Resistant hypertension has been linked to chronic excessive sympathetic drive, especially elevation of renal sympathetic activity in some groups of patients. Against this background, renal artery ablation selectively denervating the kidneys emerges as an alternative treatment for such patients. Although initial trials showed promising results with regard to large reductions in blood pressure (BP), disappointingly, the potential therapeutic role of renal denervation (RDN) in lowering BP is being challenged after the failure of recent SYMPLICITY HTN-3 trial to show a benefit of RDN over the optimal medical therapy.

The concept of RDN is supported by both experimental and early human evidence when surgical sympathectomy procedures were done and found to have huge effect on BP. However, for the time being, the BP-lowering effect of catheter-based RDN is highly variable (the rates of nonresponse to RDN vary between 8% and 37%). Kaiser et al reported that repeated RDN could significantly decrease BP in non-responders to previous radiofrequency ablation procedure. These findings suggest that lack of BP reductions after RDN may be the results of incomplete denervation. Therefore, to minimize treatment failure of blind ablation, a method that could convert what is currently a purely anatomic procedure to one that involves quantifying the efficacy of RDN intraprocedurally and mapping the renal nerves to enable a targeted therapy is of great clinical significance.

**Background**—Electric stimulation has been proved to be available to monitor the efficacy of renal denervation (RDN). This study was to evaluate the effectiveness of high-frequency stimulation (HFS)–guided proximal RDN.

**Methods and Results**—A total of 13 Chinese Kunming dogs were included and allocated to proximal RDN group (n=8) and control group (n=5). HFS (20 Hz, 8 V, pulse width 2 ms) was performed from proximal to distal renal artery in all dogs. Radiofrequency ablations were delivered in proximal RDN group and only at the proximal positive sites where systolic blood pressure (BP) increased ≥10 mm Hg during HFS. Postablation HFS was performed over the previously stimulated sites. BP, heart rate, and plasma norepinephrine were analyzed. In 8 denervated dogs, preablation HFS caused significant BP increases of 6.0±5.0/3.4±5.5, 16.9±11.7/11.1±8.5, and 17.1±8.4/8.5±5.3 mm Hg during the first, second, and third 20 s of HFS at the proximal positive sites. After ablation, these sites showed a negative response to postablation HFS with increases of BP by 1.3±3.0/1.0±2.5, 0.8±3.9/1.5±3.4, and 1.5±4.5/0.7±3.8 mm Hg. Of note, no radiofrequency applications were delivered at the positive sites of middle renal artery, repeated HFS increased BP only by 3.3±5.3/2.8±4.2, 5.3±6.6/3.8±4.7, and 2.9±4.6/1.3±3.2 mm Hg, failed to reproduce the previous BP increases of 6.2±5.6/5.3±4.4, 15.0±9.3/10.2±6.2, and 14.9±7.8/8.4±4.7 mmHg. At 3 months, BP and plasma norepinephrine substantially decreased in proximal RDN group. Whereas controls showed minimal BP decreases and had similar plasma norepinephrine concentrations as baseline.

**Conclusions**—Renal afferent nerves can be mapped safely, and HFS-guided targeted proximal RDN can achieve apparent BP reduction and sympathetic inhibition. (Circ Cardiovasc Interv. 2015;8:e001847. DOI: 10.1161/CIRCINTERVENTIONS.115.001847.)

**Key Words:** blood pressure ■ catheter ablation ■ heart rate ■ hypertension ■ renal artery

Resistant hypertension has been linked to chronic excessive sympathetic drive, especially elevation of renal sympathetic activity in some groups of patients. Against this background, renal artery ablation selectively denervating the kidneys emerges as an alternative treatment for such patients. Although initial trials showed promising results with regard to large reductions in blood pressure (BP), disappointingly, the potential therapeutic role of renal denervation (RDN) in lowering BP is being challenged after the failure of recent SYMPLICITY HTN-3 trial to show a benefit of RDN over the optimal medical therapy.

The concept of RDN is supported by both experimental and early human evidence when surgical sympathectomy procedures were done and found to have huge effect on BP. However, for the time being, the BP-lowering effect of catheter-based RDN is highly variable (the rates of nonresponse to RDN vary between 8% and 37%). Kaiser et al reported that repeated RDN could significantly decrease BP in non-responders to previous radiofrequency ablation procedure. These findings suggest that lack of BP reductions after RDN may be the results of incomplete denervation. Therefore, to minimize treatment failure of blind ablation, a method that could convert what is currently a purely anatomic procedure to one that involves quantifying the efficacy of RDN intraprocedurally and mapping the renal nerves to enable a targeted therapy is of great clinical significance.
WHAT IS KNOWN

- A better understanding of the pathophysiological roles of the renal sympathetic nerves in resistant hypertension and a detailed knowledge of neuroanatomy has allowed catheter-based renal denervation to emerge as a promising alternative treatment for patients with resistant hypertension; however, the blood pressure-lowering effect of denervation procedures performed by nonelective ablation of the renal artery wall is highly variable.
- Although electric stimulation has been shown to monitor the efficacy of renal denervation, there has been no report of transcatheter electric stimulation as a guide for renal denervation procedures.

WHAT THE STUDY ADDS

- Blood pressure and plasma norepinephrine were decreased 3 months after high-frequency stimulation-guided targeted radiofrequency ablation compared with nondenervated controls.
- Specific high-frequency stimulation-guided ablations over the proximal electric stimulation responsive sites not only abolished the blood pressure response in the postablation period but also attenuated the blood pressure response to repeated high-frequency stimulation.

A vagal response elicited by endocardial high-frequency stimulation (HFS) was used to reflect the contribution of ablation of epicardial autonomic ganglia in patients with atrial fibrillation. Chinushi et al successfully applied this concept to the field of RDN. In their study, HFS to renal artery before RDN could elicit an orthosympathetic response when stimulated renal afferent nerves activate central sympathetic nervous activity. In addition, the absence of BP-increasing response to postablation HFS was an indication to confirm the actual disruption of renal nerve fibers. The precise anatomic information in human from Sakakura et al indicated that the renal afferent nerves converge on the renal arteries closer to aorta. On the basis of these considerations, we applied HFS to map the renal afferent nerves and assessed the efficacy of HFS-guided proximal RDN.

Methods

Animals

The Chinese Kunming dog breed was originally developed from the local hybrid dogs by crossing local native dogs with working dogs. They have natural high BP, and are therefore ideal experimental animals used for this study. Eligible dogs were older than 3 years and had a systolic BP (SBP) of ≥140 mm Hg (under anesthesia and via invasive BP monitoring). After selection, a total of 24 healthy Chinese Kunming dog breed was originally developed from the local hybrid dogs by crossing local native dogs with working dogs. They have natural high BP, and are therefore ideal experimental animals used for this study. Eligible dogs were older than 3 years and had a systolic BP (SBP) of ≥140 mm Hg (under anesthesia and via invasive BP monitoring). After selection, a total of 24 healthy Chinese Kunming dogs, weighing between 30 and 35 kg, were enrolled in the present study. Twelve animals were assigned to proximal RDN group, which underwent HFS and proximal renal artery ablation guided by the autonomic responses evoked via HFS (n=12). Another 12 animals were assigned to control group, which underwent HFS but no ablation on the proximal arteries was performed (n=12).

The experiment was approved by the animal experimentation ethics committee of the Chongqing Medical University, following the guidelines of the National Institutes of Health for the care and use of laboratory animals. Standard food and water feedings were given throughout the experimental period.

Animal Preparation and Renal Angiogram

The dogs were anesthetized with 3% sodium pentobarbital of 30 mg/kg by intraperitoneal injection, followed by a maintenance dose of 5 mg/kg per hour. Penicillin was given intramuscularly after the ablation for the prevention of infection. The surface ECG was continuously recorded throughout the study by a multichannel recorder (Sichuan Jinjiang Electronic Science and Technology Company, China). Both sides of the femoral artery were punctured under sterile conditions, and an amount of 2000 IU unfractionated heparin was administered. The continuous invasive BP monitoring was recorded via the left femoral artery. A dedicated 6F bipolar micropores irrigated ablation catheter (AquaSense, Synaptic Medical Limited, Beijing, China) was introduced via the right femoral artery and placed in the right or left renal artery for delivering HFS and radiofrequency energy. The bipolar AquaSense catheter consists of a 3.5-mm-long tip electrode, and the electrode spacing is 2 mm. There are 44 micropores located around the catheter tip that are open irrigated through a central lumen with heparinized saline to maintain lower tip-to-tissue temperature at the ablation site (Figure 1).

Bilateral renal angiography was performed using a 6F JR4 Judkins catheter (Cordis Corporation Miami, FL). If renal artery abnormalities were found, diameters were below the minimum acceptable size (<4 mm, using inner diameter of 6F JR4 Judkins catheter as reference), or main renal artery length was shorter than 20 mm, the dog would be eliminated from the study.

HFS Protocol and Autonomic Responses

HFS was applied from the proximal (5–10 mm from the ostium of renal artery) to the distal (5–10 mm from first distal main renal artery bifurcation) segments of the left or right renal artery (Figure 2), via the bipolar electrodes of AquaSense catheter, as described earlier. The number of attempts of HFS on proximal, middle, and distal segments of renal arteries depended on the ease of access of the target with the electrode. Rectangular electric stimuli were delivered at a frequency of 20 Hz, amplitude to 8 V, and pulse duration of 2 ms for 60 s using a Nerve and Muscle Stimulator (XinNuo B100; Sichuan Jinjiang Electronic Science and Technology Company). The positive autonomic response was defined as elevation of SBP by ≥10 mm Hg during HFS (study by Chinushi et al indicated that when electric stimulation activated the renal nerves, SBP would increase at least 10 mm Hg). After ablation, under fluoroscopic guidance, the catheter was repositioned over the previously stimulated sites and postablation HFS was performed (using the same threshold as baseline). For analysis, each HFS of 60-s phases was subdivided into 20-s phases.

Radiofrequency Catheter Ablation

In proximal RDN group, after electric stimulation of renal arteries, radiofrequency ablation was performed over HFS-positive sites of proximal renal artery where autonomic responses evoked by transcatheter HFS were obtained. Radiofrequency ablation used the AquaSense catheter, as described earlier, with saline irrigated manually to decrease the temperature of tissue–electrode interface. Radiofrequency energy delivery and ablation time were always started at 8 W and 60 s, and increased to 15 W and 300 s if the autonomic response still occurred during postablation HFS. The end point of ablation was defined as the failure to reproduce the positive BP-increasing response with repeated HFS.

Blood Sampling and Follow-Up

Baseline blood sampling was obtained from the right femoral vein. Three milliliter of blood sampling to measure the plasma norepinephrine was obtained in EDTA. After high-speed centrifugation, the blood samples were stored at −80°C until assay. The plasma norepinephrine was assayed by high-performance liquid chromatography.
During the follow-up period, the condition of all experimental dogs was observed daily. After the ablation or sham procedure of 3 months, follow-up assessments were conducted, which consisted of invasive BP measurements via the femoral artery, bilateral renal angiography, and plasma norepinephrine assay, as described above.

**Statistical Analysis**

All continuous data were presented as mean±SD, and categorical variables were expressed as proportions. Differences of BP, heart rate (HR), ΔBP, and ΔHR during HFS were analyzed by 1-/2-way repeated measures ANOVA. Multiple pairwise comparisons were
performed with the Bonferroni test. Paired \( t \) tests were used to compare BPs and plasma norepinephrine concentrations before and 3 months after RDN, and these changes between proximal RDN group and controls were compared using Student \( t \) tests. Correlation between changes in systolic BP and plasma norepinephrine 3 months after RDN was assessed with Pearson test. A 2-tailed value of \( P < 0.05 \) was regarded as statistically significant. All the statistical analyses were performed with SPSS statistical software (version 17.0; Chicago, IL).

**Results**
Twenty-four Chinese Kunming dogs in preparation for renal artery stimulation and ablation underwent bilateral renal angiography. Angiograms of all the renal arteries revealed that 2 dogs in proximal RDN group and 4 dogs in control group were excluded, and the remaining 18 dogs were anatomically eligible to perform HFS and RDN. No renal artery dissection, stenosis, or other complications associated with electric stimulation and radiofrequency ablation procedure occurred in either group.

### Autonomic Responses to HFS

**Localization of Renal Afferent Nerves Innervation**
HFS to renal arteries caused significant increases in SBP (Figures 3 and 4). These changes often began during 20 to 40 s after the onset of HFS and returned to baseline within 2 to 5 minutes after its cessation. The number of attempts of HFS in each dog were 11±3 (range, 7–15). Autonomic responses evoked by HFS were elicited in 13 of 18 (72.2%) dogs with an average of 6±2 sites per dog, including 8 of 10 (80%) dogs in proximal RDN group and 5 of 8 (62.5%) in control group. The proximal and the middle segment of renal artery each required only a mean of 2±1 HFS attempts to determine a positive BP-increasing response. No BP elevation was induced by HFS at the distal portion of renal artery.

**Autonomic Responses in Proximal RDN Group Versus Control Group**
When compared with the values before electric stimulation, HFS to renal arteries of 8 HFS responsive dogs in proximal

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**Figure 3.** Blood pressure (BP) response at high-frequency stimulation (HFS) positive sites of proximal renal artery. A, HFS-positive site at proximal renal artery (red dot, also indicated by arrow). White dots represented HFS-negative sites, whereas red and yellow dots represented HFS-positive sites. The radiofrequency (RF) energy was delivered only over red dot, the proximal responsive site. As shown, before ablation, HFS increased BP significantly (B\(_1\) and B\(_2\)), whereas after ablation, repeated HFS was not associated with BP-increasing response (C\(_1\) and C\(_2\)). RDN indicates renal denervation.
Lu et al
Proximal RDN With Electric Stimulation

Before Proximal Renal Artery Ablation

In 8 HFS responsive dogs, preablation electric stimulation caused immediate and significant increases in BP at both the proximal and the middle segment of renal artery (Figures 3 and 4). At the proximal positive sites, compared with values before electric stimulation, HFS increased BP by 6.0±5.0/3.4±5.5, 16±11.7/11.1±8.5, and 17.1±8.4/8.5±5.3 mmHg during the initial, middle, and last 20 s of HFS (P<0.001 for ΔSBP; P<0.001 for ΔDBP, respectively; Figure 5). There were similar changes at the positive sites on the middle segment of renal artery. Mean BP increases from prestimulus baseline level were 6.2±5.6/5.3±4.4, 15.0±9.3/10.2±6.2, and 14.9±7.8/4±7.4 mmHg during the first, second, and third 20 s of HFS (P<0.001 for ΔSBP; P<0.001 for ΔDBP, respectively; Figure 5). There were similar changes at the positive sites on the middle segment of renal artery.

RDN group before RDN increased the BP by 6.1±5.3/4.3±5, 16±10.4/10.7±7.4, and 16±8/8.5±4.9 mmHg (P<0.001 for all; Figure 5) and HR by 0.9±6.1, 3.2±6.6, and 3.1±7.7 bpm (P=1, P=0.077, P=0.24 for ΔHR, respectively) at first, second, and third 20 s of HFS. Similarly, HFS increased the SBP/diastolic BP (DBP) of 5 HFS responsive dogs in control group by 1.2±6.3/1.6±5.7, 14.3±8/8.1±5.6, and 13.5±8.3/7.8±5 mmHg (P=1, P<0.001, and P<0.001 for ΔSBP; P=0.13, P<0.001, and P<0.001 for ΔDBP, respectively; Figure 5) and HR by 0.2±5.7, 6.6±12.5, and 6.9±13 bpm (P=1, P=0.025, and P=0.022 for ΔHR, respectively).

With the exception of initial 20 s of HFS, the BP increases during electric stimulation show no difference between HFS responsive dogs of 2 groups (Tables 1 and 2).

Autonomic Responses to HFS in Proximal RDN Group

Before Proximal Renal Artery Ablation

In 8 HFS responsive dogs, preablation electric stimulation caused immediate and significant increases in BP at both the proximal and the middle segment of renal artery (Figures 3 and 4). At the proximal positive sites, compared with values before electric stimulation, HFS increased BP by 6.0±5.0/3.4±5.5, 16±11.7/11.1±8.5, and 17.1±8.4/8.5±5.3 mmHg during the initial, middle, and last 20 s of HFS (P<0.001 for ΔSBP; P<0.001, and P<0.001 for ΔDBP, respectively; Figure 5). There were similar changes at the positive sites on the middle segment of renal artery. Mean BP increases from prestimulus baseline level were 6.2±5.6/5.3±4.4, 15.0±9.3/10.2±6.2, and 14.9±7.8/4±7.4 mmHg during the first, second, and third 20 s of HFS (P<0.002, P<0.001, and P<0.001 for ΔSBP; P=0.013, P<0.001, and P<0.001 for ΔDBP, respectively; Figure 5). There were similar changes at the positive sites on the middle segment of renal artery. Mean BP increases from prestimulus baseline level were 6.2±5.6/5.3±4.4, 15.0±9.3/10.2±6.2, and 14.9±7.8/4±7.4 mmHg during the first, second, and third 20 s of HFS (P<0.002, P<0.001, and P<0.001 for ΔSBP; P=0.013, P<0.001, and P<0.001 for ΔDBP, respectively; Figure 5). There were similar changes at the positive sites on the middle segment of renal artery. Mean BP increases from prestimulus baseline level were 6.2±5.6/5.3±4.4, 15.0±9.3/10.2±6.2, and 14.9±7.8/4±7.4 mmHg during the first, second, and third 20 s of HFS (P<0.002, P<0.001, and P<0.001 for ΔSBP; P=0.013, P<0.001, and P<0.001 for ΔDBP, respectively; Figure 5). There were similar changes at the positive sites on the middle segment of renal artery. Mean BP increases from prestimulus baseline level were 6.2±5.6/5.3±4.4, 15.0±9.3/10.2±6.2, and 14.9±7.8/4±7.4 mmHg during the first, second, and third 20 s of HFS (P<0.002, P<0.001, and P<0.001 for ΔSBP; P=0.013, P<0.001, and P<0.001 for ΔDBP, respectively; Figure 5).
HFS revealed significant changes in ΔHR, with increases of 3.5±4.6 bpm (*P*=0.035) and 4.4±5.5 bpm (*P*=0.027).

**After Proximal Renal Artery Ablation**

Radiofrequency ablation was performed over the proximal positive sites of the 8 HFS responsive dogs. These sites showed a negative response to each phase of postablation HFS (Figure 3), with increases of BP by 1.3±3.0/1.0±2.5, 0.8±3.9/1.5±3.4, and 1.5±4.5/0.7±3.8 mm Hg (*P*=0.33 for ΔSBP; *P*=0.26 for ΔDBP; Figure 5) when compared with values before electric stimulation. Of note, no radiofrequency applications were delivered at the middle segment of renal artery, but the HFS-positive sites located in the middle region of renal artery still failed to reproduce the same BP-increasing response with repeated HFS after ablation (Figure 4). Only during the second 20 s of HFS, SBP and DBP were increased from prestimulus baseline level by 0.8±3.5, 0.5±3.0, and 0.4±3.6 bpm (*P*=0.83).

**Autonomic Response Before Versus After Radiofrequency Ablation**

In 8 HFS responsive dogs, when compared with the autonomic response to HFS in the preablation state, a significant and substantial lower BP response was observed after RDN at positive sites on both proximal and middle segment of renal artery. When compared with the values before ablation, both mean and change of SBP and DBP during HFS after RDN were markedly attenuated (Tables 3 and 4).

**Proximal Radiofrequency Ablation**

Radiofrequency ablation was successfully obtained in 8 dogs responsive to HFS, obliterating the BP-increasing response to repeated HFS. A total of 3±1 radiofrequency applications were delivered in each dog, and average radiofrequency duration for each lesion was 215±73 s (range, 60–300 s). The mean delivered radiofrequency energy was 11±2 W (range, 8–15 W), and maximum temperature of the catheter tip was 43±2°C (range, 40–45°C). Tissue impedance decreased from 214±42 ohm before to 185±42 ohm after (*P*<0.001).

Electric current-induced BP elevation occurred not only during electric stimulation but also when radiofrequency energy was delivered. Preablation HFS increased BP of proximal responsive sites from an average of 189.8±16/131.2±16.6 bpm to 194.8±16/136±16.6 bpm (*P*=0.027).

**Figure 5.** Blood pressure (BP) response to high-frequency stimulation (HFS). A, Systolic BP increases during electric stimulation in 8 HFS responsive denervated dogs before ablation and 5 HFS responsive nonstenanered controls. B, and B, BP response to HFS before and after ablation of 8 HFS responsive dogs in proximal renal denervation group. B, HFS-positive sites on proximal renal artery. B, HFS-positive sites on the middle segment of renal artery. Differences of systolic BP were analyzed by 1-way repeated measures ANOVA. When significance between groups was detected, the Bonferroni test was used to compute post hoc comparisons of significant values. *P*<0.05 for before vs multiple time points during electric stimulation (1st 20 s, 2nd 20 s, and 3rd 20 s). RDN indicates renal denervation.

### Table 1. Comparisons of Autonomic Response to HFS Between Proximal RDN Group and Control Group: Mean of BP

<table>
<thead>
<tr>
<th></th>
<th>Proximal RDN Group</th>
<th>Control Group</th>
<th><em>P</em> Value</th>
<th>Proximal RDN Group</th>
<th>Control Group</th>
<th><em>P</em> Value</th>
<th>Proximal RDN Group</th>
<th>Control Group</th>
<th><em>P</em> Value</th>
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</thead>
<tbody>
<tr>
<td>SBP, mm Hg</td>
<td>188.7±16.2</td>
<td>175.6±16.3</td>
<td>0.001</td>
<td>130.2±16.4</td>
<td>119.8±8.7</td>
<td>0.001</td>
<td>179±20.5</td>
<td>178.2±21.4</td>
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<tr>
<td>DBP, mm Hg</td>
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<td>HR, bpm</td>
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<tr>
<td>Before HFS</td>
<td>188.7±16.2</td>
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<td>130.2±16.4</td>
<td>119.8±8.7</td>
<td>0.001</td>
<td>179±20.5</td>
<td>178.2±21.4</td>
<td>0.88</td>
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<tr>
<td>1st 20 s</td>
<td>194.8±16</td>
<td>176.8±17.7</td>
<td>&lt;0.001</td>
<td>134.5±15.5</td>
<td>121.4±10.6</td>
<td>&lt;0.001</td>
<td>179.9±18.5</td>
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<tr>
<td>2nd 20 s</td>
<td>204.6±14</td>
<td>189.8±17.2</td>
<td>&lt;0.001</td>
<td>140.8±13.7</td>
<td>127.9±12</td>
<td>&lt;0.001</td>
<td>182.2±20.8</td>
<td>184.8±22.3</td>
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<td>3rd 20 s</td>
<td>204.7±14.7</td>
<td>189.1±14.4</td>
<td>&lt;0.001</td>
<td>138.6±15.6</td>
<td>127.6±10.9</td>
<td>0.001</td>
<td>182.1±21.1</td>
<td>185.1±22.8</td>
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<td>After HFS</td>
<td>189±18.1</td>
<td>176.5±17.6</td>
<td>0.004</td>
<td>129.1±18.8</td>
<td>119.8±12.4</td>
<td>0.015</td>
<td>176.9±20.5</td>
<td>180.5±22.4</td>
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Mean of BP and HR during HFS at positive sites of 13 HFS responsive dogs, including 8 in proximal RDN group and 5 in control group. Differences of BP, HR, ΔSBP, and ΔHR between 2 groups during HFS were analyzed by 2-way repeated measures ANOVA. Multiple pairwise comparisons were performed with the Bonferroni test. DBP indicates diastolic blood pressure; HFS, high-frequency stimulation; HR, heart rate; *P* value, control group vs proximal RDN group; RDN, renal denervation; and SBP, systolic BP.
to $203.1 \pm 13.1/138.8 \pm 14.7$ mm Hg ($P<0.001$). Consistent with the change during HFS, an immediate and substantial BP-increasing response was also seen during ablation. Radiofrequency application increased BP from $186.8 \pm 19.4/128.4 \pm 19.6$ to $207.2 \pm 17.2/141.5 \pm 16.8$ mm Hg ($P<0.001$). BP showed no significant difference between HFS and radiofrequency ablation period (Figure 6).

**Plasma Norepinephrine and BP of Follow-Up**

Thirteen dogs responsive to HFS underwent ablation or sham procedure and completed 3-month follow-up. The mean baseline BPs were $194 \pm 13.2/130.1 \pm 13.5$ mm Hg in proximal RDN group and $174.4 \pm 17.2/141.5 \pm 16.8$ mm Hg in control group. After 3 months, significant BP decreases from baseline were observed in dogs underwent RDN, with reductions of $-24.4 \pm 12.6/-10.7 \pm 9.9$ mm Hg ($P=0.001$ for ΔSBP and $P=0.018$ for ΔDBP, respectively; Figure 7). Whereas controls showed minimal BP decreases, with reductions of $-5.5 \pm 7.7/-0.4 \pm 4.7$ mm Hg ($P=0.21$ for ΔSBP and $P=0.87$ for ΔDBP, respectively; Figure 7).

The plasma norepinephrine concentrations at baseline were $1.27 \pm 0.38$ nmol/L in proximal RDN group and $1.29 \pm 0.1$ nmol/L in control group. Proximal RDN resulted in a marked reduction in plasma norepinephrine 3 months after radiofrequency ablation, with a decrease of $-0.86 \pm 0.32$ nmol/L ($P<0.001$; Figure 8). The decrease in plasma norepinephrine correlated with SBP reduction after 3 months, but the correlation was not statistically significant ($r=0.632; P=0.18$). In contrast, controls at 3 months of follow-up had similar concentrations as baseline, with a reduction of $0.1 \pm 0.25$ nmol/L ($P=0.4$; Figure 8).

**Discussion**

The main findings of this study were as follows: (1) before RDN, HFS resulted in a positive autonomic response of an immediate increase in SBP $\geq 10$ mm Hg in both proximal and middle regions of renal artery. No BP elevation was induced by HFS at the distal segment of renal artery; (2) radiofrequency applications were only performed over the proximal positive sites, but after ablation repeated HFS failed to reproduce

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**Table 2. Comparisons of Autonomic Response to HFS Between Proximal RDN Group and Control Group: Change of BP**

<table>
<thead>
<tr>
<th></th>
<th>Proximal RDN Group</th>
<th>Control Group</th>
<th>P Value</th>
<th>Proximal RDN Group</th>
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<th>Proximal RDN Group</th>
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<tbody>
<tr>
<td></td>
<td>ΔSBP, mm Hg</td>
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<tr>
<td>1st 20 s</td>
<td>6.1±5.3</td>
<td>4.3±5</td>
<td>0.9±6.1</td>
<td>16±10.4</td>
<td>10.7±7.4</td>
<td>0.1</td>
<td>3.2±6.6</td>
<td>6.8±12.5</td>
<td>0.16</td>
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<tr>
<td>2nd 20 s</td>
<td>16.8±8</td>
<td>8.5±4.9</td>
<td>3.1±7.7</td>
<td>13.5±8.3</td>
<td>7.8±5</td>
<td>0.55</td>
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</table>

Change of BP and HR during HFS at positive sites of 13 HFS responsive dogs, including 8 in proximal RDN group and 5 in control group. Differences of BP, HR, ΔBP, and ΔHR between 2 groups during HFS were analyzed by 2-way repeated measures ANOVA. Multiple pairwise comparisons were performed with the Bonferroni test. DBP indicates diastolic blood pressure; HFS, high-frequency stimulation; HR, heart rate; P value, control group vs proximal RDN group; RDN, renal denervation; and SBP, systolic BP.

**Table 3. Autonomic Response to HFS Before vs After Radiofrequency Ablation in Proximal Renal Denervation Group: Mean of BP**

<table>
<thead>
<tr>
<th></th>
<th>Before Ablation</th>
<th>After Ablation</th>
<th>P Value</th>
<th>Before Ablation</th>
<th>After Ablation</th>
<th>P Value</th>
<th>Before Ablation</th>
<th>After Ablation</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SBP, mm Hg</td>
<td>DBP, mm Hg</td>
<td></td>
<td>HR, bpm</td>
<td></td>
<td></td>
<td>DBP, mm Hg</td>
<td></td>
<td></td>
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<tr>
<td>Before HFS</td>
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<tr>
<td>Ablated HFS-positive sites</td>
<td>189.8±16.0</td>
<td>186.7±18.1</td>
<td>0.6</td>
<td>131.2±16.6</td>
<td>130.3±17.7</td>
<td>0.88</td>
<td>186.4±22.5</td>
<td>185.3±20.8</td>
<td>0.88</td>
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<tr>
<td>Nonablated HFS-positive sites</td>
<td>187.6±16.9</td>
<td>183.1±18.0</td>
<td>0.46</td>
<td>129.2±16.6</td>
<td>126.9±17.5</td>
<td>0.7</td>
<td>171.6±15.5</td>
<td>175.1±14.6</td>
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<tr>
<td>Ablated HFS-positive sites</td>
<td>195.8±14.0</td>
<td>188.0±18.8</td>
<td>0.18</td>
<td>134.5±15.3</td>
<td>131.3±17.9</td>
<td>0.58</td>
<td>186.5±19.7</td>
<td>186.1±20.15</td>
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<td>Nonablated HFS-positive sites</td>
<td>193.8±18.1</td>
<td>186.4±18.4</td>
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<td>134.5±16.3</td>
<td>128.7±17.7</td>
<td>0.42</td>
<td>173.3±14.9</td>
<td>175.9±13.1</td>
<td>0.59</td>
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<td>Ablated HFS-positive sites</td>
<td>206.7±13.5</td>
<td>187.5±18.4</td>
<td>0.001</td>
<td>142.3±14.3</td>
<td>131.8±17.6</td>
<td>0.065</td>
<td>189.3±22.5</td>
<td>186.2±22.3</td>
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<td>Nonablated HFS-positive sites</td>
<td>202.6±14.6</td>
<td>188.4±18.3</td>
<td>0.018</td>
<td>139.4±13.4</td>
<td>130.7±17.3</td>
<td>0.14</td>
<td>175.1±16.8</td>
<td>175.6±14.4</td>
<td>0.92</td>
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<td>Ablated HFS-positive sites</td>
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<td>187.5±23.9</td>
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<td>202.5±15.5</td>
<td>186.0±18.5</td>
<td>0.008</td>
<td>137.6±15.6</td>
<td>128.2±18.0</td>
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<td>176.0±16.8</td>
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<td>0.94</td>
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<td>After HFS</td>
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</tr>
<tr>
<td>Ablated HFS-positive sites</td>
<td>190.4±17.8</td>
<td>184.8±18.1</td>
<td>0.37</td>
<td>130.4±19.8</td>
<td>128.2±17.7</td>
<td>0.73</td>
<td>182.3±23.7</td>
<td>186.8±21.8</td>
<td>0.57</td>
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<tr>
<td>Nonablated HFS-positive sites</td>
<td>187.6±18.8</td>
<td>182.4±19.1</td>
<td>0.42</td>
<td>127.8±18.2</td>
<td>125.7±18.6</td>
<td>0.74</td>
<td>171.4±15.4</td>
<td>174.2±15.3</td>
<td>0.6</td>
</tr>
</tbody>
</table>

Eight dogs responsive to HFS in proximal renal denervation group underwent RF ablation. Mean of BP and HR during HFS at HFS-positive sites of the 8 dogs before and after RF ablation. RF ablation was performed over proximal HFS-positive sites, and no RF applications were delivered at HFS-positive sites on the middle segment of renal artery. Differences of BP, HR, ΔBP, and ΔHR before and after RF ablation during HFS were analyzed by 2-way repeated measures ANOVA. Multiple pairwise comparisons were performed with the Bonferroni test. DBP indicates diastolic blood pressure; HFS, high-frequency stimulation; HR, heart rate; P value, before vs after RF ablation; RF, radiofrequency; and SBP, systolic blood pressure.
the same BP-increasing response at the positive sites of both proximal and middle segment of renal artery; (3) BP and plasma norepinephrine substantially decreased 3 months after HFS-guided proximal RDN. However, nondenervated controls showed minimal BP and plasma norepinephrine changes; (4) consistent with the change during HFS, an immediate and substantial BP-increasing response was seen during ablation.

**HFS-Guided RDN**

Both renal afferent nerves and efferent sympathetic fibers are distributed in close proximity to the lumen–intima interface of the renal artery and should thus be accessible via catheter ablation, but the interventionalists still have to accept a black box during the procedure. Previous studies indicated that stimulation of renal afferent nerves could activate central cardiovascular nuclei and subsequently increase systemic sympathetic nervous activity and BP. To date, there has been no report of transcatheter HFS-guided RDN, only several recent studies investigating whether the transcatheter HFS could be available to confirm the degree of denervation of renal nerves immediately after catheter-based RDN. In our study, besides real-time evaluation of denervation, we applied HFS to detect the location of renal afferent nerves. BP-increasing responses elicited by HFS occurred on the proximal and not on the distal parts of renal artery. These findings corroborate anatomic study by Sakakura et al showing sensory fibers converge on the proximal parts of renal arteries.

**Figure 6. Blood pressure (BP) during high-frequency stimulation (HFS) and radiofrequency (RF) ablation.** Eight HFS responsive dogs in proximal renal denervation group underwent RF ablation. Changes of BP and HR during HFS at HFS-positive sites of the 8 dogs before and after RF ablation. RF ablation was performed over proximal HFS-positive sites, and no RF applications were delivered at HFS-positive sites on the middle segment of renal artery. Differences of BP, HR, DBP, and ΔHR before and after RF ablation during HFS were analyzed by 2-way repeated measures ANOVA. Multiple pairwise comparisons were performed with the Bonferroni test. DBP indicates diastolic blood pressure; HFS, high-frequency stimulation; HR, heart rate; P value, before vs after of renal artery. Differences of BP, HR, and DBP in proximal and middle segment of renal artery; (3) BP and ΔHR immediately after catheter-based RDN. In our study, besides real-time evaluation of denervation, we applied HFS to detect the location of renal afferent nerves. BP-increasing responses elicited by HFS occurred on the proximal and not on the distal segments of renal artery. These findings corroborate anatomic study by Sakakura et al showing sensory fibers converge on the proximal parts of renal arteries.

**Proximal RDN**

From anatomic study, distal renal arteries have abundant sympathetic nerves, whereas renal afferent fibers converge on proximal segments. In daily clinical practice, radiofrequency ablations are applied discretely form the first distal main renal artery bifurcation all the way back to the ostium (although the number of ablation points is not a strictly fixed variable, 6 is recommended as routine procedure to provoke a BP-lowering effect).
response. Extensive ablation may be useful to improve the procedural success, but it might increase the potential risk of vascular complication of RDN. Recent data from a human study using optical coherence tomography demonstrated the possible occurrence of intimal edema and thrombus formation in the acute phase immediately after ablation.21

The effect of HFS to renal artery was different from site to site in this study. BP elevation evoked by HFS easily occurred on the proximal and middle segments of renal artery but not on the distal portions. Interestingly, radiofrequency ablation over proximal responsive sites could not only abolish their own BP responses to postablation HFS but also attenuate the BP-increasing responses on the middle segments of the renal artery. These observations suggested that ablation of proximal target sites could be adequate for the effectiveness of RDN, and no other ablation is necessary. In fact, there is no functional evidence that ablations on distal segment of renal arteries are effective and necessary for BP reductions. Some may argue that the density of renal nerves is indeed higher in the proximal parts, but they are located more remote from the lumen and are therefore less accessible to radiofrequency energy. However, this seems less likely in the HFS-guided proximal RDN because the efficacy of radiofrequency ablation and ablated sites is evaluated by the BP response to HFS. Besides the BP-lowering efficacy of proximal RDN, ablation of just the proximal segments would reduce the total procedure time and limit the total amount of endothelial injury.

BP-Increasing Response During Radiofrequency Ablation

The concept of renal nerves mapping via monitoring hemodynamic response elicit by site-specific HFS came from the efficacy of vagal reflex evoked by electric stimulation at mapping cardiac ganglionated plexuses. In addition, previous studies on atrial vagal denervation to treat patients with paroxysmal atrial fibrillation indicated that not only the electric current of HFS but also the thermal stimulation of radiofrequency energy delivery to afferent vagus nerve fibers could provoke the vagal reflex.11 In patients with paroxysmal atrial fibrillation, a vagal response during ablation has been shown to significantly reduce recurrence of atrial fibrillation during follow-up.11 To our knowledge, little is known about the BP changes during renal artery ablation, in particularly, the BP response to the radiofrequency energy delivery.

In the present study, with the exception of a substantial BP increase during HFS, a similar BP-increasing response was seen during radiofrequency ablation. This finding suggests not only high-frequency electric stimulation but also radiofrequency current could activate renal afferent nervous activity. Contrary to the animal experiments, in our ongoing clinical trials, some patients also experienced inappropriate bradycardia-hypotension episodes during radiofrequency ablation. In regards to this discrepancy, we reckoned that the effect of radiofrequency energy delivery on BP may be the same, and the only difference was the way of anesthesia. The animal experiments were conducted under general anesthesia, but our patients were just under conscious sedation. Visceral pain at the time of radiofrequency energy delivery may, in part, contribute to the BP-lowering response. On the basis of this consideration, we found that BP response during radiofrequency ablation may be another surrogate to evaluate the ablation procedure on spot.

Limitations

Some limitations should be considered in interpreting the present results. First, the relatively small data are a potential limitation, and our experimental facility did not allow measurement of renal or whole-body norepinephrine spillover,
which is the gold standard to estimate the sympathetic nervous activity. Second, in our study, HFS in 5 dogs produced either no effect on BP or a slight decrease in BP. The electric stimuli in the current study were the maximum stimulation settings of the stimulator (20 Hz, 8 V, and pulse width 2 ms). Therefore, we did not have sufficient data to explain this observation by whether different stimulation thresholds would be applied or whether these sites lack renal afferent nerves. However, considering that no BP-increasing response to HFS occurred at any site of the renal artery in these 5 dogs and HFS (20 Hz, 15 V, and pulse width 10 ms) caused a consistent BP increase in humans, we suggested that the HFS (20 Hz, 8 V, and pulse width 2 ms) unable to activate the renal afferent sensory nerves in these HFS nonresponsive dogs might contribute to this phenomenon. Finally, the present study was conducted in healthy and young animals. Atherosclerosis and calcification predominantly occur at the ostial sites of renal arteries. Limiting ablation at the proximal part of renal artery may have a higher risk for restenosis after ablation. Therefore, whether our findings in HFS-guided proximal RDN can be directly applied into the human situation remains unclear. However, our previous clinical study did not suggest such an outcome. It indicated that proximal RDN has a similar safety and efficacy profile compared with full-length RDN (unpublished data).

Conclusions
Renal afferent nerves predominantly reside in proximal renal arteries and can be mapped using HFS safely. HFS-guided targeted proximal RDN can achieve apparent BP reduction and central sympathetic inhibition.

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Disclosures
None.

References
Selective Proximal Renal Denervation Guided by Autonomic Responses Evoked via High-Frequency Stimulation in a Preclinical Canine Model

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