Effects of Nebivolol Versus Metoprolol on Sodium Sensitivity and Renal Sodium Handling in Hypertensive Hispanic Postmenopausal Women

Richard A. Preston, David Afshartous, Barry J. Materson, Rolando Rodco, Alberto B. Alonso

Abstract—Several consistent lines of evidence indicate an association between sodium sensitivity and impaired nitric oxide bioactivity. Nevertheless, whether restoring nitric oxide in humans by pharmacological means can ameliorate sodium sensitivity has not been investigated. Because nebivolol has been demonstrated to increase nitric oxide bioactivity in both laboratory and clinical investigations, we hypothesized that nebivolol might ameliorate sodium sensitivity and improve renal sodium handling in comparison to metoprolol. We therefore conducted a randomized, 2-treatment-period crossover trial in 19 Hispanic postmenopausal women with hypertension to determine the comparative effects of nebivolol versus metoprolol on (1) 24-hour ambulatory blood pressure response to an increase in dietary sodium from 5 days of low sodium to 5 days of high sodium, (2) renal natriuretic response to a 1-L saline challenge, and (3) asymmetrical dimethylarginine. Clinical blood pressure and heart rate were significantly reduced after 4 weeks of treatment with both nebivolol and metoprolol. Twenty-four–hour mean systolic blood pressure increased sharply from low sodium to high sodium for both nebivolol and metoprolol. Nevertheless, the increases in blood pressure did not differ between the 2 drugs: 7.7 (3.1, 12.3) mm Hg with metoprolol and 9.3 (4.6, 13.9) mm Hg with nebivolol (P=0.63). Furthermore, we observed no differences between the drugs in natriuretic response to saline challenge or asymmetrical dimethylarginine. In a sodium-sensitive population, at doses sufficient to produce reductions in blood pressure and heart rate, nebivolol did not demonstrate a significant effect on sodium sensitivity or sodium handling compared with metoprolol. (Hypertension. 2014;64:287-295.) ● Online Data Supplement

Key Words: hypertension ■ kidney ■ nitric oxide ■ sodium

Blood pressure is considered to be sodium sensitive when it varies directly with the intake of sodium. Sodium sensitivity is considered to be an important intermediate phenotype or precursor of arterial hypertension in humans. Furthermore, sodium-sensitive hypertensive patients are considered more likely to develop significant target organ damage than their sodium-resistant counterparts. Menopause is accompanied by an increase in the prevalence of both sodium sensitivity and hypertension, which may partially explain the corresponding increased cardiovascular risk observed in postmenopausal women.

Several consistent lines of laboratory and clinical investigation indicate an association between impaired nitric oxide (NO) bioactivity and sodium sensitivity. Despite this association, it is not known whether restoring NO bioactivity could reduce the blood pressure response to sodium in sodium-sensitive hypertensive humans. The central hypothesis tested by this clinical investigation is whether restoring NO bioactivity by pharmacological means can ameliorate sodium sensitivity.

Nebivolol is a β1 selective β-blocker with intrinsic vasodilating activity. A large number of experimental and clinical investigations suggest that nebivolol may stimulate endothelial NO bioactivity and improve endothelial function. Because sodium sensitivity is linked to impaired NO bioactivity, and because nebivolol has been demonstrated to increase NO, we hypothesized that nebivolol could ameliorate sodium sensitivity and improve renal sodium handling in comparison to metoprolol, a β1 cardioselective β-blocker that does not seem to increase NO bioactivity.

To test this hypothesis, we conducted a randomized 2-period crossover physiological trial to determine the comparative effects of nebivolol versus metoprolol titrated to an equal blood pressure goal on the blood pressure response to an abrupt increase in dietary sodium in 19 hypertensive postmenopausal women. In our clinical pharmacology research unit (CPRU),

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This trial is registered at ClinicalTrials.gov with trial identifier NCT00992056 (http://www.clinicaltrials.gov/ct/show/NCT00992056).

The online-only Data Supplement is available with this article at http://hyper.ahajournals.org/lookup/suppl/doi:10.1161/HYPERTENSIONAHA.114.03476/-/DC1.

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we used 24-hour ambulatory blood pressure monitoring (ABPM) to determine the pressor response produced by an abrupt change from 5 days of low sodium to 5 days of high sodium. In addition, we determined the comparative effects of nebivolol versus metoprolol on the renal natriuretic response to an intravenous 1-L normal saline challenge and changes in plasma asymmetrical dimethylarginine (ADMA).

Attenuation of the pressor response to an increase in dietary sodium by nebivolol versus metoprolol and improved renal sodium handling would provide key supportive evidence that restoring NO bioactivity could lead to attenuation of sodium sensitivity and improvement in renal sodium handling.

### Methods

All study procedures were conducted in the 20-bed inpatient and outpatient CPRU of the Division of Clinical Pharmacology of the University of Miami, Miller School of Medicine, under the direct supervision of the study principal investigator.

### Research Objectives

The objectives of this randomized, 2-treatment, 2-period crossover trial conducted in Hispanic postmenopausal women with hypertension were to determine the comparative effects of nebivolol versus metoprolol titrated to an equal target blood pressure on (1) the 24-hour ABPM response to an increase in dietary sodium from 5 days of low sodium to 5 days of high sodium, (2) renal natriuretic response to an acute 1-L saline challenge, and (3) changes in ADMA (nM/L).

### Primary End Points

1. Change in 24-hour mean systolic blood pressure (SBP) and diastolic blood pressure (DBP) by ABPM from final day (day 5) of low sodium period to the final day (day 10) of high sodium.
2. Renal sodium excretion (UnaV, mmol/h) in response to a 1-L intravenous saline challenge administered over 2 hours (CPRU day 11).

### Secondary End Points

1. Ambulatory clinic cuff blood pressure during 4 weeks of nebivolol versus metoprolol.
2. ADMA (nmol/L).
3. Cumulative renal sodium excretion (cUnaV, mmol) and fractional excretion of sodium after 1-L intravenous saline challenge

### Study Participants

Recruitment and all study procedures were performed by the principal investigator and study team in accordance with the guidelines of the University of Miami, Miller School of Medicine Human Subjects Research Office (Institutional Review Board) who approved this clinical study and the Principles of the Declaration of Helsinki. Written informed consent was obtained by study coordinators directly from all participants prior to their entry into the study and prior to any study procedures. The study population consisted of postmenopausal women >45 years who were self-identified as being of Hispanic origin and had SBP 140 to 159 mm Hg and DBP 90 to 99 mm Hg off antihypertensive medications. Inclusion and exclusion criteria are detailed in the online-only Data Supplement.

### Study Design and Overview

This investigation consisted of a randomized, open-label, 2-treatment, 2-period (metoprolol versus nebivolol) crossover clinical trial conducted in 19 participants who completed all phases of the protocol (Figure S1 in the online-only Data Supplement). Each randomized 4-week treatment period with either nebivolol 5 mg daily for 1 week increased by forced titration to 10 mg daily or metoprolol 50 mg daily increased to 100 mg daily. If goal blood pressure (<140/90 mm Hg) was not achieved at week 3, the nebivolol (or metoprolol) was titrated to 20 mg (or 200 mg).

Participants returned at weekly (7 days±4 days) intervals during all outpatient study phases. On the day of a scheduled visit, participants were instructed not to take their medication at home, but to bring the medication with them to the visit. Participant visits were scheduled in the morning, and all evaluations were performed at drug trough (24±2 hours after the previous day’s dose). Medications for that day were administered after all evaluations planned for that visit had been completed. At each visit, participants were questioned directly about compliance and any missed or delayed doses. In addition, pill counts were conducted at each visit.

Nebivolol was provided in 5, 10, and 20 mg tablets by Forest Laboratories, New York, NY. Metoprolol ER was purchased as the generic product in 25, 50, and 100 mg tablets.

### Eleven-Day Inpatient Confinement Period

Both 4-week outpatient treatment periods were followed by an 11-day inpatient confinement period in the CPRU. During days 1 to 5, participants received a diet designed to contain ≤30 mmol Na and 50 mmol K. On day 5, a determination of 24-hour ABPM was performed while continuing to receive this low-sodium diet. During days 6 to 10, participants received a diet designed to contain ≥200 mmol Na and 50 mmol K. On day 10 of this high sodium period, ABPM was performed while the subjects continued to receive the 200 mmol Na and 50 mmol K diet. The response to increased dietary sodium was defined as the difference between the ABPM measurements conducted on day 5 and day 10. On day 11, a sodium handling study was performed to determine the effects of nebivolol versus metoprolol on sodium excretion (UnaV) after 1-L 0.9% saline administered over 2 hours. All meals were designed with the supervision of a certified dietitian, provided by the University of Miami Hospital Food Service, and directly supervised by CPRU staff.

### Renal Sodium Handling After a 1-L Saline Challenge

On day 11 of the inpatient confinement period, while continuing to receive the 200 mmol Na and 50 mmol K diet, an intravenous saline load test was performed. Beginning at ≈6:00 AM, baseline urine for sodium, potassium, and creatinine was collected for 2 hours. At the midpoint of this 2-hour urine collection, blood was taken for sodium, potassium, and creatinine. At ≈8 AM, 1-L 0.9% normal saline was infused over 2 hours. Urine collections were performed hourly for six 1-hour periods for sodium, potassium, and creatinine. Hour 1 and 2 collections were during the saline infusion. Hour 3 to 6 collections were post saline infusion. Blood pressure and heart rate were determined at baseline and at each hour during the collection period.

At hour 1 midpoint (30 minutes after the infusion was started), and the midpoint of each hourly urine collection for a total of 6 collection periods, blood was taken for creatinine, sodium, and potassium. Immediately after the six 1-hour collection periods, the subject resumed the standard diet. Meals were identical and served at the same time on day 11 for both periods. Urine was then collected and volume recorded at intervals of 6 to 8 hours, 8 to 12 hours, and 12 to 24 hours. Aliquots were sent for creatinine, sodium, and potassium.

### Determination of Plasma ADMA (nM/L)

Plasma ADMA was determined by the Vanderbilt University School of Medicine Mass Spectrometry Core. Details are included in the online-only Data Supplement.

### Blood Pressure Measurements

Outpatient standard cuff blood pressure measurements and 24-hour blood pressure (ABPM) performed on day 5 (low sodium) and day 10 (high sodium) of the inpatient confinement period are detailed in the online-only Data Supplement.
Statistical Methods

A sample size of n=16 subjects was calculated to yield a power of 0.8 to detect a mean SBP/DBP change of 0.75 standard deviations in the change from low sodium to high sodium between nebivolol and metoprolol (α=0.05). For detecting mean changes of 0.65 and 0.55 standard deviations, the corresponding sample sizes are 21 and 28 subjects. Given sample sizes of previous studies of human sodium sensitivity (11 to 15), we estimated 1 standard deviation in the change in blood pressure with increased sodium uptake to translate to ±5.4 mmHg.11 Thus, our calculated sample size of n=16 subjects completing all ABPM determinations would provide a sufficient power of 0.8 with α=0.05 to detect a difference of 4.05 mmHg in the change in blood pressure with increased sodium intake. Because of our crossover design, the use of ABPM to reduce blood pressure variability, and the completion of 19 subjects, we consider this a conservative estimate.

Randomization was conducted in blocks of 2 via a computer-generated randomization table. The statistical significance of the primary end points was assessed via paired t tests and bootstrap t tests, combined with robust measures of location.24–26 Data were also tested for time and carryover effects.27 P<0.05 was considered significant (1-tailed), and data in the results section are presented as means with corresponding 95% confidence intervals in parentheses. Results involving multiple comparisons for the primary end points were assessed via stepwise multiple comparison procedures (eg, Holm’s method) given a familywise error rate of α=0.05. Analogous statistical methods were used for all secondary end points.

Results

Baseline and Demographic Characteristics

Twenty-four Hispanic postmenopausal women qualified for enrollment and were entered into the trial. Three withdrew informed consent during the trial. One was withdrawn because of blood pressure elevation during the washout period, and one completed all study procedures but had a faulty ABPM determination on the final reading. Nineteen women completed all phases of the study, including both outpatient treatment phases, both inpatient confinement periods, and all 4 ABPM determinations. Baseline and demographic characteristics are summarized in the Table.

Clinic Blood Pressures for 4-Week Outpatient Treatment Periods

Clinic (cuff) SBP and DBP were significantly reduced after initiation of both nebivolol and metoprolol (Figure 1A–1C). Clinic SBP at randomization was 142.1 (138.2, 145.9) mm Hg for metoprolol and 143.9 (139.2, 148.6) mm Hg for nebivolol. Although SBP did not differ for visits 2 to 4, on the final outpatient visit, SBP was 129.7 (126.2, 133.2) mm Hg for metoprolol and 123.8 (119.6, 128.1) mm Hg for nebivolol (P=0.03). Clinic DBP at randomization was 87.2 (83.6, 90.8) mm Hg for metoprolol and 90.7 (88.3, 93.0) mm Hg for nebivolol. DBP did not differ on the final outpatient visit (P=0.10).

Heart rate was significantly reduced for both nebivolol (77.9 [73.1, 82.7] to 67.4 [64.1, 71.3]) beats per minute and metoprolol (76.7 [71.6, 81.8] to 68.6 [64.1, 73.2]), but there were no differences between the 2 drugs.

Blood Pressure Response to an Increase in Dietary Sodium

Mean 24-hour ambulatory SBP increased sharply from day 5, the last day of low sodium, to day 10, the last day of high sodium, during treatment with both nebivolol (120.4 [115.7, 125.0] to 129.6 [123.2, 136.0] mm Hg; P<0.001) and metoprolol (120.9 [115.2, 126.6] to 128.6 [122.9, 134.4] mm Hg; P<0.001) (Figure 2A–2D). Nevertheless, the increases from low to high sodium did not differ between the 2 drugs: 7.7 (3.1, 12.3) mm Hg with metoprolol and 9.3 (4.6, 13.9) mm Hg with nebivolol (P=0.63). Twenty-four–hour ambulatory DBP increased significantly from day 5 of low sodium to day 10 of high sodium for nebivolol (69.8 [67.0, 72.6] to 74.0 [70.8, 77.19] mm Hg) and metoprolol (70.2 [67.4, 73.1] to 73.5 [70.1, 76.9] mm Hg). The increases from low to high sodium did not differ between the 2 drugs: 3.3 (0.19, 6.3) mm Hg with metoprolol and 4.2 (1.3, 7.0) mm Hg with nebivolol (P=0.66).

Twenty-four–hour mean arterial pressure increased significantly from day 5 of low sodium to day 10 of high sodium during treatment with both nebivolol and metoprolol, but the increases from low sodium to high sodium did not differ between the 2 drugs (P=0.78). Nocturnal variation in blood pressure as determined by day and night ABPM results did not differ between the nebivolol and metoprolol treatment periods (Figure 2C and 2D).

Sodium Excretion in Response to a 1-L Normal Saline Challenge

After a 1-L saline infusion, we observed no differences in sodium excretion rates (UnaN, mmol/h), cumulative sodium excretion (cUnaN, mmol), or fractional excretion of sodium for nebivolol versus metoprolol (Figure 3A–3C).

Twenty-Four–Hour Sodium Excretion to Estimate Dietary Intake

Twenty-four–hour urine sodium excretion was determined on admission (baseline), after 5 days of low sodium, and after 5 days of high sodium (Figure S2). After 5 days on the lowsodium diet, the 24-hour sodium excretion was 65.4 (48.0, 82.8) mmol for nebivolol and 66.6 (54.7, 78.4) mmol for metoprolol (P=0.39). After 5 days on the high sodium intake, the 24-hour sodium excretion was 177.6 (150.1, 205.1) mmol for nebivolol and 160.3 (136.3, 184.4) mmol for metoprolol (P=0.33). Although the goal intake of <30 mmol and >200 mmol day was approximated but not fully achieved based on the 24-hour collections, there were no differences in dietary sodium intake between the 2 groups as estimated by 24-hour urine collections. Both groups had a sharp 9- to 10-mmHg increase in blood pressure with the abrupt change from low to high sodium intake. Therefore, the ultimate goal of the increase from low to high sodium was successfully achieved.

Plasma ADMA

We observed no differences between nebivolol and metoprolol in the response of ADMA to treatment with either drug or the response to low or high sodium intake (Figure 4).

Discussion

Because sodium sensitivity is linked to impaired NO bioactivity, and because nebivolol has been demonstrated to increase NO bioactivity in a wide array of experimental and clinical investigations, we hypothesized that nebivolol could ameliorate sodium sensitivity of blood pressure and improve renal sodium handling in comparison to metoprolol. To test this hypothesis, we
conducted a randomized, 2-treatment-period crossover trial in 19 postmenopausal hypertensive Hispanic women to determine the comparative effects of nebivolol versus metoprolol on the blood pressure response to an abrupt increase in dietary sodium and the natriuretic response to an acute 1-L saline challenge.

As anticipated, we observed a large pressor response to the increase in dietary sodium during the CPRU inpatient phase of the study. Nevertheless, we did not observe an attenuated blood pressure response to increased dietary sodium with nebivolol compared with metoprolol. In addition, we observed no differences between nebivolol and metoprolol in their comparative effects on renal sodium handling after a 1-L saline challenge or plasma ADMA. Our results, therefore, do not support an effect of nebivolol on sodium sensitivity or sodium handling in postmenopausal Hispanic women.

We designed our study to achieve adequate statistical power to detect a clinically significant difference in blood pressure change (≈4.05 mmHg) from low to high sodium with a sample size of 16 study subjects. To ensure that there was adequate power to detect a difference, we conservatively enrolled 24 subjects, 19 of whom completed all study periods and all 4 ABPM determinations. Furthermore, we studied a population known to be characterized by a high prevalence of sodium sensitivity. Indeed, we observed a substantial increase in blood pressure with a change from low- to high-sodium diet.

Our sample size is consistent with that of similarly designed studies of sodium sensitivity in humans. For example, Morris et al demonstrated a substantial and statistically significant difference between 24 blacks and 14 whites in the effects of dietary potassium on sodium sensitivity. Although we used a similar study design with this approximate sample size in mind, our crossover design and the reduced blood pressure measurement variability produced by ABPM both served to further increase statistical power. Metoprolol and nebivolol were administered in adequate doses. We used a titration protocol in which all subjects received at least 10 mg nebivolol and 100 mg metoprolol. At these doses, both drugs produced substantial reductions of blood pressure and heart rate.

Our hypothesis was based on the connection between impaired NO bioactivity and sodium sensitivity. Schmidlin et al observed in blacks that progressive increases of blood pressure in salt-sensitive subjects were reflective of impaired vascular function and concluded that in many normotensive blacks, vascular dysfunction (impaired vasodilation) is critical to the initiation of a pressor response to dietary sodium. Bragulat et al compared endothelium-dependent and endothelium-independent vasodilation in 19 sodium-resistant versus 26 sodium-sensitive hypertensives. Compared with sodium-resistant hypertensives, salt-sensitive patients presented a significantly lower maximal acetylcholine-induced vasodilation, whereas maximal sodium nitroprusside–induced vasodilation did not significantly differ between the 2 groups, supporting the hypothesis that sodium-sensitive hypertension is associated with endothelial dysfunction characterized by a defective NO-dependent endothelium-dependent vasodilation.

In designing our study, we considered that nebivolol would be a logical candidate drug to test the hypothesis that restoring impaired NO activity could improve sodium sensitivity.

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B indicates black; BMI, body mass index; C-G, Cockroft–Gault equation estimated clearance; DBP, diastolic blood pressure; H, Hispanic; MDRD eGFR, modification of diet in renal disease equation estimated glomerular filtration rate; SBP, systolic blood pressure; and W, white.

*Final dose at the end of outpatient treatment period.
Laboratory and clinical studies of vascular function suggest that nebivolol increases NO bioactivity. Brachial artery infusion of nebivolol in 40 healthy volunteers increased forearm blood flow by 91% (P<0.01), compared with baseline, whereas an equimolar dose of atenolol had no effect. Infusion of 1-NG-monomethyl L-arginine inhibited the nebivolol-induced vasodilation by 65%. Nebivolol was infused in the forearm brachial artery of 8 men with untreated primary hypertension and resulted in an increase in forearm blood flow compared with saline vehicle, an effect that was inhibited by 1-NG-monomethyl...
L-arginine. In a double-blind, randomized, crossover study, 12 patients with hypertension received nebivolol 5 mg plus bendrofluazide 2.5 mg for 8 weeks versus atenolol 50 mg plus bendrofluazide 2.5 mg. Both the nebivolol–bendrofluazide and the atenolol–bendrofluazide regimens lowered blood pressure significantly and to the same extent. The nebivolol–bendrofluazide regimen significantly increased the brachial artery vasodilatory response to acetylcholine, as measured by forearm blood flow with venous occlusion plethysmography, compared with baseline (P<0.001); the atenolol–bendrofluazide combination had no effect. Furthermore, the nebivolol–bendrofluazide regimen significantly attenuated the vasoconstrictive response to L-NG-monomethyl L-arginine in the brachial artery (P<0.01), whereas the atenolol–bendrofluazide treatment did not. Both treatments had similar effects on the brachial artery response to the endothelium-independent vasodilatory effects of sodium nitroprusside.

The results of this study have alternative potential explanations. Our results could simply reflect that the pathogenesis of human sodium-sensitive hypertension is complex and may be related to more than a single mechanism. On the other hand, it is possible that nebivolol administered in the doses used for hypertension or clinical studies of vascular function may not restore NO sufficiently to ameliorate sodium sensitivity.

Although we have focused this investigation on the connection between impaired NO bioactivity and sodium sensitivity, we readily acknowledge that there are compelling lines of evidence supporting several alternative plausible mechanisms. Sodium sensitivity has been associated with polymorphisms in the sodium bicarbonate cotransporter SLC4A5, functional upregulation of the renin–angiotensin system, inhibition of vasodilatation by ADMA, modulation of NO directly by excessive sodium intake itself, reduced dietary potassium intake, and mutations in the epithelial sodium channel or cytochrome CYP11B2. The potential mechanisms may not be exclusive but may work in parallel with impaired NO to produce sodium sensitivity in different subsets of patients.

There are limitations to our investigation. Because of the complexity of sodium sensitivity, our negative results may not apply to other populations such as men, premenopausal women, and other races/ethnicities. A baseline determination of sodium sensitivity would have strengthened our study and allowed for selection of the most sodium-sensitive subjects for our investigation. Although we used doses of nebivolol that have been demonstrated to improve markers of endothelial function and NO bioactivity, a corroborating assessment of endothelial function could have strengthened our conclusions.
To our knowledge, this is the first study directly comparing the effects of a β-blocker with NO enhancing properties with a β-blocker without effects on NO on sodium sensitivity and renal sodium handling. We consider that the approach of directly administered sodium under controlled conditions combined with ABPM may be of use in studying the effects of a wide array of therapeutic agents and clinical maneuvers on sodium sensitivity. We selected a hypertensive study population with a high prevalence of sodium sensitivity, administered both study medications in doses sufficient to produce significant

Figure 3. Sodium excretion after 1-L saline challenge. A, Sodium excretion (UnaV). B, Cumulative UnaV. C, Fractional excretion of sodium (FENa).
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**Disclosures**

None.

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**What Is New?**

- The blood pressure response to dietary sodium varies in humans. Some are sensitive to sodium: the blood pressure increases as the dietary sodium increases. Sodium sensitivity is believed to be a precursor of hypertension and is associated with an increased risk for target organ damage. Recent studies suggest that sodium sensitivity is related to reduced endothelial nitric oxide (NO) production. Because nebivolol seems to stimulate NO production, we studied whether nebivolol could improve sodium sensitivity in comparison to metoprolol, which does not seem to affect NO bioactivity.

**What Is Relevant?**

- We found no differences between nebivolol and metoprolol in their effects on sodium sensitivity. The results of this study suggest either that the mechanisms of sodium-sensitive hypertension are complex and multiple and may be related to more than an isolated reduction in NO or that nebivolol in doses used for hypertension does not restore NO sufficiently to ameliorate sodium sensitivity.

**Summary**

In a sodium-sensitive population, at doses sufficient to reduce blood pressure, nebivolol did not demonstrate an effect of sodium sensitivity compared with metoprolol.
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Effects of Nebivolol versus Metoprolol on Sodium Sensitivity and Renal Sodium Handling in Hypertensive Hispanic Postmenopausal Women

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Methods

Inclusion and Exclusion Criteria
The study population consisted of postmenopausal women more than 45 years of age who were self-identified as being of Hispanic origin and had systolic blood pressure 140-159 mm Hg and diastolic blood pressure 90-99 mm Hg off antihypertensive medications. Potential subjects were postmenopausal for at least 1 year (no menstruation for 1 year) prior to screening or had a bilateral oophorectomy at least 3 months prior to study participation. Potential subjects were excluded if they had a history of adverse effects or intolerance to any beta-blocker, significant or unstable medical illness besides hypertension, clinically significant abnormalities of physical examination or laboratory data, or were receiving treatment with diuretics, non-steroidal anti-inflammatory drugs (NSAIDs), aspirin, metformin, steroids, or other agents known to influence blood pressure, renal function, or sodium sensitivity. Grapefruit and Seville oranges were not allowed at any time during the study. Subjects were also excluded for current alcohol or drug abuse or smoking of more than 10 cigarettes per day.

Blood pressure measurements

Standard cuff pressure
All blood pressure measurements were performed using the American Heart Association/JNC 7 protocol while the subject was sitting with back and arm support using a calibrated sphygmomanometer with an appropriate cuff size on the non-dominant arm. The blood pressure was taken as mean of 3 measurements.

Ambulatory blood pressure monitoring (ABPM)
ABPM determination of 24-hour mean systolic, diastolic, and mean arterial pressure (MAP) was performed on Day 5 (low sodium) and Day 10 (high sodium) of the inpatient confinement period using SpaceLabs 90207 programmable monitors (SpaceLabs, Redmond, WA). The units were programmed to record BP every 20 minutes during the day and every 30 min during the night. The same ABPM unit was used for each of the four determinations on a given subject.

Determination of Plasma Asymmetrical Dimethylarginine (ADMA, nM/L)
Plasma ADMA was determined by the Vanderbilt University School of Medicine Mass Spectrometry Core. Samples for ADMA were taken at baseline, following each 4 week treatment period, and at the end of low sodium and high sodium periods during CPRU confinement. Plasma samples were processed by protein precipitation, centrifugal membrane dialysis (5 kDa MWCO), and chemical derivatization with dansyl chloride. Components were separated by HPLC using an Agilent Rapid Resolution HT 1.8 mm (2.1mm x 50mm) column. MS/MS detection was done using an LTQ linear ion trap mass spectrometer (Thermo-Fisher, Waltham, MA) equipped with an Ion Max electrospray source. Quantitation was based on the multiple reaction monitoring transitions of m/z 436 to 391 (dansyl-ADMA-d0) and m/z 439 to 391 (dansyl-ADMA-d3), and m/z 443 to 398 (dansyl-ADMA-d7). Data acquisition and quantitative spectral analysis were conducted using Thermo-Finnigan Xcaliber (v. 2.0 Sur 1) and Thermo-Finnigan LCQuan (v. 2.5.5), respectively.
S1. Study Schematic

Treatment Period 1

Randomization

Screening

M 50 mg

M 100 mg

N 5 mg

N 10 mg

11 Days in CPRU

Washout

Treatment Period 2

M 50 mg

N 5 mg

N 10 mg

M 100 mg

11 Days in CPRU

Weeks

1

2-4

5-6

7-8

9

10-12

13-14

N=Nebivolol; M=Metoprolol; CPRU=Clinical Pharmacology Research Unit
Figure S2. 24-hour Urine Sodium Excretion Following 5 Days of Standardized Diet