Differential Effects of Nebivolol Versus Metoprolol on Functional Sympatholysis in Hypertensive Humans


Abstract—In young healthy humans, sympathetic vasoconstriction is markedly blunted during exercise to optimize blood flow to the metabolically active muscle. This phenomenon known as functional sympatholysis is impaired in hypertensive humans and rats by angiotensin II–dependent mechanisms, involving oxidative stress and inactivation of nitric oxide (NO). Nebivolol is a β1-adrenergic receptor blocker that has NO-dependent vasodilatory and antioxidant properties. We therefore asked whether nebivolol would restore functional sympatholysis in hypertensive humans. In 21 subjects with stage 1 hypertension, we measured muscle oxygenation and forearm blood flow responses to reflex increases in sympathetic nerve activity evoked by lower body negative pressure at rest, and during rhythmic handgrip exercise at baseline, after 12 weeks of nebivolol (5–20 mg/d) or metoprolol (100–300 mg/d), using a double-blind crossover design. We found that nebivolol had no effect on lower body negative pressure–induced decreases in oxygenation and forearm blood flow in resting forearm (from −29±5% to −30±5% and from −29±3% to −29±3%, respectively; P=NS). However, nebivolol attenuated the lower body negative pressure–induced reduction in oxygenation and forearm blood flow in exercising forearm (from −14±4% to −1±5% and from −15±2% to −6±2%, respectively; both P<0.05). This effect of nebivolol on oxygenation and forearm blood flow in exercising forearm was not observed with metoprolol in the same subjects, despite a similar reduction in blood pressure. Nebivolol had no effect on sympathetic nerve activity at rest or during handgrip, suggesting a direct effect on vascular function. Thus, our data demonstrate that nebivolol restored functional sympatholysis in hypertensive humans by a mechanism that does not involve β1-adrenergic receptors.

Clinical Trial Registration—URL: http://www.clinicaltrials.gov. Unique identifier: NCT01502787. (Hypertension. 2013;61:00-00.)

Key Words: exercise ■ hypertension ■ muscle blood flow ■ sympathetic nervous system

Essential hypertension is a major public health problem in the United States and worldwide. Patients with hypertension display a blunted decline in systemic vascular resistance during exercise, which may contribute to impaired exercise tolerance even in the absence of heart failure. Exercise triggers reflex activation of the sympathetic nervous system, resulting in elevated cardiac output, while simultaneously producing vasoconstriction in many vascular beds, including the viscera and inactive skeletal muscles. In the working muscles, sympathetically mediated vasoconstriction is greatly attenuated by vasodilator substances released during muscle contraction, thereby optimizing muscle blood flow to meet metabolic demand. We recently found that this protective mechanism, known as functional sympatholysis, is impaired in hypertensive humans. The mechanisms responsible for impaired functional sympatholysis in hypertension are incompletely understood. Our previous studies in hypertensive rats indicated a deleterious role of angiotensin II (Ang II) to blunt functional sympatholysis attributable to excessive production of superoxide and inactivation of nitric oxide (NO) in the exercising muscles. We recently extended these findings to hypertensive humans by showing that functional sympatholysis is restored by short-term treatment with the angiotensin receptor blocker irbesartan, but not with the thiazide-type diuretic chlorthalidone, despite an equivalent reduction in blood pressure (BP). These findings indicate that lowering BP alone is not sufficient to restore functional sympatholysis. Therefore, on the basis of our previous work, we anticipate that antihypertensive agents that inhibit the renin–angiotensin system, mitigate oxidative stress, or increase NO bioavailability would also normalize sympathetic regulation of muscle blood flow during exercise. Nebivolol is a third-generation selective β1-adrenergic receptor (AR) blocker that also possesses vasodilator and antioxidant properties. The vasodilator effect is attributed largely to activation of endothelial NO synthase and increased NO release, whereas the antioxidant effect is...
thought to be attributable to inhibition of NADPH oxidase and reduced superoxide formation.\textsuperscript{12–14} Given the unique \(\beta_1\)-AR–independent properties of nebivolol to improve NO signaling and reduce oxidative stress, we hypothesized that treatment with nebivolol would restore functional sympatholysis and improve muscle perfusion during exercise in hypertensive humans. In this study, we therefore sought to determine whether short-term treatment with nebivolol would attenuate sympathetically mediated vasoconstriction in the exercising muscles of subjects with primary hypertension. To determine whether the effect of nebivolol could be explained by its distinct pharmacological profile independent of \(\beta_1\)-AR antagonism, we compared nebivolol with metoprolol, a selective \(\beta_1\)-AR blocker without vasodilator or antioxidant properties, using a randomized double-blind crossover design. Because our previous work implicated a major role for Ang II–induced oxidative stress to impair functional sympatholysis in hypertensive rats, we also asked whether nebivolol treatment would mitigate the increases in oxidative stress and BP evoked by Ang II infusion in hypertensive humans.

\section*{Methods}

Twenty-one subjects with untreated stage 1 hypertension participated in the study, after providing written informed consent. The study was approved by the institutional review board of the University of Texas Southwestern Medical Center at Dallas. All subjects had BP between 140 to 159 and 90 to 99 mm Hg on 3 determinations by oscillometric sphygmomanometry (CE0050, Welch Allyn, Skaneateles Falls, NY). Respiration was monitored with a strain-gauge pneumograph, and subjects were instructed to avoid sympathoexcitatory maneuvers, including cold pressor testing. Blood pressures were recorded continuously by electrocardiography, and systolic and diastolic blood pressures (SBPs and DBPs) were measured by automated oscillometric sphygmomanometry (Stoelting, Chicago, IL). Subjects performed intermittent handgrip to reach 80% of the subject’s maximal squeezes of a handgrip dynamometer (Stoelting, Chicago, IL). Subjects performed intermittent handgrip to reach 80% of the subject’s maximal squeezes of a handgrip dynamometer (Stoelting, Chicago, IL).

\section*{Skeletal Muscle Oxygenation}

Near infrared (NIR) spectroscopy (NIR-500, Hamamatsu Photonics, Hamamatsu, Japan) was used to measure changes in tissue concentrations of oxygenated hemoglobin and myoglobin (HbO\(_2\)+MbO\(_2\)) in the forearm, as previously described.\textsuperscript{16,15} To monitor NIR light absorption, 2 fiber-optic bundles spaced 2 cm apart were placed over the flexor digitorum profundus muscle, which is the main muscle recruited during handgrip.\textsuperscript{16} NIR signals were sampled at a rate of 1 Hz, converted to chromophore concentrations using established algorithms, and digitally stored for later analysis. Changes in the NIR signals were quantified as a percentage of the total labile signal, which was defined in each experiment as the maximal decrease in HbO\(_2\)+MbO\(_2\) achieved during inflation of a pneumatic cuff on the upper arm to 220 mm Hg for 3 minutes. Because blood vessels >1 mm in diameter maximally absorb NIR light, changes in HbO\(_2\)+MbO\(_2\) reflect changes occurring mainly in the microvessels.\textsuperscript{17}

\section*{Forearm Blood Flow}

Brachial artery diameter and mean blood velocity were measured by Duplex Doppler ultrasonography (Philips i33, Bothell, WA), using an 11-MHz probe in the nondominant arm. The probe was placed in a holder and fixed to the skin over the brachial artery throughout the entire experiment. Diameter measurements were obtained at end-diastole. Blood velocity was acquired with a probe insonation angle of 60°. The output of the handgrip dynamometer was transferred to the auxiliary input of the ultrasound system and displayed simultaneously with the ultrasound images during handgrip exercise. Images were stored on DVD discs and were analyzed offline, using edge detection software (Brahial Analyzer, Medical Imaging Applications LLC, Coralville, IA). Forearm blood flow (FBF, mL min\(^{-1}\)) was calculated as mean blood velocity\(\times\)cross-sectional area (cross-sectional area of the brachial artery)\(\times\)60. Because motion artifact during handgrip produced distortion of the Doppler waveforms, images acquired during muscle contraction were excluded from analysis. Forearm vascular conductance (FVC; mL min\(^{-1}\)×100 mm Hg) was calculated as (FBF×MAP)/100.

\section*{Reflex Activation of Sympathetic Nerves}

Lower body negative pressure (LBNP) was used to produce reflex sympathetic vasoconstriction in the forearm. The subject’s lower body was enclosed to the level of the iliac crest in a negative pressure chamber. LBNP at −20 mm Hg was used to unload mainly the cardiopulmonary baroreceptors and trigger increases in muscle sympathetic nerve activity (SNAn).\textsuperscript{1} Multiunit recordings of SNAn were obtained with unipolar tungsten microelectrodes inserted into muscle fascicles of the peroneal nerve by microneurography.\textsuperscript{19} Neural signals were amplified, filtered (bandwidth 700–2000 Hz), rectified, and integrated to obtain mean voltage neuromgrams. Recordings were considered acceptable based on well-defined criteria that discriminate muscle SNAn from other neural signals, including skin SNAn and muscle spindle activity.\textsuperscript{1} Muscle SNAn was expressed as burst frequency (bursts min\(^{-1}\) and total activity (burst frequency×mean burst amplitude). Changes in SNAn (total activity) during the course of each experimental protocol were expressed as relative increases from the baseline activity at rest. Changes in SNAn specifically in response to LBNP were expressed as the relative increases from the pre-LBNP baseline at rest or during handgrip.

\section*{Handgrip Exercise}

Maximal voluntary contraction for each subject was designated as the greatest of 2 maximal squeezes of a handgrip dynamometer (Stoelting, Chicago, IL). Subjects performed intermittent handgrip to the rhythm of a metronome (20 handgrips min\(^{-1}\); 50% duty cycle) at 30% maximal voluntary contraction for 6 minutes. Force production was displayed on an oscilloscope to provide subjects with visual feedback. This level of handgrip alone does not increase muscle SNAn in healthy subjects.\textsuperscript{7}

\section*{Plasma F2-Isoprostanes (F2-IsopPs)}

F2-IsopPs (plasma F2-isoprostanes), a prostaglandin F2α-like compound, was measured by gas chromatography–mass spectrometry.\textsuperscript{19} Lower limit of detection of F2-IsopPs is 4 pg/mL. The precision of this assay in biological fluids is ±6% and the accuracy 94%.\textsuperscript{19}

\section*{Experimental Protocols}

\section*{Protocol 1. Effects of Nebivolol Versus Metoprolol on Functional Sympatholysis in Hypertensive Subjects (n=21)}

BP, HR, forearm muscle oxygenation, FBF, FVC, and SNAn were measured in response to 2 minutes of LBNP at −20 mm Hg applied at rest and during 3 to 5 minutes of handgrip in all hypertensive subjects at baseline. After the baseline study, subjects were randomized to receive 12 weeks of nebivolol (5–20 mg/d) or metoprolol succinate (100–300 mg/d), using a double-blind crossover design with 2-week washout between each treatment phase. Every 4 weeks, clinic BP was measured using an oscillometric device (CE0050, Welch Allyn) after participants had been sitting quietly for at least 5 minutes. BP measurements were repeated twice after 1 minute between each reading, and data were averaged with the first reading. The doses of nebivolol and metoprolol were titrated to achieve clinic BP of <140/90 mm Hg. After 12-week treatment with each study drug, measurement of BP, HR, forearm muscle oxygenation, FBF, FVC, and SNAn during LBNP and handgrip were repeated, and these variables were compared with those obtained at baseline.
Protocol 2. Assessment of Vasoconstrictor Response to Ang II During Nebivolol Versus Metoprolol (n=21)

To determine whether nebivolol attenuates Ang II–induced vasoconstriction and BP elevation in hypertensive humans by preventing Ang II–induced increase in oxidative stress, we assessed changes in MAP, FBF, forearm vascular resistance (which was calculated as MAP×80/FBF) at baseline and in response to intravenous infusion of Ang II at the dose of 1, 2, and 3 ng/kg per min each for 15 minutes in the same subjects participated in protocol 1 after completion of protocol 1 for ≥30 minutes. Plasma F2-IsoPs were also obtained at baseline and at the end of Ang II infusion protocol in the same subjects.

Statistical Analysis

For protocol 1, drug treatment and experiment condition responses were compared with linear mixed model repeated measures analyses that included repeated factors to assess treatment phase (baseline, nebivolol, metoprolol), experimental condition (LBNP, rest, handgrip), and the treatment-by-condition interaction. In our analysis, the study subject is modeled as a random effect and, moreover, the mixed model approach permits flexibility in modeling the covariance structure of the repeated measures and can handle unbalanced data.20 In the presence of a statistically significant main effect or interaction test, planned pairwise comparisons were made from the least squares means contrasts derived from the mixed models. For study in protocol 2, data were also analyzed with linear mixed models to test the treatment phase (baseline, nebivolol, metoprolol), Ang II dose, and the treatment-by-dose interaction effects. In all analyses, variables with skewed distributions were log or rank transformed before analysis. Statistical analyses were conducted using SAS version 9.2 (SAS Institute, Cary, NC). All tests were 2-sided, and a P value <0.05 was considered statistically significant. Data are presented as means±SEM.

Results

Baseline characteristics of our hypertensive subjects are shown in Table 1. The average daily dose of nebivolol used in the study was 9±1 mg, and the average dose of metoprolol succinate was 174±20 mg. Both nebivolol and metoprolol had no effect on fasting plasma glucose, and there were no differences in fasting plasma insulin or HOMA-IR during treatment with nebivolol compared with metoprolol (P>0.05; Table S1 in the online-only Data Supplement).

Protocol 1: Effects of Nebivolol Versus Metoprolol on Sympathetically Mediated Vasoconstriction During Exercise in Hypertensive Subjects

At baseline, LBNP at −20 mm Hg evoked significant decreases in muscle oxygenation (−29±5%, FBF (−29±3%), and FVC (−28±4%) in the resting forearms of untreated hypertensive subjects that were accompanied by significant increases in muscle SNA (+55±15%) (all P<0.05 rest + LBNP versus rest; Table 2; Figures 1 and 2). When applied during rhythmic handgrip, this same level of LBNP caused reductions in muscle oxygenation, FBF, and FVC (−14±4%, −15±2%, and −18±2%, respectively, P<0.05 handgrip + LBNP versus handgrip) that were only partially attenuated compared with the responses observed at rest (Table 2; Figures 1 and 2).

Treatment with nebivolol for 12 weeks had no effect on the LBNP-induced decreases in forearm muscle oxygenation (−30±5%), FBF (−29±3%), or FVC (−28±3%) or increase in muscle SNA (+71±19%) at rest (all P=NS nebivolol versus baseline). However, nebivolol markedly attenuated the LBNP-induced reductions in oxygenation (−1±5%), FBF (−6±2%), and FVC (−6±1%) in the exercising forearms (all P<0.05; Table 1). The average daily dose of nebivolol used was 54±3 mg.

Figure 2). Although nebivolol attenuated the LBNP-induced vasoconstrictor responses during handgrip, it did not attenuate the increase in SNA during handgrip alone or handgrip plus LBNP (P=NS versus baseline [no drug period]; Table 2). In contrast to nebivolol, metoprolol had no effect on LBNP-induced vasoconstriction or decrease in muscle oxygenation during handgrip in the same hypertensive subjects (Figures 1 and 2; and Table 2).

This beneficial effect of nebivolol on sympathetic vasoconstriction during exercise was not explained by the magnitude of BP reduction because a similar decrease in resting MAP was achieved by treatment with either nebivolol or metoprolol succinate (7±3 versus 8±2 mm Hg; P=NS; Table 2 and Figure S1). Nebivolol also caused a similar reduction in MAP and HR during handgrip when compared with metoprolol succinate (P=NS; Table 2 and Figure S1).

Protocol 2: Effects of Nebivolol Versus Metoprolol on Ang II–Mediated Vasoconstriction

In untreated hypertensive subjects at baseline, Ang II infusion caused a dose-dependent increase in MAP and forearm vascular resistance (P<0.05; Figure 3 and Table S2), but had no effect on FBF or SNA (Figure 3 and Table S2). Although nebivolol and metoprolol cause similar reductions in resting MAP, neither affected the MAP or forearm vascular resistance responses to Ang II. Before Ang II infusion, plasma F2-IsoP levels were lower during nebivolol than metoprolol treatment (34.4±2.7 versus 41.1±3.9 pg/mL, respectively; P<0.05, although not significantly different from baseline (38.8±4.5 pg/mL; P>0.05). Ang II infusion caused a significant increase in plasma F2-IsoP within 45 minutes of infusion during all phases of treatment (P<0.01), but the increase in plasma F2-IsoP was unaffected by nebivolol or metoprolol (Figure 3 and Table S2).

Discussion

The major new findings of the present study are 3-fold. First, nebivolol attenuated sympathetic vasoconstriction in the exercising forearm muscles of individuals with mild, uncomplicated hypertension that was not observed with metoprolol, despite a similar reduction in resting BP. Second, this
improvement in muscle blood flow regulation during exercise was not explained by a reduction in central sympathetic outflow, implicating a direct effect of nebivolol on the peripheral vasculature. Third, nebivolol did not ameliorate the increases in vascular resistance or plasma F2-isoprostanes evoked by short-term Ang II infusion, suggesting that the nebivolol-induced restoration of functional sympatholysis was likely not attributable to inhibition of Ang II–induced oxidative stress. Collectively, these findings indicate that the ability of nebivolol to improve functional sympatholysis in hypertensive individuals cannot be attributed to its conventional β-blocking and BP-lowering effects but is likely attributable to its ancillary vasodilating properties.

In young and middle-aged healthy subjects, our group and others have shown that reflex sympathetic activation induced by mild orthostatic stress or infusion of sympathomimetic drugs evokes vasoconstriction in the resting forearm, which is greatly attenuated during mild to moderate levels of rhythmic handgrip exercise. In hypertensive subjects, however, we previously reported that sympathetic activation produces equivalent decreases in muscle blood flow and oxygenation in resting and exercising forearm, indicating impaired functional sympatholysis. We now confirm this finding of impaired sympatholysis in the present study in another group of mildly hypertensive, middle-aged subjects as evidenced by the persistent forearm vasoconstriction evoked by reflex sympathetic activation during handgrip exercise. Our new data show that this impairment is readily reversed by treatment with the third-generation vasodilating β1-AR blocker nebivolol, but not with the more traditional nonvasodilating β1-AR blocker metoprolol. Taken together with our previous work showing restoration of sympatholysis in hypertensive subjects treated with an angiotensin receptor blocker, but not with a thiazide-type diuretic, these findings strengthen the conclusion that BP reduction alone is not sufficient to improve functional sympatholysis. Only those drugs with specific pharmacological properties that intersect with mechanistic pathways involved in sympatholysis are likely to normalize muscle blood flow regulation during exercise in hypertension.

Our findings provide some clues about the potential β1-AR independent mechanisms underlying effect of nebivolol to attenuate sympathetically mediated vasoconstriction during

### Table 2. Hemodynamic and Sympathetic Responses to LBNP at Rest and During Handgrip in Hypertensive Subjects Treated With Nebivolol vs Metoprolol

<table>
<thead>
<tr>
<th>Variables</th>
<th>Baseline (no drug)</th>
<th>Rest</th>
<th>Rest+LBNP</th>
<th>Handgrip</th>
<th>Handgrip+LBNP</th>
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</thead>
<tbody>
<tr>
<td><strong>MAP, mm Hg</strong></td>
<td>104±2</td>
<td>102±2</td>
<td>110±4*</td>
<td>110±2*</td>
<td></td>
</tr>
<tr>
<td><strong>HR, bpm</strong></td>
<td>65±2</td>
<td>65±2</td>
<td>71±1*</td>
<td>72±2*</td>
<td></td>
</tr>
<tr>
<td><strong>SNA, bursts min⁻¹</strong></td>
<td>40±3</td>
<td>45±3*</td>
<td>45±4</td>
<td>50±3*</td>
<td></td>
</tr>
<tr>
<td><strong>ΔSNA, % total activity</strong></td>
<td>0±0</td>
<td>52±15*</td>
<td>40±15*</td>
<td>100±24 *†</td>
<td></td>
</tr>
<tr>
<td><strong>FBF, mL min⁻¹</strong></td>
<td>114±15</td>
<td>80±11*</td>
<td>469±50*</td>
<td>405±49†</td>
<td></td>
</tr>
<tr>
<td><strong>FVC, units</strong></td>
<td>110±14</td>
<td>78±10*</td>
<td>441±45*</td>
<td>367±39*†</td>
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<td><strong>Nebivolol</strong></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td><strong>MAP, mm Hg</strong></td>
<td>97±3‡</td>
<td>96±3‡</td>
<td>103±3‡</td>
<td>104±3‡</td>
<td></td>
</tr>
<tr>
<td><strong>HR, bpm</strong></td>
<td>56±2‡</td>
<td>56±3‡</td>
<td>62±2‡</td>
<td>63±3‡</td>
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</tr>
<tr>
<td><strong>SNA, bursts min⁻¹</strong></td>
<td>38±3</td>
<td>44±3*</td>
<td>47±5*</td>
<td>52±4*</td>
<td></td>
</tr>
<tr>
<td><strong>ΔSNA, % total activity</strong></td>
<td>0±0</td>
<td>71±19*</td>
<td>33±10*</td>
<td>81±22 †</td>
<td></td>
</tr>
<tr>
<td><strong>FBF, mL min⁻¹</strong></td>
<td>105±13</td>
<td>75±9*</td>
<td>427±37*</td>
<td>404±37†</td>
<td></td>
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<tr>
<td><strong>FVC, units</strong></td>
<td>109±13</td>
<td>78±9*</td>
<td>413±99*</td>
<td>389±33†</td>
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<td><strong>Metoprolol</strong></td>
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<tr>
<td><strong>MAP, mm Hg</strong></td>
<td>96±2‡</td>
<td>95±3‡</td>
<td>101±3‡</td>
<td>100±3‡</td>
<td></td>
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<tr>
<td><strong>HR, bpm</strong></td>
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<td>55±2‡</td>
<td>60±2‡</td>
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<td></td>
</tr>
<tr>
<td><strong>SNA, bursts min⁻¹</strong></td>
<td>39±3</td>
<td>46±3*</td>
<td>45±4*</td>
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<td><strong>ΔSNA, % total activity</strong></td>
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<td>77±18*</td>
<td>30±10*</td>
<td>99±22 †</td>
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<td><strong>FBF, mL min⁻¹</strong></td>
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<td>75±12*</td>
<td>388±43*</td>
<td>340±40*†∥</td>
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<tr>
<td><strong>FVC, units</strong></td>
<td>99±14</td>
<td>79±12*</td>
<td>392±39*</td>
<td>353±35†∥</td>
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</table>

**FBF** indicates forearm blood flow; **FVC** indicates forearm vascular conductance; **HR** indicates heart rate; **LBNP** indicates lower body negative pressure; **MAP** indicates mean arterial pressure; and **SNA** indicates sympathetic nerve activity.

*P<0.05 vs baseline; †P<0.05 vs handgrip; ‡P<0.05 vs baseline, ||P<0.05 vs rest; †P<0.05 vs baseline, ‡P<0.05 vs handgrip.
most likely to be mediated by its unique β2 agonist properties which are β1
inert and α1-adrenergic receptor antagonist. However, in our study, we were unable to
measure β2 agonist properties or α1-adrenergic receptor antagonist activity. It is also
possible that the beneficial effects of nebivolol seen in our study could have been due
to its putative antioxidant properties, as it is known to directly scavenge reactive oxi-

dant species (ROS), as well as reduce ROS production, by inhibiting the activity and
expression of NADPH oxidase. We previously implicated NADPH oxidase as a
potential source of ROS in the contracting muscles. Infusion of the antioxidant
tempol normalized sympatholysis in these hypertensive animals. Ang II also plays a role
in the impaired functional sympatholysis in hypertensive humans because treatment with an angiotensin receptor blocker readily reverses this abnormality. In the current
study, we therefore asked whether nebivolol would mitigate Ang II–induced oxidative stress in the hypertensive subjects. To address this question, we measured plasma levels
of F2-isoprostanes in response to a brief systemic infusion of Ang II. Our finding that Ang II–induced increases in F2-isoprostanes in response to intravenous infusion
of angiotensin II (3 ng/kg per min) in hypertensive subjects at baseline (no drug), after treatment with nebivolol for 12 weeks, and after metoprolol for 12 weeks. *P<0.05 vs baseline (no drug); †P<0.05 vs baseline (no drug); ‡P<0.05 vs metoprolol.

excessive production of ROS in the contracting muscles.

Figure 3. Summary data showing the changes in mean arterial pressure (MAP), forearm vascular resistance (FVR), and plasma F2-isoprostanes (F2-IsoP) in response to intravenous infusion of angiotensin II (3 ng/kg per min) in hypertensive subjects at baseline (no drug), after treatment with nebivolol for 12 weeks, and after metoprolol for 12 weeks. *P<0.05 vs baseline (no drug); †P<0.05 vs baseline (no drug); ‡P<0.05 vs metoprolol.

Figure 2. Summary data showing changes in (A) muscle oxygenation, (B) forearm blood flow (FFB), (C) forearm vascular conductance (FVC), and (D) sympathetic nerve activity (SNA) in response to lower body negative pressure (LBNP) at rest and during handgrip (HG) in 21 hypertensive subjects. *P<0.05 vs rest; †P<0.05 vs baseline (no drug) or metoprolol. FVC indicates forearm vascular conductance; and TLS, total stable signal.

exercise. This could not be explained by a reduction in central sympathetic drive because LBNP evoked similar increases in SNA at rest and during handgrip in subjects at baseline (no treatment) and during treatment with either nebivolol or metopro-

It is also unlikely that nebivolol reduced sympathetic neurotransmission or directly inhibited α-AR because LBNP caused similar decreases in FFB and FVC at rest during the baseline and treatment phases of our study. These data are consistent with studies in pithed rats, showing that nebivolol did not attenuate the pressor responses to selective α1- or α2-AR agonists or to electric stimulation of the spinal cord.22 Finding no evidence to suggest that nebivolol restored functional sympatholysis in hypertensive individuals by reducing sympathetic drive or inhibiting α-AR, we then turned our attention to its putative antioxidant properties.

One of the unique features of nebivolol that could explain its beneficial effect on functional sympatholysis is its ability to inhibit oxidative stress. Nebivolol may directly scavenge reactive oxygen species (ROS), as well as reduce ROS production, by inhibiting the activity and expression of NADPH oxidase. We previously implicated a mechanistic role for oxidative stress to impair functional sympatholysis in rat models of Ang II–dependent hypertension attributable to upregulation of NADPH oxidase and
sympatholysis in hypertension. A growing body of evidence indicates that vasodilating effect of nebivolol is mediated by its ability to stimulate NO production in blood vessels.\textsuperscript{27,28} Interestingly, in the renal microvasculature, this effect of nebivolol seems to be mediated by increased ATP efflux resulting in activation of endothelial P2Y receptors and subsequent calcium-dependent activation of endothelial NO synthesis.\textsuperscript{27} Nebivolol has also been shown to reduce circulating levels of the endogenous NO synthase inhibitor, asymmetric dimethylarginine, in hypertensive patients by increasing the expression and activity of the asymmetric dimethylarginine-degrading enzyme, dimethylarginine dimethylaminohydrolase.\textsuperscript{29} Whether nebivolol restores functional sympatholysis by stimulating NO or ATP release in the human skeletal muscle remains to be investigated.

Our study is limited by a lack of placebo arm for comparison because it is unethical to withhold antihypertensive treatment for an extended duration of 12 weeks. However, it is unlikely that the restoration of functional sympatholysis during nebivolol treatment is a random occurrence. In our previous study, we showed that sympatholysis was normalized in hypertensive subjects during treatment with an angiotensin receptor blocker, but that sympatholysis was similarly impaired in the same subjects before treatment and 4 weeks after withdrawal from treatment. Our study is also limited by the lack of normotensive control subjects to verify that functional sympatholysis is impaired in the hypertensive cohort. However, in our previous study, we showed that sympathetic vasocostriction is significantly enhanced in the exercising, but not the resting, forearm muscles of hypertensive subjects compared with age-matched normotensive controls, thereby documenting impaired sympatholysis in the hypertensive group. Given this finding, we recruited hypertensive subjects with similar characteristics for the present study.

**Perspectives**

Use of older generation β-blockers without ancillary vasodilating properties is associated with exercise intolerance and fatigue.\textsuperscript{30–32} This is thought to be a result of attenuation of β-AR–mediated increase in HR and cardiac output during exercise.\textsuperscript{32} These side effects seem to be much less common in patients treated with newer generation β-blockers like nebivolol because of its tendency to improve oxygen uptake or augment the reduction in systemic vascular resistance at peak exercise.\textsuperscript{31,33} Although nebivolol has been shown to improve left ventricular function and functional capacity in hypertensive patients with systolic heart failure,\textsuperscript{34} beneficial effects of nebivolol on exercise performance was also observed in patients with heart failure with normal ejection fraction,\textsuperscript{35} suggesting additional benefit beyond myocardial function. Impaired functional sympatholysis is increasingly recognized as a cause of skeletal muscle malperfusion not only in hypertension but also in the normotensive elderly population and individuals with physical inactivity.\textsuperscript{35,36} Thus, inhibition of sympathetic vasoconstriction may constitute an additional mechanism by which exercise tolerance is improved with nebivolol. Clinical trials with long-term follow-up are needed to determine whether nebivolol can improve skeletal muscle perfusion and exercise capacity in hypertensive subjects without heart failure.

**Acknowledgments**

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**Disclosures**

None.

**References**

Broeders MA, Doevendans PA, Bekkers BC, Bronsaer R, van Gorsel E, van der Zee R.


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Differential Effects of Nebivolol vs Metoprolol on Functional Sympatholysis in Hypertensive Humans

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Table S1. Glucose, insulin, and HOMA-IR responses to nebivolol vs. metoprolol

<table>
<thead>
<tr>
<th>Variables</th>
<th>Baseline</th>
<th>Nebivolol</th>
<th>Metoprolol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose, mg/dL</td>
<td>101 ± 3</td>
<td>103 ± 3</td>
<td>104 ± 4</td>
</tr>
<tr>
<td>Insulin, µU/L, median</td>
<td>4.1</td>
<td>4.6</td>
<td>4.2</td>
</tr>
<tr>
<td>Interquartile range</td>
<td>2.3-6.8</td>
<td>3.65-9.25</td>
<td>2.7-10.0</td>
</tr>
<tr>
<td>HOMA-IR, median</td>
<td>0.96</td>
<td>1.16</td>
<td>0.97</td>
</tr>
<tr>
<td>Interquartile range</td>
<td>0.52-1.84</td>
<td>0.84-2.24</td>
<td>0.60-2.42</td>
</tr>
</tbody>
</table>
Table S2. Hemodynamic and sympathetic responses to Ang II infusion in hypertensive subjects treated with nebivolol vs. metoprolol

<table>
<thead>
<tr>
<th>Variables</th>
<th>Pre infusion</th>
<th>Ang 1 ng/kg/min</th>
<th>Ang 2 ng/kg/min</th>
<th>Ang 3 ng/kg/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP, mmHg</td>
<td>105 ± 2</td>
<td>112 ± 2*</td>
<td>119 ± 2*</td>
<td>122 ± 2*</td>
</tr>
<tr>
<td>HR, bpm</td>
<td>66 ± 2</td>
<td>66 ± 1</td>
<td>67 ± 1*</td>
<td>68 ± 1*</td>
</tr>
<tr>
<td>SNA, bursts min⁻¹</td>
<td>41 ± 4</td>
<td>40 ± 4</td>
<td>42 ± 4</td>
<td>40 ± 4</td>
</tr>
<tr>
<td>FBF, ml min⁻¹</td>
<td>141 ± 16</td>
<td>137 ± 14</td>
<td>128 ± 13</td>
<td>142 ± 15</td>
</tr>
<tr>
<td>FVR, resistance units</td>
<td>76 ± 10</td>
<td>82 ± 10*</td>
<td>91 ± 12*</td>
<td>85 ± 10*</td>
</tr>
<tr>
<td>Plasma F2-IsoP, pg/ml</td>
<td>38.8 ± 4.5</td>
<td>52.9 ± 6.1*</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Nebivolol

<table>
<thead>
<tr>
<th>Variables</th>
<th>Pre infusion</th>
<th>Ang 1 ng/kg/min</th>
<th>Ang 2 ng/kg/min</th>
<th>Ang 3 ng/kg/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP, mmHg</td>
<td>99 ± 3‡</td>
<td>105 ± 3*‡</td>
<td>112 ± 3*‡</td>
<td>113 ± 3*‡</td>
</tr>
<tr>
<td>HR, bpm</td>
<td>58 ± 2‡</td>
<td>58 ± 2‡</td>
<td>59 ± 2‡</td>
<td>61 ± 2‡</td>
</tr>
<tr>
<td>SNA, bursts min⁻¹</td>
<td>41 ± 4</td>
<td>39 ± 4</td>
<td>37 ± 4*</td>
<td>37 ± 5*</td>
</tr>
<tr>
<td>FBF, ml min⁻¹</td>
<td>118 ± 12</td>
<td>117 ± 15</td>
<td>118 ± 12</td>
<td>117 ± 13</td>
</tr>
<tr>
<td>FVR, resistance units</td>
<td>81 ± 10</td>
<td>97 ± 14*</td>
<td>99 ± 15*</td>
<td>98 ± 13*</td>
</tr>
<tr>
<td>Plasma F2-IsoP, pg/ml</td>
<td>34.4 ± 2.7</td>
<td></td>
<td></td>
<td>53.7 ± 4.9*</td>
</tr>
</tbody>
</table>

Metoprolol

<table>
<thead>
<tr>
<th>Variables</th>
<th>Pre infusion</th>
<th>Ang 1 ng/kg/min</th>
<th>Ang 2 ng/kg/min</th>
<th>Ang 3 ng/kg/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP, mmHg</td>
<td>98 ± 3‡</td>
<td>104 ± 3*‡</td>
<td>110 ± 3*‡</td>
<td>114 ± 3*‡</td>
</tr>
<tr>
<td>HR, bpm</td>
<td>57 ± 2‡</td>
<td>57 ± 2‡</td>
<td>59 ± 2‡</td>
<td>60 ± 2‡</td>
</tr>
<tr>
<td>SNA, bursts min⁻¹</td>
<td>42 ± 2</td>
<td>39 ± 2</td>
<td>41 ± 2</td>
<td>41 ± 2</td>
</tr>
<tr>
<td></td>
<td>Pre-infusion 124 ± 16</td>
<td>Pre-infusion 119 ± 20</td>
<td>Pre-infusion 108 ± 12</td>
<td>Pre-infusion 101 ± 11‡</td>
</tr>
<tr>
<td>--------------------------</td>
<td>-----------------------</td>
<td>-----------------------</td>
<td>-----------------------</td>
<td>------------------------</td>
</tr>
<tr>
<td>FBF, ml min⁻¹</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FVR, resistance units</td>
<td>81 ± 9</td>
<td>97 ± 11*</td>
<td>101 ± 11*</td>
<td>112 ± 15*</td>
</tr>
<tr>
<td>Plasma F2-IsoP, pg/ml</td>
<td>41.1 ± 3.9</td>
<td></td>
<td></td>
<td>60.9 ± 6.3*</td>
</tr>
</tbody>
</table>

LBNP, lower body negative pressure; MAP, mean arterial pressure; HR, heart rate; SNA, sympathetic nerve activity; FBF, forearm blood flow; FVC, forearm vascular conductance.

* P < 0.05 vs pre-infusion; ‡ P < 0.05 vs baseline (no drug); || p < 0.05 vs metoprolol.
Figure S1: Pressor and sympathoexcitatory responses to rhythmic handgrip alone in hypertensive subjects (n = 21) during nebivolol vs metoprolol treatment

Summary data showing MAP, HR, and SNA (expressed as % change in total activity from resting baseline) during the first 3 min of rhythmic handgrip at 30% MVC. Handgrip evoked a similar increase in SNA during nebivolol and metoprolol treatment compared to baseline. Nebivolol and metoprolol caused a similar reduction in MAP and HR at rest and during handgrip. * P < 0.05 vs min 0 (rest); † P < 0.05 vs Baseline (no drug) or metoprolol.