Tetrahydrobiopterin ameliorates the exaggerated exercise pressor response in patients with chronic kidney disease: a randomized controlled trial

Ann M. Lin,1,2 Peizhou Liao,3 Erin C. Millson,4 Arshed A. Quyyumi,5 and Jeannie Park1,2

1Renal Division, Department of Medicine, Emory University School of Medicine, Atlanta, Georgia; 2Research Service Line, Department of Veterans Affairs Medical Center, Decatur, Georgia; 3Department of Biostatistics and Bioinformatics, Rollins School of Public Health, Emory University, Atlanta, Georgia; 4Clinical Research Network, Atlanta Clinical and Translational Science Institute, Emory University School of Medicine, Atlanta, Georgia; and 5Cardiology Division, Department of Medicine, Emory University School of Medicine, Atlanta, Georgia

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LIN AM, Liao P, Millson EC, Quyyumi AA, Park J. Tetrahydrobiopterin ameliorates the exaggerated exercise pressor response in patients with chronic kidney disease: a randomized controlled trial. Am J Physiol Renal Physiol 310: F1016–F1025, 2016. First published March 9, 2016; doi:10.1152/ajprenal.00527.2015.—Chronic kidney disease (CKD) patients have an exaggerated increase in blood pressure (BP) during rhythmic handgrip exercise (RHG 20%) and static handgrip exercise (SHG 30%). Nitric oxide levels increase during exercise and help prevent excessive hypertension by both increasing vasodilation and reducing sympathetic nerve activity (SNA). Therefore, we hypothesized that tetrahydrobiopterin (BH4), an essential cofactor for nitric oxide synthase, would ameliorate the exaggerated exercise pressor response in CKD patients. In a randomized, double-blinded, placebo-controlled trial, we tested the effects of 12 wk of BH4 treatment on BP and muscle SNA (MSNA) responses during RHG 20% and SHG 30% in CKD patients. The BH4-treated group had a significantly lower systolic BP (+6 ± 1 vs. +13 ± 2 mmHg, P = 0.002) and mean arterial pressure response (+5 ± 1 vs. +10 ± 2 mmHg, P = 0.020) during RHG 20% and a significantly lower systolic BP response (+19 ± 3 vs. +28 ± 3 mmHg, P = 0.043) during SHG 30%. Under baseline conditions, there was no significant difference in MSNA responses between the groups; however, when the BP response during exercise was equalized between the groups using nitropusslide, the BH4-treated group had a significantly lower MSNA response during RHG 20% (BH4 vs. placebo, +12 ± 1 vs. +21 ± 2 bursts/min, P = 0.004) but not during SHG 30%. These findings suggest that BH4 ameliorates the augmented BP response during RHG 20% and SHG 30% in CKD patients. A reduction in reflex activation of SNA may contribute to the decreased exercise pressor response during RHG 20% but not during SHG 30% in CKD patients.

sympathetic nerve activity; handgrip exercise; exercise pressor reflex

Address for reprint requests and other correspondence: J. Park, Renal Division, Emory Univ. School of Medicine, 1639 Pierce Drive, WMB 338, Atlanta, GA 30322 (e-mail: jeanie.park@emory.edu).

PAtIENTS WITH CHRONIC RENAL FAILURE have exercise intolerance and poor physical capacity, which contribute to increased cardiovascular risk (22, 50). One important contributor to exercise intolerance in this patient population is an abnormality of hemodynamic and sympathetic nerve responses during exercise. Our prior work has shown that both patients with end-stage renal disease and chronic kidney disease (CKD) have an exaggerated increase in blood pressure (BP) during rhythmic handgrip exercise (RHG) and static handgrip exercise (SHG) (30, 33). Although an increase in BP during exercise is a physiological response to meet the increased metabolic demand of exercising skeletal muscle (23, 51), an exaggerated increase in BP during exercise could contribute to exercise dysfunction and increase the likelihood of adverse cardiovascular events during physical activity.

The mechanisms underlying the exaggerated exercise pressor response in patients with kidney disease have not been fully elucidated but may be due to a combination of increased sympathetic nervous system (SNS) activation and impaired vasodilation. Our prior work has shown that the exaggerated exercise pressor reflex in CKD patients is at least in part due to augmented reflex activation of sympathetic nerve activity (SNA) mediated by a heightened muscle mechanoreflex (33). The final hemodynamic response during exercise, however, is determined both by the vasoconstriction mediated by this reflex activation of SNA (23) as well as local vasodilation within exercising skeletal muscle mediated in part by nitric oxide (NO) (13, 24, 40). CKD patients are known to have reduced NO bioavailability (59) that could potentially lead to impaired vasodilation during exercise, contributing to an augmented BP response. Furthermore, NO has an inhibitory effect on central SNS activation, and, therefore, reduced NO bioavailability in CKD could contribute to the exaggerated increases in the SNA response during exercise (2, 41). Therefore, strategies to improve NO bioavailability could ameliorate the exaggerated BP response during exercise by reducing augmented SNA responses during exercise as well as potentially increasing local vasodilation.

One potential novel approach for improving NO bioavailability in CKD patients is via treatment with sapropterin dihydrochloride (6R-BH4; BioMarin). 6R-BH4 is the synthetic form of the naturally occurring enzyme tetrahydrobiopterin (BH4), an essential cofactor for NO synthase in the catalytic reaction that forms NO (29, 45, 48). BH4 levels have been found to be significantly lower in humans with CKD (58), and BH4 supplementation has been shown to increase NO bioavailability in animal models of chronic renal failure (34, 36, 56). We have previously reported that BH4 supplementation significantly reduced resting levels of muscle SNA (MSNA) and improved baseline measures of vascular stiffness in CKD patients (32) in a 12-wk randomized, double-blinded placebo-controlled clinical trial (NCT 1356966). An additional primary aim of the trial was to determine the effect of BH4 on the augmented BP response during exercise in CKD patients. Therefore, we now extend the results of the trial to test the hypothesis that treatment with BH4 ameliorates the exaggerated BP response during exercise in CKD. We further...
hypothesized that the reduction in the exercise pressor response is mediated in part by a reduction in the reflex activation of SNA.

**METHODS**

**Study Population**

The clinical trial enrolled 49 male subjects with hypertension and CKD stage 2 (estimated glomerular filtration rate [eGFR] between 60 and 89 ml·min⁻¹·1.73 m⁻²) with a concomitant urate microalbumin-to-creatinine ratio of >30 mg/g or stage 3 (eGFR between 30 and 59 ml·min⁻¹·1.73 m⁻²), as previously described (32). Inclusion criteria were stable renal function and a stable medication regimen for 3 mo before enrollment. Exclusion criteria included severe CKD (eGFR < 30 ml/min); diabetes; human immunodeficiency virus infection; symptomatic heart failure or ejection fraction below 35%; coronary, cerebrovascular, aortic, or peripheral vascular disease; symptomatic heart disease; liver function abnormalities; hemoglobin level < 10 g/dL; history of nephrolithiasis; any serious systemic disease that might influence survival; current treatment with clonidine, levodopa, or metoclopramide; or surgery for compliance (32). Absolute values of BP were internally calibrated using an upper arm BP reading and were measured noninvasively using finger cuffs that detect digital blood flow and translate blood flow oscillations into continuous pulse pressure waveforms and beat-to-beat values of BP (CNAP, CNSystems) (20, 47, 52, 55).

**Study Design**

This was a prospective, randomized, placebo-controlled, double-blinded, parallel group clinical trial (clinicaltrials.gov identifier NCT 1356966). The primary outcomes were to test the effects of 6R-BH₄ treatment on resting measures of MSNA, flow-mediated dilation (FMD), and vascular stiffness, as previously reported (32), as well as on exercise-induced changes in MSNA and BP, which are currently presented in this report. Randomization and assignment to treatment group were conducted by the investigational pharmacist using a simple randomization scheme (coin toss method). The investigational pharmacist was not present during laboratory procedures or data analysis and was responsible for allocation concealment and dispensing study drugs from the central investigational pharmacy. Both the study investigators (including data collectors and analysts) and participants were blinded to treatment allocation. Screening and enrollment were conducted by the Principal Investigator (J. Park) and approved study coordinators.

After obtaining baseline measurements as described below in Experimental Protocol, participants were randomly assigned to receive 6R-BH₄ at 200 mg twice daily with folic acid at 1 mg daily or an identical placebo twice daily with folic acid at 1 mg daily. Participants were assessed at weeks 1, 3, 6, 9, and 12 for compliance via pill count, adverse effects, hemodynamic and laboratory measurements, and to ensure that no changes were made to medication regimens, dietary, or exercise habits throughout the duration of the trial. The experimental protocol, as described below, was repeated at the end of the trial. This study was approved by the Emory University Institutional Review Board and the Atlanta Veterans Affairs Medical Center Research and Development Committee.

**Measurements and Procedures**

**BP.** Office BP was measured at baseline at each visit before study procedures by a single study staff member using standard techniques. After 5 min of rest in a seated position with the arm supported at the heart level using an appropriately sized cuff, BP was measured with an automated device (Dinamap). Each data point of BP was obtained as an average of three consecutive BP measurements separated by 5 min.

**MSNA.** Multifiber recordings of postganglionic SNA directed to muscle (MSNA) were obtained by microneurography, as previously described (27). A tungsten microelectrode (tip diameter: 5–15 µm, Bioengineering, University of Iowa) was inserted into the peroneal nerve with a reference electrode in close proximity and was manipulated to obtain a satisfactory nerve recording that met previously established criteria (27). The signals were amplified (total gain: 50,000–100,000), filtered (700–2,000 Hz), rectified, and integrated (time constant: 0.1 s) to obtain a mean voltage display of SNA (model 662C-4, Nerve Traffic Analyzer, Bioengineering, University of Iowa) that was recorded by the LabChart 7 program (PowerLab 16sp, AD Instruments). Continuous ECG was recorded simultaneously with the neurogram using a bioamp system. Beat-to-beat arterial BP was measured noninvasively using finger cuffs that detect digital blood flow and translate blood flow oscillations into continuous pulse pressure waveforms and beat-to-beat values of BP (CNAP, CNSystems) (20, 47, 52, 55). Absolute values of BP were internally calibrated using a concomitant upper arm BP reading and were calibrated at the start and every 15 min throughout the study.

**Low-level RHG (RHG 20%).** This low-level rhythmic exercise primarily engages muscle mechanoreceptors, without the engagement of muscle metaboreceptors (1). The participant was asked to squeeze a hand dynamometer (Stoelting) with maximal force. The highest force attained from three attempts was considered the maximum voluntary contraction (MVC). The participant squeezed the hand dynamometer intermittently at 20% MVC for 3 min, at a rate of 1 contraction/2 s.

**Moderate SHG (SHG 30%).** Moderate SHG elicits an increase in MSNA (28) by activating muscle metaboreceptors and mechanoreceptors. Moderate SHG was performed by squeezing the hand dynamometer at 30% MVC (SHG 30%) in a sustained manner for 3 min. The participant was instructed to avoid inadvertentValsalva and to maintain normal breathing patterns.

**Sodium nitroprusside.** At the end of the 12-wk trial, RHG 20% and SHG 30% were repeated during concomitant intravenous sodium nitroprusside (NTP) infusion in a subset of participants treated with placebo and 6R-BH₄. The purpose of infusing intravenous NTP during the handgrip exercises was to further examine the MSNA responses during the handgrip exercises when the BP responses were equalized between the treatment groups. The initial dose of 0.3 µg·kg⁻¹·min⁻¹ was titrated to maintain a mean arterial pressure (MAP) response of 0 mmHg during RHG 20% and 10 mmHg during SHG 30% in both placebo- and 6R-BH₄-treated groups.

**Exercise capacity.** Participants underwent maximal treadmill exercise until exhaustion using the modified Balke protocol to determine maximal O₂ uptake (V̇O₂max) as previously described (3). The protocol consisted of a series of 3-min stages in which speed and incline were increased in a stepwise manner. Levels of mixed expired O₂, CO₂, and ventilation were recorded at rest and every 30 s during exercise using a breathing apparatus with a metabolic cart (Sensormedics VMax Spectra 29, Rugby, UK). V̇O₂max was defined as the highest O₂ uptake rate observed during maximal exercise.

**Experimental Protocol**

During the study visit, all participants presented in the early morning after abstaining from food, caffeine, and alcohol for at least 12 h and exercise for at least 24 h. The study room was quiet, semidark, and temperate (~21°C). Blood and urine samples were collected. Participants were placed in a supine position, and a 20-gauge intravenous catheter was placed into the antecubital vein of the dominant (nonexercising) arm. Finger cuffs were placed for continuous beat-to-beat arterial BP measurements, and an upper arm cuff was placed for intermittent automatic BP calibrations. ECG patch electrodes were placed for continuous heart rate (HR) recordings. The leg was positioned for microneurography, and the tungsten microelectrode was inserted and manipulated to obtain a satisfactory nerve recording. After 10 min of rest, baseline BP, HR, and MSNA were recorded continuously for 10 min. Participants then performed the following two maneuvers in random order: 1) RHG 20% for 3 min
and 2) SHG 30% for 3 min. Each exercise task was performed in the nondominant arm. MSNA, BP, and HR were recorded continuously throughout the maneuvers. Thirty minutes of recovery time were given between the exercise tasks, allowing sufficient time for hemodynamics and MSNA to return to baseline levels before each new maneuver.

The procedures above were performed once at baseline and repeated again at the end of the 12-wk clinical trial. During the posttrial microneurography study visit, a subset of participants randomized to both 6R-BH4 and placebo treatment repeated the handgrip exercises during intravenous NTP infusion. The intravenous NTP infusion was begun at a dose of 0.3 μg·kg⁻¹·min⁻¹ and titrated to maintain a mean change in MAP of 0 mmHg during RHG 20% and a mean change in MAP of +10 mmHg during SHG 30% in all participants, thereby providing uniformity in hemodynamic changes between the groups by equalizing the BP response during the handgrip maneuvers in all participants. Forty-five minutes of rest were given between the handgrip maneuvers with infusions to ensure sufficient washout of drugs.

Maximal treadmill exercise testing was performed on a separate day at baseline and at the end of the 12-wk clinical trial to assess \( \dot{V}O_2 \)max as described above.

### Data Analysis

MSNA. MSNA, ECG, and arterial BP data were exported from the Labchart 7 program into WinCPRS (Absolute Aliens, Turku, Finland) for analysis. R waves were automatically marked by the program from the continuous ECG recording. MSNA bursts were automatically detected and marked using the following criteria: a 3:1 burst-to-noise ratio within a 0.5-s search window, with an average latency of 1.2–1.4 s from the previous R wave. After automatic detection, the ECG and MSNA neurograms were visually inspected for accuracy of detection by a single investigator (J. Park). For total activity measurements, burst amplitudes were normalized by assigning the largest burst amplitude under baseline conditions a measurement of 1,000 arbitrary units (AU). MSNA was expressed as burst frequency (in bursts/min), amplitude under baseline conditions a measurement of 1,000 arbitrary units (AU). MSNA was expressed as burst frequency (in bursts/min), amplitude under baseline conditions a measurement of 1,000 arbitrary units (AU). MSNA bursts were automatically detected and marked using the following criteria: a 3:1 burst-to-noise ratio within a 0.5-s search window, with an average latency of 1.2–1.4 s from the previous R wave. After automatic detection, the ECG and MSNA neurograms were visually inspected for accuracy of detection by a single investigator (J. Park). For total activity measurements, burst amplitudes were normalized by assigning the largest burst amplitude under baseline conditions a measurement of 1,000 arbitrary units (AU). MSNA was expressed as burst frequency (in bursts/min), amplitude under baseline conditions a measurement of 1,000 arbitrary units (AU). MSNA was expressed as burst frequency (in bursts/min), amplitude under baseline conditions a measurement of 1,000 arbitrary units (AU). MSNA was expressed as burst frequency (in bursts/min), amplitude under baseline conditions a measurement of 1,000 arbitrary units (AU). MSNA was expressed as burst frequency (in bursts/min), amplitude under baseline conditions a measurement of 1,000 arbitrary units (AU).

### Statistical analysis

Statistical analysis was performed using the SAS 9.2 program (SAS Institutes). Baseline characteristics were compared using two-tailed two-sample \( t \)-tests. Repeated-measures ANOVA was performed using PROC GLM to determine differences between groups (6R-BH4 vs. placebo-treated groups) with respect to changes in MSNA, systolic BP (SBP), diastolic BP (DBP), MAP, and HR with time during each intervention: SHG without and with intravenous NTP and RHG without and with intravenous NTP. When the overall \( F \)-test was significant, the contrast option for post hoc analysis was used to compare the difference from baseline to each time point between groups. Results are expressed as means ± SE. \( P \) values of <0.05 were considered statistically significant.

### RESULTS

#### Study Enrollment and Baseline Characteristics

Forty-nine participants who met the eligibility criteria were enrolled. As previously described (32), 17 participants were excluded due to inability to obtain adequate baseline MSNA \( n = 6 \), not following up to start study pills \( n = 5 \), screening BP outside inclusion parameters \( n = 5 \), and withdrawal of consent \( n = 1 \). Thirty-two participants were ultimately randomized to receive 6R-BH4 \( n = 18 \) versus placebo \( n = 14 \) treatment (32). Table 1 shows baseline and clinical characteristics of the study population, as previously reported (32). All participants were men and primarily African-American. Mean serum creatinine and eGFR were similar between groups, and the cause of CKD was hypertension in the majority of both groups. The mean number of antihypertensive medications was 2.2 among the 6R-BH4-treated group and 2.4 among the placebo-treated group, and the majority of both groups were treated with Ca²⁺ channel blockers and angiotensin-converting enzyme inhibitors or angiotensin receptor blockers. Baseline handgrip strength measured as MVC, resting hemodynamics, and MSNA before handgrip exercises was similar between the groups. The enrollment dates were from May 2011 to June 2013, and final study procedures were performed in September 2013. The study was unblinded in November 2013 after all study procedures and initial analyses were complete. No interim analyses were performed.

The primary outcomes were changes in resting MSNA, FMD, and vascular stiffness, as previously reported (32), as well as changes in MSNA and BP responses during handgrip exercise, as currently reported. A power analysis performed

### Table 1. Baseline characteristics of the study population, stratified by treatment group

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>6R-BH4</th>
<th>Placebo</th>
<th>( P ) Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>( n )</td>
<td>18</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>Age, yr</td>
<td>57.5 ± 1.3</td>
<td>55.2 ± 2.2</td>
<td>0.386</td>
</tr>
<tr>
<td>Race, n (%)</td>
<td>17 (94%)</td>
<td>13 (93%)</td>
<td>0.854</td>
</tr>
<tr>
<td>Black</td>
<td>1 (6%)</td>
<td>1 (7%)</td>
<td>0.824</td>
</tr>
<tr>
<td>White</td>
<td>108.3 ± 4.6</td>
<td>102.5 ± 4.2</td>
<td>0.373</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>32.7 ± 1.2</td>
<td>32.3 ± 1.3</td>
<td>0.824</td>
</tr>
<tr>
<td>Creatinine, mg/dl</td>
<td>1.81 ± 0.087</td>
<td>1.62 ± 0.070</td>
<td>0.107</td>
</tr>
<tr>
<td>Estimated glomerular filtration rate, ml·min⁻¹·1.73 m⁻²</td>
<td>49.8 ± 3.5</td>
<td>54.3 ± 2.7</td>
<td>0.350</td>
</tr>
<tr>
<td>Urinary microalbumin-to-creatinine ratio, mg/g</td>
<td>148 ± 54</td>
<td>163 ± 58</td>
<td>0.854</td>
</tr>
<tr>
<td>Cause of chronic kidney disease, n (%)</td>
<td>10 (56)</td>
<td>8 (57)</td>
<td>0.928</td>
</tr>
<tr>
<td>Hypertension</td>
<td>2 (11)</td>
<td>1 (7)</td>
<td>0.702</td>
</tr>
<tr>
<td>Nephrotoxic drugs</td>
<td>1 (6)</td>
<td>1 (7)</td>
<td>0.854</td>
</tr>
<tr>
<td>Autosomal dominant polycystic kidney disease</td>
<td>1 (6)</td>
<td>1 (7)</td>
<td>0.854</td>
</tr>
<tr>
<td>Glomerulonephritis</td>
<td>1 (6)</td>
<td>0 (0)</td>
<td>0.370</td>
</tr>
<tr>
<td>Unknown</td>
<td>4 (22)</td>
<td>4 (29)</td>
<td>0.681</td>
</tr>
<tr>
<td>Antihypertensive medications, n (%)</td>
<td>11 (61)</td>
<td>8 (57)</td>
<td>0.821</td>
</tr>
<tr>
<td>Ca²⁺ channel blockers</td>
<td>11 (61)</td>
<td>11 (79)</td>
<td>0.290</td>
</tr>
<tr>
<td>Angiotensin-converting enzyme inhibitors/angiotensin receptor blockers</td>
<td>6 (33)</td>
<td>8 (57)</td>
<td>0.178</td>
</tr>
<tr>
<td>β-Blockers</td>
<td>6 (33)</td>
<td>5 (36)</td>
<td>0.888</td>
</tr>
<tr>
<td>Aldosterone receptor blockers</td>
<td>2 (11)</td>
<td>1 (7)</td>
<td>0.702</td>
</tr>
<tr>
<td>α-Blockers</td>
<td>3 (17)</td>
<td>0 (0)</td>
<td>0.109</td>
</tr>
<tr>
<td>Hydralazine</td>
<td>1 (6)</td>
<td>1 (7)</td>
<td>0.854</td>
</tr>
<tr>
<td>Number of antihypertensive medications</td>
<td>2.2 ± 0.2</td>
<td>2.4 ± 0.2</td>
<td>0.489</td>
</tr>
<tr>
<td>Lipid-lowering drugs, n (%)</td>
<td>4 (22)</td>
<td>4 (29)</td>
<td>0.681</td>
</tr>
<tr>
<td>Baseline hemodynamics</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Systolic blood pressure, mmHg</td>
<td>134 ± 3</td>
<td>140 ± 4</td>
<td>0.184</td>
</tr>
<tr>
<td>Diastolic blood pressure, mmHg</td>
<td>84 ± 1</td>
<td>87 ± 2</td>
<td>0.448</td>
</tr>
<tr>
<td>Mean arterial pressure, mmHg</td>
<td>101 ± 3</td>
<td>105 ± 2</td>
<td>0.284</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>62 ± 2</td>
<td>64 ± 3</td>
<td>0.608</td>
</tr>
<tr>
<td>Baseline muscle sympathetic nerve activity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Burst frequency, bursts/min</td>
<td>47 ± 2</td>
<td>45 ± 3</td>
<td>0.664</td>
</tr>
<tr>
<td>Burst incidence, bursts/100 heart beats</td>
<td>79 ± 4</td>
<td>75 ± 4</td>
<td>0.535</td>
</tr>
<tr>
<td>Baseline peak ( O_2 ) uptake, ml/kg·min⁻¹·1.73 m⁻²</td>
<td>20.8 ± 1.5</td>
<td>24.5 ± 1.7</td>
<td>0.126</td>
</tr>
<tr>
<td>Maximum voluntary contraction, kg</td>
<td>43 ± 2</td>
<td>41 ± 3</td>
<td>0.381</td>
</tr>
</tbody>
</table>

Values are means ± SE or n (%), where n is the number of subjects/group. 6R-BH4: sarcoprotein dihydrochloride.
before enrollment specified that a sample size of 30 would give >95% power to detect a difference of 5 bursts/min in resting MSNA with a common SD of 5 bursts/min and 80% power to detect a 75% difference in FMD between groups with a common SD of 70% using a two-tailed t-test with a 0.05 two-sided significance level. Of the 14 participants allocated to placebo treatment, 3 participants were further excluded from primary analysis due to inability to obtain end-of-study MSNA (n = 1), withdrawal due to dysgeusia (n = 1), and withdrawal due to unrelated trauma (n = 1), leaving 11 participants for analysis of MSNA and BP changes during handgrip exercises. Of the 18 participants allocated to 6R-BH4 treatment, 4 participants were further excluded due to inability to obtain end-of-study MSNA (n = 1), withdrawal due to nausea (n = 1), and withdrawal by the Principal Investigator for acute kidney injury (n = 1), leaving 14 participants for primary analysis.

Change in Resting Hemodynamics and MSNA

As previously reported (32), after 12 wk of treatment, there were no significant differences in change in resting measurements of SBP, DBP, MAP, or HR from baseline to the end of the trial period between the treatment groups (Table 2). We did observe a significant difference in change in resting MSNA between groups (P = 0.003). Whereas MSNA decreased significantly (by −7.5 ± 2.1 bursts/min, P = 0.007) in the 6R-BH4-treated group, there was no significant change in MSNA in the placebo-treated group (±3.2 ± 1.3 bursts/min, P = 0.175). Results were similar when analyzed as burst incidence (in bursts/100 heart beats).

Changes in MSNA and Hemodynamic Responses During RHG 20%

The purpose of the present study was to study MSNA and hemodynamic responses during low-level RHG in which muscle contractions occur without generation of ischemic metabolites (54). At baseline pretreatment, there were no statistically significant differences in change in SBP, DBP, MAP, or HR between the treatment groups during 3 min of RHG 20% (Fig. 1). At the end of the 12-wk treatment period (posttreatment), there was a significantly lower increase in SBP (P = 0.002 by ANOVA F-test) and MAP (P = 0.020 by ANOVA F-test) during RHG 20% in the 6R-BH4-treated group compared with the placebo-treated group. During the third minute of RHG 20%, the 6R-BH4-treated group compared with the placebo-treated group had a significantly lower SBP response (+6 ± 1 vs. +13 ± 2 mmHg, P = 0.002) and MAP response (+5 ± 1 vs. +10 ± 2 mmHg, P = 0.020, Fig. 3) after 12 wk of treatment. There were no significant differences in DBP or HR responses during RHG 20% between the groups posttreatment.

At baseline pretreatment, there was no statistically significant difference in the change in MSNA burst frequency between the treatment groups during 3 min of RHG 20% (Fig. 1). At the end of the 12-wk treatment period (posttreatment), there were no significant differences in MSNA burst frequency or total activity between the treatment groups. RHG 20% was repeated during concomitant intravenous NTP infusion at the end of the 12-wk study period (posttreatment) in a subset of participants randomized to 6R-BH4 (n = 9) and placebo (n = 4) treatment. This subset of participants was chosen randomly without knowledge of treatment allocation. The purpose of the intravenous NTP infusion was to eliminate the difference in BP responses between groups and observe the changes in MSNA during exercise between the groups when BP changes and therefore baroreflex-mediated influences on MSNA were made uniform. The intravenous NTP infusion was successfully titrated to produce a mean change in MAP of 0 mmHg during RHG 20% in both groups, eliminating the difference in BP responses during RHG20% between the treatment groups. When the BP response was equalized between the groups using intravenous NTP, the increase in MSNA burst frequency was significantly lower during RHG 20% in the 6R-BH4-treated group compared with the placebo-treated group. The change in MSNA when quantified as total activity during RHG 20% with intravenous NTP was not significantly different between groups (data not shown).

Changes in MSNA and Hemodynamic Responses During SHG 30%

The purpose of the present study was to study MSNA and hemodynamic responses during moderate-intensity SHG that is intense enough to generate ischemic metabolites. During SHG 30% muscle mechanoreceptors, metaboreceptors, and central command are all engaged. At baseline pretreatment, there were no statistically significant differences in changes in SBP, DBP, MAP, or HR between the treatment groups during 3 min of SHG 30% (Fig. 2). At the end of the 12-wk treatment period (posttreatment), there was a significantly lower increase in SBP (P = 0.043 by ANOVA F-test) during SHG 30% in the 6R-BH4-treated group compared with the placebo-treated group. During the third minute of SHG 30%, the 6R-BH4-treated group compared with the placebo-treated group had a significantly lower SBP response (6R-BH4 vs. placebo treatment, +19 ± 3 vs. +28 ± 3 mmHg, P = 0.043; Fig. 3) after
At baseline pretreatment, there was no statistically significant differences in changes in MSNA burst frequency between groups. Posttreatment, there were no significant differences in DBP, MAP, or HR responses during SHG 30% between the groups.

12 wk of treatment. There were no significant differences in DBP, MAP, or HR responses during SHG 30% between the groups posttreatment.

At baseline pretreatment, there was no statistically significant differences in changes in MSNA burst frequency between 6R-BH4- and placebo-treated groups after treatment. *P < 0.05 indicates a significant difference between the groups posttreatment at that time point.
the treatment groups during 3 min of SHG 30% (Fig. 2). At the end of the 12-wk treatment period (posttreatment), there were no significant differences in MSNA burst frequency or total activity between the treatment groups.

SHG 30% was repeated during concomitant intravenous NTP infusion at the end of the 12-wk study period (posttreatment) in a subset of participants randomized to 6R\textsuperscript{BH}_4 (n = 9) and placebo (n = 4) treatment. Again, this subset of participants was chosen randomly without knowledge of treatment allocation. The intravenous NTP infusion was successfully titrated to equalize BP responses between the groups and produce a mean change in MAP of +10 mmHg during SHG 30% in both groups, eliminating the difference in BP responses during SHG 30% between the treatment groups (Fig. 4). When the BP response was equalized between the groups using intravenous NTP, there were no significant differences in changes in MSNA burst frequency or burst incidence during SHG 30% between the treatment groups.

### Change in Exercise Capacity

As shown in Table 2, there were no significant changes in exercise capacity measured as VO\textsubscript{2max} from baseline to after treatment in both 6R\textsuperscript{BH}_4- and placebo-treated groups.

### Adverse Events

As previously described, 6R\textsuperscript{BH}_4 was well tolerated with few adverse events (32). Among patients assigned to 6R\textsuperscript{BH}_4 treatment, one patient withdrew from the study due to nausea and gastroesophageal reflux and another patient was withdrawn due to acute kidney injury after week 3 of the study. The cause of the acute kidney injury was deemed likely secondary to decreased renal perfusion from low BP, and the patient’s BP and renal function returned to baseline after cessation of the study drug. Among patients assigned to placebo treatment, one patient withdrew due to dysgeusia and a second patient withdrew due to trauma unrelated to the study.
In this randomized, double-blinded, placebo-controlled, parallel-group trial, we observed that after 12 wk of treatment, CKD patients randomized to 6R-BH₄ treatment had a significantly lower SBP and MAP responses during low-level RHG (RHG 20%) and a significantly lower SBP response during moderate-intensity SHG (SHG 30%) compared with CKD patients treated with placebo. Concomitantly, under baseline conditions, there were no significant differences in MSNA responses between the treatment groups during RHG 20% and SHG 30%. However, equalizing the BP response using intravenous NTP in a subset of participants, and thereby equalizing the baroreflex-mediated restraint of SNS activity during exercise between the groups after treatment, revealed that 6R-BH₄ significantly lowered MSNA responses during RHG 20% but not during SHG 30%.

Our prior studies have shown that patients with reduced renal function have exaggerated increases in BP during handgrip exercise (30, 33). Augmented exercise pressor responses have been shown to correlate with an increased risk of cardiovascular disease in treadmill exercise studies (19, 49). Therefore, interventions to ameliorate the exaggerated exercise pressor response in CKD patients could have the potential to impact cardiovascular risk in patients with reduced renal function. Our findings suggest that chronic 6R-BH₄ treatment ameliorates the augmented BP response during RHG and SHG in CKD patients.

The mechanisms by which 6R-BH₄ treatment ameliorates the exaggerated exercise pressor response in CKD patients are unclear. One potential mechanism is by reducing the augmentation in reflex activation of SNA during exercise, characteristic of CKD. CKD patients have chronic overactivation of SNS activity, which contribute to increased cardiovascular risk and hypertension (31, 60). Although the mechanisms that underlie chronic SNS overactivation are multifactorial, decreased NO bioavailability is an important contributing factor to chronic SNS overactivity in CKD (57). NO tonically inhibits central SNS activity, and part of the BP-raising effect of NO inhibition is due to an increase in central sympathetic outflow (2, 41, 42). In CKD, a disease state characterized by decreased NO bioavailability, NO is important for modulating central norepinephrine turnover (57). Therefore, strategies to increase NO bioavailability have the potential to ameliorate SNS overactivation both at rest and during exercise in CKD.

BH₄, an essential cofactor for the function of NO synthase that has been shown to be relatively deficient in CKD patients (43, 58), increases NO bioavailability in animal models of CKD (34–36, 56).

We previously published results from this randomized clinical trial that showed 6R-BH₄ treatment significantly reduced resting levels of SNS activity in humans with CKD (32). These results showed that there was a significant reduction in resting MSNA in the 6R-BH₄-treated group but no difference in changes in resting BP or HR between the treatment groups (32). We also showed that there was a significant reduction in the central augmentation index, a measure of vascular stiffness, after 12 wk of 6R-BH₄ treatment. An additional primary aim of the trial was to determine whether 6R-BH₄ might modulate reflex sympathetic responses in CKD patients. Our prior work has shown that SNS overactivation during exercise is one important mechanism that contributes to the exaggerated exercise pressor response in patients with CKD (33). When BP responses during exercise were normalized using intravenous NTP, CKD patients had a significantly higher MSNA response during both RHG 20% and SHG 30% compared with control subjects without kidney disease, suggesting that an exaggerated increase in SNS activation contributes to the exaggerated pressor response during RHG and SHG in CKD patients (33).

In the present study, we observed a significant reduction in the SBP response but not the DBP response during handgrip exercises among patients randomized to 6R-BH₄ treatment. This pattern of an isolated reduction in the SBP response during both RHG 20% and SHG 30% suggests that amelioration in the BP response with 6R-BH₄ treatment may be less likely due to inhibition of SNS activation and potentially more related to a reduction in vascular stiffness, as shown in our previous study (32). However, to investigate whether SNS responses may differ between treatment groups when the differential influence of the arterial baroreflexes was removed, we used intravenous NTP to equalize the BP response during RHG 20% and SHG 30% in a small subset of CKD patients after 12 wk of treatment with 6R-BH₄ or placebo. Patients with chronic renal failure have intact arterial baroreflex sensitivity (5, 25, 26), and arterial baroreflexes continue to modulate SNS activation during exercise (44). Therefore, since CKD patients treated with 6R-BH₄ had lower BP responses during handgrip exercises compared with CKD patients treated with placebo, our goal was to eliminate any differential effects of arterial baroreflex-mediated modulation of SNS activation during exercise by equalizing the BP response between the groups. Although under baseline conditions there were no differences in MSNA responses during RHG 20% and SHG 30% between the treatment groups in the setting of differential BP responses, equalization of the BP response during exercise between the groups revealed that CKD patients treated with 6R-BH₄ had significantly lower increases in MSNA during RHG 20% but not during SHG 30% compared with the placebo-treated group. This observation suggests that amelioration of the SNS response may play a contributory role to the lowering of exercise-induced increases in BP among CKD patients treated with 6R-BH₄ during low-level RHG (RHG 20%) but not during moderate-intensity SHG (SHG 30%).

The reasons for the differential effect of 6R-BH₄ treatment on MSNA responses during RHG 20% versus SHG 30% are unclear but may be due to differential effects of 6R-BH₄ treatment on the type of exercise and engagement of the exercise pressor reflex. Although we found that 6R-BH₄ treatment ameliorated BP responses during both RHG 20% and SHG 30%, we observed after equalization of BP responses that 6R-BH₄ treatment lowered MSNA activation during RHG 20% but not during SHG 30%. RHG 20% is a rhythmic maneuver that does not generate ischemic metabolites and thereby primarily engages muscle mechanoreceptors (1). SHG 30%, on the other hand, is intense enough to generate ischemic metabolites and thereby engages mechanoreceptors and metaboreceptors (28). In our prior studies, we observed that CKD patients had a significantly higher MSNA response during both RHG 20% and SHG 30% but not during posthandgrip circulatory arrest, a maneuver that traps ischemic metabolites within the skeletal muscle after exercise and thereby isolates metabo-
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receptor control. Taken together, our findings suggest that 6R-BH4 may modulate reflex SNS activation during muscle mechanoreflex engagement but not during muscle metaboreflex engagement.

It is unclear whether 6R-BH4 treatment ameliorates the exaggerated BP response during handgrip exercise in CKD patients by also increasing vasodilatory capacity during exercise. Exercise induces local vasodilation within contracting muscles that increases blood flow to match the increased O2 demand within working skeletal muscles. Several studies have shown that NO plays a major role in this exercise-induced vasodilation (9, 13–15, 17), whereas others have shown that NO is not essential for exercise hyperemia in working skeletal muscle (10, 12, 38). In addition to direct vasodilation, sympathetically mediated vasoconstriction within the exercising skeletal muscle is inhibited to preserve muscle blood flow, termed functional sympatholysis. Some (4, 11, 53) but not all (8, 39) studies have demonstrated that NO attenuates sympathetic vasoconstriction during exercising skeletal muscle, thus playing an important role in functional sympatholysis. In our previously published results from this study population, 6R-BH4 treatment lowered resting MSNA and improved the central augmentation index, a measure of vascular stiffness. In contrast, there was no improvement in endothelium-mediated vasodilation assessed as brachial artery FMD, suggesting that 6R-BH4 may have had more central effects than peripheral effects on the endothelium. Although we did not assess functional sympatholysis or muscle blood flow in this study, we observed that 6R-BH4 supplementation ameliorated the increase in BP without a concomitant reduction in sympathetic activity, at least under baseline conditions. These findings suggest that other mechanisms besides a reduction in SNA, such as improvements in functional sympatholysis, may have played a role. Interestingly, acute BH4 supplementation has been shown to blunt sympathetic vasoconstrictor responsiveness in resting and contracting skeletal muscle in rats (21). Whether long-term 6R-BH4 supplementation improves functional sympatholysis in humans with CKD remains to be tested.

Despite a reduction in the exercise pressor response during handgrip exercise, there was no improvement in exercise capacity assessed using a maximal treadmill test. Although pharmacological treatment alone with 6R-BH4 improved augmented BP and SNA responses during low-level RHG and improved augmented BP responses during moderate-intensity SHG, these effects may not extend to whole body exercise or high-intensity exercise. Whether 6R-BH4 might have an additive role to aerobic exercise training for improving exercise capacity and tolerance in CKD patients should be tested in the future.

Limitations

NO bioavailability was not measured directly in this study; therefore, whether 6R-BH4 exerts improvements in exaggerated BP and SNS reactivity during exercise due to an increase in NO bioavailability is unclear. Previous experimental studies have shown that BH4 supplementation improves NO bioavailability in CKD (34–36, 56), and 6R-BH4 treatment has been shown to increase NO bioavailability in humans with other diseases (6, 7, 16, 18, 37). Second, muscle blood flow measurements and assessments of functional sympatholysis were not made during this study. Therefore, whether 6R-BH4 improves functional sympatholysis or skeletal muscle blood flow during exercise remains unknown and should be tested in future studies. Third, BH4 levels were not checked in participants; thus, the degree to which the current dose raises blood BH4 levels is not known. The dosage used in this study was based on a prior report (37) that 200 mg twice daily of 6R-BH4 led to significant improvements in hemodynamics in a group of hypertensive patients. In addition, adherence to the study drug was monitored regularly and was excellent throughout the trial. Fourth, noninvasive beat-to-beat BP measures were obtained using the CNAP device rather than intraarterial catheters. Although CNAP measurements have been shown to reflect intra-arterial BP, particularly MAP, during rest and during hypotension, validation studies of CNAP measures during exercise have not been previously performed. Fifth, antihypertensive medications were not held during the study. However, there were no significant differences in the antihypertensive regimen between the groups, and all participants maintained a stable regimen of medications throughout the study. Sixth, the study may have been underpowered to detect differences in MSNA responses during handgrip exercise under baseline conditions, and, in particular, the NTP experiments included a small number of subjects in each group. Finally, the study was conducted primarily in African-Americans and in men, and the results may not be generalizable to women and other ethnic groups.

Conclusions

The present study demonstrates that CKD patients treated with 12 wk of 6R-BH4 had significantly decreased BP responses during RHG 20% and SHG 30% compared with patients treated with placebo. Under baseline conditions, there were no significant differences in MSNA responses between the groups during handgrip exercises; however, when the BP response during exercise was equalized between the groups using intravenous NTP, CKD patients treated with 6R-BH4 had a significantly lower MSNA response during RHG 20% but not during SHG 30%. These findings suggest that 6R-BH4 ameliorates the augmented BP response during RHG and SHG in CKD patients. A reduction in the reflex activation of SNA may contribute to the decreased exercise pressor response during low-level RHG but not during SHG exercise in CKD. Whether improvements in the exaggerated exercise pressor response and SNS activation during exercise with 6R-BH4 treatment improves long-term cardiovascular risk or exercise capacity remains to be tested.

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