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Differences in Vascular Nitric Oxide and Endothelium-Derived Hyperpolarizing Factor Bioavailability in African Americans and Whites

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Abstract

Objectives—Abnormalities in nitric oxide (NO) bioavailability have been reported in African Americans. Whether there are differences in endothelium-derived hyperpolarizing factor (EDHF) in addition to NO between African Americans and whites, and how these affect physiologic vasodilation remains unknown. We hypothesized that the bioavailability of vascular NO and

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EDHF, at rest and with pharmacologic and physiologic vasodilation, varies between white and African Americans.

Approach and Results—In 74 white and 86 African American subjects without known cardiovascular disease risk factors, forearm blood flow (FBF) was measured using plethysmography at rest and during inhibition of NO with N^G-monomethyl-L-arginine (L-NMMA) and/or of K⁺_{Ca} channels (EDHF) with tetraethylammonium (TEA). The reduction in resting FBF was greater with L-NMMA (p=0.019) and similar with TEA in whites compared to African Americans. Vasodilation with bradykinin, acetylcholine, and sodium nitroprusside was lower in African Americans compared to whites (all p<0.0001). Inhibition with L-NMMA was greater in whites compared to African Americans with bradykinin, acetylcholine, and exercise. Inhibition with TEA was lower in African Americans with bradykinin, but greater during exercise and with acetylcholine.

Conclusions—The contribution to both resting and stimulus-mediated vasodilator tone of NO is greater in whites compared to African Americans. EDHF partly compensates for the reduced NO release in exercise and acetylcholine-mediated vasodilation in African Americans. Preserved EDHF but reduced NO bioavailability and sensitivity characterizes the vasculature in healthy African Americans.

Keywords

vasodilation; exercise; African American; endothelial function; endothelium-derived hyperpolarizing factor; nitric oxide

Introduction

Endothelial dysfunction predisposes to the development of hypertension and atherosclerosis that together inflict significant morbidity and mortality. African Americans (AA) suffer from a higher incidence of premature stroke and myocardial infarction when compared to white counterparts.¹ Although these disparities are often attributed to a higher prevalence of cardiovascular risk factors, and socioeconomic burden, underlying endothelial abnormalities may play a crucial underlying role.²⁻⁴ Abnormalities in nitric oxide (NO) bioavailability in the microcirculation and conductance vessels, reduced sensitivity of exogenous NO, increased endothelin bioavailability, and structural abnormalities in the vascular wall have all been observed in AAs, but the results have been controversial.⁵⁻¹⁶

The endothelium contributes to the maintenance of vasodilator tone by releasing NO, prostacyclin, and endothelium derived hyperpolarizing factors (EDHF).¹⁷⁻²⁰ NO contributes to resting vasodilator tone,²¹ and to physiologic vasodilation during exercise,¹⁸ and its bioavailability is significantly impaired in individuals with cardiovascular risk factors.²¹⁻²³ We have demonstrated an important contribution of EDHF via activation of K⁺_{Ca} channels to (1) resting vascular tone, (2) vasodilation in response to the endothelium-dependent agonist, bradykinin, and (3) exercise-induced vasodilation in the healthy human forearm vasculature.^{24, 25} Whether racial differences in EDHF exist remains unknown. We hypothesized that there is a differential racial contribution of the two endogenous agonists,

NO and EDHF to vasodilator tone at rest and to vasodilation during pharmacologic stimulation and with exercise.

Methods

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

Results

Baseline Characteristics

AA subjects had a greater body mass index and whites had a higher fasting glucose level, but the remaining characteristics were similar (Table 1). There were no significant changes in the FBF and FVR in the non-infused arm and mean arterial pressure and heart rate remained unchanged in both groups throughout the study period.

Contribution of NO and K^+_{Ca} channel activation to resting vasodilator tone

Effect of L-NMMA on resting forearm vascular tone—Resting FBF and FVR were similar in AA and white subjects. NO blockade with L-NMMA reduced resting FBF and increased FVR in both groups, but the 21.3% reduction in FBF ($p=0.019$) and 35% increase in FVR ($p=0.023$) in AA were both lower than in whites (32.1% decrease in FBF and 54% increase in FVR), indicating reduced contribution of NO to resting vasodilator tone in AAs (Figure 2a).

Effect of TEA on resting forearm vascular tone—Blockade of K^+_{Ca} channels with TEA decreased resting FBF and increased FVR in both subsets and the magnitude of change was similar in whites and AAs, indicating similar contribution of K^+_{Ca} channel activation to resting vasodilator tone in AAs and whites (Figure 2b).

Effect of L-NMMA combined with TEA on resting forearm vascular tone—Combined blockade of NO and K^+_{Ca} channels with L-NMMA and TEA had an additive effect in both subsets and resulted in significantly greater reduction in FBF and increase in FVR than with either antagonist alone (Figure 2c). The reduction in FBF ($p=0.003$) and increase in FVR ($p=0.006$) was greater in whites than in AAs, indicating that there is increased contribution to resting vasodilator tone of NO and EDHF together in whites compared to AAs.

Bradykinin-stimulated vasodilation

Bradykinin infusion resulted in dose-dependent vasodilation in both ethnic subsets, however, the response was attenuated in AA compared to white subjects, with a 27.3% ($p<0.0001$) lower mean FBF and 32% ($p<0.0001$) greater mean FVR in AAs (Figure 3a).

Effects of L-NMMA on bradykinin-mediated vascular responses—Bradykinin-induced dilation was attenuated with L-NMMA in both ethnic subsets, however, the reduction in FBF (-32% vs -26% , $p=0.012$) and the increase in FVR (78% vs 45% , $p=0.002$) was greater in whites than in AAs, respectively (Figure 4a), indicating reduced contribution of NO to bradykinin-mediated microvascular dilation in AA subjects.

Effects of TEA on bradykinin-mediated vascular responses—Bradykinin-induced dilation was attenuated with TEA in both ethnic subsets, however, the reduction in FBF (–31% vs –22%, $p<0.0001$) and the increase in FVR (40% vs 32%, $p=0.008$) was greater in whites than in AAs (figure 4b), indicating a decreased contribution of K^+_{Ca} channel activation to bradykinin-stimulated vasodilation in AAs compared to whites.

Effects of combined infusions of L-NMMA and TEA on bradykinin-mediated vascular responses—After combined infusion of L-NMMA and TEA, bradykinin-induced vasodilation was inhibited additively and equally in both subsets; in AA, 45% ($p<0.0001$) mean reduction in FBF and 94% ($p<0.0001$) increase in mean FVR; and in whites, 57%, $p<0.0001$ lower FBF and 155%, $p=0.0001$ higher FVR.

Acetylcholine-stimulated vasodilation

Acetylcholine infusion produced dose-dependent vasodilation in both ethnic subsets, however, the response was attenuated in AA compared to whites with a 34% ($p<0.0001$) lower mean FBF and 66% ($p<0.0001$) greater mean FVR in AAs (Figure 3b).

Effects of L-NMMA on acetylcholine-mediated vascular responses—

Acetylcholine-induced vasodilation was significantly inhibited by L-NMMA in both ethnic subsets, however, the reduction in FBF (–36% vs –20%, $p=0.042$) and the increase in FVR (81% vs 39%, $p=0.004$) were greater in whites than in AAs, indicating greater contribution of NO to acetylcholine-mediated dilation in white compared to AA subjects (Figure 5a).

Effects of TEA on acetylcholine-mediated vascular responses—Acetylcholine-induced vasodilation was inhibited in AAs with TEA (–15%, $p=0.06$ reduction in FBF and 14%, $p=0.03$ increase in FVR) but remained unchanged in whites (Figure 5B), indicating an important contribution of K^+_{Ca} channel activation to acetylcholine-stimulated vasodilation in AAs and none in whites.

Exercise-induced vasodilation

Intermittent handgrip exercise resulted in progressive vasodilation that was not significantly different between the two ethnic groups ($p=0.15$), although at the two higher exercise levels, there was a trend toward a lower increase in FBF in AA (–11%, $p<0.06$) (Figure 3c).

Effects of L-NMMA on exercise-induced vasodilation—Exercise-induced vasodilation was significantly inhibited by L-NMMA in both ethnic subsets, however, the reduction in FBF was greater in whites than in AAs (–21% vs –9% ($p<0.0001$), indicating decreased contribution of NO to exercise-induced vasodilation in AAs (Figure 6a).

Effects of TEA on exercise-induced vasodilation—Exercise-induced vasodilation was significantly inhibited by TEA in both ethnic subsets, however, the reduction in FBF was greater in AAs than in whites (–20% vs –16%, $p=0.013$), indicating greater contribution of K^+_{Ca} channel activation to exercise-stimulated vasodilation in AAs (Figure 6b). Moreover, in AA subjects, exercise-induced vasodilation was attenuated to a greater extent with TEA than with L-NMMA (–20% vs –9%, $p<0.0001$ reduction in FBF, respectively),

indicating that K^+_{Ca} channel activation and not NO is the predominant mechanism of exercise-induced vasodilation in AAs.

Effect of combined infusions of L-NMMA and TEA on exercise-induced vasodilation—Exercise-induced vasodilation was attenuated in an additive manner in both ethnic subsets with similar reductions in FBF in AAs and whites (–26% and –33%, $p=0.56$, respectively).

Sodium nitroprusside-induced vasodilation

Sodium nitroprusside produced vasodilation in both ethnic subsets, however, the response was attenuated in AAs compared to whites (–22%, $p=0.0002$ lower mean FBF and 12%, $p=0.0015$ greater mean FVR) (Figure 3d). This indicates reduced sensitivity of the smooth muscle cells to NO in AAs. Importantly, L-NMMA and TEA did not alter the vasodilator responses to sodium nitroprusside in either group.

Discussion

In the most comprehensive comparative investigation of microvascular function in a healthy bi-racial population to date, we investigated the relative contributions of NO and EDHF to basal vasodilator tone, to agonist-stimulated endothelium-dependent and -independent vasodilation, and to physiologic vasodilation. Important novel findings are: 1) there is greater contribution of NO than of EDHF to resting vasodilator tone in white compared to AA subjects, with similar EDHF bioavailability in both races; 2) there is reduced endothelium-dependent vasodilation with both acetylcholine and bradykinin in AA compared to white subjects, and this is secondary to reduced contribution of both NO and EDHF to bradykinin-mediated dilation, and to reduced contribution of NO to acetylcholine-mediated dilation in AA. EDHF partly compensates for the reduced NO release with acetylcholine; 3) there is reduced contribution of NO and relatively increased contribution of EDHF to exercise-induced vasodilation in AA compared to white subjects; and 4) there is reduced sensitivity of the vascular smooth muscle to NO, demonstrable as a reduced responses to sodium nitroprusside in AAs compared to whites.

Contribution of NO and EDHF to resting vasodilator tone—We have demonstrated an important contribution of both NO and EDHF, measured as TEA-inhibitable K^+_{Ca} channel activation, to resting forearm microvascular dilator tone in both ethnic subsets.²⁶⁻²⁸ There were no differences in the contribution of EDHF whether TEA was administered before or after L-NMMA was given. Although resting FBF was similar in the two groups, AAs had reduced contribution of NO, demonstrated by the decreased response to L-NMMA, and preserved contribution of EDHF in comparison to white subjects. Conductance vessel NO bioavailability has been reported to be either lower,^{5, 10, 14} or not different in AAs compared to whites.⁹ This is the first demonstration of reduced tonic basal NO activity in the microcirculation of healthy AA subjects, and may provide an explanation for the proportionally higher risk and prevalence of hypertension and cardiovascular disease in this group.¹ Whether other endogenous vasodilators such as prostacyclin, adenosine, carbon

monoxide etc. are differentially bioavailable in AA compared to White subjects needs to be studied.

Contribution of NO and EDHF to bradykinin-stimulated vasodilation—Not only did we confirm that bradykinin-mediated vasodilation is attenuated in AAs, but show that this is due to lower contribution of NO and EDHF.^{8, 15} Bradykinin is a potent endogenous vasodilator that stimulates NO, prostaglandins, and EDHF release by stimulating endothelial B₂ receptors. We confirmed that bradykinin-mediated vasodilation is both NO- and EDHF-mediated,^{22, 24, 26, 29-31} but whether their reduced contribution in the AA vasculature is due to enhanced metabolism need further study.¹⁵

Contribution of NO and EDHF to acetylcholine-stimulated vasodilation—Previous studies have reported either impaired^{15, 16} or intact¹¹ endothelial responses to muscarinic receptor stimulation with methacholine or acetylcholine in healthy AAs compared to whites. We recently demonstrated that acetylcholine-mediated vasodilation is largely due to stimulation of release of NO, and unlike bradykinin, acetylcholine does not stimulate EDHF release, a finding consistent with previous reports.^{27, 32} Now we demonstrate that not only is acetylcholine-mediated vasodilation depressed in AAs compared to whites, but that there is some compensatory increase in release of EDHF with acetylcholine from the AA microvasculature, a finding also observed in experimental models.^{33, 34} These findings in healthy AAs are similar to those observed in patients with multiple risk factors.²⁴

Contribution of NO and EDHF to exercise-induced vasodilation—We have recently shown that both NO and K⁺_{Ca} channel activation (EDHF) contribute individually and in concert to exercise-induced microvascular vasodilation.²⁵ Here, we demonstrate that the contribution to exercise-induced forearm microvascular dilation of NO is lower, and of EDHF is greater, in AAs compared to white subjects. Thus, even in healthy AAs, EDHF is the predominant mechanism responsible for exercise-induced forearm vasodilation, whereas NO is the predominant endothelium-dependent agonist in white subjects. These abnormalities are similar to the reduced mental stress-induced vasodilation we previously reported in normotensive AAs.³⁵

Mechanisms underlying vascular dysfunction in AAs—Oxidative stress from upregulation of superoxide anions and its effects on NO metabolism and endothelial nitric oxide synthase uncoupling and inhibition, play a central role in risk factor-mediated vascular pathophysiology.³⁶ Decreased NO bioavailability and increased production of peroxynitrite has been demonstrated in isolated human umbilical vein endothelial cells obtained from AAs,³⁷ and we have also demonstrated increased systemic oxidative stress measured as higher oxidized aminothiols levels and increased endothelin activity in AAs compared to whites which provides an explanation for the observed reduced NO bioavailability.^{38, 39} Treatment with nebivolol appears to normalize the increased free radical production in cells from AA and restores NO bioavailability to that observed in cells from Whites.⁴⁰ Other studies have demonstrated higher asymmetric dimethylarginine in AA compared to Whites.⁴¹

Differences in endothelium-independent vasodilation—We confirmed previous findings of reduced microvascular dilatory responses to sodium nitroprusside, an NO donor, in AAs, likely due to decreased responsiveness of the vascular smooth muscle to NO.⁴² Our previous findings of reduced cyclic nucleotide-mediated responses in AA forearm microcirculation involving both the adenylate cyclase and guanylate cyclase systems suggest either a generalized decrease in responsiveness of multiple vasodilator systems or involvement of a common distal step in the vasodilating pathways.^{16, 42} Modulation of cytosolic calcium concentration is one potential final pathway for regulating smooth muscle tone, and racial differences in its regulation may be one mechanism.⁴² Paradoxically, an enhanced response of conductance vessels to sublingual nitroglycerin was observed in AAs.⁹

Limitations

Although we found that the vasoconstrictor response to L-NMMA was lower in AAs, we did not examine effects of other non-specific vasoconstrictors to investigate whether this is a reflection of reduced sensitivity of the vascular smooth muscle to vasoconstrictors. However, the fact that the constrictor response to TEA was similar to whites suggests that the response to L-NMMA is specific for reduced NO bioavailability. The reduced sensitivity to exogenous NO (sodium nitroprusside) complicates the interpretation of the reduced dilator responses observed with acetylcholine and bradykinin in AAs. However, because basal NO and the contribution of NO during exercise is lower in AAs, it is likely that in addition to reduced sensitivity, there is also an endothelial defect in NO release in AAs.

L-NMMA and TEA are competitive inhibitors and thus our results may underestimate the physiological contribution of both NO and K^+_{Ca} channels to vasodilation. Our investigation was conducted on a background of cyclooxygenase inhibition because of its negligible contribution in the healthy human circulation.^{43, 44} Although we employed intermittent isometric handgrip exercise, other muscle beds and different intensities of exercise may need to be explored in future studies. Using plethysmography, we have assessed the microcirculatory responses and not the conductance vessel changes in this study, however, it is known that the contribution of EDHF is less in conductance vessels than in the microvessels.⁴⁵

Significance

Although NO and EDHF contribute to resting blood flow, to agonist-mediated vasodilation, and to vasodilation during exercise, the contribution of NO is reduced and of EDHF is preserved or increased in the microcirculation of healthy AAs with these stimuli. Preserved EDHF but reduced NO bioavailability in AAs may underlie their increased risk of hypertension and cardiovascular disease and have crucial therapeutic implications that need to be explored.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

none

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Abbreviations

AA	African American
EDHF	Endothelium-Derived Hyperpolarizing Factor
NO	Nitric Oxide

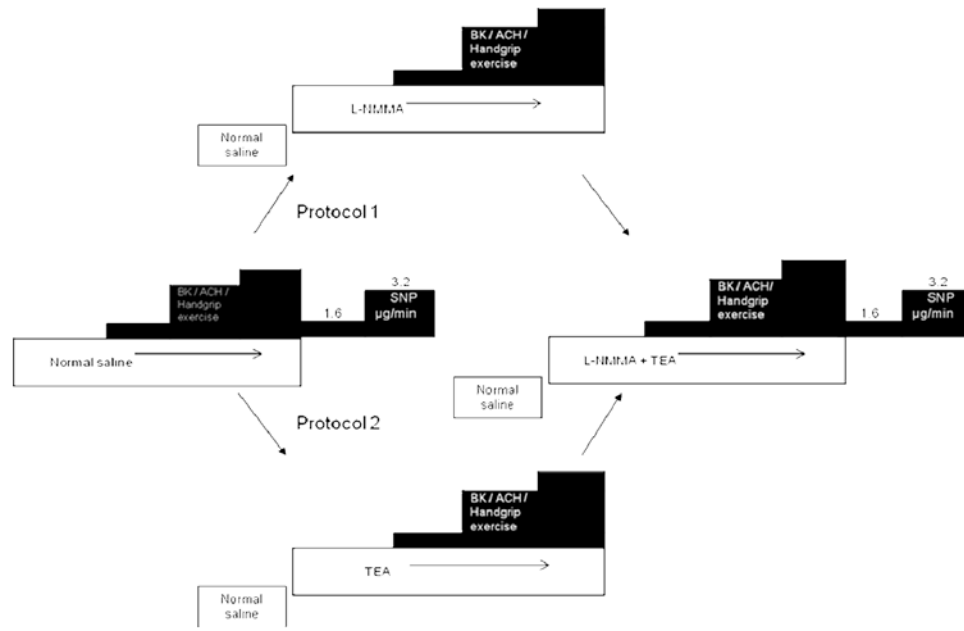


Figure 1.

Aspirin (975mg) was administered 1hour prior to commencement of the study. In separate protocols, FBF measurements were performed after either intra-arterial infusions of bradykinin (100, 200 and 400 ng/min), or intra-arterial acetylcholine (7.5, 15 and 30 µg/min), or handgrip exercise (15%, 30% and 45% of maximum grip strength) followed by endothelium-independent vasodilation with sodium nitroprusside (1.6, 3.2 µg/min). Measurements were repeated after NO blockade with L-NMMA, K^+_{Ca} channel blockade with TEA, and after combined blockade with L-NMMA and TEA.

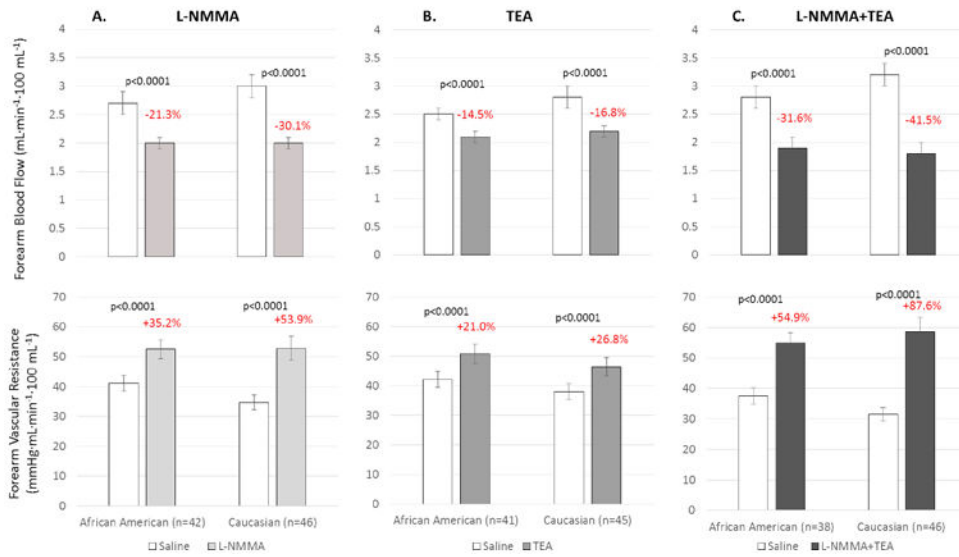


Figure 2. Change in resting FBF and FVR in response to (a) L-NMMA in 42 healthy AA and 46 white subjects, (b) in response to TEA in 41 healthy AA and 45 white subjects, and (c) to combined L-NMMA and TEA infusions in 38 healthy AA and 46 white subjects. Data is shown as mean \pm SEM.

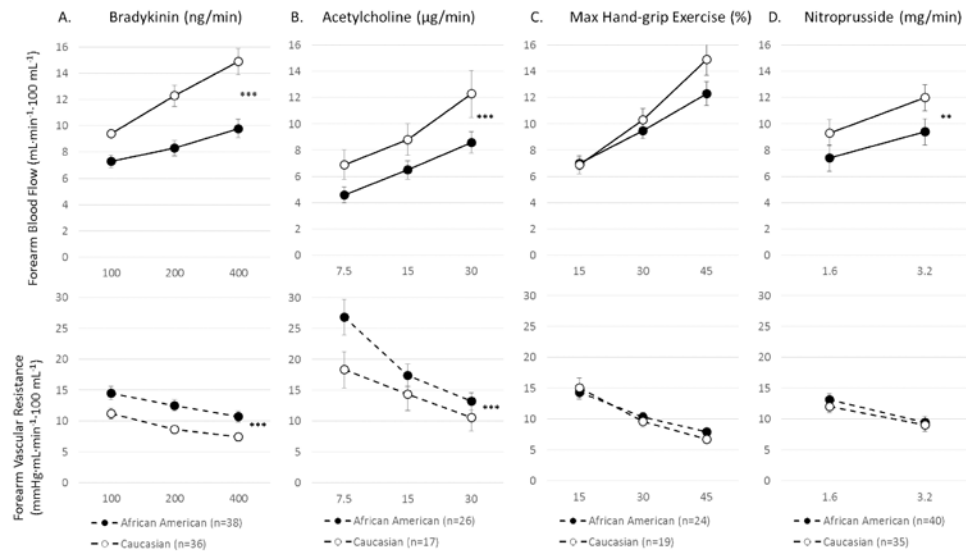


Figure 3. Forearm vasodilator response to (a) bradykinin, (b) acetylcholine, (c) hand-grip exercise, and (d) sodium nitroprusside in healthy African American and Caucasian subjects. Data shown as mean + SEM. Data shown as mean + SEM. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

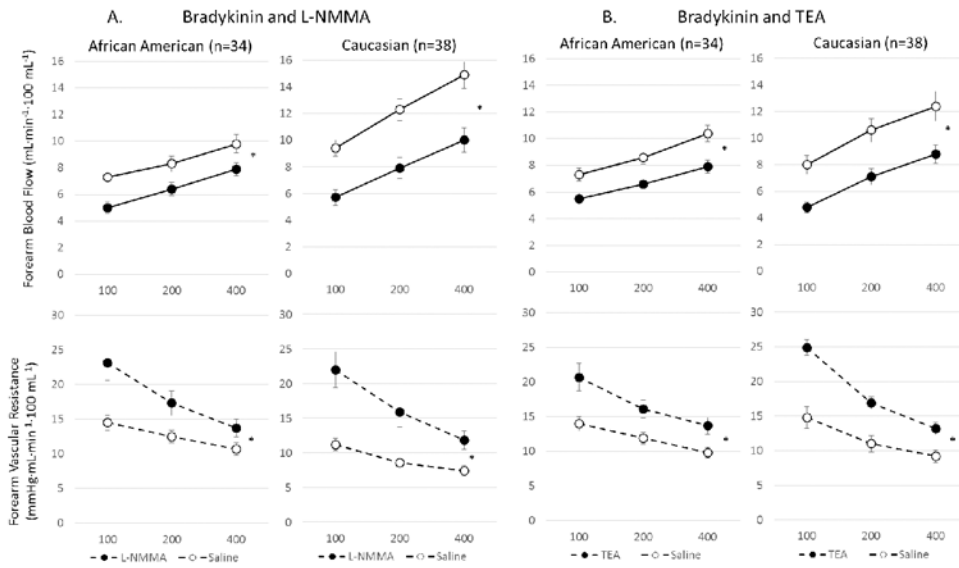


Figure 4. Vasodilation with bradykinin before and after inhibition with (a) L-NMMA or (b) TEA in African American and Caucasian subjects. Change in FBF and FVR in response to bradykinin after inhibition with (a) L-NMMA in AA (n=34) and white (n=38) subjects, and (b) with TEA in AA (n=32) and white (n=28) subjects. Data is shown as mean \pm SEM. * $p < 0.0001$

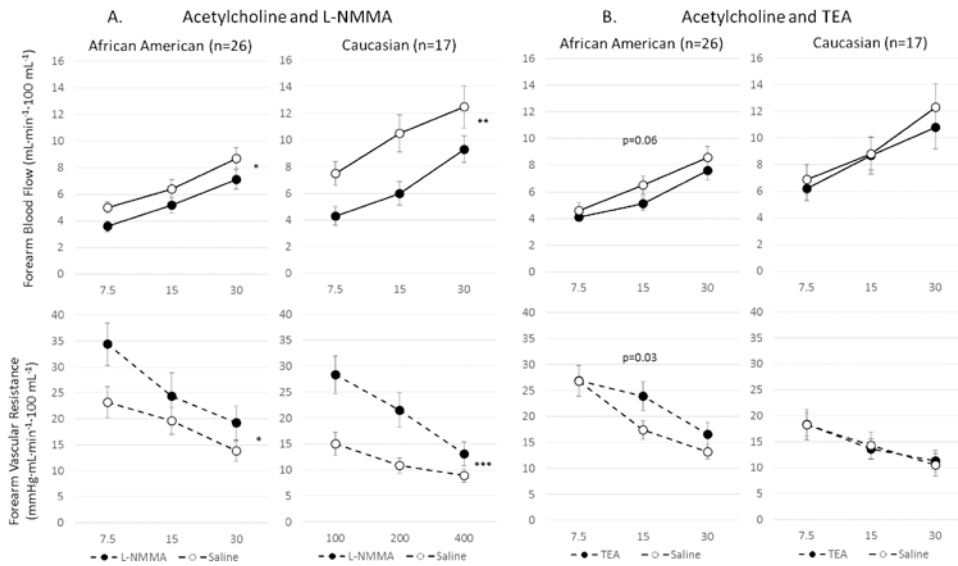


Figure 5. Vasodilation with acetylcholine before and after inhibition with L-NMMA or TEA in African Americans and Caucasians subjects. Change in FBF and FVR in response to acetylcholine after inhibition with (a) L-NMMA in AA (n=26) and white (n=17) subjects, and (b) after inhibition with TEA in African American (n=26) and Caucasian (n=17) subjects. Data is shown as mean \pm SEM. * $p < 0.01$; ** $p < 0.001$; *** $p < 0.0001$

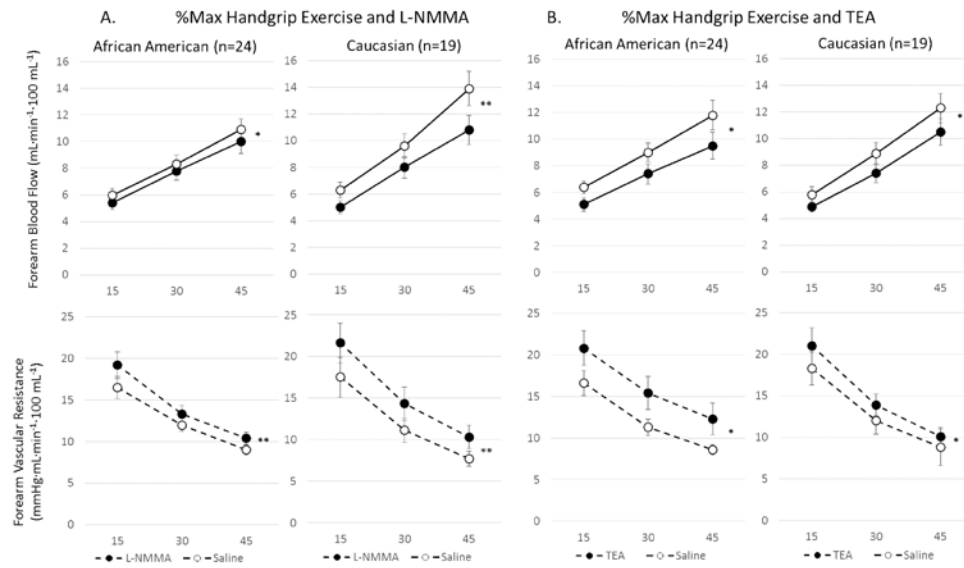


Figure 6. Vasodilation with forearm exercise before and after inhibition with (a) L-NMMA or (b) TEA in African American and Caucasian subjects. Change in FBF and FVR in response to increasing handgrip exercise (15, 30 and 45% of maximal handgrip) alone and (a) after L-NMMA, 16 μ mol/min in AA (n=24) and Caucasian (n=19) subjects, and (b) after TEA in AA (n=24) and Caucasian (n=19) subjects. Data shown as mean \pm SEM. *p=0.01; **p<0.0001

Table 1
Characteristics of Study Subjects

	African American (n=86)	Caucasians (n=74)
Age, years	39±11	40±13
Male, n (%)	46 (53.5)	38 (51.4)
Systolic BP, mmHg	118±13	117±11
Diastolic BP, mmHg	72±10	69±12
Body Mass Index, kg/m ²	28.8±6	26.8±5*
Creatinine, mg/dL	0.9±0.2	0.9±0.2
Glomerular Filtration Rate, ml/min	100.6±17.7	101.1±19.0
Glucose, mg/dL	85±10	89±10*
Triglycerides, mg/dL	102±49	127±109
High Density Lipoprotein, mg/dL	55±13	54±13
Low Density Lipoprotein, mg/dL	126±39	122±43

Glomerular Filtration Rate calculated using Cockcroft-Gault equation. Data are mean ± SD.

* Indicates a statistically significant ($p < 0.05$; t test) difference between groups.