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PRECLINICAL RESEARCH

Improvement of Myocardial Function Following Catheter-Based Renal Denervation in Heart Failure

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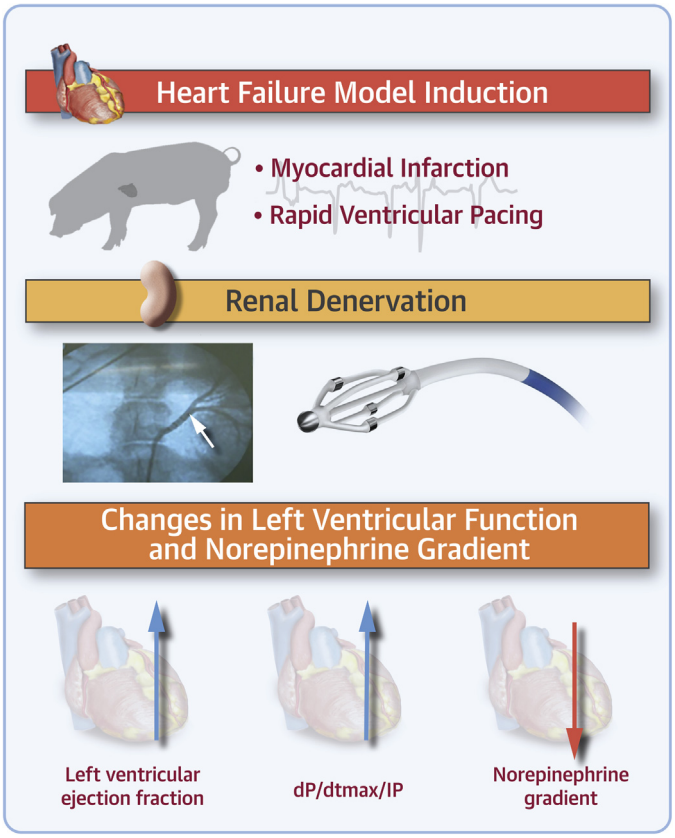
VISUAL ABSTRACT



Liao, S.-Y. et al. J Am Coll Cardiol Basic Trans Science. 2017;2(3):270–81.

HIGHLIGHTS

- A porcine model of heart failure was induced by myocardial infarction followed by rapid ventricular pacing for 4 weeks.
- Catheter-based renal denervation was performed using an expandable basket with 4 electrodes to deliver radiofrequency energy. Histological examination showed significant denervation of the renal arteries after the procedure.
- Compared with the control group, animals that received renal denervation showed significant improvement of cardiac function as determined by LV ejection fraction, maximum rate of LV pressure rise normalized to instantaneous developed pressure, and reduction of myocardial and renal norepinephrine gradient at 10 weeks after procedure.

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SUMMARY

Renal denervation (RD) is a potential novel nonpharmacological therapy for heart failure (HF). We performed bilateral catheter-based RD in 10 adult pigs and compared them with 10 control subjects after induction of HF to investigate the long-term beneficial effects of RD on left ventricular (LV) function and regional norepinephrine gradient after conventional HF pharmacological therapy. Compared with control subjects, animals treated with RD demonstrated an improvement in LV function and reduction of norepinephrine gradients over the myocardium and kidney at 10-week follow-up. Our results demonstrated that effective bilateral RD decrease regional norepinephrine gradients and improve LV contractile function compared with medical therapy alone. (J Am Coll Cardiol Basic Trans Science 2017;2:270–81) © 2017 The Authors. Published by Elsevier on behalf of the American College of Cardiology Foundation. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

ABBREVIATIONS AND ACRONYMS

ACE = angiotensin-converting enzyme

BNP = B-type natriuretic peptide

dP/dt_{max} = the maximum rate of left ventricular pressure rise

HF = heart failure

IP = instantaneous developed pressure

LV = left ventricular

MI = myocardial infarction

RD = renal denervation

TH = tyrosine hydroxylase

Heart failure (HF) is a major medical problem with substantial health care burden, and the associated mortality and morbidity of HF remain high despite advances in pharmacological and device management (1,2). There is thus a compelling need for new therapies that target the underlying pathophysiology of HF and further improve clinical outcome. In patients with HF, increased activity of the sympathetic nervous system is associated with progression of left ventricular (LV) dysfunction and increased mortality (3,4). Both renal afferent and efferent sympathetic nerves have been proposed to contribute to the sympathoexcitation in HF (5,6). In HF, increased central sympathetic neural outflow is associated with cardiac and renal efferent sympathetic nerve activations, and thus, cardiac and renal norepinephrine spillover (7–9). However, activation of renal afferent sympathetic nerves caused by renal hypoxemia and ischemia may also directly induce increased central sympathetic outflow (9,10). Recent development of catheter-based renal nerve ablation for treatment of refractory hypertension has led to a growing interest in renal denervation (RD) for treatment of HF (6,11–16).

SEE PAGE 282

Prior studies showed that surgical RD improves LV function and prevents HF in rats after myocardial infarction (MI) (17–19). In large animal models of rapid pacing-induced HF, catheter-based RD also reduced systemic neurohormonal activation and renal norepinephrine content, and improved LV function and dyssynchrony (20–22). Nevertheless, the long-term beneficial effects of RD on cardiac function after conventional pharmacological therapy for HF have not been addressed in these studies. Here, we hypothesized that RD reduces the myocardial and renal norepinephrine discharge and improves LV function in HF. Accordingly, we investigated the long-term effect of bilateral catheter-based RD, using a multipolar electrode catheter, on LV contractile function and regional norepinephrine release in a porcine model of mixed cardiomyopathy treated with angiotensin-converting enzyme (ACE) inhibitor and beta-blocker.

METHODS

STUDY PROTOCOL. Female farm pigs weighing 35 to 45 kg (age 9 to 12 months) were used for this study. An animal model of HF induced by myocardial ischemia and rapid pacing (MI + HF) was created as described previously (23,24). In brief, all animals underwent baseline assessment of LV function using echocardiographic and invasive hemodynamic assessments. Acute MI was induced in all animals by coronary artery embolization to the left circumflex artery, followed by 4 weeks of rapid right ventricular pacing (150 beats/min) using a VVI pacemaker to induce HF. After ventricular pacing had ceased for 24 h, repeat echocardiographic and invasive hemodynamic assessments were performed. Animals with impaired left ventricular ejection fraction (LVEF) <45% were randomized to receive medication alone (control group, n = 10) or catheter-based bilateral RD (RD group, n = 10) after invasive

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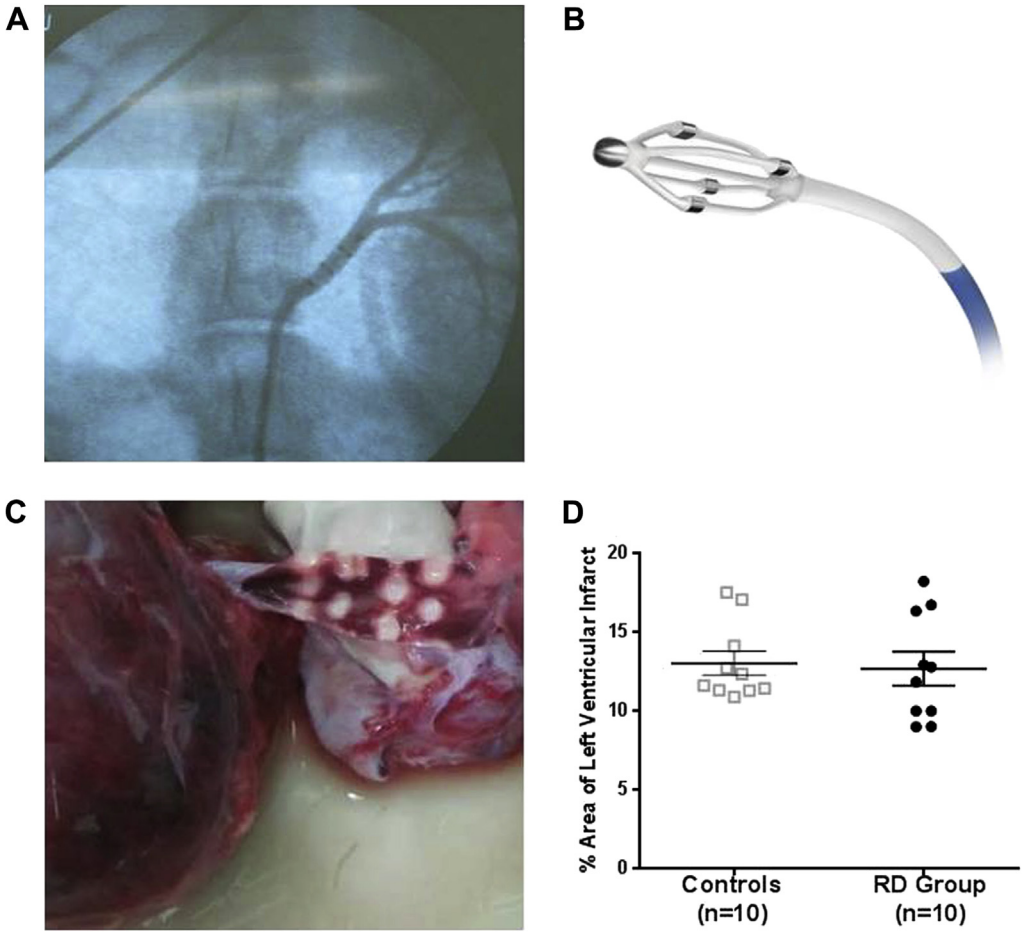
FIGURE 1 Renal Denervation Procedure and Histological Assessment



(A) Renal angiogram of left renal artery in anteroposterior view before renal denervation (RD). **(B)** A 5-F quadripolar multiple electrode array ablation catheter (EnligHTNTM, St. Jude Medical, St. Paul, Minnesota) that consists of an expandable basket with 4 electrodes was used for RD. **(C)** An example of acute gross histology after RD to show the placement of lesions inside the renal artery. **(D)** Percentage area of left ventricular infarct as measured by histological examination in the control group (n = 10) and RD group (n = 10).

hemodynamic studies and were followed for 10 weeks. During this period, all animals with or without RD were treated with daily oral metoprolol succinate (25 mg) plus ramipril (2.5 mg).

Serial echocardiographic examinations were performed at 2, 6, and 10 weeks following RD. At 10 weeks, final invasive hemodynamic studies were performed, and all animals were sacrificed for histological and immunohistochemical assessment.

Electrocardiographic parameters, including resting heart rate, corrected QT interval, and systolic and diastolic blood pressure, as well as serum biomarkers, including angiotensin II, B-type natriuretic peptide (BNP), and norepinephrine levels, were measured at baseline, immediately post-MI, and before and after 10 weeks of RD. Moreover, the regional venoarterial gradient of norepinephrine from the myocardium and kidneys was determined by measuring serum norepinephrine levels from the femoral arteries and coronary sinus and from the renal arteries and veins, respectively (7).

RENAL ANGIOGRAM AND CATHETER-BASED RD PROTOCOL. The animals were laid in a supine position following induction of anesthesia. Renal angiography was performed through a 6-F JR4 guiding catheter (Cordis Corp., Miami, Florida) via a femoral artery cutdown. Intra-arterial nitroglycerin (200 ug) was injected into the renal arteries via the guiding catheter and direct renal angiogram was performed to guide the RD procedure (Figure 1A). In both the control and RD group, catheterization was

performed to collect blood samples from the femoral arteries and coronary sinus ostium and then from the bilateral renal arteries and veins to measure the myocardial and renal veno-arterial gradient (venous-arterial levels) of norepinephrine, respectively. In the RD group, catheter-based RD was performed using a 5-F quadripolar multiple electrode array ablation catheter (EnligHTNTM, St. Jude Medical, St. Paul, Minnesota), which consists of an expandable basket with 4 electrodes that were used for endovascular delivery of radiofrequency energy into the renal arteries (**Figure 1B**). The catheter was connected to a temperature controlled radiofrequency generator that automatically controls energy delivery using a proprietary algorithm based on temperature and impedance feedback. In each main stem of the renal artery, 1 min of radiofrequency ablation energy at 4 to 10 W with a temperature limit of 60°C was performed at each electrode. This resulted in a spiral pattern of individual treatment sites spaced rotationally and longitudinally along the artery (**Figure 1C**). After RD, renal angiogram was repeated, and blood samples were taken from the bilateral renal arteries and veins to measure the renal spillover of norepinephrine, renin, and angiotensin II. After the final invasive cardiac hemodynamic assessment at 10 weeks after RD, blood samples were taken from the bilateral renal arteries and veins to measure the renal veno-arterial gradient of norepinephrine. In the control group, renal angiogram and blood sampling were performed without RD.

INVASIVE HEMODYNAMIC ASSESSMENT. Invasive hemodynamic assessment was performed during induction of MI and before and 10 weeks after RD to assess changes in LV function (21,22). In brief, a 7-F combined catheter-micromanometer (Millar Instruments, Houston, Texas) was advanced into the carotid or femoral artery to measure peripheral blood pressure and then advanced into the LV for measurement of LV pressure, maximum rate of left ventricular pressure rise ($+dP/dt_{max}$), and maximum rate of left ventricular pressure reduction ($-dP/dt_{max}$) (23,24). Moreover, dP/dt_{max} normalized to instantaneous developed pressure (IP) (dP/dt_{max}/IP) was also used to determine LV contractile function, as the pre-load condition can be altered during general anesthesia (25). Pulmonary capillary wedge pressure (PCWP) and cardiac output were measured with a Swan-Ganz catheter. Body surface area of each farm pig was calculated as previously described (26). Stroke volume index was calculated by the following formula:

$$\text{stroke volume index} = (\text{cardiac output})/(\text{heart rate} \times \text{body surface area})$$

ECHOCARDIOGRAPHIC MEASUREMENTS. Standard transthoracic echocardiograms including 2-dimensional and M-mode imaging were performed using a commercially available echocardiographic system (Vivid i, GE Vingmed, Horten, Norway) equipped with a 3-9 MHz transducer. In each animal, standard 2-dimensional and M-mode echocardiograms were used to measure the LV volume. To obtain the LV volume using M-mode echocardiograms, the maximum minor axis of the LV at end-diastole and -systole were measured on the parasternal long- or short-axis view, on the assumption that the LV is a spheroid at the parasternal long- and short-axis views, to calculate LVEF (24). All echocardiographic measurements were interpreted off-line in a blinded fashion by another independent operator using a computer workstation (GE Medical, EchoPac, Horten, Norway). The intra-observer variability of the measurement of LVEF based on M-mode measurement was 4% using 20 repeated random measurements.

BIOMARKERS ASSESSMENT. Serum porcine angiotensin II (Peninsula Laboratories, San Carlos, California), BNP (Phoenix Pharmaceuticals, Burlingame, California), and norepinephrine (Rocky Mountain Diagnostics, Boulder, Colorado) levels were measured in peripheral arterial blood using enzyme-linked immunosorbent assay kits to assess HF status and peripheral neurohormonal activation. For the measurement of norepinephrine level, all blood samples were placed in ice immediately after collection followed by extraction. Then, all the extracted samples were stored at −80°C, and were measured in a single batch with calibration using standard control samples. The coefficients of variation for angiotensin II, BNP, and norepinephrine were 8.8%, 7.9%, and 11.2%, respectively, based on repeated measurements in 20 random samples. Femoral arterial and coronary sinus blood samples and renal arterial and venous blood samples were also obtained in the control and RD groups to measure myocardial and kidney norepinephrine veno-arterial gradients, respectively, at MI + HF status and at 10-week follow-up. Coronary sinus blood sampling was performed by using a 6-F JR4 guiding catheter (Cordis Corp.) positioned at the mid-portion of the coronary sinus. Moreover, serial measurement of serum creatinine level was measured as the assessment of kidney function before and after RD.

HISTOLOGY AND IMMUNOHISTOCHEMICAL ASSESSMENT. The animals were sacrificed at 10-week follow-up after RD. After euthanasia, the heart was cut into 8 sections (7-to 10-mm thick) perpendicular to the apical-basal axis. The sections of the LV were traced, and color photographs of each section were obtained

to serve as a permanent record. Planimetry of the tracings was performed to measure the size of infarction (as a percentage of the LV mass) (23,24). Detailed histological and immunohistochemical assessments of the renal arteries were performed to determine the degree of denervation after catheter-based RD. After 10 weeks of RD, and while under general anesthesia after hemodynamic assessment and renal angiogram, the pigs' abdominal aorta and kidneys were rapidly harvested. The renal arteries were dehydrated in ascending concentrations of alcohol and embedded in paraffin. Transverse sections of the renal artery were made every 5 to 8 mm from its origin up to just distal to the main bifurcation of the renal artery, resulting in 3 sectioning levels (proximal, mid, and distal segments). On each level, 5-μm serial sections were cut, and per artery, all sectioning levels were mounted on a single glass slide. The sections were immunohistochemically stained for tyrosine hydroxylase (TH), which are markers for sympathetic nerve fibers. Endogenous peroxidase was quenched using 0.03% H_2O_2 for 30 min, and then the sections were incubated with a rabbit anti-TH primary antibody (1:1,000 dilution) (Merck Millipore, Temecula, California) followed by a horseradish peroxidase–conjugated antirabbit secondary antibody (1:500 dilution) (DAKO, Glostrup, Demark) as previously described (27). For amplification of the signal, a tyramide signal amplification kit (Perkin Elmer, Waltham, Massachusetts) was applied. The sections were incubated in the fluorogenic substrate Alexa Fluor 488 tyramide (1:100) (Life Technologies, Eugene, Oregon) for 10 min and then coverslipped with mounting medium with DAPI (Sigma-Aldrich, St. Louis, Missouri). Image analysis was undertaken using a fluorescent microscope (Leica DMLB2, Leica, Wetzlar, Germany). The percentage of TH area staining from each image was quantified with Image J software (National Institutes of Health). For each slide, 5 regions were randomly selected and examined at ×10 magnification. All measurements were counted in a blinded fashion.

STATISTICAL ANALYSIS. All data are expressed as mean ± SEM, and analysis was performed using SPSS software (SPSS Inc., Chicago, Illinois). Comparisons of parameters between different groups before and after RD were made using the Mann-Whitney *U* test or 1-way analysis of variance with Tukey's test, as appropriate. Serial changes in echocardiographic and invasive LV hemodynamic parameters and biomarkers at different time points were compared by 1-way repeated analysis of variance with Tukey's test. Statistical significance was defined at a value of $p < 0.05$.

RESULTS

A total of 26 pigs with MI and impaired LVEF documented on echocardiogram underwent rapid ventricular pacing to induce HF, and 20 animals who survived after induction of MI + HF were studied. These animals were randomized to the control group (n = 10) and the RD group (n = 10). Histological examination demonstrated that there were no significant differences in the LV infarct size between the 2 groups (**Figure 1D**).

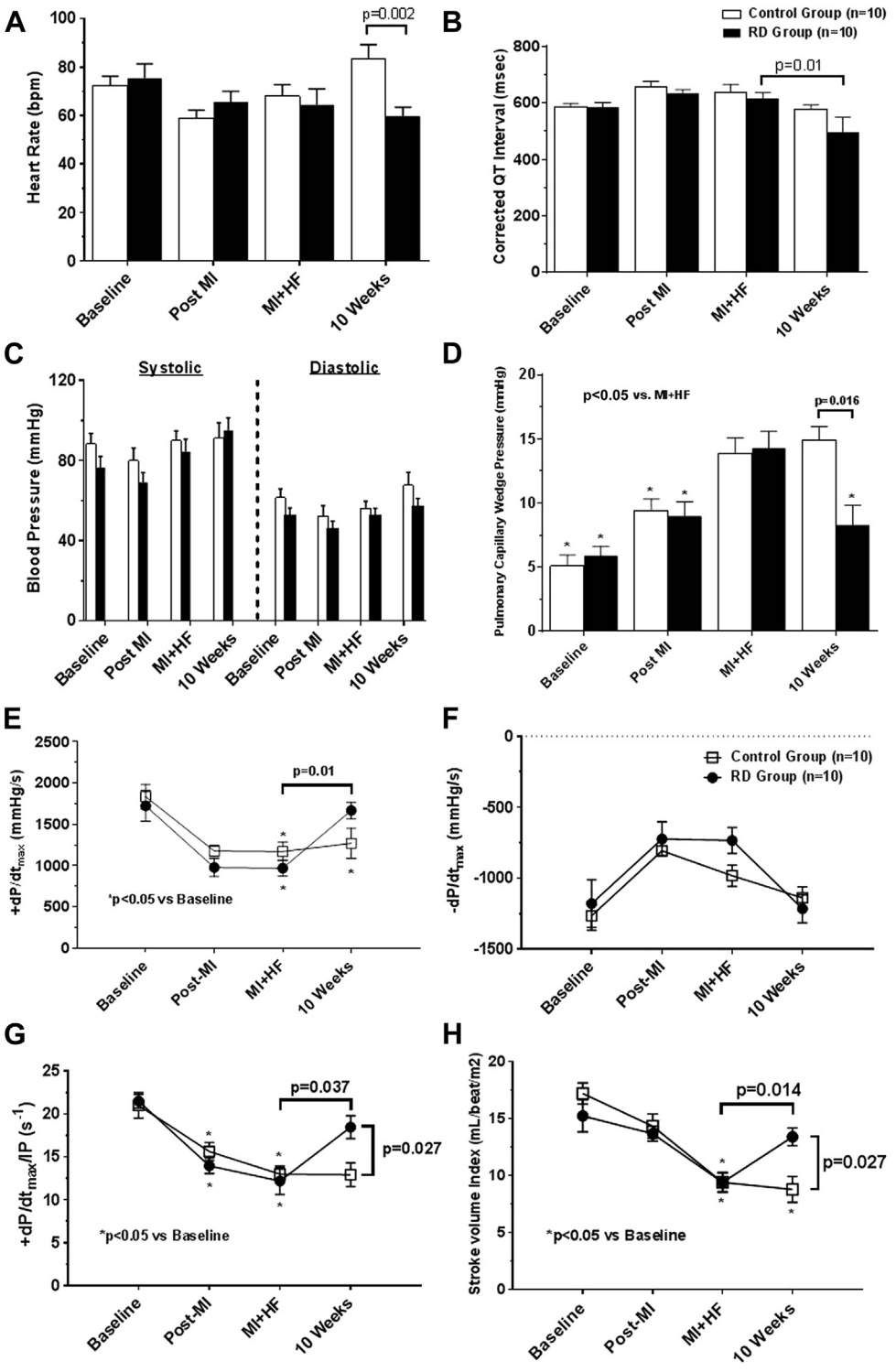
ELECTROCARDIOGRAPHIC DATA. There were no significant differences in the resting heart rate (**Figure 2A**) and corrected QT interval (**Figure 2B**) at baseline, after MI, and during MI + HF among the control and the RD groups (all $p > 0.05$). However, the resting heart rate was significantly lower and the corrected QT interval was shorter in the RD group at 10 weeks compared with the control group (**Figures 2A and 2B**) ($p < 0.05$).

HEMODYNAMIC DATA. There were no significant differences in the systolic and diastolic blood pressure (**Figure 2C**) at baseline, after MI, during MI + HF, and at 10 weeks between the control and the RD groups (all $p > 0.05$). Similarly, there were no significant differences in the systolic and diastolic blood pressure at baseline, after MI, during MI + HF, and at 10-week follow-up in each group. The PCWP was significantly elevated during MI + HF compared with baseline and after MI in both groups. Moreover, in the RD group, the PCWP was significantly lower at 10 weeks compared with during MI + HF and the control group at 10 weeks (**Figure 2D**).

Compared with baseline, LV systolic contractile function as determined by $+dP/dt_{max}$, $+dP/dt_{max}$/IP, and stroke volume index significantly decreased after MI and during MI + HF in the control and RD groups, but there were no differences in $-dP/dt_{max}$ (**Figures 2E to 2H**). In the control group, there was no change in $+dP/dt_{max}$, dP/dt_{max}/IP, and stroke volume index at 10 weeks compared with MI + HF ($p > 0.05$). Contrary to this, $+dP/dt_{max}$, dP/dt_{max}/IP, and stroke volume index significantly increased at 10 weeks in the RD group compared with their MI + HF status (**Figures 2E, 2G, and 2H**) (all $p < 0.05$). Moreover, dP/dt_{max}/IP and stroke volume index were also significantly higher at 10 weeks in the RD group compared with the control group (**Figures 2G and 2H**) (all $p < 0.05$).

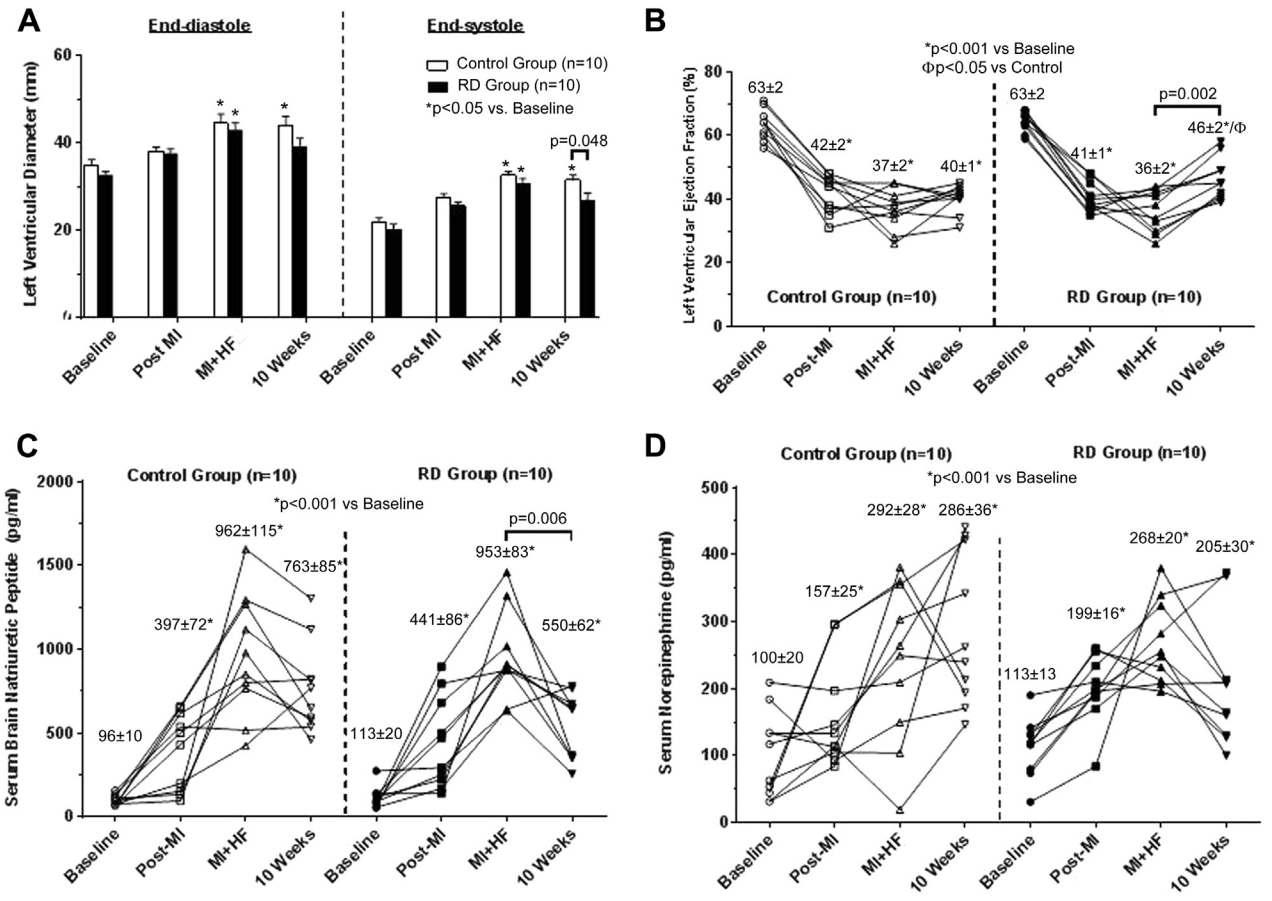
ECHOCARDIOGRAPHIC DATA. After pacing was turned off for 24 h, echocardiogram showed that LVEF decreased from 65.3 ± 0.9% at baseline to 38.7 ± 2.1% after induction of MI + HF ($p < 0.001$). As

FIGURE 2 Hemodynamic Assessment



Serial changes in **(A)** resting heart rate; **(B)** corrected QT interval; **(C)** systolic and diastolic blood pressure; **(D)** pulmonary capillary wedge pressure; **(E)** + maximum rate of left ventricular pressure rise (dP/dt_{max}); **(F)** −dP/dt_{max}; **(G)** +dP/dt_{max} normalized to instantaneous developed pressure (IP) (dP/dt_{max}/IP); and **(H)** stroke volume index at baseline, post myocardial infarction (post-MI), after induction of heart failure (MI + HF), and at 10-week follow-up in the control group (n = 10) and renal denervation (RD) group (n = 10).

FIGURE 3 Echocardiographic and Neurohormonal Parameters



Serial changes in **(A)** left ventricular (LV) end-diastolic and -systolic diameter; **(B)** LV ejection fraction; and serum levels of **(C)** brain natriuretic peptide and **(D)** peripheral blood norepinephrine at baseline, post-MI, after MI + HF, and at 10-week follow-up in the control group (n = 10) and RD group (n = 10). **(E)** Changes in the venoarterial gradients of norepinephrine over the myocardium and left and right kidney during MI + HF and at 10-week follow-up in the control group (n = 10) and RD group (n = 10). Serial changes in serum levels of **(F)** angiotensin II and **(G)** creatinine at baseline, post-MI, after MI + HF, and at 10-week follow-up in the control group (n = 10) and RD group (n = 10). Abbreviations as in Figure 2.

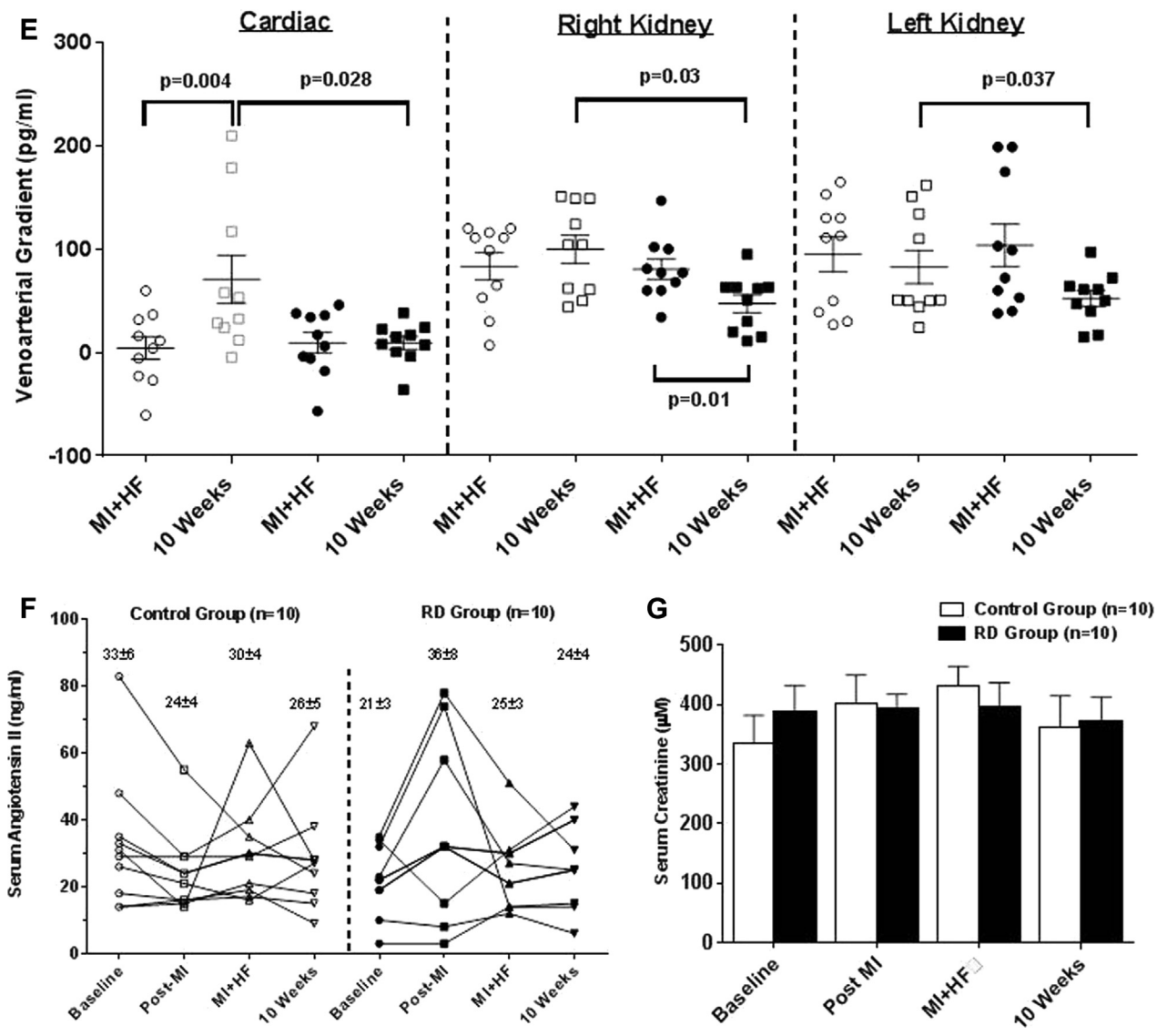
Continued on the next page

shown in Figure 3A, maximum minor axis of the LV at end-diastole and -systole during MI + HF was significantly increased compared with baseline in both groups. At 10 weeks, the LVDs were smaller in the RD group compared with the control group ($p < 0.05$).

There were no significant differences in LVEF at baseline, after MI, and during MI + HF between the control and the RD groups (Figure 3B) ($p > 0.05$). Serial echocardiogram showed no changes in LVEF at 10 weeks compared with MI + HF in the control group (Figure 3A) ($p > 0.05$). However, LVEF was significantly increased at 10 weeks in the RD group compared with during MI + HF, and was higher than the control group at 10 weeks (Figure 3B) ($p < 0.05$).

BIOMARKER DATA. Serial changes in serum BNP (Figure 3C) and norepinephrine (Figure 3D) in the control and RD groups showed similar trends: their levels progressively increased after induction of MI + HF compared with baseline ($p < 0.05$). At 10 weeks, the serum BNP was significantly lower compared with MI + HF in the RD group (Figure 3C) ($p < 0.05$), but not in the control group ($p > 0.05$). However, there was no difference in the serum BNP level between the control and RD groups at 10 weeks. At 10 weeks, the serum norepinephrine level was still persistently elevated in both groups compared with their baseline ($p < 0.05$), and there was no significant difference between the control and RD group ($p > 0.05$).

FIGURE 3 Continued



Interestingly, the cardiac veno-arterial gradient of norepinephrine was significantly elevated in the control group at 10 weeks compared with during MI + HF ($p < 0.05$), but not in the RD group ($p > 0.05$) (Figure 3E). Moreover, the cardiac veno-arterial gradient of norepinephrine was significantly elevated in the control group at 10 weeks compared with the RD group (Figure 3E) ($p < 0.05$). Both the right and left kidney veno-arterial gradient of norepinephrine remained unchanged at 10 weeks compared with during MI + HF in the control group (Figure 3E) ($p > 0.05$). However, the right and left kidney veno-arterial gradient of norepinephrine was lower at 10 weeks compared with during MI + HF in the RD group (Figure 3E) ($p < 0.05$ for right kidney). Furthermore, the right and left kidney veno-arterial gradient of norepinephrine was significantly lower in the RD group at 10 weeks compared with the control group (Figure 3E) ($p < 0.05$). Taken together, these findings show that RD prevents the increasing myocardial norepinephrine gradient and reduces the renal norepinephrine gradient in HF.

There were no significant changes in serum angiotensin II (Figure 3F) and creatinine (Figure 3G) levels at baseline, after MI, during MI + HF, and at 10 weeks in both groups (all $p > 0.05$). Moreover, there were also no significant differences in serum angiotensin II and creatinine levels at baseline, after MI,

during MI + HF, and at 10 weeks between the control and the RD groups (Figures 3F and 3G) (all $p > 0.05$).

HISTOLOGY AND IMMUNOHISTOCHEMICAL DATA. Histological examination demonstrates that catheter-based RD denervated the renal sympathetic nerves around the renal arteries (Figure 4A), and semi-quantitative examination revealed a similar percentage area of TH positive sympathetic nerves at the proximal, mid, and distal segment of renal arteries in the control group (Figure 4B). Moreover, in the RD group, the percentage area of TH-positive sympathetic nerves at the proximal, mid, and distal segments of renal arteries was significantly lower than in the control group (Figure 4B) ($p < 0.05$). These results demonstrate that catheter-based RD significantly denervated the renal sympathetic nerves around the renal arteries.

DISCUSSION

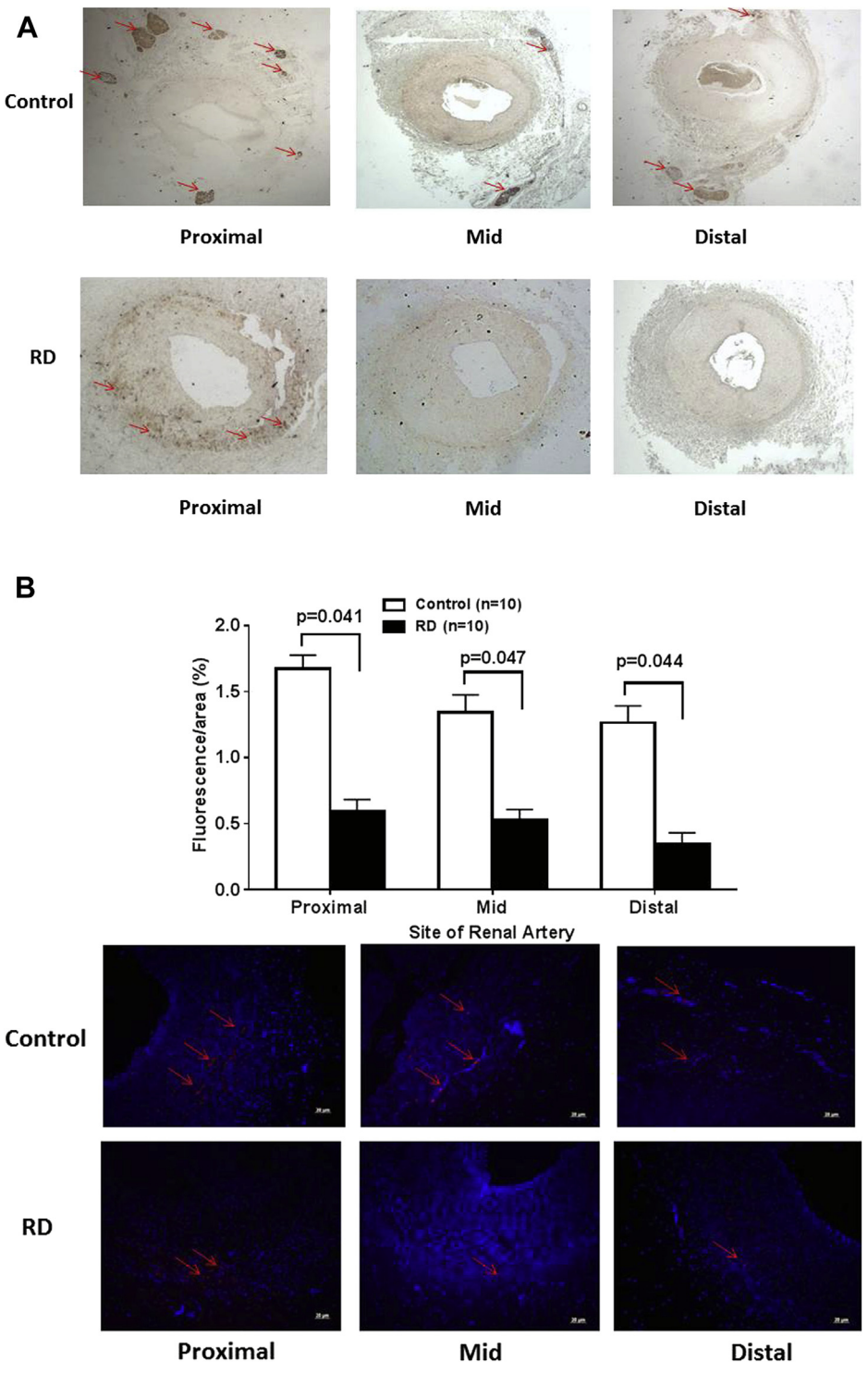
In this study, we investigated the long-term effects of bilateral RD using a catheter-based multielectrode catheter in a porcine model of HF induced by MI and rapid pacing. Our results showed that the catheter-based multielectrode catheter can effectively denervate renal sympathetic nerves to prevent elevation of myocardial norepinephrine gradient, reduce renal norepinephrine gradient, and improve LV function, as measured by serial echocardiographic measurement of LVEF and invasive hemodynamic assessments of dP/dt_{max}/IP and stroke volume index, on top of conventional pharmacological therapies with ACE inhibitor and β-blocker in HF. However, there was no significant change in serum angiotensin II and creatinine level after RD.

Catheter-based RD has emerged as a non-pharmacological approach for treatment of refractory hypertension via decreasing sympathetic activities (6,11–14). In HF, sympathoexcitation is associated with increased mortality and progression of LV dysfunction (3,4). As a result, RD has been proposed as a novel therapy for HF via suppression of sympathetic activities. Prior experimental studies (17–19) showed that surgical RD can prevent adverse LV remodeling after MI in rats. In an ovine model of pacing-induced HF, short-term catheter-based RD reduced renal norepinephrine content (22). Moreover, long-term catheter-based RD has also been shown to reduce serum BNP, angiotensin II, and aldosterone levels and improve LV function in a canine model of pacing-induced HF (20,21). Nevertheless, the potential effect of RD in HF after optimal evidence-based pharmacological therapies and the extent of RD after the procedure were not addressed in these studies. Moreover, the effect of RD on sympathetic overactivation in HF remains unclear.

In this study, we confirm that catheter-based RD can improve LV function in a clinically relevant large-animal model of MI with HF. Our results demonstrated that bilateral RD reduced PCWP and improved LV contractile function, as measured by echocardiogram and detailed invasive hemodynamic assessment. Moreover, reverse remodeling of the LV dimensions, as shown by significant reduction of LV end-systolic and -diastolic diameters after RD compared with control, were observed. However, a major concern about the use of RD in HF is the risk of hypotension and kidney injury after the procedure. In contrast to previous experimental studies (20–22), we did not observe any significant change in blood pressure or angiotensin II level after RD. This may be attributed to the concomitant use of ACE inhibitors in this study, and thus, the effect of RD on blood pressure via the suppression of the renin-angiotensin-aldosterone system is masked. Indeed, recent human pilot studies on RD in HF patients treated with ACE inhibitors or angiotensin II blockers also demonstrated no significant change in blood pressure after the procedure (28). However, it is also possible that the blood pressure response to RD involves a more complex mechanism in which the beneficial effects on HF are mediated through attenuation of adverse neurohormonal feedback loops, such as regional changes in the norepinephrine gradient, as observed in this study. In this study, the lack of significant differences in serum norepinephrine and angiotensin II between the control and RD groups might also be related to the use of ACE inhibitors and beta-blockers, albeit at relatively low doses. Importantly, we did not observe any significant change in kidney function as measured by the serum creatinine level after RD. Therefore, our data provide further reassurance to support the safety of RD in HF.

One of the important findings of this study is that effective bilateral RD, as confirmed by histological examination, is associated with the prevention of elevation of myocardial norepinephrine gradient as observed in the control group, and significantly reduced the renal norepinephrine gradient in HF. In the control group, treatment with ACE inhibitors and β-blockers was associated with a reduction in the peripheral but not coronary sinus and renal norepinephrine levels. These findings suggest persistent sympathetic overactivation in the heart and kidney despite conventional pharmacological therapies for HF. In contrast, decreased cardiac and renal norepinephrine gradients were observed after

FIGURE 4 Histological Assessment of Renal Denervation



(A) Representative histological image of the renal sympathetic nerve as determined by tyrosine hydroxylase (TH) staining **(red arrows)** to show its distribution at the proximal, mid, and distal segments of renal arteries in the control and renal denervation (RD) groups. **(B)** Histological examination on the renal sympathetic nerve as determined by the immunofluorescence staining of TH-positive nerve in the control and RD groups. **(Top)** The percent area of TH-positive nerve at the proximal, mid, and distal segments of the renal arteries in the control and RD groups. **(Bottom)** The representative image of the immunofluorescence staining of TH-positive nerve **(red arrows)** at the proximal, mid, and distal segments of the renal arteries in the control and RD groups.

bilateral RD. It is possible that elimination of renal afferent and efferent sympathetic nerves reduces not only the renal sympathetic activation as shown in previous studies (22), but also the central sympathetic neural outflow to decrease cardiac norepinephrine gradient.

STUDY LIMITATIONS. First, there is a potential effect of renal nerve reinnervation after RD, as observed in recent experimental studies (28). Second, the direct causal relationships between the change in cardiac and renal norepinephrine gradient and improvement in LV function remain unclear. Third, the doses of ACE inhibitors and beta-blockers were low without titration. Therefore, our results cannot be extrapolated to include patients with truly maximally tolerated medical therapies. Final, the renal excretion of norepinephrine in the urine was not measured in this study due to the difficulty in collecting 24-h urine in pigs. Therefore, the overall changes in the kidney norepinephrine levels could not be determined, as a substantial portion of norepinephrine is excreted from the kidney.

CONCLUSIONS

Our findings provide further evidence to support the application of catheter-based RD for treatment of HF. Several ongoing clinical studies will assess the long-term safety and efficacy of catheter-based RD in HF (15,29).

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PERSPECTIVES

COMPETENCY IN MEDICAL KNOWLEDGE: RD has been proposed as novel therapy for treatment of HF. In a preclinical large-animal model of HF, we have demonstrated that successful bilateral RD using catheter-based radiofrequency ablation decreased myocardial and renal norepinephrine gradients and improved LV contractile function on top of conventional medical therapies with ACE inhibitor and beta-blocker.

TRANSLATIONAL OUTLOOK: Our findings provide further support for future clinical investigation on the use of catheter-based RD for treatment of HF.

REFERENCES

1. Bui AL, Horwich TB, Fonarow GC. Epidemiology and risk profile of heart failure. Nat Rev Cardiol 2011;8:30–41.

2. Roger VL. Epidemiology of heart failure. Circ Res 2013;113:646–59.

3. Floras JS. Sympathetic nervous system activation in human heart failure: clinical implications of an updated model. J Am Coll Cardiol 2009;54:375–85.

4. Lymperopoulos A, Rengo G, Koch WJ. Adrenergic nervous system in heart failure: pathophysiology and therapy. Circ Res 2013;113:739–53.

5. Booth LC, May CN, Yao ST. The role of the renal afferent and efferent nerve fibers in heart failure. Front Physiol 2015;6:270.

6. Madanieh R, El-Hunjul M, Alkhawam H, Kosmas CE, Madanieh A, Vittorio TJ. A perspective on sympathetic renal denervation in chronic congestive heart failure. Heart Fail Rev 2016;21:1–10.

7. Hasking GJ, Esler MD, Jennings GL, Burton D, Johns JA, Korner PI. Norepinephrine spillover to plasma in patients with congestive heart failure: evidence of increased overall and cardiorenal sympathetic nervous activity. Circulation 1986;73:615–21.

8. Petersson M, Friberg P, Eisenhofer G, Lambert G, Rundqvist B. Long-term outcome in relation to renal sympathetic activity in patients with chronic heart failure. Eur Heart J 2005;26:906–13.

9. May CN, Yao ST, Booth LC, Ramchandra R. Cardiac sympathoexcitation in heart failure. Auton Neurosci 2013;175:76–84.

10. Sobotka PA, Mahfoud F, Schlaich MP, Hoppe UC, Böhm M, Krum H. Sympatho-renal axis in chronic disease. Clin Res Cardiol 2011;100:1049–57.

11. Bhatt DL, Kandzari DE, O'Neill WW, et al. A controlled trial of renal denervation for resistant hypertension. N Engl J Med 2014;370:1393–401.

12. Mircheva M, Neuhofer W, Popa C, Krämer BK, Heitzmann D. Renal denervation for resistant hypertension. Lancet 2015;386:1239–40.

13. Mahfoud F, Böhm M, Azizi M, et al. Proceedings from the European clinical consensus conference for renal denervation: considerations on future clinical trial design. Eur Heart J 2015;36:2219–27.

14. Chatterjee NA, Singh JP. Novel interventional therapies to modulate the autonomic tone in heart failure. J Am Coll Cardiol HF 2015;3:786–802.

15. Tsioufis C, Papademetriou V, Dimitriadis K, et al. Effects of multielectrode renal denervation on cardiac and neurohumoral adaptations in resistant hypertension with cardiac hypertrophy: an EnligHTN I substudy. J Hypertens 2015;33:346–53.

16. Tsioufis C, Papademetriou V, Tsiachris D, et al. Drug-resistant hypertensive patients responding to multielectrode renal denervation exhibit improved heart rate dynamics and reduced arrhythmia burden. J Hum Hypertens 2014;28:587–93.

17. Nozawa T, Igawa A, Fujii N, et al. Effects of long-term renal sympathetic denervation on heart failure after myocardial infarction in rats. Heart Vessels 2002;16:51–6.

18. Hu J, Li Y, Cheng W, et al. A comparison of the efficacy of surgical renal denervation and pharmacologic therapies in post-myocardial infarction heart failure. PLoS One 2014;9:e96996.

19. Hu J, Yan Y, Zhou Q, et al. Effects of renal denervation on the development of post-myocardial infarction heart failure and cardiac autonomic nervous system in rats. Int J Cardiol 2014;172:e414–6.

20. Zhao Q, Huang H, Wang X, et al. Changes of serum neurohormone after renal sympathetic denervation in dogs with pacing-induced heart failure. Int J Clin Exp Med 2014;7:4024–30.

21. Hu W, Zhao QY, Yu SB, et al. Renal sympathetic denervation inhibits the development of left ventricular mechanical dyssynchrony during the

progression of heart failure in dogs. Cardiovasc Ultrasound 2014;12:47.

22. Booth LC, Schlaich MP, Nishi EE, et al. Short-term effects of catheter-based renal denervation on cardiac sympathetic drive and cardiac baroreflex function in heart failure. Int J Cardiol 2015;190:220–6.

23. Liao SY, Siu CW, Liu Y, et al. Attenuation of left ventricular adverse remodeling with epicardial patching after myocardial infarction. J Card Fail 2010;16:590–8.

24. Liao SY, Liu Y, Zuo M, et al. Remodelling of cardiac sympathetic re-innervation with thoracic spinal cord stimulation improves left ventricular function in a porcine model of heart failure. Europace 2015;17:1875–83.

25. Chakir K, Daya SK, Aiba T, et al. Mechanisms of enhanced beta-adrenergic reserve from cardiac resynchronization therapy. Circulation 2009;119:1231–40.

26. Swindle MM, Makin A, Herron AJ, et al. Swine as models in biomedical research and toxicology testing. Vet Pathol 2012;49:344–56.

27. Booth LC, Nishi EE, Yao ST, et al. Reinnervation of renal afferent and efferent nerves at 5.5 and 11 months after catheter-based radiofrequency renal denervation in sheep. Hypertension 2015;65:393–400.

28. Davies JE, Manisty CH, Petraco R, et al. First-in-man safety evaluation of renal denervation for chronic systolic heart failure: primary outcome from REACH-Pilot study. Int J Cardiol 2013;162:189–92.

29. McArdle MJ, deGoma EM, Cohen DL, Townsend RR, Wilensky RL, Giri J. Beyond blood pressure: percutaneous renal denervation for the management of sympathetic hyperactivity and associated disease states. J Am Heart Assoc 2016;4:e001415.

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