

Florida International University FIU Digital Commons

Department of Biostatistics Faculty Publications

Robert Stempel College of Public Health & Social
Work

3-2016

Nitric Oxide Contributes to Vasomotor Tone in Hypertensive African Americans Treated With Nebivolol and Metoprolol

Robert B. Neuman
Emory University

Salim Hayek
Emory University

Joseph C. Poole
Emory University

Ayaz Rahman
Emory University

Vivek Menon
Emory University

See next page for additional authors

Follow this and additional works at: https://digitalcommons.fiu.edu/biostatistics_fac

 Part of the [Medicine and Health Sciences Commons](#)

Recommended Citation

Neuman, Robert B.; Hayek, Salim; Poole, Joseph C.; Rahman, Ayaz; Menon, Vivek; Kavtaradze, Nino; Polhemus, David; Veledar, Emir; Lefer, David J.; and Quyyumi, Arshed A., "Nitric Oxide Contributes to Vasomotor Tone in Hypertensive African Americans Treated With Nebivolol and Metoprolol" (2016). *Department of Biostatistics Faculty Publications*. 31.
https://digitalcommons.fiu.edu/biostatistics_fac/31

This work is brought to you for free and open access by the Robert Stempel College of Public Health & Social Work at FIU Digital Commons. It has been accepted for inclusion in Department of Biostatistics Faculty Publications by an authorized administrator of FIU Digital Commons. For more information, please contact dcc@fiu.edu.

Authors

Robert B. Neuman, Salim Hayek, Joseph C. Poole, Ayaz Rahman, Vivek Menon, Nino Kavtaradze, David Polhemus, Emir Veledar, David J. Lefer, and Arshed A. Quyyumi



Published in final edited form as:

J Clin Hypertens (Greenwich). 2016 March ; 18(3): 223–231. doi:10.1111/jch.12649.

Nitric Oxide Contributes to Vasomotor Tone in Hypertensive African Americans Treated With Nebivolol and Metoprolol

Robert B. Neuman¹, Salim Hayek¹, Joseph C. Poole¹, Ayaz Rahman¹, Vivek Menon¹, Nino Kavtaradze¹, David Polhemus², Emir Veledar¹, David J. Lefer², and Arshed A. Quyyumi¹

¹Emory University School of Medicine, Division of Cardiology, Atlanta, GA

²Louisiana State University Health Sciences Center, Department of Pharmacology, New Orleans, LA

Abstract

Endothelial dysfunction is more prevalent in African Americans (AA) compared to whites. We hypothesized that nebivolol, a selective β -1 antagonist that stimulates NO, will improve endothelial function in AA with hypertension when compared to metoprolol. In a double-blind, randomized, cross-over study, 19 AA hypertensive subjects were randomized to a 12-week treatment period with either nebivolol 10mg or metoprolol succinate 100mg daily. Forearm blood flow (FBF) was measured using plethysmography at rest and after intra-arterial infusion of acetylcholine, and sodium nitroprusside to estimate endothelium-dependent and independent vasodilation, respectively. Physiologic vasodilation was assessed during hand-grip exercise. Measurements were repeated after NO blockade with L-N^G-monomethylarginine (L-NMMA), and after inhibition of endothelium-derived hyperpolarizing factor (EDHF) with tetraethylammonium chloride (TEA). NO blockade with L-NMMA produced a trend toward greater vasoconstriction during nebivolol compared to metoprolol treatment period (21% vs 12% reduction in FBF, $p=0.06$, respectively). This difference was more significant after combined administration of L-NMMA and TEA ($p<0.001$). Similarly, there was a contribution of NO to exercise-induced vasodilation during nebivolol but not during metoprolol treatment. There were significantly greater contributions of NO and EDHF to resting vasodilator tone and of NO to exercise-induced vasodilation with nebivolol compared to metoprolol in AA with hypertension.

Keywords

nebivolol; metoprolol; nitric oxide; hypertension; black; african american; vasodilation; acetylcholine; exercise

Corresponding Author: Arshed A. Quyyumi, MD, Professor of Medicine, Division of Cardiology, Co-Director, Emory Clinical Cardiovascular Research Institute, 1462 Clifton Road N.E. Suite 510, Atlanta GA 30322, Tel: 404 727 3655, Fax: 404 712 8785, aquyyum@emory.edu.

Disclosures: None of the authors have conflicts of interests to disclose.

Conflicts of Interests: None

Clinicaltrials.gov Identifier: NCT01049009

INTRODUCTION

Endothelial dysfunction precipitated by loss of nitric oxide (NO) bioavailability is associated with exposure to cardiovascular risk factors. African Americans (AA) shoulder a higher burden of hypertension and its associated target organ damage including nephropathy, stroke, myocardial infarction and cardiovascular mortality.¹ In comparison to their white counterparts, AAs have decreased NO bioavailability and worse endothelial function that has been attributed to both decreased production and increased degradation of NO.²⁻⁵

Nebivolol is a third-generation, β_1 -adrenergic receptor antagonist with vasodilatory properties that appear to be independent of β_1 -receptor antagonism and related to β_3 -receptor agonist effects.⁶⁻⁸ It stimulates nitric oxide release through β_3 -receptor and ATP-dependent, P2Y-receptor activation.^{6,9-13} In hypertensive patients, vasodilation with nebivolol is evident after a single dose and persists after chronic administration.^{14,15} Furthermore, nebivolol increased endothelium-dependent vasodilation with acetylcholine when compared to atenolol in whites with hypertension.¹⁶

Endothelium-dependent vasodilation in response to agonists such as acetylcholine and bradykinin is due to the release of several factors including NO, endothelium-derived hyperpolarization factor (EDHF), prostaglandins, and others.¹⁷ EDHF release may compensate for reduced NO bioavailability in certain disease states and contributes to physiologic vasodilation due to exercise.¹⁷⁻²⁰ Although there may be several EDHFs, they all relax vascular smooth muscle via activation of calcium-dependent potassium channels that can be inhibited with tetraethylammonium chloride (TEA).²⁰ Using these antagonists, we and others have shown that EDHF contributes to resting vasodilator tone and to bradykinin-mediated vasodilation in healthy subjects.¹⁷ Furthermore, we found that NO but not EDHF activity is reduced in the forearm vasculature of AA compared to whites.⁴

Because of the reduction in NO bioavailability in the vasculature of AA, we sought to determine whether nebivolol, compared to metoprolol, can selectively improve endothelial function by modulating NO and EDHF activities in AAs with hypertension. We tested the hypothesis that nebivolol, by increasing NO bioavailability, would improve endothelial function compared to matched hypotensive doses of sustained release metoprolol in AA with hypertension.

METHODS

Study design

In a randomized, double-blind, crossover study, subjects with resting blood pressure (BP) >135/85 (GE Dynamap) were randomized to receive either nebivolol or metoprolol succinate in addition to their current regimen of anti-hypertensive medications (Figure 1A). Subjects with hypertension and BP <135/85 at randomization had their regimen altered by decreasing the dose of concomitant medications. The study drug was initiated as either nebivolol 5mg or metoprolol succinate 50mg daily. After 2 weeks, the dose of the study drug was increased to either nebivolol 10mg or metoprolol 100mg if BP remained >125/80. Subjects continued the highest titrated dose of study drug for an additional 10 weeks prior to

performance of vascular studies. At the end of the first treatment phase, subjects crossed over into the alternate treatment arm. BP was measured at each visit after a 10-minute rest period using a mean of 3 measurements taken 5 minutes apart. The study was reviewed and approved by the Emory University Institutional Review Board. All subjects provided written informed consent.

Subjects

Self-identified AA subjects aged 22–80 years old with a history of essential hypertension were recruited. Exclusion criteria included initiation or change in statin or anti-hypertensive therapy, occurrence of stroke or acute coronary syndrome within 2 months prior to randomization, presence of chronic stable angina, current neoplasm, symptoms of heart failure, aortic stenosis, chronic kidney or liver diseases (creatinine >2.5mg/dL, liver enzymes > twice upper limit of normal), and premenopausal females with the potential for pregnancy. Subjects with contra-indications to beta blockade (i.e. second or third degree AV block, bradycardia, severe reactive airways disease) were also excluded. Concurrent therapy with angiotensin antagonists (ACE inhibitors or ARBs) was not permitted. Allowable concurrent anti-hypertensive therapy included thiazide diuretics, calcium channel antagonists, clonidine, and vasodilators. Subjects on beta-adrenergic blockers had their drug changed to the study drug at time of enrollment. Subjects with co-morbid cardiovascular risk factors including hyperlipidemia, diabetes and smoking were included as long as there was no recent or planned change in therapy within 2 months of randomization and during the course of the study.

Materials

L-NMMA: *N*^G-mono- methyl-L-arginine (L-NMMA; Bachem, Laufelfingen, Switzerland) is an analogue of L-arginine which competitively and irreversibly inhibits the generation of NO from arginine by nitric oxide synthases (NOS1, NOS2 and NOS3). Given at 8 μmol/min it attenuates agonist- and exercise-stimulated FBF, respectively.^{21,22} *TEA*: Tetraethylammonium (Sigma Aldrich) is a quaternary ammonium compound which selectively blocks voltage-sensitive potassium channels. When given at 1 mg/min, TEA is known to selectively inhibit K⁺_{Ca} channels and inhibits bradykinin-mediated vasodilation.^{23–25} Sodium nitroprusside is an endothelium-independent vasodilator that acts as a direct nitric oxide donor.²⁶ Its infusion serves to detect alterations in vascular smooth muscle sensitivity to nitric oxide. Acetylcholine (Novartis, East Hanover, NJ) is an endothelium-dependent vasodilator that stimulates nitric oxide release from endothelial cells.²⁷

Measurement of forearm blood flow

Subjects refrained from exercise, alcohol, tobacco and caffeine for at least 24 hours before the study admission. After an overnight fast in a quiet temperature-controlled (22 to 24°C) room, subjects received 975mg of aspirin to inhibit prostacyclin synthesis at least 1 hour prior to the study.²⁸ A 20-gauge catheter (Teleflex Inc, Research Triangle Park, NC) was inserted in the brachial artery of the non-dominant arm under direct ultrasound guidance for intra-arterial drug infusions, pressure monitoring, and blood sampling. Simultaneous

forearm blood flow (FBF) measurements were obtained in both arms using a dual-channel venous occlusion strain gauge plethysmograph (model EC6, DE Hokanson, Bellevue, WA) as described previously.^{17,21} Flow measurements were recorded for approximately 7 seconds, every 15 seconds up to eight times and a mean FBF value in $\text{mL}\cdot\text{min}^{-1}\cdot 100\text{ mL}^{-1}$ was computed. Forearm vascular resistance (FVR) was calculated as the mean arterial pressure \div FBF and expressed as $\text{mmHg per mL}\cdot\text{min}^{-1}\cdot 100\text{ mL}^{-1}$.

All agents were administered intra-arterially. Resting FBF measurements were made after 15 minutes of normal saline infusion (1.5ml/min) and repeated during intra-arterial infusion of the acetylcholine at 7.5, 15 and 30 $\mu\text{g}/\text{min}$ for 5 minutes each. Physiologic forearm vasodilation was investigated using intermittent handgrip exercise where the evaluated forearm was exercised by squeezing an inflated pneumatic bag as previously described.^{21,29} Exercise was performed at 15%, 30%, and 45% of the subject's maximum voluntary grip strength. Each contraction lasted for 5 seconds followed by relaxation for 10 seconds and was repeated for 5 minutes at each workload. FBF was measured in the final 2 minutes of each dose/exercise workload.

After recovery, the acetylcholine and exercise protocol was repeated during inhibition of NO synthesis with the concomitant infusion of L-NMMA at 8 $\mu\text{mol}/\text{min}$ during acetylcholine infusion and 16 $\mu\text{mol}/\text{min}$ during exercise. After recovery, while continuing L-NMMA, TEA was infused intra-arterially at 1 mg/min and acetylcholine and exercise protocols were repeated. Thus, we measured resting vasomotor tone, acetylcholine- and exercise-mediated vasodilation under control conditions, during NO blockade, and during combined NO and EDHF blockade allowing quantification of NO- and EDHF-dependent vasodilation. Finally, sodium nitroprusside was infused intra-arterially at 1.6 and 3.2 $\mu\text{g}/\text{min}$ for 5 minutes each with FBF and FVR measured with each dose. L-NMMA and TEA have both been shown to not alter vasodilator responses to nitroprusside (Figure 1B).¹⁷

Analysis of inorganic nitrite and nitrate levels

Plasma nitrite and nitrate were measured from blood samples collected prior to FBF studies into distilled water-rinsed centrifuge tubes containing 100 mL of 100 mmol/L *N*-ethylmaleimide and 5 mL of 0.5 mmol/L EDTA. Extracted plasma was flash frozen and stored at -80°C and subsequently analyzed for nitrite and nitrate levels by ion chromatography (ENO20, Eicom USA, San Diego, CA) as previously described.³⁰

Statistical Analysis

Descriptive subject characteristics were reported as means, standard deviations (SD), standard errors (in figures), and percentages. A paired student's *t*-test was used to compare BP between treatment phases. Analysis of FBF and FVR measurements was performed using linear mixed effects modeling with repeated measures, after log-transformation of the non-normal and positively skewed variables. An unstructured covariance form was assumed for the repeated measures. In the model, treatment period (metoprolol or nebivolol) and inhibitor (L-NMMA, TEA, L-NMMA and TEA) were added as fixed effects, and subject ID as a random effect. Based on previous studies, with a sample size of 20 we should detect a

10% or greater change between the two drugs in FBF with L-NMMA or TEA with $\alpha=0.05$ and power=0.8.^{15,16}

RESULTS

Subjects

Of the 19 subjects (13 men and 6 women) recruited, 74% were on one or more anti-hypertensive medications, most commonly diuretics and calcium channel antagonists (Table 1). Fourteen subjects (74%) reached the target doses of nebivolol 10mg and metoprolol 100mg. Of the 5 subjects who did not require up-titration, 2 were controlled on nebivolol 5mg and metoprolol 50mg, 1 required nebivolol 5mg and metoprolol 100mg, and 2 required nebivolol 10mg and metoprolol 50mg.

Resting forearm vascular tone

The heart rate and blood pressure was similar during both nebivolol and metoprolol treatment periods (Table 1). Resting vasodilator tone was also similar with the two study agents; FBF 2.8 ± 1.3 and 2.9 ± 1.2 mL·min⁻¹·100 mL⁻¹, $p=0.6$ and FVR 40.8 ± 17 and 37.5 ± 12 mmHg/mL·min⁻¹·100 mL⁻¹, $p=0.8$ with nebivolol and metoprolol, respectively (Figure 2).

NO blockade with L-NMMA reduced resting FBF by 21% with nebivolol and 12% with metoprolol (both $p<0.001$ compared to baseline, and $p=0.06$ between groups for comparison of absolute values, and $p=0.053$ for comparison of % change in FBF). Similarly, FVR increased by 26% with nebivolol and 18% with metoprolol (both $p<0.001$ compared to baseline, $p=0.1$ between groups for comparison of absolute values, and $p=0.1$ for comparison of % change in FVR) (Figure 2). Addition of, K⁺_{Ca} channel blockade with TEA to L-NMMA resulted in further significant reduction in FBF and an increase in FVR with nebivolol but not metoprolol ($p<0.001$) (Figure 2). Thus, there was a greater contribution of NO and EDHF combined to resting blood flow during therapy with nebivolol compared to metoprolol with NO accounting for the majority of the difference.

Acetylcholine-mediated vasodilation

Acetylcholine produced a similar dose-dependent increase in FBF and a concurrent decrease in FVR with both nebivolol and metoprolol (both $p=0.6$) (Figure 3). L-NMMA co-infusion attenuated acetylcholine-mediated vasodilation during treatment with both drugs, resulting in a $23.1 \pm 27.3\%$, $p<0.001$ and a $14.8 \pm 35.5\%$, $p=0.002$ decrease in FBF with nebivolol and metoprolol, respectively (Figure 4). The difference between the groups did not reach statistical significance ($p=0.4$). Addition of TEA to L-NMMA did not further impact either the FBF or FVR during either the nebivolol or metoprolol treatment periods, indicating lack of contribution of EDHF to acetylcholine-mediated vasodilation with either drug (Figure 4).

Exercise-induced vasodilation

Graded exercise produced progressive forearm vasodilation that was similar during treatment with nebivolol and metoprolol ($p=0.3$ and $p=0.4$, respectively) (Figure 3C, D). With nebivolol however, NO antagonism with L-NMMA resulted in a significant decrease

in FBF ($p=0.001$) and increase in FVR ($p<0.001$) (Figure 5). There was no significant change in FBF or FVR with L-NMMA during the metoprolol treatment period (Figure 5). This suggests a significant contribution of NO to exercise-mediated vasodilation with nebivolol, but not with metoprolol treatment. Combined administration of L-NMMA and TEA produced no further reduction in FBF or increase in FVR during either the metoprolol or nebivolol treatment periods, indicating lack of contribution of EDHF to exercise-mediated vasodilation with either beta-antagonist (Figure 5).

Sodium nitroprusside-induced vasodilation

Sodium nitroprusside infusion produced similar vasodilation during treatment with nebivolol and metoprolol (Figure 3E and 3F) indicating no differences in endothelium-independent vasodilation with these agents.

Plasma nitrite and nitrate levels

Plasma nitrite and nitrate levels were similar during treatment with nebivolol (0.17 ± 0.1 and 9.3 ± 3.5 $\mu\text{mol/L}$) and metoprolol (0.17 ± 0.1 and 8.7 ± 4.1 $\mu\text{mol/L}$, $p= 0.7$ and 0.5 , respectively).

DISCUSSION

Compared to whites, both healthy and hypertensive AA have diminished basal NO activity and reduced endothelium-dependent and -independent vasodilation.^{4,31-36} In this study, we found evidence for NO bioavailability at rest during treatment with both nebivolol and metoprolol succinate in hypertensive AA subjects, with a clear trend for a greater contribution of NO during nebivolol therapy. Moreover, after combined blockade of NO and EDHF, there was a significantly greater vasoconstriction during nebivolol compared metoprolol therapy, suggesting greater contribution of both NO and EDHF combined to resting vasomotor tone during nebivolol treatment. Moreover, the contribution of NO to exercise-induced vasodilation was greater during treatment with nebivolol compared to metoprolol succinate. This is the first study to explore the role of nebivolol compared to metoprolol in AA hypertensives who have profound abnormalities in NO bioavailability. Specifically, we demonstrate a greater contribution of endothelium-derived vasodilators to resting FBF and greater contribution of NO to vasodilation during exercise with nebivolol compared to metoprolol. We found no differences in acetylcholine-mediated vasodilation between these agents, a finding that is different from that reported in white hypertensive subjects who received either nebivolol or atenolol.¹⁶

We have previously shown that there is *no* contribution of NO at rest and after acetylcholine in hypertensive subjects (no effect of L-NMMA).³⁷⁻⁴³ Here we demonstrate that there is some contribution of NO after AA hypertensives are treated with beta adrenergic blockade, and further show that this is higher with nebivolol at rest compared to metoprolol. In healthy subjects, L-NMMA reduces resting flow by 30 to 40%. After beta blockade, we show that even in hypertensives, it is restored to approximately half of what it is in healthy subjects.

Exercise-induced vasodilation is a complex process involving multiple vasodilator mechanisms including a modest contribution of NO and various putative EDHFs.^{4,21,44,45}

We have previously demonstrated that both NO and EDHF contribute individually and in concert to exercise-induced microvascular vasodilation in the healthy human forearm circulation. Nebivolol, but not metoprolol restored functional sympatholysis that is impaired in working muscle in hypertensive patients.⁴⁶ In this study, while there was no difference in the magnitude of vasodilation during exercise between treatments, we observed a significant contribution of NO to exercise-induced vasodilation during the nebivolol but not the metoprolol treatment period. This suggests that nebivolol restores contribution of NO to exercise-induced vasodilation in AA with hypertension. Interestingly, neither drug increased the contribution of EDHF to exercise-induced vasodilation.

In previous studies in hypertensive subjects from both ethnicities, we and others found that acetylcholine-mediated NO release was lower compared to normotensive controls.^{4,34–36} In fact, L-NMMA did not inhibit acetylcholine responses in *untreated* hypertensive subjects. Herein, we show significant NO release in response to acetylcholine during treatment with both beta-receptor antagonists. In a previous study of European subjects with hypertension, increased acetylcholine-mediated NO activity was reported with nebivolol but not after atenolol.¹⁶ Improved NO activity with endothelium-dependent vasodilators long-acting metoprolol may thus account for the benefits of this preparation compared to atenolol.⁴⁷

Greater NO activity was found in internal mammary artery and vein specimens from subjects pre-treated with nebivolol compared to metoprolol.⁴⁸ In subjects with coronary artery disease or hypertension, nebivolol, but not atenolol improved flow-mediated dilation (FMD) and lowered asymmetric dimethylarginine (ADMA) levels, a naturally occurring amino acid that inhibits eNOS.^{15,49} Finally, in AA with stage 1 hypertension, nebivolol monotherapy increased FMD and improved arterial wave reflections compared to untreated state.⁵⁰ In contrast, no differences between metoprolol succinate and nebivolol were observed in aortic compliance indices in a largely AA population of diabetics.⁵¹

Moreover, these experiments were performed in the setting of prostacyclin inhibition. The contribution of the prostacyclin pathway was thus not investigated in this study and may potentially be also affected by beta-blockade. Whether other endogenous vasodilators such as prostacyclin, adenosine, carbon monoxide and others are affected differentially by nebivolol versus other beta-blockers needs to be studied.

Strengths and limitations

Strengths of our study include the blinded crossover design and investigation in AA hypertensive subjects who are at particularly high cardiovascular risk and have profound NO abnormalities. We have also examined the contribution of both NO and EDHF to resting, acetylcholine-mediated and exercise-induced vasodilation. Limitations include the small sample size, the heterogeneity of the population, which was largely driven by the requirement of repeated intra-arterial cannulation. The lack of placebo treatment phase would have allowed comparison of either agent with no therapy, however this was precluded by potential hazards of performing three invasive studies in the same subject. This study also did not include other beta-receptor antagonist comparators such as atenolol. In addition, the interaction of concomitant medications such as thiazides or diuretics with the study drugs on

NO bioavailability, while largely controlled by the cross-over design and stable dosing, could not be examined.

CONCLUSIONS

In conclusion, there was significant contribution of NO and EDHF to resting vasodilator tone and a significant contribution of NO to exercise-induced vasodilation with nebivolol compared to an equipotent dose of metoprolol succinate. These findings demonstrate selective effects of nebivolol on NO activity in AA with hypertension and provide mechanistic insights into endothelial dysfunction in this at-risk group. Further study is needed to establish if these observations translate into clinically meaningful outcomes.

Acknowledgments

The authors wish to thank the dedicated nursing and support staff of the Emory Clinical Research Network and referring physicians without whom this study would not have been possible.

Sources of Funding: This work was supported by an investigator initiated grant from Forest Pharmaceuticals and in part by American Heart Association Postdoctoral Fellowship Grant 11POST7140036 (R.B.N.) and by the National Center for Advancing Translational Sciences of the National Institutes of Health under Award Number UL1TR000454.

References

1. Cooper R, Cutler J, Desvigne-Nickens P, Fortmann SP, Friedman L, Havlik R, Hogelin G, Marler J, McGovern P, Morosco G, Mosca L, Pearson T, Stamler J, Stryer D, Thom T. Trends and disparities in coronary heart disease, stroke, and other cardiovascular diseases in the United States: findings of the national conference on cardiovascular disease prevention. *Circulation*. 2000; 102(25):3137–3147. [PubMed: 11120707]
2. Cowie CC, Port FK, Wolfe RA, Savage PJ, Moll PP, Hawthorne VM. Disparities in incidence of diabetic end-stage renal disease according to race and type of diabetes. *N Engl J Med*. 1989; 321(16):1074–1079. [PubMed: 2797067]
3. Houghton JL, Philbin EF, Strogatz DS, Torosoff MT, Fein SA, Kuhner PA, Smith VE, Carr AA. The presence of African American race predicts improvement in coronary endothelial function after supplementary L-arginine. *J Am Coll Cardiol*. 2002; 39(8):1314–1322. [PubMed: 11955849]
4. Ozkor MA, Murrow JR, Rahman A, Kavtaradze N, Arshad S, Syed H, Lin J, Manatunga A, Quyyumi AA. The contribution of nitric oxide and endothelium-derived hyperpolarizing factor to resting and stimulated vasodilator tone in African Americans and whites. *ATVB*. 2014 in press.
5. Kalinowski L, Dobrucki IT, Malinski T. Race-specific differences in endothelial function: predisposition of African Americans to vascular diseases. *Circulation*. 2004; 109(21):2511–2517. [PubMed: 15159296]
6. Mason RP, Jacob RF, Corbalan JJ, Szczesny D, Matysiak K, Malinski T. The favorable kinetics and balance of nebivolol-stimulated nitric oxide and peroxynitrite release in human endothelial cells. *BMC pharmacology & toxicology*. 2013; 14:48. [PubMed: 24074014]
7. Mason RP, Kubant R, Jacob RF, Walter MF, Boychuk B, Malinski T. Effect of nebivolol on endothelial nitric oxide and peroxynitrite release in hypertensive animals: Role of antioxidant activity. *J Cardiovasc Pharmacol*. 2006; 48(1):862–869. [PubMed: 16891916]
8. Van de Water A, Janssens W, Van Neuten J, Xhonneux R, De Cree J, Verhaegen H, Reneman RS, Janssen PA. Pharmacological and hemodynamic profile of nebivolol, a chemically novel, potent, and selective beta 1-adrenergic antagonist. *J Cardiovasc Pharmacol*. 1988; 11(5):552–563. [PubMed: 2455841]
9. Kalinowski L, Dobrucki LW, Szczepanska-Konkel M, Jankowski M, Martyniec L, Angielski S, Malinski T. Third-generation beta-blockers stimulate nitric oxide release from endothelial cells

through ATP efflux: a novel mechanism for antihypertensive action. *Circulation*. 2003; 107(21): 2747–2752. [PubMed: 12742996]

10. Mollnau H, Schulz E, Daiber A, Baldus S, Oelze M, August M, Wendt M, Walter U, Geiger C, Agrawal R, Kleschyov AL, Meinertz T, Munzel T. Nebivolol prevents vascular NOS III uncoupling in experimental hyperlipidemia and inhibits NADPH oxidase activity in inflammatory cells. *Arterioscler Thromb Vasc Biol*. 2003; 23(4):615–621. [PubMed: 12692005]
11. Cominacini L, Fratta Pasini A, Garbin U, Nava C, Davoli A, Criscuoli M, Crea A, Sawamura T, Lo Cascio V. Nebivolol and its 4-keto derivative increase nitric oxide in endothelial cells by reducing its oxidative inactivation. *J Am Coll Cardiol*. 2003; 42(10):1838–1844. [PubMed: 14642697]
12. Fratta Pasini A, Garbin U, Nava MC, Stranieri C, Davoli A, Sawamura T, Lo Cascio V, Cominacini L. Nebivolol decreases oxidative stress in essential hypertensive patients and increases nitric oxide by reducing its oxidative inactivation. *J Hypertens*. 2005; 23(3):589–596. [PubMed: 15716701]
13. Maffei A, Vecchione C, Aretini A, Poulet R, Bettarini U, Gentile MT, Cifelli G, Lembo G. Characterization of nitric oxide release by nebivolol and its metabolites. *Am J Hypertens*. 2006; 19(6):579–586. [PubMed: 16733229]
14. Arosio E, De Marchi S, Prior M, Zannoni M, Lechi A. Effects of nebivolol and atenolol on small arteries and microcirculatory endothelium-dependent dilation in hypertensive patients undergoing isometric stress. *J Hypertens*. 2002; 20(9):1793–1797. [PubMed: 12195121]
15. Lekakis JP, Protogerou A, Papamichael C, Vamvakou G, Ionomidis I, Fici F, Mavrikakis M. Effect of nebivolol and atenolol on brachial artery flow-mediated vasodilation in patients with coronary artery disease. *Cardiovasc Drugs Ther*. 2005; 19(4):277–281. [PubMed: 16187009]
16. Tzemos N, Lim PO, MacDonald TM. Nebivolol reverses endothelial dysfunction in essential hypertension: a randomized, double-blind, crossover study. *Circulation*. 2001; 104(5):511–514. [PubMed: 11479245]
17. Ozkor MA, Murrow JR, Rahman AM, Kavtaradze N, Lin J, Manatunga A, Quyyumi AA. Endothelium-derived hyperpolarizing factor determines resting and stimulated forearm vasodilator tone in health and in disease. *Circulation*. 2011; 123(20):2244–2253. [PubMed: 21555712]
18. Halcox JP, Narayanan S, Cramer-Joyce L, Mincemoyer R, Quyyumi AA. Characterization of endothelium-derived hyperpolarizing factor in the human forearm microcirculation. *American Journal of Physiology - Heart & Circulatory Physiology*. 2001; 280(6):H2470–2477. [PubMed: 11356600]
19. Cohen RA, Vanhoutte PM. Endothelium-dependent hyperpolarization. Beyond nitric oxide and cyclic GMP. *Circulation*. 1995; 92(11):3337–3349. [PubMed: 7586323]
20. Quyyumi AA, Ozkor M. Vasodilation by hyperpolarization: beyond NO. *Hypertension*. 2006; 48(6):1023–1025. [PubMed: 17088444]
21. Gilligan DM, Panza JA, Kilcoyne CM, Waclawiw MA, Casino PR, Quyyumi AA. Contribution of endothelium-derived nitric oxide to exercise-induced vasodilation. *Circulation*. 1994; 90(6):2853–2858. [PubMed: 7994830]
22. Gilligan DM, Panza JA, Garcia CE, Quyyumi AA, Cannon RO 3rd. Selective loss of microvascular endothelial function in human hypercholesterolemia. *Circulation*. 1994; 90(1):35–41. [PubMed: 8026018]
23. Honing ML, Smits P, Morrison PJ, Rabelink TJ. Bradykinin-induced vasodilation of human forearm resistance vessels is primarily mediated by endothelium-dependent hyperpolarization. *Hypertension*. 2000; 35(6):1314–1318. [PubMed: 10856283]
24. Langton PD, Nelson MT, Huang Y, Standen NB. Block of calcium-activated potassium channels in mammalian arterial myocytes by tetraethylammonium ions. *Am J Physiol*. 1991; 260(3 Pt 2):H927–934. [PubMed: 1900393]
25. Inokuchi K, Hirooka Y, Shimokawa H, Sakai K, Kishi T, Ito K, Kimura Y, Takeshita A. Role of Endothelium-Derived Hyperpolarizing Factor in Human Forearm Circulation. *Hypertension*. 2003; 42(5):919–924. [PubMed: 14557280]
26. Palmer RF, Lasseter KC. Drug therapy. Sodium nitroprusside. *The New England journal of medicine*. 1975; 292(6):294–297. [PubMed: 1089194]

27. Chowienczyk PJ, Watts GF, Cockcroft JR, Ritter JM. Impaired endothelium-dependent vasodilation of forearm resistance vessels in hypercholesterolaemia. *Lancet*. 1992; 340(8833): 1430–1432. [PubMed: 1360559]
28. Heavey DJ, Barrow SE, Hickling NE, Ritter JM. Aspirin causes short-lived inhibition of bradykinin-stimulated prostacyclin production in man. *Nature*. 1985; 318(6042):186–188. [PubMed: 3903519]
29. Zelis R, Longhurst J, Capone RJ, Mason DT. A comparison of regional blood flow and oxygen utilization during dynamic forearm exercise in normal subjects and patients with congestive heart failure. *Circulation*. 1974; 50(1):137–143. [PubMed: 4835259]
30. Bryan NS, Calvert JW, Gundewar S, Lefer DJ. Dietary nitrite restores NO homeostasis and is cardioprotective in endothelial nitric oxide synthase-deficient mice. *Free Radic Biol Med*. 2008; 45(4):468–474. [PubMed: 18501719]
31. Cardillo C, Kilcoyne CM, Cannon RO III, Panza JA. Racial Differences in Nitric Oxide-Mediated Vasodilator Response to Mental Stress in the Forearm Circulation. *Hypertension*. 1998; 31(6): 1235–1239. [PubMed: 9622135]
32. Campia U, Choucair WK, Bryant MB, Waclawiw MA, Cardillo C, Panza JA. Reduced endothelium-dependent and -independent dilation of conductance arteries in African Americans. *J Am Coll Cardiol*. 2002; 40(4):754–760. [PubMed: 12204507]
33. Rosenbaum DA, Pretorius M, Gainer JV, Byrne D, Murphey LJ, Painter CA, Vaughan DE, Brown NJ. Ethnicity affects vasodilation, but not endothelial tissue plasminogen activator release, in response to bradykinin. *Arterioscler Thromb Vasc Biol*. 2002; 22(6):1023–1028. [PubMed: 12067915]
34. Panza J, Quyyumi AA, Brush J Jr, Epstein S. Abnormal Endothelium-dependent vascular relaxation in patients with essential hypertension. *N Engl J Med*. 1990; 323:22–27. [PubMed: 2355955]
35. Panza JA, Casino PR, Kilcoyne CM, Quyyumi AA. Role of endothelium-derived nitric oxide in the abnormal endothelium-dependent vascular relaxation of patients with essential hypertension. *Circulation*. 1993; 87(5):1468–1474. [PubMed: 8491001]
36. Taddei S, Virdis A, Ghiadoni L, Magagna A, Pasini AF, Garbin U, Cominacini L, Salvetti A. Effect of calcium antagonist or beta blockade treatment on nitric oxide-dependent vasodilation and oxidative stress in essential hypertensive patients. *J Hypertens*. 2001; 19(8):1379–1386. [PubMed: 11518845]
37. Panza JA, Quyyumi AA, Callahan TS, Epstein SE. Effect of antihypertensive treatment on endothelium-dependent vascular relaxation in patients with essential hypertension. *J Am Coll Cardiol*. 1993; 21(5):1145–1151. [PubMed: 8459069]
38. Panza JA, Quyyumi AA, Brush JE Jr, Epstein SE. Abnormal endothelium-dependent vascular relaxation in patients with essential hypertension. *N Engl J Med*. 1990; 323(1):22–27. [PubMed: 2355955]
39. Panza JA, Garcia CE, Kilcoyne CM, Quyyumi AA, Cannon RO 3rd. Impaired endothelium-dependent vasodilation in patients with essential hypertension. Evidence that nitric oxide abnormality is not localized to a single signal transduction pathway. *Circulation*. 1995; 91(6): 1732–1738. [PubMed: 7882481]
40. Panza JA, Casino PR, Kilcoyne CM, Quyyumi AA. Impaired endothelium-dependent vasodilation in patients with essential hypertension: evidence that the abnormality is not at the muscarinic receptor level. *J Am Coll Cardiol*. 1994; 23(7):1610–1616. [PubMed: 7515084]
41. Cardillo C, Kilcoyne CM, Quyyumi AA, Cannon RO 3rd, Panza JA. Selective defect in nitric oxide synthesis may explain the impaired endothelium-dependent vasodilation in patients with essential hypertension. *Circulation*. 1998; 97(9):851–856. [PubMed: 9521333]
42. Cardillo C, Kilcoyne CM, Quyyumi AA, Cannon RO, Panza JA. Reduced nitric oxide-dependent forearm vasodilation in normotensive blacks compared to whites. *Journal of the American College of Cardiology*. 1997; 29(2):7062–7062.
43. Cardillo C, Kilcoyne CM, Cannon RO 3rd, Panza JA. Impairment of the nitric oxide-mediated vasodilator response to mental stress in hypertensive but not in hypercholesterolemic patients. *J Am Coll Cardiol*. 1998; 32(5):1207–1213. [PubMed: 9809927]

44. Schrage WG, Joyner MJ, Dinunno FA. Local inhibition of nitric oxide and prostaglandins independently reduces forearm exercise hyperaemia in humans. *J Physiol.* 2004; 557(Pt 2):599–611. [PubMed: 15047770]
45. Shoemaker JK, Halliwill JR, Hughson RL, Joyner MJ. Contributions of acetylcholine and nitric oxide to forearm blood flow at exercise onset and recovery. *Am J Physiol.* 1997; 273(5 Pt 2):H2388–2395. [PubMed: 9374776]
46. Price A, Raheja P, Wang Z, Arbique D, Adams-Huet B, Mitchell JH, Victor RG, Thomas GD, Vongpatanasin W. Differential effects of nebivolol versus metoprolol on functional sympatholysis in hypertensive humans. *Hypertension.* 2013; 61(6):1263–1269. [PubMed: 23547240]
47. Basile JN. One size does not fit all: the role of vasodilating beta-blockers in controlling hypertension as a means of reducing cardiovascular and stroke risk. *Am J Med.* 2010; 123(7 Suppl 1):S9–15. [PubMed: 20609697]
48. Bayar E, Ilhan G, Furat C, Atik C, Arslanoglu Y, Kuran C, Ozpak B, Durakoglugil ME. The Effect of Different beta-Blockers on Vascular Graft Nitric Oxide Levels: Comparison of Nebivolol Versus Metoprolol. *Eur J Vasc Endovasc Surg.* 2014; 47 (2):204–208. [PubMed: 24309401]
49. Pasini AF, Garbin U, Stranieri C, Boccioletti V, Mozzini C, Manfro S, Pasini A, Cominacini M, Cominacini L. Nebivolol treatment reduces serum levels of asymmetric dimethylarginine and improves endothelial dysfunction in essential hypertensive patients. *Am J Hypertens.* 2008; 21(11):1251–1257. [PubMed: 18772860]
50. Merchant N, Searles CD, Pandian A, Rahman ST, Ferdinand KC, Umpierrez GE, Khan BV. Nebivolol in high-risk, obese African Americans with stage 1 hypertension: effects on blood pressure, vascular compliance, and endothelial function. *J Clin Hypertens (Greenwich).* 2009; 11(12):720–725. [PubMed: 20021529]
51. Briasoulis A, Oliva R, Kalaitzidis R, Flynn C, Lazich I, Schlaffer C, Bakris G. Effects of nebivolol on aortic compliance in patients with diabetes and maximal renin angiotensin system blockade: the EFFORT study. *J Clin Hypertens (Greenwich).* 2013; 15(7):473–479. [PubMed: 23815535]

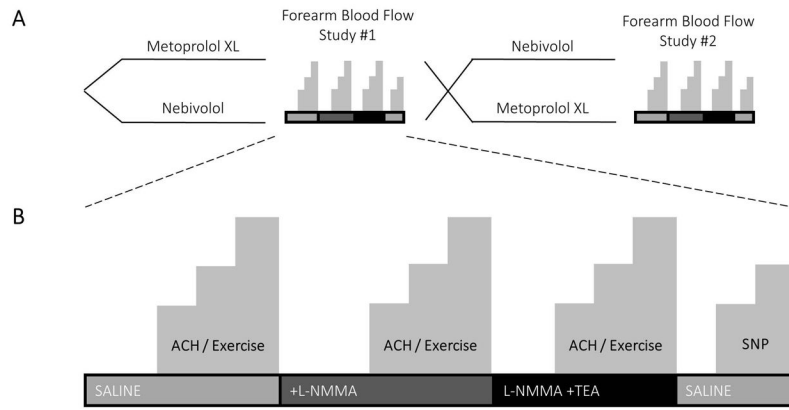


Figure 1.
Study design (A) and protocol (B)

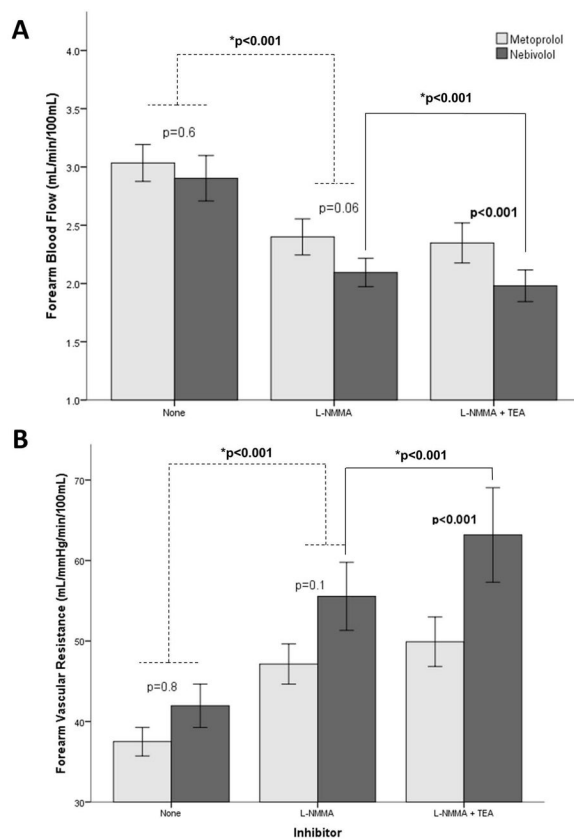


Figure 2. Contribution of nitric oxide and K^+_{Ca} channel activation to resting forearm blood flow and vascular resistance during treatment with nebivolol (A and C) and metoprolol (B and D). Responses to infusion of L-NMMA and combined infusions of L-NMMA and TEA are shown. P values for effect of L-NMMA and TEA on FBF and FVR. Data presented as mean \pm SEM.

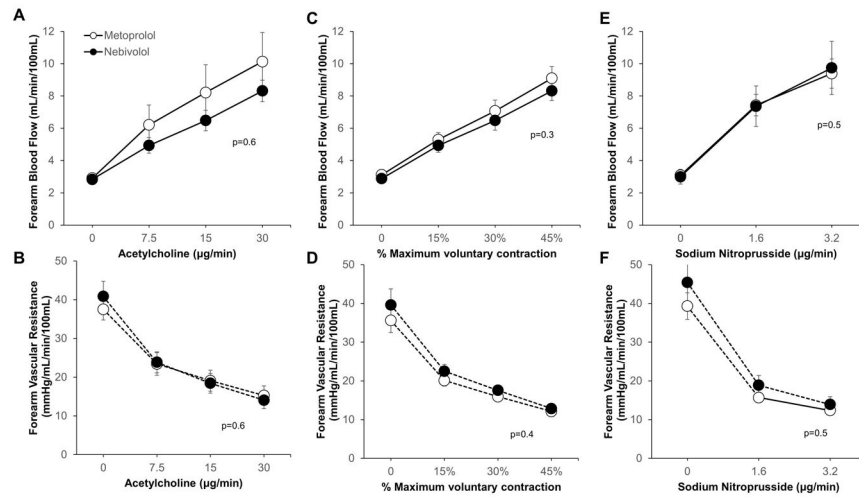


Figure 3. Endothelium-dependent FBF (A) and FVR (B) changes with acetylcholine, endothelium-independent changes in FBF (C) and FVR (D) with sodium nitroprusside, and forearm exercise induced changes in FBF (E) and FVR (F) during treatment with nebivolol and metoprolol. P values are for treatment effect of nebivolol/metoprolol by mixed model. Data presented as mean±SEM.

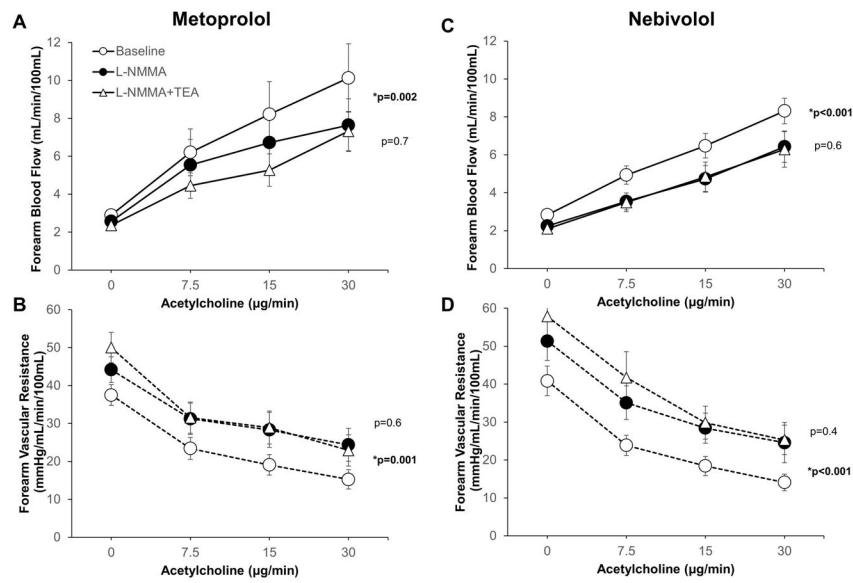


Figure 4. Contribution of nitric oxide and K^+_{Ca} channel activation to acetylcholine-mediated vasodilation during treatment with nebivolol (A and C) and metoprolol (B and D). FBF and FVR responses to increasing doses of acetylcholine alone after initial infusion of L-NMMA, and combined blockade with L-NMMA and TEA are shown. P values are for effect of L-NMMA and TEA by mixed model. *p denotes p-value for comparison of L-NMMA and control, non-starred p reflects p-value for comparison of L-NMMA and L-NMMA+TEA. Data presented as mean±SEM.

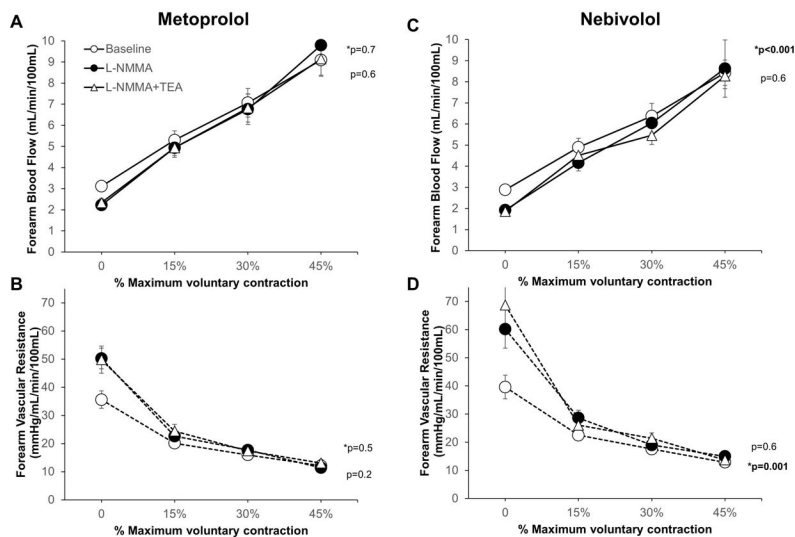


Figure 5. Contribution of nitric oxide and K+Ca channel activation to forearm exercise mediated vasodilation during treatment with nebivolol (A and C) and metoprolol (B and D). FBF and FVR responses to increasing doses of acetylcholine alone, after initial infusion of L-NMMA, and combined blockade with L-NMMA and TEA are shown. P values are for effect of L-NMMA and TEA by mixed model. *p denotes p-value for comparison of L-NMMA and control, non-starred p reflects p-value for comparison of L-NMMA and L-NMMA +TEA. Data presented as mean±SEM.

Table 1

Characteristics of Study Subjects

Hypertensive African-Americans (n=19)			
Age, years			51±8.6
Male/Female			13/6
Diabetes mellitus, n (%)			1(5.1%)
Smoker, n (%)			7 (36.8%)
Hypercholesterolemia, n (%)			6 (31.5%)
Family History of CAD, n (%)			6 (31.5%)
Statin Therapy, n (%)			4 (21%)
Weight, kg			96.5±22.3
Body mass index, kg/m ²			32.5±7.4
Concurrent anti-hypertensive drugs			
Diuretic (thiazide)			8 (42.1%)
Calcium Channel Antagonist			5 (26.3%)
On-Treatment Hemodynamic Data			
	Nebivolol	Metoprolol succinate	p-value
Systolic Blood Pressure, mmHg	135±15	134±15	0.8
Diastolic blood pressure, mmHg	81±14	81±21	0.8
Resting heart rate, bpm	63±8	64±9	0.8

* Data are Mean ±SD