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Racial Differences in Nitric Oxide-Dependent Vasorelaxation

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Abstract

Along with the growing heterogeneity of the American population, ethnic/racial disparity is becoming a clear health issue in the United States. The awareness of ethnic/racial disparities has been growing because of considerable data gathered from recent clinical and epidemiological studies. These studies have highlighted the importance of addressing these differences in the diagnosis and treatment of various diseases potentially according to race. It is becoming particularly clear that there is a 2- to 3-fold racial difference in certain cardiovascular diseases (eg, preeclampsia) associated with dysfunctional nitric oxide-mediated vasodilation. In this review, the authors summarize the current literature on racial disparities in nitric oxide-mediated vasodilation in relation to cardiovascular health with an emphasis on vascular nitric oxide bioavailability as a balance between production via endothelial nitric oxide synthase and degradation through reactive oxygen species. The major hypotheses postulated on the biological basis of these differences are also highlighted.

Keywords

Race; nitric oxide; endothelial nitric oxide synthase

Nitric oxide (NO) was discovered in 1988 as the vascular endothelial cell-derived factor that causes relaxation of the underlying vascular smooth muscle.^{1–3} NO dilates all types of blood vessels by stimulating soluble guanylate cyclase that produces cyclic guanosine monophosphate in smooth muscle cells.³ In addition to its vasodilatory properties, NO exerts some anti-inflammatory effects in the blood vessel wall by inhibiting leukocyte adhesion to the endothelium.⁴ NO also inhibits platelet adhesion and activation, thereby acting as an antithrombotic factor.⁵ NO inhibits smooth muscle cell proliferation and migration and regulates the expression of certain proteins involved in vessel wall remodeling, thereby contributing to the control of vascular compliance.⁶ Other functions of NO not related to the cardiovascular system include regulation of neuronal proliferation/differentiation,⁷ sexual function,⁸ insulin release,⁹ and immune responses in inflammation.¹⁰ NO participates in a broad spectrum of physiologic and pathophysiologic processes,¹¹ such as neurodegeneration and memory function,^{12,13} pulmonary vascular remodeling and apoptosis,¹⁴ atherosclerosis,¹⁵ and exercise-induced cardioprotection.¹⁶ Impairment of NO bioavailability leads to endothelial dysfunction and is a pivotal event in the pathogenesis of many cardiovascular diseases such as hypertension, heart failure, and coronary artery disease.^{17–19} NO-dependent endothelial dysfunction has also been associated with a range of other disorders such as pulmonary hypertension,²⁰ asthma,^{14,20} erectile dysfunction,⁸ preeclampsia,²¹ and insulin resistance and diabetes mellitus.⁹

The ethnic/racial impact on health has been receiving considerable attention in recent years. The phenomenon of ethnic health disparities in cardiovascular and many other diseases is becoming clear, and awareness of the importance in addressing these differences in the

diagnosis and treatment of various diseases has grown because of the increasing heterogeneity of the American population. Ethnic diversity is a factor in a variety of pathophysiological processes, including responses to stress, psychological issues, and communication between health care professionals and patients. In particular, African Americans and Africans (AA) are disproportionately affected by hypertension, which causes an estimated 30% and 20% of all deaths in AA men and women, respectively.^{18,22} African Americans have a more virulent course of hypertension, with higher rates of target organ damage, including heart failure, end-stage renal disease, myocardial infarct, and stroke as compared with non-African Americans.^{23–25} Hypertension in AA is associated with salt sensitivity, in which a decrease in NO-dependent vasodilation is also evident.²⁶ In addition to a higher incidence in cardiovascular diseases, AAs also have a higher incidence of other diseases in which NO plays an important role such as asthma²⁷ and type 2 diabetes mellitus associated with insulin resistance.^{28,29} Other races such as Asian Indians (AIs) are comparable to AAs in that they have a higher incidence of type 2 diabetes correlated with insulin resistance and cardiovascular disease as compared with Caucasians or European Americans (EAs).^{30,31}

The mechanisms underlying these racial disparities are multifactorial and involve genetic factors, socioeconomic status, psychosocial stressors/risks, and other environmental factors. Despite significant efforts being made to decipher the pathogenesis of complex diseases, such as hypertension, stroke, diabetes, and asthma, our knowledge regarding these diseases and, needless to say, its relationship to racial disparity remains destitute. Socioeconomical factors have been shown to play a role; however, these cannot fully account for the racial disparities. Obviously, more studies are required to elucidate the mechanisms leading to these diseases and to discover whether certain biological targets are more prevalent in some races as compared with others. It has been proposed that the observed racial differences in the prevalence in cardiovascular diseases, diabetes associated with renal failure, and even preeclampsia occurring in pregnancy could be, at least in part, attributed to decreased NO-mediated vasodilation in various vascular beds.^{18,32} NO-dependent vasodilation can be affected at the levels of either diminished NO production or increased NO degradation, thus rendering less NO bioavailable to target cells. In this review, we mainly cover the literature on the differences in vascular reactivity to different stimulants of NO production and potential causes of decreased NO production and/or bioavailability between AAs and other races, in particular, EAs. We emphasize those studies showing physiological and molecular differences in relation to NO among races, and we then highlight the major hypothesis postulated on the biological basis of these differences.

REGULATION OF NITRIC OXIDE PRODUCTION AND DEGRADATION

NO is a gaseous molecule derived from conversion of L-arginine to L-citrulline via a family of NO synthases (NOS), including neuronal NOS (nNOS/NOS1), inducible NOS (iNOS/NOS2), and endothelial NOS (eNOS/NOS3).³³ eNOS is the predominantly expressed NOS isoform responsible for most of the NO produced in the vasculature.³⁴ Catalytically active eNOS is in homodimer composition, where the C-terminal reductase domain (which binds nicotinamide-adenine dinucleotide phosphate [NADPH], flavin mononucleotide, and flavin-adenine dinucleotide) is linked to the N-terminal oxygenase domain of the other monomer (which carries a prosthetic heme group and also binds to [6R]-5,6,7,8-tetrahydrobiopterin [BH₄], molecular oxygen, and L-arginine). The reaction involves flavin-mediated electron transfer from the COOH-terminally bound NADPH to the heme on the NH₂ terminus. At the heme, the electrons are used to reduce and activate oxygen. Two cycles are needed to synthesize NO. In the first cycle, NOS hydroxylates L-arginine to N^w-hydroxy-L-arginine, which remains bound to the enzyme, and in the second step, N^w-hydroxy-L-arginine is oxidized to L-citrulline and NO.³³

The regulation of eNOS function is complex and involves mobilization of intracellular calcium, protein-protein interactions, and phosphorylation of eNOS by various signaling pathways.^{35,36} Calcium-activated calmodulin increases the rate of electron transfer from NADPH via the reductase domain flavins to the heme center, thereby linking intracellular levels of calcium to eNOS activation. Studies have shown that calcium removal or antagonism of calmodulin with inhibitors blocks the generation of NO from eNOS.³⁶ Protein-protein interactions also regulate eNOS activity.³⁶ For instance, in its less active state, eNOS interacts with caveolin-1 at cholesterol-rich plasmalemmal sites, the specialized membranous microdomain caveolae.³⁷ Upon stimulation, Ca²⁺-calmodulin activation leads to caveolin-1:eNOS dissociation and recruitment of heat shock protein 90 (HSP90). Proposed mechanisms by which HSP90 increases eNOS activity include inducing a conformational change in the enzyme, stabilizing the activation complex, increasing calmodulin affinity for eNOS, or acting as a scaffold for the recruitment of protein kinase B (PKB/Akt) to phosphorylate eNOS.³⁶ Phosphorylation via various protein kinases has been identified as an important mechanism for eNOS activation.³⁸ Six phosphorylation sites have been identified on eNOS. The most studied sites in eNOS are Serine¹¹⁷⁷ and Threonine⁴⁹⁵. Serine¹¹⁷⁷ has been shown to be phosphorylated by a series of different kinases, including PKB/Akt,³⁹ AMP-activated kinase,⁴⁰ protein kinase A, and protein kinase G,⁴¹ which is normally associated with increased enzymatic activity. Threonine⁴⁹⁵ can be phosphorylated by protein kinase C commonly in conjunction with decreased activity.⁴² Activation of these protein kinases can occur via the activation of multiple signaling pathways. Nevertheless, the main physiologically relevant signaling pathway activated by hormones (ie, estrogen/glucocorticoids),^{43,44} growth factors (insulin/vascular endothelial growth factor [VEGF]),^{45,46} and blood flow-generated laminar shear stress^{47,48} is the phosphotydylinositol-3 kinase-phosphoinositide-dependent protein kinase-1 (PDK1)-Akt/PKB pathway. Activated Akt directly phosphorylates Serine¹¹⁷⁷ on eNOS protein, which results in upregulation of eNOS activity, thereby increasing NO production.

The levels of bioavailable vascular NO depend on the balance between NOS-dependent NO production and NO degradation or sequestering by interaction with superoxide (O²⁻) to yield peroxynitrate (ONOO⁻). Decreased NO bioavailability is most commonly seen in oxidative stress conditions in which O²⁻ production is augmented. Oxidative stress refers to as an imbalance between reactive oxygen species (ROS), in particular O²⁻, production and the antioxidant defense mechanism to scavenge them, which shifts to left overwhelming ROS production.^{49,50} Oxidative stress has been shown to be one of the key factors that reduce NO bioavailability in the cardiovascular system, which may be a leading mechanism causing vasoconstriction of the vessels, hypertension, and other cardiovascular diseases.⁵¹⁻⁵³ Pregnancy-induced hypertension and preeclampsia are examples that decreased NO bioavailability causes maternal dysfunctional endothelium largely due to increased ROS mainly O²⁻ production.^{54,55}

Several mechanisms have been proposed for increased oxidative stress, including increased ROS formation via activation of NADPH oxidases or xanthine oxidases in the vascular wall.^{56,57} In addition, some studies have shown that, under certain conditions, the ferrous-dioxygen complex of eNOS becomes unstable and liberates O²⁻ instead of NO. This is referred to as eNOS uncoupling.³³ Many factors can lead to eNOS uncoupling, including decreased bioavailability of tetrahydrobiopterin or decreased recruitment or activity of HSP90.^{33,58} Decreased bioavailability of L-arginine (due to increased arginase expression) or increased levels of the endogenous competitor asymmetric dimethyl-L-arginine (ADMA) have also been correlated with eNOS uncoupling in vivo.⁵⁸ Oxidative stress is also linked to eNOS uncoupling, as it can increase ADMA levels and decrease BH₄ levels. Increased levels of ONOO⁻ generated from uncoupled eNOS have been associated with myriad events important in the pathogenesis of cardiovascular diseases, such as oxidation of tyrosine residues, inhibition/activation of enzyme activity, and lipid peroxidation.^{56,57} Therefore, it is the

balance between NO and O_2^{2-} that has been shown to be pivotal in maintaining vascular homeostasis.

RACIAL DIFFERENCES IN VASCULAR REACTIVITY

Most of the available data on the effects of race on NO-mediated vasodilation have been obtained by studies of vascular reactivity in various populations surveyed. A few studies have directly measured NO metabolites in plasma or urine, and only a couple of studies have investigated the molecular basis of racial differences in NO levels in vitro by comparing human umbilical vein endothelial cells (HUVEC) from AAs or EAs. We have summarized those studies of differences between AAs or AIs and non-AAs/non-AIs (mostly European originated) in Tables 1 to 3. As summarized, some studies involved healthy normotensive (NTS) persons, while others studied those with hypertension (HTS) with or without left ventricular hypertrophy, heart failure, and type 2 diabetes mellitus with or without microalbuminuria or chest pain. Some studies involved young adolescent to middle-aged individuals, while others investigated the differences in obese and lean individuals.

Five common methodologies were used to investigate endothelium-dependent and -independent vasorelaxation (Table 1). On the venous occlusion plethysmography, vasodilation was induced by endothelium-dependent or -independent agonists or by ischemia, and the main end point was changes in blood flow that reflect vasodilation of resistant vessels in the forearm. Among the agonists studied were acetylcholine and methacholine, both of which activate muscarinic receptors on endothelial cells leading to increases in intracellular calcium and eNOS activation^{1,59}; bradykinin, which activates eNOS via G-coupled receptor activation⁶⁰; and isoproterenol, which activates eNOS via β -adrenoreceptor activation.⁶¹ Acetylcholine/methacholine and isoproterenol also affect smooth muscle cells in different manners: the muscarinic agonists increase smooth muscle contraction via changes in intracellular calcium, while isoproterenol induces smooth muscle relaxation via activation of β -adrenoreceptors, leading to increases in cyclic AMP.⁶² Other stimuli studied include ischemia (or hyperemia), exercise, and mental stress. These stimuli have been shown to exert vasodilatory effects via NO-dependent mechanisms but also can induce vasodilation via other vasoactive substances.^{63–67} We found 15 studies on racial differences in endothelial function using this methodology: 14 studies on comparisons between AAs and non-AAs^{68–81} and 1 study comparing AIs with EAs⁸² (Table 1). With the exception of Kahn's and Androne's studies, all other studies were performed on otherwise healthy NTS persons. Normotensive AAs show a blunted response to endothelium-dependent vasodilation as induced by acetylcholine, methacholine, bradykinin, isoproterenol, exercise, mental stress, and, in some studies, also to ischemia (Table 1). Studies on small vessel reactivity using other methodologies have revealed similar results. For instance, healthy lean AAs also showed decreased vasodilation to methacholine (detected by thermodilution)⁸³ and a decreased response to albuterol (detected by reflective index)^{84,85} compared with EAs (Table 1). Normotensive AIs also showed a blunted response to a low dose of acetylcholine as compared with normotensive EAs.⁸² In the study by Kahn et al,⁸⁰ a blunted response to methacholine was observed in AAs with HTS but not in NTS AAs. Few studies demonstrated a direct role of NO in these blunted responses. In one study, Cardillo et al⁷⁴ tested the effect of the eNOS antagonist N^G -monomethyl-L-arginine (L-NMMA) on isoproterenol-induced vasodilation to elucidate the importance of direct smooth muscle versus NO-mediated endothelium-dependent vasodilation.⁷⁴ In that study, L-NMMA decreased isoproterenol-induced vasodilation in both races, leading the authors to conclude that AA had a blunted response to isoproterenol effects on smooth muscle relaxation. However, in another study from the same group, L-NMMA decreased mental stress-induced vasodilation in EAs only,⁷³ indicating that a blunted endothelial response to NO-dependent mental stress is present in AAs. Therefore, AAs exhibit decreased NO-dependent vasorelaxation in response to mental stress but not to isoproterenol. These data suggest that a particular signaling pathway

activated by mental stress is blunted in AAs while the β -adrenoreceptor signaling pathway in the endothelium of AAs might be intact. In conclusion, from this segment of studies, healthy AAs exhibited blunted responses to NO-dependent stimuli, such as mental stress, bradykinin, and acetylcholine/methacholine, in all but 1 study.⁸⁰ Moreover, while hypertension and heart failure are associated with endothelial dysfunction leading to decreased NO-dependent vasodilation, AAs have an even higher NO-dependent dysfunction than EAs do.^{80,81} Interestingly, Kahn et al⁸⁰ also observed that the addition of ascorbic acid did not correct for the observed racial differences in vascular reactivity to methacholine in HTS AAs versus HTS EAs. The authors suggested that a short-term correction of oxidative stress might not suffice to correct the impaired NO-dependent vasodilation observed in HTS AAs.

Another research group investigated racial differences in endothelial function by measuring the coronary microvasculature resistance with Doppler-tipped guide wire inserted in the left coronary artery.^{86–89} In contrast to the studies performed in the forearm microvasculature, studies on coronary microvasculature resistance have shown that there are no racial differences between NTS AAs and NTS EAs in response to acetylcholine-induced (endothelium-dependent) or adenosine-induced (endothelium-independent) vasodilation.^{87–89} Also, there are no differences in vascular reactivity to acetylcholine or adenosine between AA and EA women with chest pain,⁸⁶ although this study had a very small study population. However, when comparing AA and EA persons with hypertension and left ventricular hypertrophy, AAs exhibited decreased vasodilatory responses to acetylcholine in comparison to matched EAs but similar responses to the endothelium-dependent vasodilator adenosine.^{88,89} These results show that although hypertension is correlated with endothelial dysfunction in both races, AA hypertensives show a greater dysfunction on NO-dependent vasodilation as compared with matched EA hypertensives. In addition, while there were no racial differences in NTS subjects to acetylcholine-induced vasorelaxation, supplementary L-arginine significantly enhanced acetylcholine-induced vasodilation in NTS AA subjects but not in NTS EA subjects.⁸⁷ These results suggest that there are racial differences in the L-arginine bioavailability and correlate well with another study⁹⁰ showing that healthy NTS AAs have significantly higher levels of ADMA, an endogenous inhibitor competing with L-arginine for NOS-mediated NO production. Moreover, in the study by Melikian et al,⁹⁰ increased levels of ADMA correlated with a blunted vasodilation in response to ischemia (Table 1).

Another set of data on racial differences in endothelial function has been derived from studies on brachial artery (a conduit vessel) vasodilation.^{90–99} In these studies, a hyperemic state is induced by 3- to 5-minute cuff occlusion of arterial flow. Flow-mediated dilation (FMD) occurs after cuff release. The diameter of the brachial artery and blood flow at baseline and during hyperemia are measured using high-resolution ultrasound. FMD has been shown to depend on NO and to correlate with endothelial dysfunction.^{11,100,101} In addition to FMD, some researchers investigated the effect of nitroglycerin (eg, an endothelium-independent vasodilator) on racial differences in conduit vessel vasodilation. As shown in Table 1, in general, AAs have a lower FMD in response to hyperemia as compared with EAs, but there were no racial differences in response to nitroglycerin. Some differences have been observed among the studies. Some studies have shown that racial differences are present in normotensive healthy individuals^{90–94} while others have not.^{96,97} However, latter studies have observed significant racial differences in subjects who manifest a disease associated with endothelial dysfunction such as hypertension,⁹⁶ cardiovascular disease/diabetes in postmenopausal women,⁹⁵ heart failure,⁸¹ or diabetes with microalbuminuria.⁹⁷ In the latter studies, AAs with cardiovascular disease or diabetes seem to have lower FMDs than matched EAs or Hispanics, indicating that disease-related endothelial dysfunction occurs sooner and/or with greater severity in AAs as compared with EAs.^{81,95–97} We also found 3 studies investigating racial differences between healthy NTS AIs and matched non-AIs^{82,98,99} (Table 1). Two studies demonstrated that AIs, similarly to AAs, have a blunted vasodilation to ischemia as compared

with EAs.^{82,99} However, in the study by Raji et al,⁹⁸ there were no differences between AIs and EAs in response to ischemia-induced vasodilation, but AIs exhibited a blunted vasodilator response to insulin as compared with EAs (Table 1). Although similar studies on insulin-dependent vasodilation in AAs have not been published, an abstract¹⁰² reported that AAs also show a blunted response to insulin-dependent vasodilation, although the methodology used was different from that in the study by Raji et al. Recent studies have attempted to investigate other parameters that can be associated with the blunted response to NO-dependent vasodilation in AAs and AIs. Among these, increased levels of ADMA,⁹⁰ lipoprotein(a),⁹³ infectious burden,⁹⁴ and inflammatory markers (vascular cell adhesion molecule, intercellular adhesion molecule, tumor necrosis factor α , and interleukin-6)^{84,94} have been found in association with decreased brachial artery and forearm vasodilation in AAs and impaired insulin secretion in both AAs and AIs.^{79,82,98} Surprisingly, 2 markers of oxidative stress, C-reactive protein and isoprostane F2 α , were not been found to be associated with decreased FMD in AAs as compared with EAs.^{84,90} In keeping with data from the study by Kahn et al⁸⁰ showing that ascorbic acid is unable to correct for the blunted vasodilator response of AAs, these data strongly oppose the theory that increased oxidative stress is potentially the primary cause of earlier endothelial dysfunction in AAs.

Available physiological data point to the fact that AAs as a group suffer from greater endothelial dysfunction (in particular, NO dependent) than other races do. However, caution should be taken in interpreting these data since vasodilation to most stimuli could be derived from the stimulation of various pathways other than eNOS stimulation. Of all the stimuli surveyed, only the muscarinic agonists (acetylcholine and methacholine), insulin, and estrogen are considered to induce vasodilation primarily via eNOS activation. Therefore, data obtained with other stimuli should be interpreted with caution unless the effect was also studied in the presence of the eNOS inhibitor L-NMMA. Nevertheless, these data strongly suggest that AAs show a blunted NO-mediated response to most stimuli, although other vasodilating mechanisms might also be blunted in this race. In the past decade, a new drug combination of isosorbide dinitrate and hydralazine has consistently shown beneficial effects in AA patients with heart failure.¹⁰³ These clinical studies have clearly demonstrated that AAs can benefit greatly from NO donor therapy and that the smooth muscle response to exogenous NO is adequate in AAs even in the presence of heart failure. On the other hand, another anti-heart failure drug, an angiotensin-converting enzyme inhibitor, has shown a lower effect on AAs as compared with EAs,^{104,105} indicating racial differences in response to drug therapy.

RACIAL DIFFERENCES IN NITRIC OXIDE PRODUCTION/BIOAVAILABILITY

Five independent studies have investigated the racial differences in NO bioavailability by measuring plasma/urine levels of nitrites/nitrates (NOx, the stable metabolites of NO)^{106–110} (Table 2). Plasma NOx levels can be affected by endothelium production of NO, oxidative stress, and diet, while urinary NOx levels reflect the production of NO at the kidney level and are therefore also affected by kidney function. One study performed on diabetic patients with microalbuminuria showed that AAs had a blunted response to a renal vasodilator stimulus that increases urinary NOx levels.¹⁰⁸ Renal function, blood pressure, and duration of diabetes together with other parameters were similar between the races, suggesting that the blunted responses to the vasodilator stimuli in AA are most likely the result of decreased production of NO.¹⁰⁸ This study correlated well with the 2006 study by Jawa et al,⁹⁷ who observed decreased brachial artery vasodilation in response to ischemia in AAs versus EAs with diabetes and microalbuminuria. In a larger study investigating the relationship between vasoactive factors and sodium excretion, a significantly decreased basal level of urinary NOx in AAs as compared with EAs was reported, although diet was not controlled among the groups.¹⁰⁹ Although this group did not study vasodilation in response to a stimulus, they did find a significant correlation between sodium excretion and urinary levels of NOx in AAs but not in

EAs.¹⁰⁹ These authors concluded that the NO natriuretic axis is more active in regulating blood pressure via sodium excretion/NO production in AAs than in EAs. Another interesting study compared premenopausal AA and EA overweight women responses to weight loss in terms of oxidative stress (6-nitrotyrosine plasma levels and myeloperoxidase levels) and NO bioavailability (NOx plasma levels).¹⁰⁷ Interestingly, overweight premenopausal AA women showed higher basal levels of plasma NOx, which is in contrast with the 2002 study on postmenopausal AA and EA women by Ke et al,¹⁰⁶ who observed no differences on basal levels of plasma NOx. These results suggest that racial differences in response to endogenous estrogen effects on NO production could exist. Premenopausal AA and EA women responded to weight loss with a significant increase in plasma NOx levels.¹⁰⁷ Therefore, there were no racial differences in response to weight loss in terms of NO bioavailability. This study correlates well with the Lteif et al study,⁸³ in which the authors observed no racial differences in methacholine-induced vasodilation in obese subjects but blunted vasodilation in lean AA subjects. These studies on obese young subjects suggest that obesity has a lower impact on endothelial function in AAs as compared with EAs, as NO-dependent vasodilation is further impaired with obesity in EAs compared with AAs.⁸³ In contrast, in the study by Ke et al,¹⁰⁶ EA post-menopausal women responded to hormone replacement therapy (estrogen and medroxyprogesterone) with a 27.5% increase in NOx levels, whereas there was no increase in AA postmenopausal women. This study also correlated well with a larger study in 2004 on post-menopausal women by Loehr et al,⁹⁵ who observed that AA postmenopausal women had a blunted vasodilatory response to ischemia as compared with EA post-menopausal women, regardless of hormone replacement therapy. Studies on EA postmenopausal women have shown that hormone replacement therapy significantly increases NO bioavailability and NO-dependent vasodilation,¹¹¹ mainly because of the effects of estrogen on eNOS activation. Therefore, these studies indicate that AA post-menopausal women lose their natural response to estrogen-induced increases in NO production, as compared with EA women, potentially because of an earlier NO-dependent endothelial dysfunction. A study on brachial artery vasodilation of young healthy women of both races⁹¹ found that FMD was significantly lower in AA pre-menopausal women as compared with EA premenopausal women. More interestingly, there were no significant differences in FMD between young AA men and women, whereas significant differences existed between EA men and women (women demonstrated higher FMDs than men did).⁹¹ Unfortunately, plasma NOx levels were not analyzed to determine whether the racial differences on FMD in young AA and EA women could be due to differences in NO bioavailability. Nevertheless, based on the previous studies,^{91,106} we could hypothesize that AA women have a lesser response than EA women to endogenous/exogenous estrogen. Finally, Malhotra et al¹¹⁰ studied the effect of mental stress on a larger population by determining the effect on blood pressure and plasma NOx. They did not observe any racial differences in NOx levels before or after the stimulus. Altogether, these studies indicate that basal NOx levels (under controlled diet conditions) do not vary among the races but that there are racial differences to various NO-dependent stimuli.

Racial differences in oxidative stress markers were also studied in addition to NOx levels. There were no racial differences in basal levels of oxidative stress markers such as 3-nitrotyrosine proteins, myeloperoxidase, and 8-isoprostane,^{106,107} which correlates with other studies on oxidative stress markers and endothelial function.^{84,90} On the other hand, Fenster et al¹⁰⁷ observed that although AA premenopausal women had higher plasma NOx levels before and after weight loss, only EA premenopausal women responded to weight loss with a decrease in oxidative stress markers (myeloperoxidase and 6-nitrotyrosine), while AA premenopausal women showed the opposite effect. These data suggest that there are racial differences in the production/degradation of ROS to certain stimuli (ie, weight loss) that could be related to a later presence of endothelial dysfunction. It could be hypothesized that increased oxidative stress could induce a compensatory increase in NO production in premenopausal women, leading to increases in plasma NOx in AAs, as observed in the study by Fenster et al.

Further studies on this area are required to elucidate the molecular basis of the racial differences observed.

MECHANISMS OF DECREASED NITRIC OXIDE PRODUCTION/ BIOAVAILABILITY

Very few studies have tried to explore the molecular basis underlying the racial disparities in NO-dependent vasodilation. A group led by Malinski studied these differences in HUVECs originating from AAs and EAs.^{112,113} They used nanosensors to detect individual-cell production of NO, O_2^{2-} , and $ONOO^-$ and found that HUVECs from AAs have lower basal and stimulated (with calcium ionophore or acetylcholine) levels of NO and higher levels of O_2^{2-} , leading to higher levels of $ONOO^-$ as compared with HUVECs from EAs (Table 3). Further studies on eNOS activity demonstrated that eNOS derived from AAs is actually more active than eNOS derived from EAs, and the levels of basal eNOS expression were higher in AAs as compared with EAs. Studies with N^G -nitro-L-arginine-methyl ester (L-NAME) showed that HUVECs from AAs and EAs produce similar levels of O_2^{2-} when eNOS is inhibited, demonstrating that eNOS derived from AAs is responsible for both NO and O_2^{2-} production, thus suspiciously working as an uncoupled enzyme, whereas eNOS derived from EAs produced mainly NO. In addition, they demonstrated that HUVECs from AAs showed higher expression of O_2^{2-} -producing enzymes such as NADPH oxidases. Altogether, they proposed that AAs have higher oxidative stress that leads to eNOS uncoupling and decreased NO bioavailability. Brilliantly, they proved their hypothesis by showing that apocynin (an NADPH oxidase inhibitor) or nebivolol (a β_1 -adrenoreceptor inhibitor), both of which have antioxidant properties, can completely restore NO bioavailability in HUVECs from AAs to levels comparable to HUVECs from EAs.

However, caution should be taken when extrapolating these in vitro results to NO bioavailability in the human body. Studies on human subjects have indicated that decreased NO-dependent vasodilation is not associated with increased markers of oxidative stress.^{90, 93} Moreover, if oxidative stress is consistently higher in AAs from birth until adulthood, then important compensatory mechanisms must exist, as physiological studies on humans have not observed racial differences in basal vascular tone, while half of the studies have also not observed racial differences in NO-dependent vasorelaxation in healthy NTS subjects. For instance, one study observed decreased superoxide dismutase activity, the main cytosolic enzyme in charge of transforming O_2^{2-} into hydrogen peroxide, in hypertensive AAs as compared with normotensive AAs,¹¹⁴ although no comparisons were made with EAs. More studies on the oxidant/antioxidant enzyme machinery with regard to racial differences are needed to further detail whether AAs suffer from higher oxidative stress and why. Many enzymes are regulated by the cellular reduction-oxidation (redox) homeostasis, including enzymes that synthesize and degrade ADMA: arginine N-methyltransferase type I and dimethylarginine dimethylaminohydrolase. Studies on the expression and activity of these enzymes are warranted as ADMA levels have been shown to be significantly elevated in healthy AAs as compared with healthy EAs.⁹⁰ In addition, studies on the oxidation status of tetrahydrobiopterin in AAs and EAs are also warranted. Altogether, the current hypothesis is that oxidative stress leads to enzyme activity changes, leading to increased ADMA levels, eNOS uncoupling, and O_2^{2-} production. Several redox-sensitive pathways may converge at the point of reduced NO bioavailability, leading to endothelial dysfunction and subsequent vascular diseases, and AAs might be predisposed to the activation/inhibition of 1 (or more) such particular pathway.

Is there a genetic component to the racial disparities in NO-dependent vasorelaxation? Complex diseases such as hypertension, asthma, and diabetes are believed to be the consequence of intricate gene-environment interactions. The Human Genome Projects suggest

that genetic variation is a continuous phenomenon and therefore cannot lead to clear boundaries among different racial groups.³² However, racial disparities in health can be useful to study gene-environment interactions as historically, American people of different races originated from different continents, thereby providing different gene-environment interactions as major factors that result in observed racial health disparities. For example, genetic variations in genes that encode for sodium channels are more common in AAs than EAs and are associated with the development of a salt-sensitive form of hypertension.¹¹⁵ Similarly, a pool of genetic variations in vasoactive genes has been identified, and significant ethnic variations in prevalence have been shown.^{116,117} Of interest in NO bioavailability, several polymorphisms in the eNOS gene have been identified that potentially can alter NO production.^{118–121} One of these, the 4a/4b polymorphism is generated by a variable number of tandem repeats in intron 4 and has been shown in some studies,^{122–125} but not in others,^{118,126,127} to lead to decreased expression of eNOS and decreased NO production and to be associated with cardiovascular disease.^{128,129} The 4a variant is consistently more common among AAs (30%–36%) than among EAs (15%–18%).^{130,131} Nevertheless, 1 study investigating the relationship of eNOS polymorphisms with blood pressure, endothelial function, and plasma nitric oxide levels did not find any correlation among them.¹³² In addition, other studies have shown that eNOS expression in healthy NTS AA subjects is similar or higher as compared with matched EA subjects.^{112,133} Similarly, an attempt to explain blunted responses to β -adrenoreceptor drugs in AAs by analyzing polymorphisms in the β 2-adrenoreceptor have equally failed.⁸⁴

Other gene candidates involved in racial differences in endothelial function/dysfunction is the glucose-6-phosphate dehydrogenase (G6PD) gene. G6PD regulates the availability of eNOS cofactors NADPH, BH_4 , and glutathione.¹³⁴ A deficiency in G6PD has been shown to be more common in AAs (~15%) than in EAs (~6%) and has been proposed to be a participant in the increased oxidative stress observed in AAs. Again, a caveat in this hypothesis is that a deficiency in G6PD would also decrease NADPH-dependent redox signaling. In addition, several investigators have observed increased NADPH oxidase activity and increased NADPH oxidase subunit expression¹¹² in AAs as compared with EAs. Nevertheless, genetic polymorphisms in NOS and/or O_2^- synthesizing enzymes that may be more prevalent in AAs as compared with EAs could account for some of the differences observed in NO-dependent vasodilation. In addition, studying the effect of genetic polymorphisms on candidate vasoactive genes together with other factors such as oxidative stress and obesity is essential, as some studies have observed racial differences in endothelial function in only certain physiological-genetic scenarios but not in others.^{77,110} Identifying new polymorphisms involved in the development of endothelial dysfunction or NO bioavailability and understanding the gene-gene and gene-environment interactions are fundamental for the prevention and treatment of vascular diseases in specific racial groups as well as in the population at large.

RACIAL DISPARITIES IN NITRIC OXIDE-MEDIATED VASCULAR DYSFUNCTION IN PREGNANCY

A particular area of health disparities poorly understood and studied is pregnancy complications, especially pregnancy-induced hypertension and preeclampsia. The mysterious disease of preeclampsia occurs in 5% to 10% of all human pregnancies, accounting for most of the maternal/neonatal morbidity and mortality in the United States as well as other countries worldwide. Endothelial dysfunction has been recognized as a hallmark of preeclampsia.^{54, 135} During normal pregnancy, the cardiovascular system of pregnant women undergoes significant adaptive changes, including increased cardiac output, decreased systemic vascular resistance, decreased mean blood pressure, and so forth.¹³⁶ A large body of evidence has suggested that bioavailable NO plays a significant role in these adaptive mandatory changes in the mother's cardiovascular system.¹³⁷ Although the etiology of preeclampsia is elusive, it

is generally accepted that decreased bioavailable NO and increased oxidative stress impairing NO-dependent vasodilation is a main causative factor.^{135,137,138} Moreover, increased levels of ADMA have been observed in preeclamptic pregnancies,^{139,140} and L-arginine supplementation has been shown to have beneficial effects on preeclampsia in some studies.¹⁴¹ Of note, significant racial differences have been observed in this human-specific, pregnancy-associated disease. For instance, AA mothers had a higher prevalence of obstetric complications than European or Asian mothers did.¹⁴² AA mothers suffering from preeclampsia, abruption placentae, placenta previa, or postpartum hemorrhage were 2 to 3 times more likely to die from these complications as compared with European women.¹⁴³ In a more recent study, it was found that AAs and EAs showed similar prevalence rates of pregnancy-induced hypertension; however, AA pregnant women showed a higher prevalence of diagnosed preeclampsia.¹⁴⁴ Other studies have shown as well that AA pregnant women suffer from a higher prevalence of preeclampsia as compared with EAs, and this trend has increased in the past decade, even though socioeconomic differences among the groups remain the same.¹⁴⁵ Finally, AAs are also more likely to experience preterm labor than Europeans or Asians.¹⁴⁶ NO has been shown to play a pivotal role in the development of placenta and the increase in the uteroplacental blood flow.^{147–150}

In addition, NO has been shown to play a role in parturition, as the levels of NO in the uterine tissue drop while NO levels in the cervix increase during labor.^{151,152} Moreover, polymorphisms in eNOS have been associated with both preeclampsia and preterm labor.^{153,154} To date, very few studies have attempted to uncover the biological basis of these racial differences in maternal/neonatal morbidity/mortality and outcomes, and no studies have been conducted to explore racial disparities in the eNOS-NO system during pregnancy. In keeping with the rapidly growing knowledge of the racial disparity of NO-mediated vasodilatation in the cardiovascular system and the important roles that NO plays in various reproductive processes, it is very likely that racial differences in the eNOS-NO system will contribute to the observed racial disparity in various pregnancy complications—a hypothesis we propose but that awaits for further investigation.

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Table 1
Nitric Oxide–Dependent Vasodilation: Studies on Racial Differences in Vascular Reactivity

Study Population Main Characteristics (n)	Stimulus	Racial Differences Observed	Reference
Method 1: Plethysmography: forearm or lower leg resistance vessel			
NTS EA male (20)	10-min ischemic handgrip exercise	↓ Vasodilator response in AAs (independent of parental history of hypertension)	Basset et al (1992) ⁶⁸
NTS AA male (21)			
Young			
NTS EA (13)	Isoproterenol	↓ Vasodilator response in AAs	Lang et al (1995) ⁶⁹
NTS AA (9)			
Young			
NTS EA (30)	10-min ischemia	↓ Vasodilator response in AAs (independent of parental history of hypertension)	Hinderliter et al (1996) ⁷⁰
NTS AA (30)			
Matched for age, gender, and CVD risks			
NTS EA male (22)	5-min ischemia	↓ Vasodilator response in AAs without parenteral history of hypertension	Bond et al (1996) ⁷¹
NTS AA male (32)			
Young			
NTS EA male (9)	Isoproterenol	↓ Vasodilator response in AAs to both stimuli	Stein et al (1997) ⁷²
NTS AA male (11)	Methacholine		
Young			
NTS EA (14)	5-min ischemia	No differences	Cardillo et al (1998) ⁷³
NTS AA (12)	Mental stress	↓ Vasodilator response in AAs to mental stress	
Middle-aged	Mental stress + L-NMMA	L-NMMA decreased vasodilation to mental stress in EAs only	
NTS EA (18)	5-min ischemia	No differences	Cardillo et al (1999) ⁷⁴
NTS AA (18)	Acetylcholine	↓ Vasodilator response in AAs to both acetylcholine and isoproterenol	
Middle-aged	Isoproterenol ± L-NMMA	L-NMMA decreased isoproterenol dilation in both races	
NTS EA male (12)	Acetylcholine	↓ Vasodilator response in AAs	Jones et al (1999) ⁷⁵
NTS AA male (16)			
Young			
NTS EA (10)	Isoproterenol	↓ Vasodilator response in AAs	Stein et al (2000) ⁷⁶
NTS AA (10)			
Young			
NTS EA (14)	Bradykinin	↓ Vasodilator response in AAs	Gainer et al (2001) ⁷⁷
NTS AA (14)	Acetylcholine		
Young			
NTS EA (19)	Bradykinin	↓ Vasodilator response in AAs to all 3 stimuli	Rosenbaum et al (2002) ⁷⁸
NTS AA (21)	Acetylcholine		
Young	Methacholine		
Healthy EA (33)	5-min ischemia	↓ Vasodilator response in AAs	Duck et al (2007) ⁷⁹
Healthy AA (25)		Associated with increased insulin secretion	
Adolescent			
NTS EA (36)	Methacholine	↓ Vasodilator response in HTS AAs, no difference in NTS	Kahn et al (2002) ⁸⁰
NTS AA (34)	Methacholine + ascorbic acid	Persistent ↓ in vasodilator response in HTS AAs after ascorbic acid	
HTS EA (26)			
HTS AA (22)			
Middle-aged			
HF non-AA (188)	3-min handgrip exercise	↓ Vasodilator response in AAs (independent of hypertension)	Androne et al (2006) ⁸¹
HF AA (69)			
Healthy male EA (25)	Acetylcholine	↓ Vasodilator response in AI	Murphy et al (2007) ⁸²
Healthy male AI (24)	Low dose	No difference	
Young	High dose		
Method 2: Thermodilution: small-vessel resistance			
Healthy lean EA (25)	Methacholine	↓ Vasodilator response in lean AA as compared to lean EA	Lteif et al (2005) ⁸³
Healthy lean AA (30)		No racial differences in obese subjects	
Healthy obese EA (17)		Associated with insulin resistance	
Healthy obese AA (25)			
Middle-aged			
Method 3: Digital volume pulse photoplethysmograph: small vessel reactivity			
NTS EA (82)	Albuterol	↓ Vasodilator response in AA	Kalra et al (2005) ⁸⁴
NTS AA (78)		Associated with levels of fasting insulin, IL-6, and TNF- α	
Middle-aged to senior			
NTS EA (101)	Albuterol	↓ Vasodilator response in AA but not in Jamaicans	Kalra et al (2006) ⁸⁵
NTS AA (197)		Associated with ↑ BMI and ↑ DBP	
NTS Jamaican (81)			
Middle-aged to senior			
Method 4: Doppler-tipped guide wire: coronary microvascular resistance			
CP EA female (9)	Acetylcholine	No differences	Houghton et al (1997) ⁸⁶
CP AA female (8)			

Study Population Main Characteristics (n)	Stimulus	Racial Differences Observed	Reference
NTS EA (33) NTS black (33) Matched for age and gender, no CVD	Acetylcholine Acetylcholine + L-arginine	No differences in NTS subjects ↑ Vasodilator response to acetylcholine with L-arginine in AAs but not in whites	Houghton et al (2002) ⁸⁷
NTS EA (21) vs NTS AA (10) HTS + LVH EA (25) vs HTS + LVH AA (24)	Acetylcholine	No difference in NTS subjects ↓ Vasodilator response in AAs with HTS + LVH	Houghton et al (1997) ⁸⁸
NTS EA (56) vs NTS AA (24) HTS + LVH EA (65) vs HTS + LVH AA (50)	Acetylcholine	No difference in NTS subjects ↓ Vasodilator response in HTS + LVH AAs	Houghton et al (2003) ⁸⁹
Method 5: Brachial artery ultrasound: flow-mediated conduit vessel dilation			
NTS EA (24) vs NTS AA (28) Young	5-min ischemia	↓ Vasodilator response in AAs (both male and female; loss of gender effect in AAs)	Perregaux et al (2000) ⁹¹
NTS EA (46) vs NTS AA (46) Middle-aged	5-min ischemia	↓ Vasodilator response in AAs	Campia et al (2002) ⁹²
NTS EA (40) NTS AA (28) NTS H (21) Middle-aged	5-min ischemia	↓ Vasodilator response in AA Associated with ↑ lipoprotein (a)	Wu et al (2004) ⁹³
NTS EA male (28) NTS AA male (30) Young	5-min ischemia	↓ Vasodilator response in AA Associated with ↑ ADMA levels	Melikian et al (2007) ⁹⁰
NTS EA male (20) NTS AA male (22) Young	4-min ischemia	↓ Vasodilator response in AA Associated with levels of infectious burden (cytomegalovirus, hepatitis B and C, herpes 1 and 2, Epstein-Barr and <i>Chlamydia pneumoniae</i>)	Marchesi et al (2007) ⁹⁴
PMPW EA (1330) PMPW AA (297) Not matched CVD, DM	4-min ischemia	↓ Vasodilator response in AAs (independent of HRT treatment, CVD, or DM)	Loehr et al (2004) ⁹⁵
NTS EA (45) vs NTS AA (44) HTS EA (46) vs HTS AA (44) Middle-aged	5-min ischemia	No difference in NTS ↓ Vasodilator response in HTS AAs	Gokce et al (2001) ⁹⁶ Reviewed in Vita (2002) ¹⁵⁵
HF non-AA (188) HF AA (69)	5-min ischemia	↓ Vasodilator response in AA	Androne et al (2006) ⁸¹
DM – MA EA (NSt) DM – MA AA (15) DM + MA EA (NSt) DM + MA AA (35)	4-min ischemia	No difference in absence of MA ↓ Vasodilator response in AA with MA	Jawa et al (2006) ⁹⁷
Healthy EA (15) Healthy AI (25) Middle-aged	5-min ischemia ± 2 h hyperinsulinemic clamp	↓ Vasodilation to insulin in AI, but no differences in response to ischemia Associated with insulin resistance, ↓ in adiponectin and ↑ in plasminogen-activator 1	Raji et al (2004) ⁹⁸
Healthy male EA (25) Healthy male AI (24) Young	5-min ischemia	↓ Vasodilator response in AI Associated with insulin resistance and reduced endothelial progenitor cells	Murphy et al (2007) ⁸²
Healthy male EA (18) Healthy male AI (26) Middle-aged	4.5-min ischemia	Vasodilator response in AI	Chambers et al (1999) ⁹⁹

Abbreviations: AA, African American; ADMA, asymmetric dimethyl-L-arginine AI, Asian Indians (South Asians); BMI, body mass index; CP, chest pain; CVD, cardiovascular disease; DBP, diastolic blood pressure; DM, diabetes mellitus; EA, European Americans (Caucasians); H, Hispanics; HF, heart failure; HRT, hormone replacement therapy; HTS, hypertensive; IL, interleukin; L-NMMA, *N*^G-monomethyl-L-arginine; LVH, left ventricular hypertrophy; MA, microalbuminuria; NSt, not stated; NTS, normotensive; PMPW, postmenopausal women; TNF- α , tumor necrosis factor α .

Table 2**Racial Differences in Nitric Oxide Levels: Clinical Studies**

Study Population Main Characteristics (n)	Stimulus	Racial Differences Observed	Reference
EA PMPW (13) AA PMPW (14)	6 wk-hormone therapy (estrogen and medroxyprogesterone)	No differences of basal plasma NOx 27.4% ↑ in plasma levels of NOx after HT in EAs but not in AAs	Ke et al (2002) 106
EA PreMPW (21) AA PreMPW (21) Normoglycemic, NTS, and similar creatinine clearance Middle-aged	1-mo weight loss regimen	↑Basal plasma NOx levels in AAs ↑Plasma NOx levels after weight loss in both races No differences in basal urinary NOx clearance Renal plasma flow and urinary NOx clearance in AAs in response to amino acid infusion	Fenster et al (2004) ¹⁰⁷
EA DM + MA (9) AA/AI DM + MA (9)	Amino acid infusion to induce renal vasodilation	Decreased basal urinary NOx in AAs, preprotocol but no differences in NOx intraprotocol Higher correlation of sodium excretion with urinary NOx excretion in AA	Earle et al (2001) 108
EA NTS (20) AA NTS (31) Healthy EA (262) Healthy AA (235) Young	None Mental stress	↑Diastolic blood pressure in AAs, but no differences in NOx levels before or after stimulus	Jackson et al (2001) ¹⁰⁹ Malhotra et al (2004) ¹¹⁰

Abbreviations: AA, African American; AI, Asian Indians; DM, diabetes mellitus; EA, European Americans (Caucasians); HT, hormone therapy; MA, microalbuminuria; NOx, nitrites and nitrates; NTS, normotensive; PMPW, postmenopausal women; PreMPW, premenopausal women.

Table 3**Racial Differences in Nitric Oxide Production/Bioavailability: In Vitro Studies**

Study Population Main Characteristics (n)	Stimulus (End Point)	Racial Differences Observed	Reference
Healthy EA (12) Healthy AA (12) Matched for clinical characteristics No perinatal complications	CaI Acetylcholine +Apocynin +L-NAME	↓NO, ↑O ₂ ⁻ , ↑ONOO ⁻ in HUVECs from blacks (both basal and after CaI or acetylcholine stimulation) Abolish racial differences (similar NO levels in both races) ↓NO, ↓O ₂ ⁻ , ↓ONOO ⁻ levels in both races (eNOS uncoupling is ↑ in AAs)	Kalinowski et al (2004) ¹¹²
Healthy EA (12) Healthy AA (12) Matched for clinical characteristics No perinatal complications	CaI and acetylcholine + Nebivolol + Apocynin	↑Response to antioxidant effects of nebivolol in AAs Nebivolol and apocynin abolish racial differences in NO, superoxide, and peroxynitrate levels	Mason et al (2005) ¹¹³

Abbreviations: AA, African American; CaI, calcium ionophore; EA, European Americans (Caucasians); eNOS, endothelial NOS; HUVEC, human umbilical vein endothelial cell; L-NAME, N^G-nitro-L-arginine-methyl ester (eNOS antagonist); NO, nitric oxide; O₂⁻, superoxide; ONOO⁻, peroxynitrate.