	−	−	−	5
12	44 M	23.3	98	119	110/75	+	−	+	0
13	44 M	34	114	46	140/78	+	+	+	36
14	45 M	24.9	142	116	170/108	+	−	+	65
15	46 F	35	121	98	216/120	+	−	+	16
16	47 M	26	100	122	146/100	+	−	−	195
17	47 F	42	154	158	158/96	+	−	+	56
18	48 M	35.6	115	120	120/85	−	−	−	26
19	49 M	24	109	154	122/85	+	−	+	22
20	50 F	29.2	102	96	134/76	+	−	−	19
21	50 F	33	67	78	150/92	−	+	−	34
22	50 F	47	88	65	200/102	+	−	−	27
23	51 F	40.2	118	165	200/120	+	−	−	9
24	53 F	35.7	142	201	185/95	+	+	−	29
25	54 M	26	104	120	138/96	+	−	−	6
26	56 F	27	103	81	150/88	+	+	−	104
27	60 F	25	90	143	140/75	−	−	+	−2
28	61 M	27	191	143	145/85	+	−	−	14
29	63 M	26	108	121	148/82	−	−	−	63
30	64 F	29.9	103	97	148/72	+	−	−	15
31	66 M	24.4	120	122	130/82	−	−	−	21
32	68 F	28.7	113	90	160/80	+	+	−	7
33	72 F	29	105	111	175/90	+	−	−	10

AChP = peak coronary blood flow response to intracoronary acetylcholine; BMI = body mass index; BP = blood pressure; LDLC = low-density lipoprotein cholesterol; DM = history of diabetes mellitus; HTN = history of hypertension; LVMI = left ventricular mass indexed by body surface area; Tob = history of tobacco use.

73% of the two groups. Chronic tobacco use, diabetes mellitus and hyperlipidemia were present in similar proportions, 30% versus 27%, 18% versus 21% and 24% versus 12%, respectively, p = NS for each of these comparisons. Risk factor score was 2.2 ± 0.2 in African Americans and 2.8 ± 0.2 in white subjects, p = 0.07. Increase in CBF in response to ACh alone was depressed (<150% increase above baseline) in 29 of 33 African Americans (88%) and in 29 of 33 white subjects. After ACh, constriction of the epicardial coronary artery under study occurred in 13 of 33 African Americans (39%) and in 13 of 33 white subjects. Of the three criteria defining endothelial dysfunction, 6 African American and 4 Caucasian subjects had only one criteria; 14 and 17, respectively, had two criteria; and 13 and 12, respectively, had all three criteria. The mean endothelial dysfunction criteria score was identical (2.2 ± 0.1) among African American and Caucasian subjects. Left ventricle mass indexed by $height^{2.7}$, an allometric approach which minimizes the contribution of obesity, was nearly identical among African American and white subjects (53.5 ± 3 $kg/m^{2.7}$ vs. 53.1 ± 3 $kg/m^{2.7}$). Left ventricular ejection fraction was similar (66 ± 2% vs. 68 ± 2%) as was mean arterial pressure (108 ± 3 mm Hg vs. 102 ± 2 mm Hg, p = 0.11). Serum creatinine (1.0 ± 0.1 mg/dl vs. 0.9 ± 0.1 mg/dl), fasting glucose (125 ± 13 mg/dl vs. 110 ± 9 mg/dl, p = 0.24) and lipoprotein(a) (42 ± 6 mg/dl vs. 27 ± 6 mg/dl, p = 0.14) were not significantly different. The only value found to significantly differ between groups was HDLC (51 ± 3 mg/dl vs. 40 ± 2 mg/dl, p = 0.001). Univariate linear regression analysis showed no significant correlation between change in ACh stimulated CBF after L-arginine and the following independent variables: baseline peak ACh response before L-arginine, age, LVMI, BMI, LDLC, HDLC, lipoprotein(a), adenosine response, mean arterial pressure during testing, risk factor score and endothelial dysfunction criteria score.

Of the 33 African American subjects, 19 (58%) were being treated with potentially vasoactive medications. In six cases (18%), vasoactive medications were taken within 12 h of the procedure. This included two receiving coronary vasodilator drugs and four receiving beta-antagonist drugs in combination with coronary dilator and/or angiotensin-

Table 2. Individual Clinical Characteristics and Coronary Blood Flow Responses After L-Arginine in 33 White Patients Matched to Those in Table 1

Patient Number	Age (yrs) and Gender	BMI (kg/m^2)	LVMI (g/m^2)	LDLC (mg/dl)	BP (mm Hg)	HTN	DM	Tob	Percentile Change in AChP After L-Arginine
1	27 M	28.9	75	103	112/86	+	−	−	−10
2	35 M	25.1	96	167	122/90	+	−	−	−35
3	28 M	36	132	101	128/100	+	+	−	−20
4	31 M	28	117	109	125/95	−	−	+	33
5	34 M	29	159	151	116/94	+	+	+	81
6	35 M	26.8	115	101	105/78	−	−	−	63
7	40 M	27	136	154	150/92	+	−	+	31
8	31 M	36	102	168	142/100	+	−	−	−14
9	37 M	30	117	112	130/95	−	−	+	48
10	51 F	31.8	165	62	152/76	+	+	−	−8
11	42 M	26.5	118	130	135/88	−	−	−	−73
12	43 M	21	91	133	115/65	−	−	+	51
13	46 M	35.6	111	136	170/106	+	−	−	0
14	47 M	29	139	121	140/95	+	−	−	4
15	47 F	34.2	107	101	140/80	+	−	−	29
16	50 M	31	80	121	120/75	−	−	−	75
17	50 F	34	131	135	150/95	+	+	−	5
18	48 M	35.6	75	145	122/70	+	−	−	−13
19	45 M	26	105	156	125/80	−	−	−	−81
20	48 F	23.6	112	72	142/78	+	−	+	−28
21	48 F	30.1	105	83	135/70	+	+	−	25
22	51 F	40	110	110	122/70	+	−	+	−5
23	59 F	35.5	103	133	155/95	+	+	−	3
24	56 F	40	140	183	190/100	+	−	+	−20
25	55 M	24.4	98	126	124/80	−	−	−	−4
26	57 F	31	86	102	162/90	+	−	−	−22
27	58 F	21	92	178	128/60	−	−	−	30
28	59 M	23	199	137	98/65	+	−	−	25
29	60 M	28.4	84	113	152/94	+	−	−	−14
30	68 F	33	84	136	125/75	+	−	−	−41
31	65 M	28.7	128	149	132/78	+	−	−	15
32	66 F	34	126	128	195/86	+	+	+	10
33	73 F	34.6	114	74	182/80	+	−	−	0

Abbreviations as in Table 1.

converting enzyme inhibitor drugs. Of the 33 white subjects, 24 (73%) were being treated with potentially vasoactive medications. In three cases (9%), vasoactive medications were taken within 12 h of the procedure. This included one receiving a coronary vasodilator drug and two receiving beta-antagonist drugs in combination with an alpha-adrenergic blocking drug or angiotensin-converting enzyme inhibitor drug. Among the 26 female subjects, 11 of 13 African Americans were postmenopausal (three taking hormone replacement therapy), one perimenopausal and one premenopausal; 10 of 13 white subjects were postmenopausal (four taking hormone replacement therapy), two perimenopausal and one premenopausal.

Table 3. Matching Clinical Characteristics in 33 Pairs of African American and White Patients

	African American Subjects (n = 33)	White Subjects (n = 33)	p Value
Age (yrs)	47.4 ± 2.2	48.2 ± 2.1	0.8
BMI (kg/m^2)	29.8 ± 1.1	30.3 ± 0.9	0.7
LVMI (g/m^2)	115.6 ± 4.2	113.7 ± 4.7	0.8
LDLC (mg/dl)	121.7 ± 6.7	125.2 ± 5.2	0.7

Abbreviations as in Table 1.

Endothelium-dependent coronary vascular relaxation after L-arginine. Figure 1 shows the individual microvascular responses of the African American and white subjects. The peak CBF response after ACh is shown at baseline and then after intracoronary L-arginine for each subject. As a group, African Americans showed significant enhancement of ACh-mediated increase in CBF above baseline after L-arginine when compared with before (120 ± 12% vs. 76 ± 9%, p = 0.004). By contrast, the matched white subjects as a group demonstrated no significant benefit after L-arginine (90.2 ± 11% vs. 86 ± 10%). Figure 2 shows absolute percentile improvement in peak ACh response after intracoronary L-arginine in African Americans versus white subjects, first partitioned by gender, then combined. This figure demonstrates greater improvement among African Americans as a group (44.5 ± 10.3% vs. 4.2 ± 6.4%, p = 0.0016), among African American men compared with matched white men (55.6 ± 16% vs. 8.1 ± 9.8%, p = 0.016) and among African American women compared

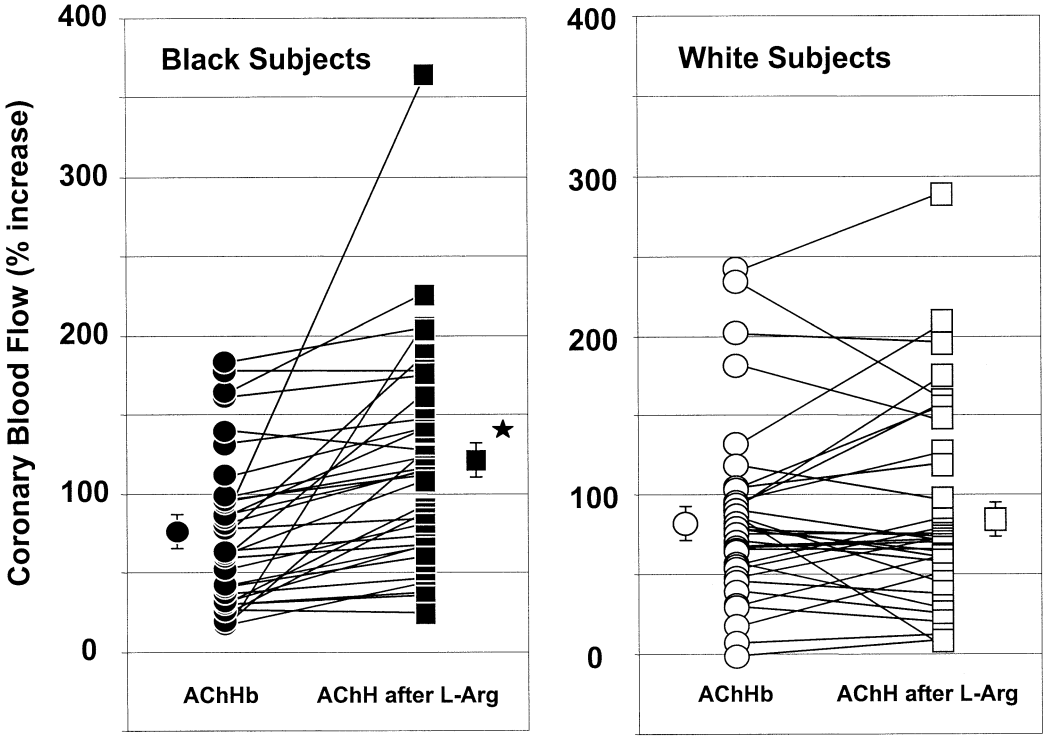

Figure 1. Individual peak percent increase in coronary blood flow above baseline in response to acetylcholine alone (AChHb) and then after repeated administration of acetylcholine following infusion of L-arginine (AChH after L-Arg) is shown in 33 African Americans **(left panel)** and in 33 matched white subjects **(right panel).** ★p = 0.004.

with matched white women (27.3 ± 7.6% vs. −1.7 ± 6.2%, p = 0.007). Using Grubbs outlier analysis, the L-arginine response of the subject with the largest improvement was >2 SD from the mean and was identified as a possible outlier. The experimental procedure and study data were of excellent quality, arguing against arbitrary removal. The analysis was, however, repeated after removal of the single largest responder after L-arginine (Table 1, Patient 4) and the paired subject from Table 2. There were no changes in the overall results. No other subject met criteria defining an outlier. L-arginine infusion alone did not change baseline CBF. Though not as dramatic an effect, L-arginine infusion also resulted in a greater epicardial dilator response (as measured by percent increase in coronary diameter) after ACh in African Americans but not white subjects (0.33 ± 0.5% vs. −2.2 ± 0.7%, p = 0.02). Finally, coronary vascular resistance index (percentile change) was decreased after L-arginine infusion followed by ACh provocation among African Americans but not among matched white subjects when compared with ACh provocation alone (−10.7 ± 2.3% vs. −0.9 ± 1.9%, p = 0.0017).

Though our study lacks a control group of subjects with none of the three criteria used to define endothelial dysfunction, four African American and four white subjects in the matched study groups had normal vasodilator responses to ACh, that is, CBF ≥150% above baseline and epicardial dilation. Despite normal vasodilator function, the four African Americans demonstrated further improvement in ACh responses after L-arginine, yet the four white subjects did not (24.8 ± 13.5% vs. −16.3 ± 25.5% increase in CBF, p = 0.21), suggesting that L-arginine is efficacious even among black, but not white, subjects with preserved endothelial function. Furthermore, we studied an additional 15 Caucasian subjects (nine men, six women) with normal coronary angiography who could not be matched to a comparable African American subject and had normal vasodilator responses to ACh but one or more risk factors for CAD. These 15 had similar age, LVMI, LDLC and BMI characteristics and demonstrated nearly identical mild improvement in peak ACh responses after L-arginine when compared with the larger matched Caucasian study group (5.2 ± 12% vs. 4.2 ± 6%, p = NS).

DISCUSSION

Our study demonstrates that intracoronary L-arginine infusion preferentially enhances microvascular relaxation after ACh stimulation in African American subjects referred for coronary angiography. This finding is independent of gender. Both African American men and women demonstrated greater augmentation of CBF after supplementary L-arginine in comparison with Caucasian men and women well matched for characteristics known to be associated with endothelial dysfunction. The mechanism of improved response was mediated through further reduction in coronary microvascular resistance. Similarly, though to a lesser degree, coronary epicardial dilation in response to ACh was

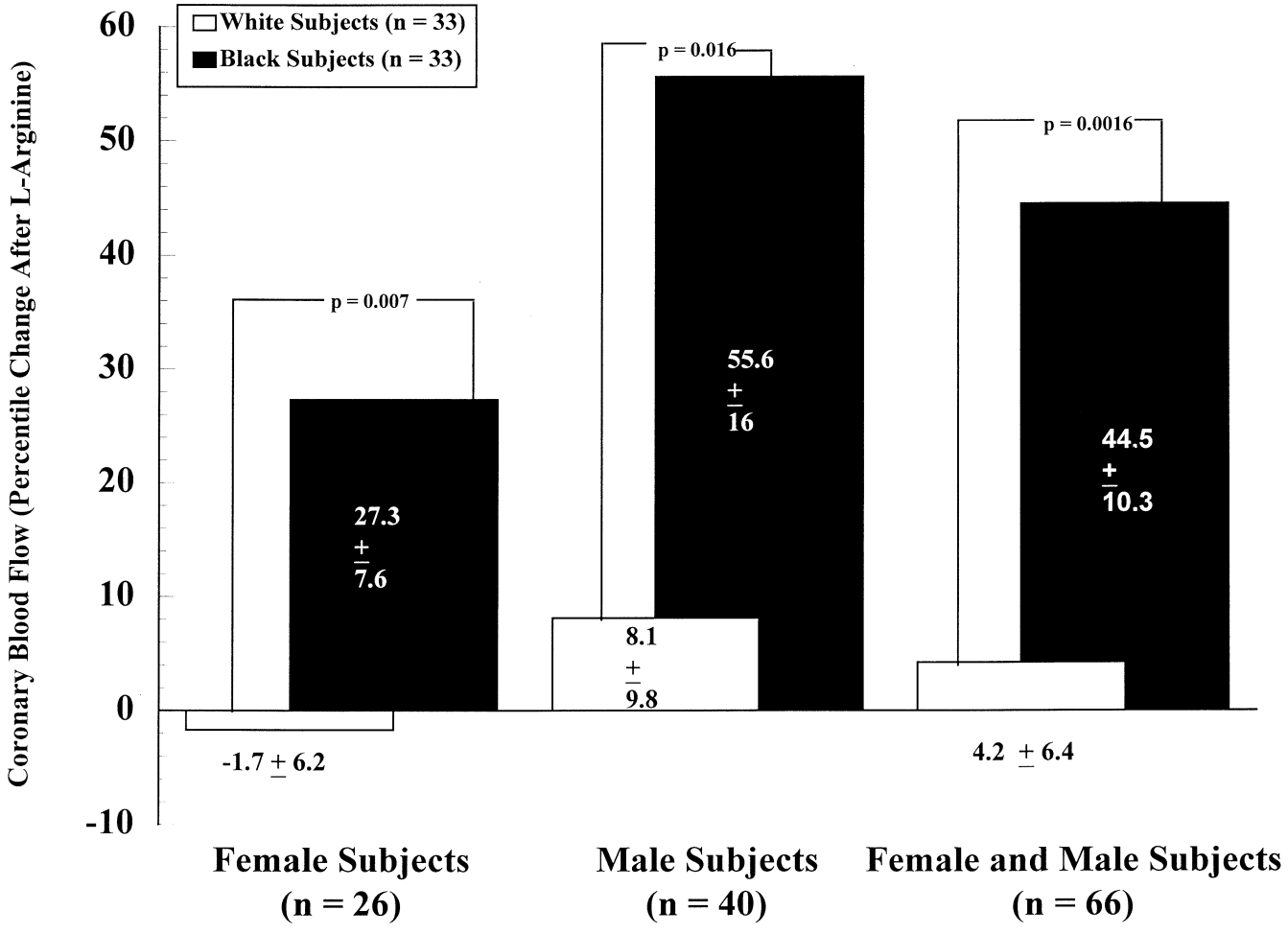

Figure 2. Absolute percentile change in coronary blood flow in response to peak acetylcholine infusion after L-arginine infusion compared with peak acetylcholine infusion alone. Data are shown for 13 African American and 13 matched white women; for 20 African American and 20 matched white men; and for the total population of 33 African American and 33 matched white subjects.

preferentially enhanced after L-arginine in African American subjects.

We studied patients with normal coronary arteries based on angiographic assessment. Because angiographic assessment of epicardial arteries is insensitive for detection of atherosclerosis and endothelial dysfunction, we used other criteria to define endothelial dysfunction: presence of coronary risk factors, coronary artery constriction in response to ACh infusion and CBF increase <150% above baseline in response to ACh (1,4,9–12). Subjects with no angiographic CAD and endothelial dysfunction are conjectured to represent an early stage in the natural history of CAD and cardiomyopathy where reparative therapy of endothelial dysfunction may serve to retard or prevent progressive disease. This is a population of enormous importance in preventive cardiology.

L-arginine-mediated augmentation of vascular relaxation. For over 10 years now, supplementary L-arginine has been reported to improve endothelium-dependent vasomotor function in patients with endothelial dysfunction. Our study is the first, to our knowledge, to investigate racial and gender patterns in L-arginine responsiveness of the coronary endothelium. Multiple candidate mechanisms for efficacy of L-arginine have been proposed, including provision of substrate for endothelial cell NO synthase, indirect antioxidant effects, competition with naturally occurring NO synthase inhibitors such as asymmetric dimethylarginine (ADMA), alteration of endothelial cell acid/base microenvironments, attenuation of norepinephrine bioactivity, release of other vasoactive agents such as insulin and histamine and provision of substrate for inducible NO synthase (32–35).

Although the exact mechanism remains unknown in our study, there are two that merit particular attention. First, accumulation of ADMA preferentially among African Americans would result in competitive inhibition of endothelial NO synthase, which could be rapidly reversed by supplemental L-arginine. Though there is no data about the prevalence of increased plasma ADMA among African Americans, it has been shown that ADMA levels are increased in the presence of renal failure, hypercholesterolemia and peripheral vascular disease (33,34). More pertinent to our study, plasma ADMA concentration increased and NO metabolites decreased after salt loading in salt-

sensitive hypertensives (35). Although salt-sensitive hypertension is present in both black and white subjects, it is significantly more prevalent among African Americans, providing a possible rationale for preferential L-arginine benefit (36). Our study was not designed to measure ADMA plasma levels or to test for salt sensitivity; therefore, we can only speculate about this possible association. The second mechanism of interest is attenuation of norepinephrine bioactivity, a non–NO-dependent mechanism. Arginine has been shown to nonstereospecifically antagonize responses to norepinephrine, a vascular smooth muscle constrictor (37). This causes shifting of the norepinephrine dose response curve to the right, thus lessening induced arterial constriction during submaximal infusions. There is a clinical basis for suspecting increased coronary contractile sensitivity among African Americans. A consistent observation in previous studies is that black subjects exhibit increased peripheral vasoconstriction in response to stimuli that activate the sympathetic nervous system, such as exercise, mental arithmetic, cold pressor stimuli and other forms of behavioral coping challenge (38–40). Studies in the brachial circulation have also found reduced vasodilation in response to agonists in African Americans (41,42). Thus, attenuation of norepinephrine bioactivity by L-arginine may be a more important mechanism in African Americans because of the propensity for increased contractile sensitivity.

Comparison with previous coronary artery studies. In our study, there was only a modest average improvement in ACh-mediated increase in CBF after L-arginine among the Caucasian subjects (4.2 ± 6.4%), though a broad range of responses (81% to −81%). A total of 50% of Caucasian subjects, but only 9% of African Americans, showed no improvement after L-arginine (Tables 1 and 2). This is in contrast to several previous studies that largely investigated Caucasian patients with significant CAD (18,19). These, together with another study that presented combined findings of 24 patients with normal coronary angiography and 15 with CAD, reported significant improvement in CBF responses to ACh after L-arginine (21). Our study group differs importantly because it consists of subjects without angiographic CAD. Another study that enrolled Japanese subjects analyzed those with normal angiography and those with mild disease separately, showing no improvement in CBF responses after L-arginine in either group (17). In addition to differing patient characteristics, previous studies have used differing doses and/or modes of administration of ACh and L-arginine, likely contributing to the divergent findings.

Study limitations. Electronic digital calipers, as employed in our study, are useful for performing serial angiographic measurements because of simplicity and low cost of the technology. Although this technique has been shown to consistently overestimate the true diameter of phantoms, when serial measurements are expressed as percent diameter change from baseline (as done in our study), comparable results were found using either digital calipers or automated quantitative coronary angiography (43).

A placebo infusion for comparison with L-arginine infusion during ACh testing was not performed because of the additional time required during an already lengthy research protocol. Consequently, reproducibility of ACh effect on CBF response cannot be determined in our study. Other studies, however, have reported excellent long-term stability of ACh responses at baseline and after six months of placebo (44–46).

Conclusions. We found that African American race predicts enhanced coronary vasodilator response to ACh after L-arginine infusion in a referral cardiac population with normal coronary arteriograms and endothelial dysfunction after matching for age, gender, LVMI, LDLC and BMI. This finding was independent of gender. Improvement in microvascular function in African Americans after L-arginine was mediated through further reduction in coronary vascular resistance. If confirmed, our study suggests that there are target populations in which L-arginine supplementation may be particularly efficacious, especially for amelioration of microvascular endothelial dysfunction. Additional investigations studying enhancement of coronary endothelium-dependent relaxation after L-arginine in African American patients are needed to confirm our findings and explore possible mechanisms.

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