



Renal Sympathetic Denervation Protects the Failing Heart Via Inhibition of Nephilysin Activity in the Kidney

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ABSTRACT

BACKGROUND Sustained sympathetic activation contributes to the progression of myocardial cell injury, cardiac fibrosis, and left ventricular (LV) dysfunction in heart failure (HF).

OBJECTIVES This study investigated the effects of radiofrequency renal nerve denervation (RF-RDN) on the pathobiology of HF and the interaction between the renal sympathetic nerves and natriuretic peptide (NP) metabolism.

METHODS Spontaneously hypertensive rats (SHR) and normotensive Wistar-Kyoto rats (WKY) were subjected to 45 min of coronary artery ligation and reperfusion for 12 weeks. At 4 weeks post-reperfusion, SHR and WKY underwent either bilateral RF-RDN or sham-RDN.

RESULTS Following RF-RDN in both strains, LV ejection fraction remained significantly above those levels in respective sham-RDN rats, and at the end of the 12-week study, rats in both strains had significantly reduced LV fibrosis and improved vascular function. RF-RDN therapy significantly improved vascular reactivity to endothelium-dependent and -independent vasodilators as well as vascular compliance in the setting of severe HF. Improvements in LV function were accompanied by significant elevations in circulating NP as compared to those associated with sham-RDN. Further investigation into the cause of increased circulating NP levels demonstrated that RF-RDN significantly inhibited renal neprilysin activity in SHR and WKY with HF. Likewise, chronic treatment with the beta₁ antagonist bisoprolol inhibited renal neprilysin activity and increased circulation NP levels in WKY with HF.

CONCLUSIONS This study identifies a novel endogenous pathway by which the renal nerves participate in the degradation of cardioprotective NP. Furthermore, removal of the influence of the renal nerves on kidney function attenuates renal neprilysin activity, augments circulating NP levels, reduces myocardial fibrosis, and improves LV function in the setting of HF. (J Am Coll Cardiol 2017;70:2139-53) © 2017 by the American College of Cardiology Foundation.

Heart failure (HF) continues to grow as an inefficiently managed health care burden that affects nearly 6 million people in the United States alone, which is projected to double by 2030 (1). In HF, activation of the sympathetic nervous system contributes to the progression of myocardial

dysfunction (2,3) and current HF pharmacotherapies that target the autonomic nervous system (i.e., beta-blockers and renin-angiotensin system [RAS] inhibitors) reduce morbidity and mortality (4-7). However, the many adverse effects and patient nonadherence rates limit optimal use of these drugs (8).



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ABBREVIATIONS AND ACRONYMS

Ach	= acetylcholine
ANP	= atrial natriuretic peptide
BNP	= B-type natriuretic peptide
CNP	= C-type natriuretic peptide
HF	= heart failure
LV	= left ventricular
LVEF	= left ventricular ejection fraction
mRNA	= messenger RNA
NE	= norepinephrine
NP	= natriuretic peptide
RAS	= renin-angiotensin system
RDN	= renal denervation
RF	= radiofrequency
SHR	= spontaneously hypertensive rat
SNP	= sodium nitroprusside
TH	= tyrosine hydroxylase
WKY	= Wistar-Kyoto

Alternative interventional therapeutic approaches are under development to replace or supplement contemporary pharmacologic strategies for the treatment of HF (9). Renal denervation (RDN) is a minimally invasive endovascular procedure whereby radiofrequency (RF) energy is used to ablate the sympathetic nerves that traverse the renal artery (10). Originally developed for the treatment of resistant hypertension, mixed clinical trial results have sparked debate over whether this strategy effectively lowers blood pressure in hypertensive patients (10-13). Despite this debate, it is likely that RF-RDN has beneficial effects on cardiovascular function beyond lowering blood pressure. In particular, RF-RDN may have cardioprotective effects by interrupting pathological afferent and central reflex mechanisms that contribute to the progression of HF.

Numerous clinical trials in HF patients report that beta-blockers augment cardioprotective and vasculoprotective circulating natriuretic peptide (NP) levels by some unidentified mechanism(s) (14-19). Beta₁-adrenergic receptors are located in the kidneys and are innervated by renal sympathetic nerves. Therefore, we propose that in HF, elevated renal nerve activity opposes the beneficial renal, vascular, and cardiac protective effects of NP by a pathway involving enhanced metabolism of NP.

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Therefore, the aim of these investigations was to test the efficacy of RF-RDN as a treatment of HF and to determine the effects of this procedure on the synthesis and metabolism of NP. For these studies, changes in cardiac and vascular function and NP levels produced by bilateral RF-RDN were examined in spontaneously hypertensive rats (SHR) or normotensive Wistar-Kyoto (WKY) rats with HF.

METHODS

Male SHR and male WKY 19 to 20 weeks of age (Charles River Laboratories, Wilmington, Massachusetts) were used in the study (Online Appendix). Myocardial ischemia reperfusion was performed as previously described (19). Following 45 min of ischemia, rats were reperfused for 12 weeks and assessed for cardiac remodeling and failure. The experimental protocol is described in Figure 1.

SHR and WKY were randomized into either a RF-RDN or sham-RDN group 4 weeks after ischemia

reperfusion using 2-h plasma cardiac troponin I concentrations to ensure equal initial cardiac injury and myocardial cell death between groups. RF-RDN was performed as previously described (19,20).

Additional SHR (n = 4 to 5 per group) and WKY (n = 7 per group) at 19 weeks of age were implanted with a radio telemetry transmitter (Data Sciences International, St. Paul, Minnesota) for measurement of blood pressure and heart rate as previously described (19).

ECHOCARDIOGRAPHY. Long-axis, 2-dimensional echocardiography was performed at baseline, 1-, 2-, 4-, 6-, 8-, 10-, and 12-week time points on a Vevo 2100 (VisualSonics, Toronto, Ontario, Canada) (Online Appendix).

PLASMA NOREPINEPHRINE MEASUREMENT. At the 12-week endpoint, plasma was collected and catecholamine levels were measured using enzyme-linked immunosorbent assay technique according to the manufacturer's recommendations (Abnova Corp., Taipei, Taiwan).

PLASMA RENIN MEASUREMENT, ANGIOTENSIN II MEASUREMENT. See the Online Appendix.

TYROSINE HYDROXYLASE STAINING OF RENAL ARTERIES. At 8 weeks following sham-RDN or RF-RDN (12 weeks after reperfusion), renal arteries distal to the RF lesion were excised, fixed, sectioned, stained, and scored as previously described (19).

MYOCARDIAL NITRITE LEVELS. Nitrite concentrations were quantified as previously described (21) using an automated ion chromatography system (ENO30 Analyzer, Eicom, San Diego, California).

RIBONUCLEIC ACID ISOLATION AND REVERSE TRANSCRIPTASE REAL-TIME POLYMERASE CHAIN REACTION, NATRIURETIC PEPTIDE QUANTIFICATION. See the Online Appendix.

NEPRILYSIN ACTIVITY ASSAY. Neprilysin enzyme activity was determined as previously described (22) (Online Appendix).

MYOCARDIAL FIBROSIS. Hearts were collected at the 12-week endpoint, tissues were paraffin embedded and stained with either Masson trichrome or picrosirius red (Online Appendix).

EX VIVO AORTIC VASCULAR REACTIVITY EXPERIMENTS. See the Online Appendix.

BISOPROLOL PREPARATION AND ADMINISTRATION. WKY rats received 1 mg/kg/day bisoprolol in the drinking water. This dose was selected based on previous rat studies to ensure cardiac improvements and heart rate reduction (23). Prior to administration, drinking studies were performed to calculate

appropriate drug concentrations in the drinking water. Bisoprolol fumarate was provided by DC Chemicals (Shanghai, China, DCAPI1411).

SACUBITRIL PREPARATION AND ADMINISTRATION. WKY rats received 37 mg/kg/day sacubitril in the drinking water. Prior to administration, drinking studies were performed to calculate appropriate drug concentrations in the drinking water, and 37 mg/kg/day was chosen based on previous rat studies (24,25). Sacubitril (AHU-337 hemicalcium salt, DC Chemicals DC8735) was dissolved in 0.5% ethanol. Animals that did not receive sacubitril were administered 0.5% ethanol in the drinking water.

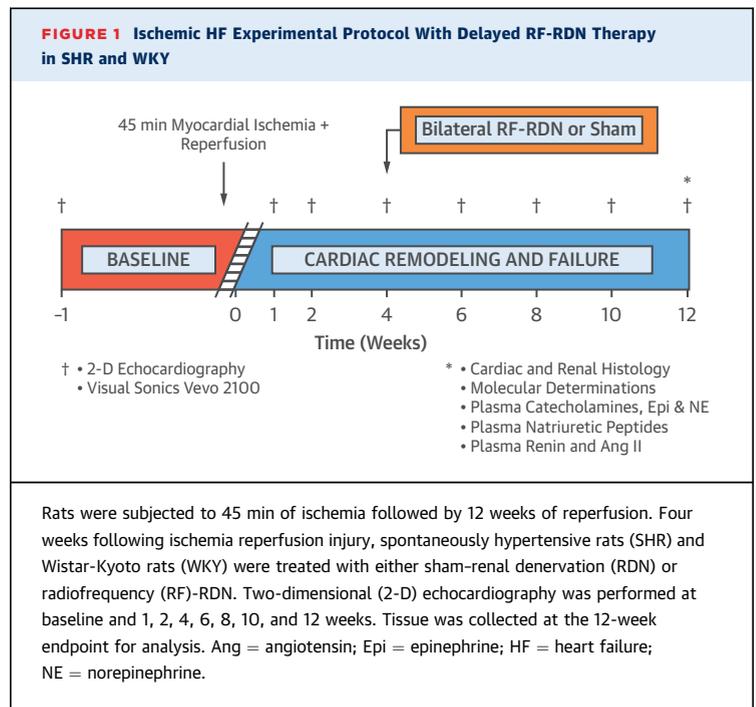
VASCULAR COMPLIANCE. See the [Online Appendix](#).

STATISTICAL ANALYSIS. All data in this study are expressed as the mean ± SEM. Differences in data between the groups were compared using Prism 6 (GraphPad Software, San Diego, California) with Student unpaired, 2-tailed *t* test when only 2 groups were compared at a single time point. A repeated measure 2-way analysis of variance with matched values and Bonferroni post-test were used for blood pressure, heart rate, and echocardiography analysis. Mann-Whitney *U* tests were used for ranked histological analysis. All *p* values of <0.05 were considered statistically significant. A limitation of the multiple comparison analyses used in this study is an increased risk of false-positive results.

RESULTS

RENAL ARTERY NERVE VIABILITY AND NOREPINEPHRINE LEVELS IN SHR IN HF FOLLOWING RF-RDN. We used renal artery sympathetic nerve tyrosine hydroxylase (TH) staining as an index of renal sympathetic nerve activity and viability in animals receiving sham-RDN or RF-RDN. Renal artery nerve TH staining at 8 weeks following RF-RDN or sham-RDN in SHR is reported in [Figure 2](#). Representative photomicrographs ([Figures 2A to 2D](#)) demonstrate significantly reduced renal nerve viability following RF-RDN as compared to sham-RDN procedures. These data are summarized in [Figure 2E](#). Kidney and plasma norepinephrine (NE) levels were measured as a biomarker for sympathetic outflow in SHR at 8 weeks following RF-RDN or sham-RDN. There was a significant reduction in kidney and circulating NE following RF-RDN compared with the sham-RDN in SHR ([Figures 2F and 2G](#)).

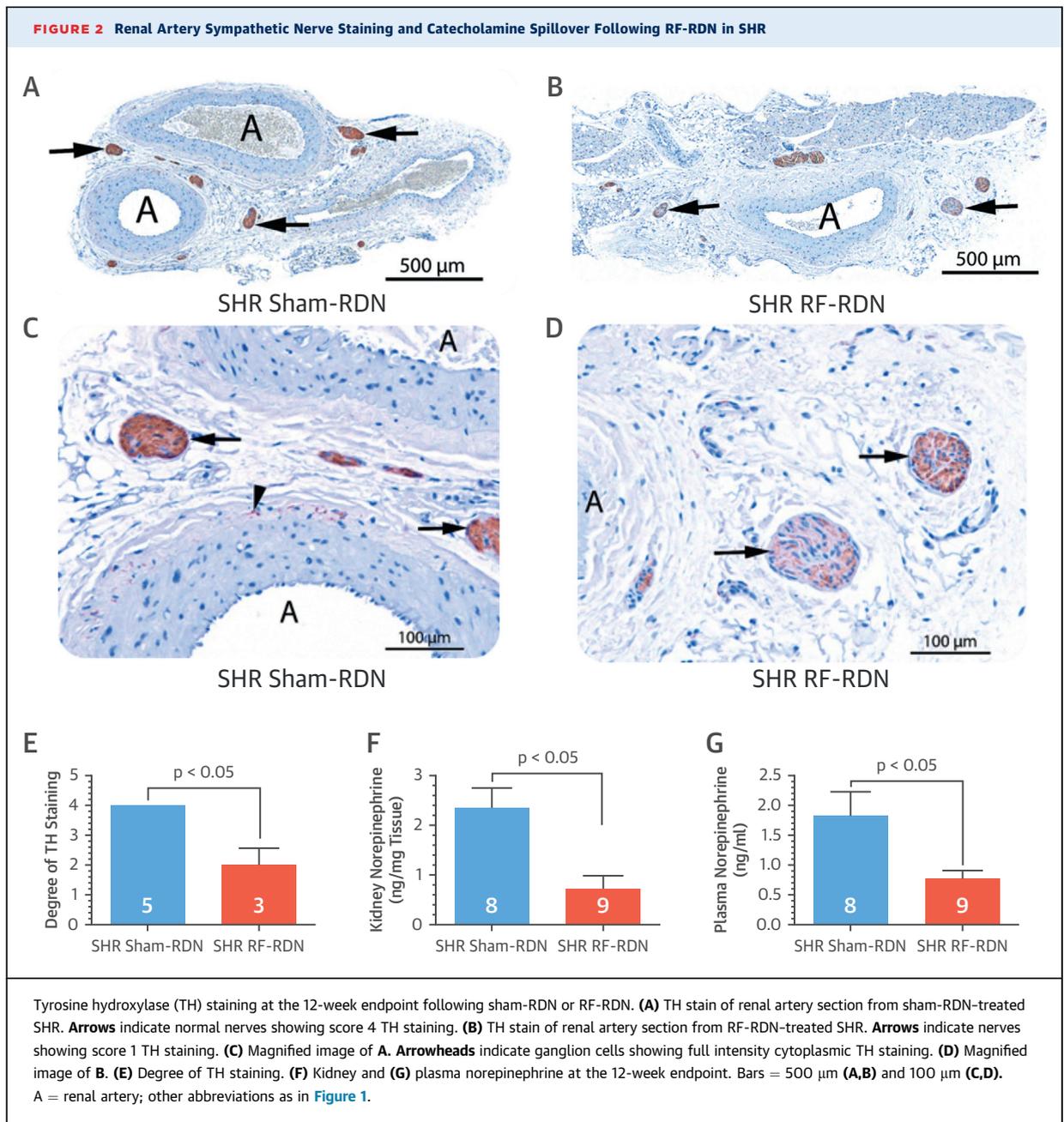
DELAYED RF-RDN THERAPY PRESERVES LV FUNCTION FOLLOWING ISCHEMIC-REPERFUSION INJURY IN SHR. Left ventricular (LV) function, as quantified by LV ejection fraction (LVEF), was



markedly improved as early as 4 weeks following therapy and was significantly improved compared with LV function of sham-treated animals through the 12-week endpoint ([Figure 3A](#)). LV end-systolic diameter was significantly reduced at 10 weeks, compared with that of sham-RDN animals ([Figure 3B](#)). There were no differences in LV end-diastolic diameter between groups at all time points ([Figure 3C](#)). These findings support a significant contributory role for the renal nerves in the progression of HF and demonstrate that RF-RDN has a major protective effect on cardiac contractile function in this pathology.

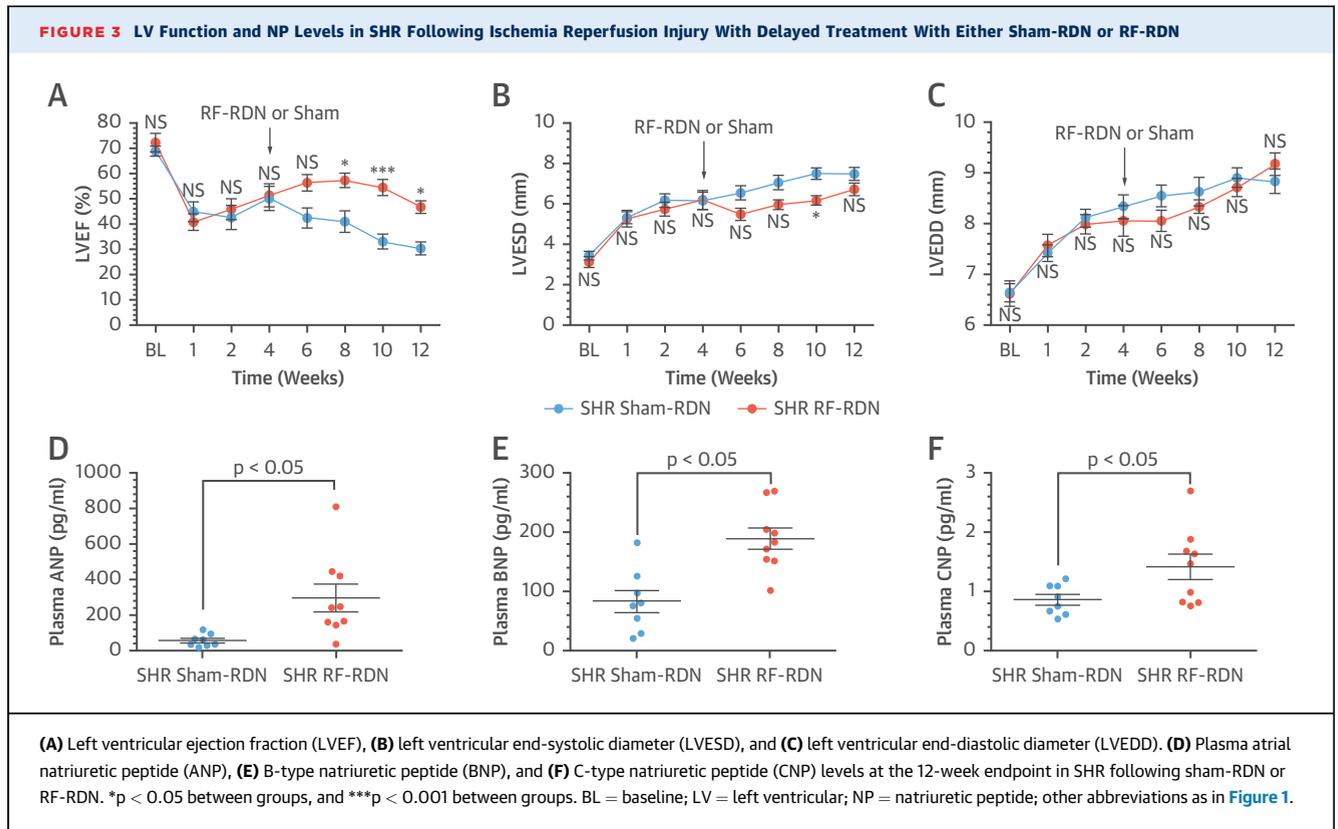
RF-RDN MODULATES CIRCULATING NP IN SHR IN HF. In addition to improved LV function, 12-week plasma atrial natriuretic peptide (ANP) ([Figure 3D](#)), B-type natriuretic peptide (BNP) ([Figure 3E](#)), and C-type natriuretic peptide (CNP) ([Figure 3F](#)) levels were significantly (*p* < 0.05) elevated in the RF-RDN-treated group compared with the levels in the sham-RDN. However, these changes were not due to increased gene transcription of ANP, BNP, and CNP in the myocardium ([Figures 4A to 4C](#)). Additionally, N-terminal pro-BNP levels in the LV and plasma indicate that RF-RDN did not enhance the release of NP from the myocardium ([Figures 4D and 4E](#)). These data clearly demonstrate that RF-RDN preserves circulating NP in the setting of severe HF.

RF-RDN INHIBITS RENAL NEPRILYSIN ACTIVITY IN SHR IN HF. Because increases in the transcription on



NP genes in the myocardium were not the cause of augmented NP levels in the circulation, we next investigated the clearance and degradation of NP. NP are primarily cleared in the kidney via the NP clearance receptor and are principally degraded into inactive fragments by the metallo-endopeptidase, neprilysin. Neprilysin is present in a wide variety of tissues, but it is particularly abundant in the kidney. Analyses of messenger RNA (mRNA) and protein levels indicate that RF-RDN does not alter circulating,

myocardial, or kidney neprilysin levels ([Online Figures 1B to 1D](#)). However, RF-RDN significantly inhibited renal neprilysin activity ([Figure 4F](#)) at 12 weeks following the onset of HF ($p < 0.05$ vs. sham-RDN). Additionally, neprilysin degrades other potentially cardioprotective and vasculoprotective peptides such as substance P and bradykinin. The mRNA levels of the NP clearance receptor in the kidney following RF-RDN indicate that reduced clearance did not contribute to elevated NP in circulation

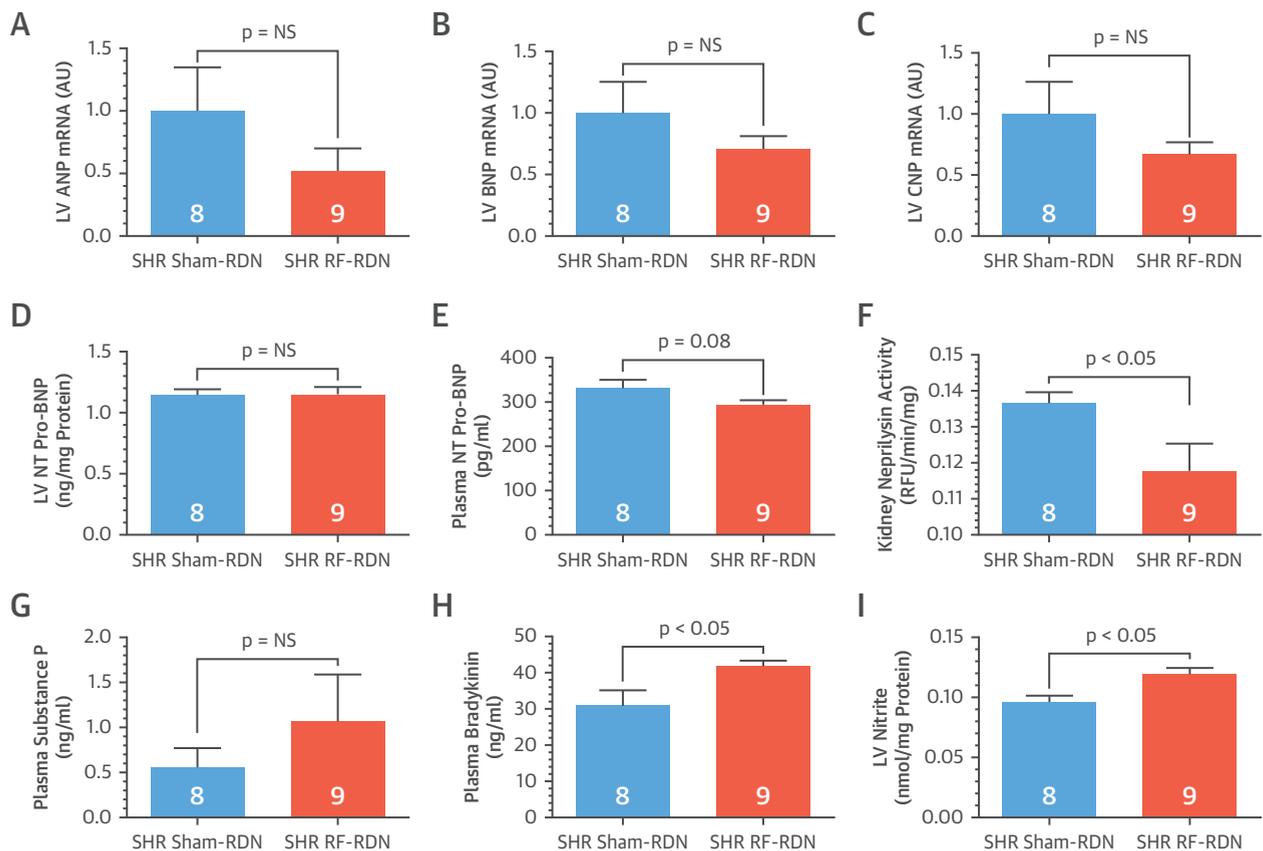


(Online Figure 1A). RF-RDN did not significantly alter circulating substance P levels (Figure 4G), but it did significantly ($p < 0.05$) increase bradykinin levels in the circulation (Figure 4H). It is well established that bradykinin augments NO levels by activating endothelial NO synthase. Increased LV nitrite levels following RF-RDN (Figure 4I) indicate improved NO signaling and suggest another possible mechanism of cardioprotection.

RF-RDN ATTENUATES CARDIAC FIBROSIS AND PROFIBROTIC SIGNALING FOLLOWING ISCHEMIC INJURY IN SHR. RF-RDN significantly reduced LV fibrosis score and percentage area of fibrosis in the LV ($29 \pm 2.7\%$ vs. $16 \pm 3.0\%$; $p < 0.05$ vs. sham-RDN) at 12 weeks following myocardial ischemia reperfusion injury (Figures 5B and 5C). Gene quantification revealed that RF-RDN mitigated profibrotic signaling, collagen type 1 alpha-1 and type 3 alpha-1, transforming growth factor-beta, interleukin-6, and connective tissue growth factor in the infarct border zone (Figure 5D). We observed no differences in matrix metalloproteinase 2, metalloproteinase inhibitor 1, or TIMP2 gene expression in the RF-RDN-treated group (Figure 5D). Additionally, RF-RDN significantly ($p < 0.05$ vs. sham-RDN group) inhibited infarct expansion compared with sham (Figures 5E and 5F).

BLOOD PRESSURE FOLLOWING MYOCARDIAL INFARCTION IN HYPERTENSIVE SHR AND NORMOTENSIVE WKY TREATED WITH RDN. RF-RDN, compared with sham-RDN, produced a small, but not statistically different decrease in systolic, diastolic, and mean arterial blood pressure (Online Figure 2). Furthermore, the RF-RDN-treated animals remained hypertensive with a systolic pressure above 150 and diastolic pressure above 110 mm Hg. RF-RDN did not significantly alter heart rate (Online Figure 2). In normotensive WKY, RF-RDN had no significant effect on systolic pressure, diastolic pressure, or heart rate when compared with sham-RDN-treated WKY (Online Figure 3).

RAS ACTIVITY FOLLOWING RF-RDN IN HF. The sympathetic nervous system can activate the RAS to further exacerbate the pathogenesis and progression of heart failure. Specifically, angiotensin II causes peripheral vasoconstriction and promotes cardiac remodeling to exacerbate heart failure. We therefore explored the role of RF-RDN on RAS in heart failure. In hypertensive rats, RF-RDN resulted in significant reductions in plasma renin and plasma angiotensin II at 12 weeks following reperfusion (Online Figures 4A and 4B). However, RF-RDN did not significantly alter the RAS system in normotensive rats

FIGURE 4 LV NP Gene Expression, Nephrylin Activity, Cardioprotective and Vasoactive Agents in SHR Following Sham-RDN or RF-RDN in HF

(A) LV ANP messenger RNA (mRNA), (B) LV BNP mRNA, and (C) LV CNP mRNA levels. (D) LV N-terminal pro-B-type natriuretic peptide (NT-proBNP) and (E) plasma NT-pro-BNP in SHR following sham-RDN or RF-RDN. (F) Kidney neprilysin activity, (G) substance P, (H) plasma bradykinin, and (I) LV nitrite levels in SHR treated with either sham-RDN or RF-RDN. AU = arbitrary units; RFU = relative fluorometric units; other abbreviations as in [Figures 1 and 3](#).

([Online Figures 4C and 4D](#)). These results suggest that the cardioprotective effects of RF-RDN extend beyond inactivation of the RAS system.

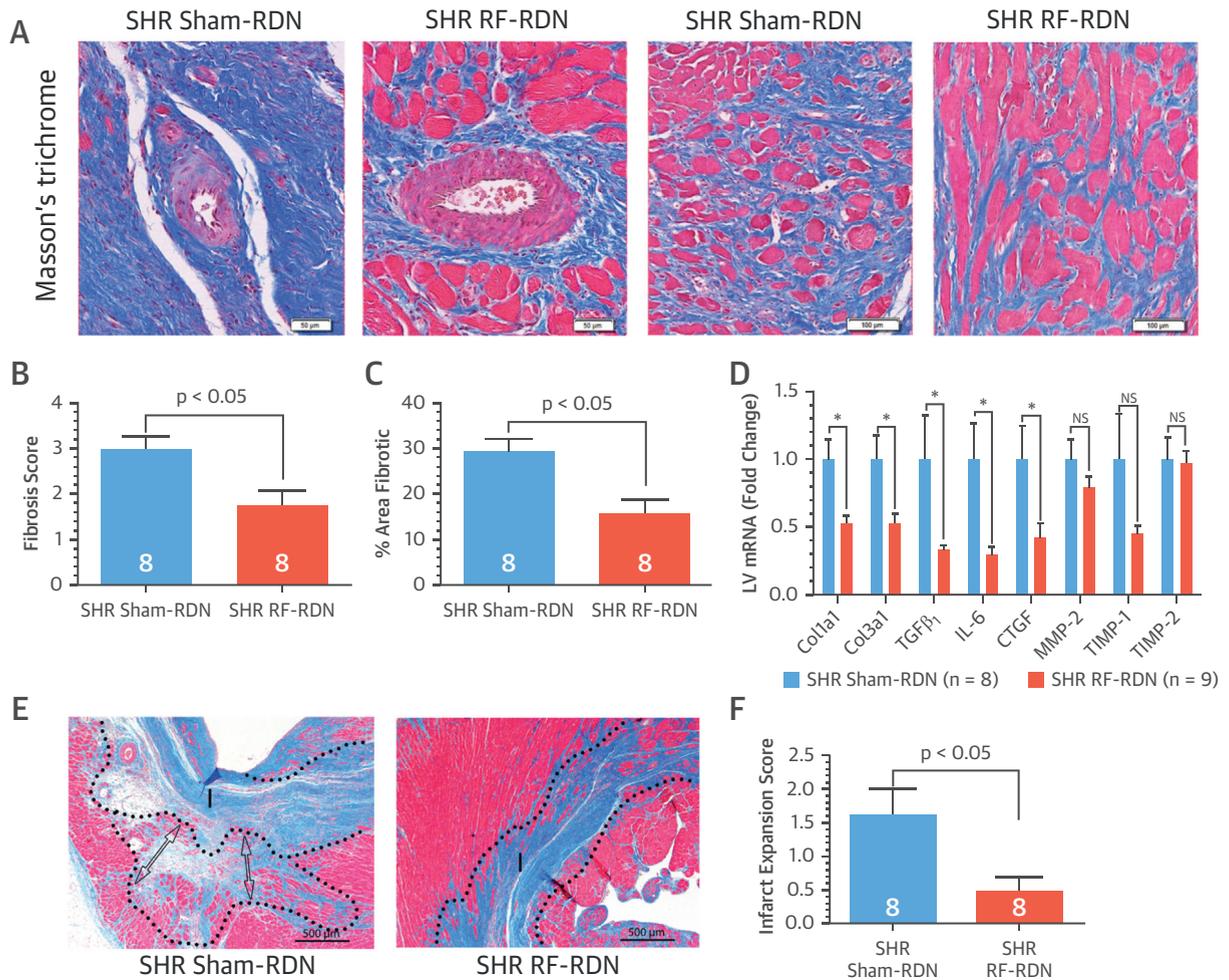
RENAL ARTERY NERVE VIABILITY AND NOREPINEPHRINE LEVELS IN WKY FOLLOWING RF-RDN IN HF

Renal artery nerve TH staining at 8 weeks following RF-RDN or sham-RDN in WKY rats revealed a significant ($p < 0.05$) reduction in renal nerve viability in RF-RDN as compared with that in sham-RDN SHR ([Figure 6E](#)). Plasma and kidney NE levels were measured 8 weeks following RF-RDN or sham-RDN and there were significant reductions in kidney and circulating NE following RF-RDN versus sham-RDN in WKY ([Figures 6F and 6G](#)).

DELAYED RF-RDN THERAPY PRESERVES LV FUNCTION IN NORMOTENSIVE WKY. The cardioprotective effects of RF-RDN therapy in HF in the SHR could be

attributed in part to a modest reduction in systolic and diastolic pressure, which reduces the afterload of the heart. To control for the effects of RF-RDN on blood pressure, we performed identical experiments in normotensive WKY. In these experiments, LV function ([Figure 7](#)) was improved in normotensive animals as early as 2 weeks following therapy and remained significantly improved when compared with LV function in the sham-treated animals through the 12-week endpoint ([Figure 7A](#)). RF-RDN also preserved LV dimensions in WKY subjected to HF. Following RF-RDN therapy, LV end-diastolic diameter was reduced at the 6-week time point compared with that of sham-RDN animals, but remained unchanged between groups throughout the remainder of the study ([Figure 7C](#)). LV end-systolic diameter was maintained below the sham-RDN dimension from week 6 through the 12-week endpoint following

FIGURE 5 Cardiac Fibrosis in SHR in HF Following Sham-RDN or RF-RDN Therapy



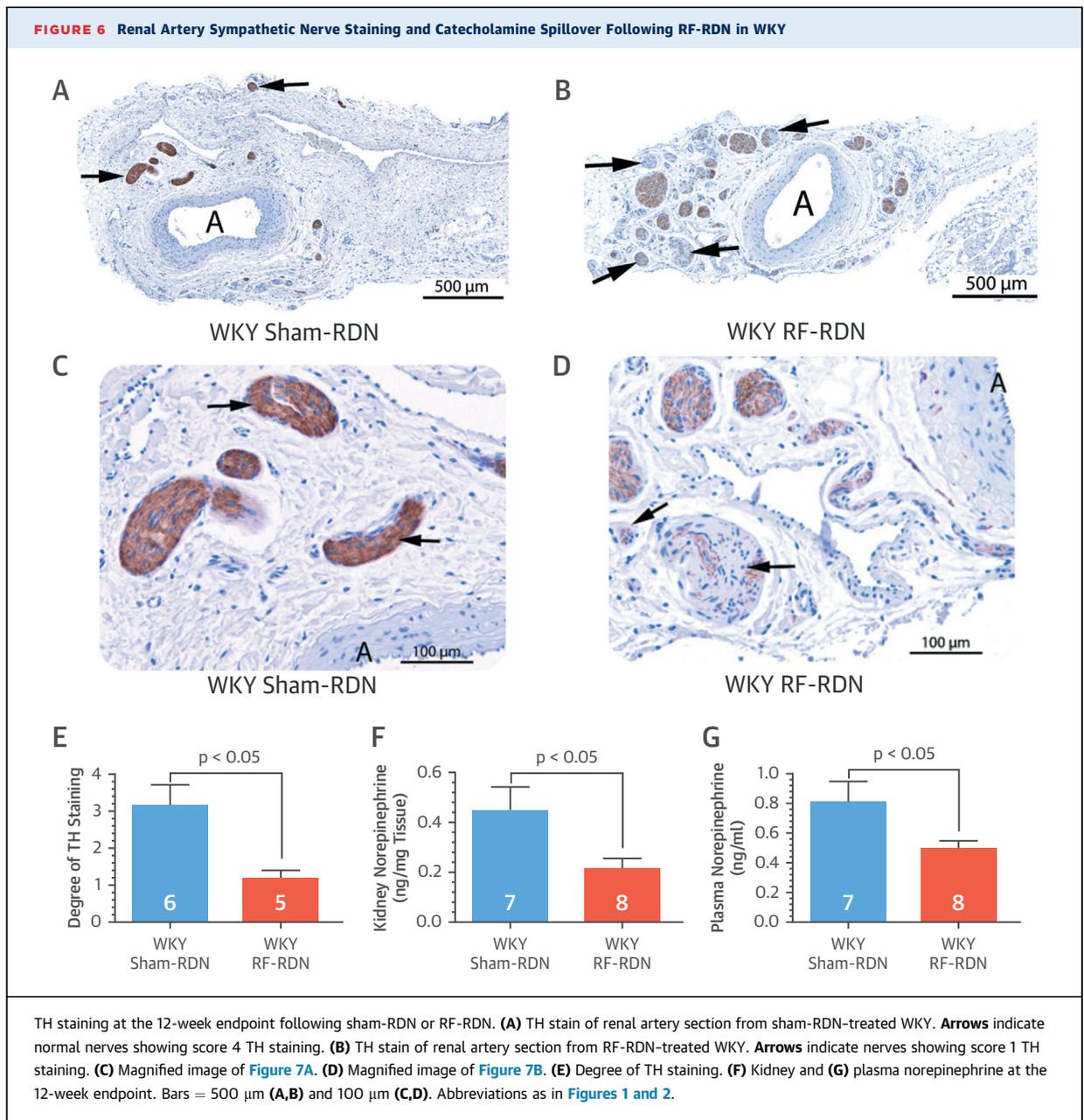
(A) Representative Masson trichrome staining of perivascular and interstitial fibrosis in the left ventricle of SHR 12 weeks following ischemia reperfusion injury. **(B)** Fibrosis score and **(C)** percentage of area that is fibrotic of the left ventricle. **(D)** The mRNA levels of fibrotic genes collagen type 1 (*Col1a1*), collagen type 3 (*Col3a1*), transforming growth factor beta, (*TGFβ*), interleukin-6 (*IL6*), connective tissue growth factor (*CTGF*), matrix metalloproteinase-2 (*MMP-2*), metalloproteinase inhibitor-1 (*TIMP-1*), and metalloproteinase inhibitor-2 (*TIMP-2*). **(E)** Representative images of infarct expansion and **(F)** infarct expansion score. **p* < 0.05 between groups. Bars = 50 μm (**A**, left and left center) and 100 μm (**A**, right center and right). Abbreviations as in **Figures 1, 3, and 4**.

RF-RDN (**Figure 7B**). These results indicate that RF-RDN may have therapeutic potential for HF in both hypertensive and normotensive patients.

RF-RDN INHIBITS RENAL NEPRILYSIN ACTIVITY AND INCREASES CIRCULATING NP IN WKY IN HF.

We investigated whether renal sympathetic denervation modulates endogenous regulation of NP in normotensive animals subjected to HF. In a similar fashion to SHR, 12-week plasma BNP (**Figure 7D**) and CNP (**Online Figure 5C**) levels were significantly elevated in the RF-RDN-treated WKY versus sham-RDN-treated animals. RF-RDN-induced BNP

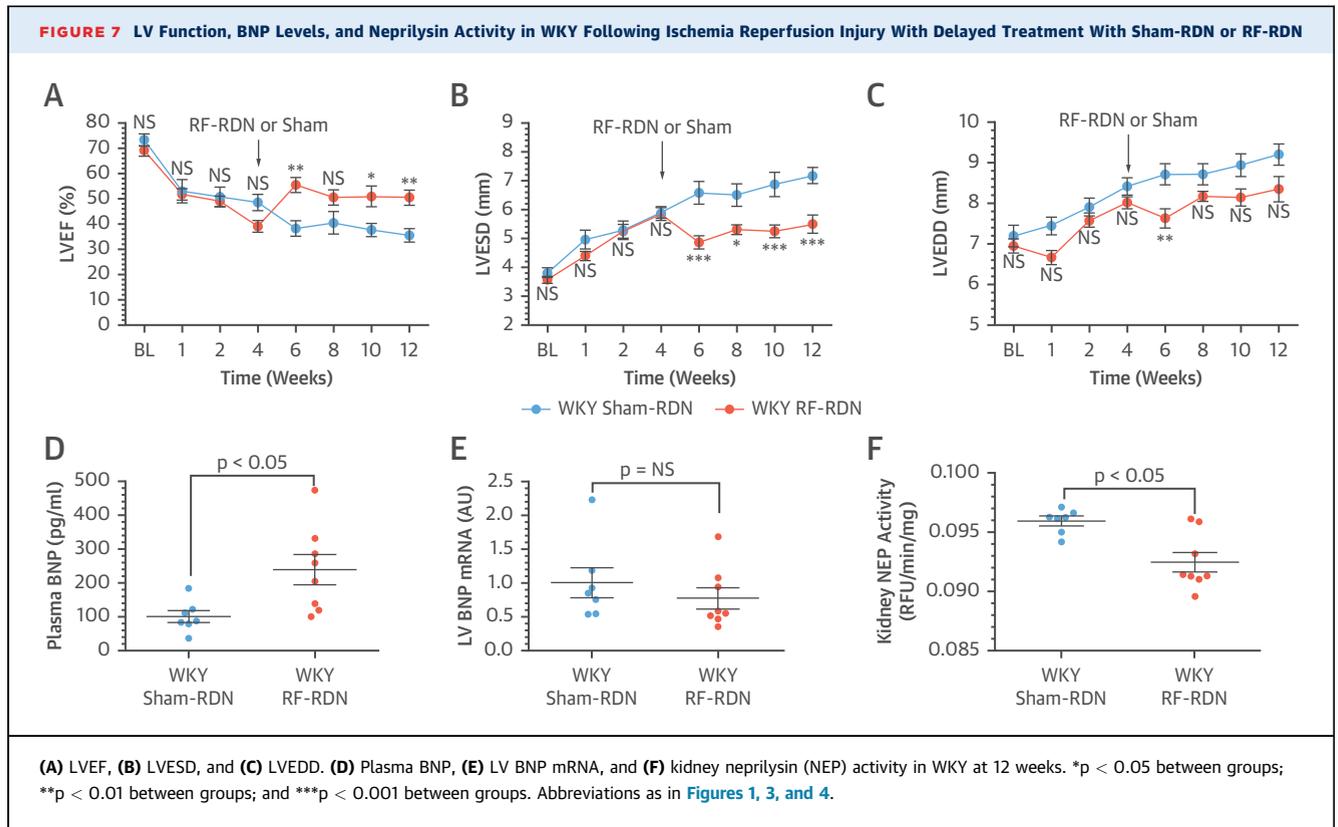
elevation peaked at the 6-week time point and remained higher than that of sham-RDN-treated animals throughout the study (**Online Figure 5G**). Plasma levels of ANP (**Online Figure 5A**) were not increased in WKY subjected to HF and RF-RDN. This disparity from the other NP is likely due to the significant reduction in ANP production in LV of the RF-RDN group as quantified by LV mRNA levels (**Online Figure 5B**). The changes in BNP and CNP were not a result of increased myocardial gene transcription (**Figure 7E**, **Online Figure 5D**). Plasma N-terminal pro-BNP levels were unchanged between the sham- and RF-RDN groups, indicating that RF-RDN did not



significantly enhance the release of NP from the cardiac myocyte (**Online Figure 5E**). As in the setting of hypertension, RF-RDN significantly inhibited renal neprilysin activity during HF (**Figure 7F**). Likewise, RF-RDN significantly increased circulating bradykinin levels following RF-RDN in WKY in the setting of HF (**Online Figure 5F**).

BETA₁ ANTAGONIST, BISOPROLOL, IMPROVES CARDIAC FUNCTION, INHIBITS NEPRILYSIN, AND INCREASES CIRCULATING BNP LEVELS IN HF. When administered 4 weeks following myocardial

infarction, bisoprolol significantly improved systolic function in WKY rats starting 4 weeks after the onset of treatment compared with systolic function in sham-RDN-treated WKY (**Figure 8A**). This improvement was preserved through the 12-week endpoint. There were no significant differences in LVEF between bisoprolol and RF-RDN-treated rats. Similar to RF-RDN, bisoprolol treatment resulted in a >2-fold increase in BNP levels ($p < 0.05$ vs. sham-RDN) and a significant ($p < 0.001$ vs. sham-RDN) reduction in renal neprilysin activity measured at the study endpoint (**Figures 8B and 8C**).



NEPRILYSIN INHIBITOR, SACUBITRIL, DOES NOT IMPROVE CARDIAC FUNCTION AS DOES RF-RDN IN WKY FOLLOWING ISCHEMIA REPERFUSION INJURY. When administered to WKY 4 weeks following myocardial infarction, sacubitril was superior to sham-RDN only at the 8-week time point. Comparatively, RF-RDN-treated animals had significantly improved LVEF at all time points following therapy compared with sham-RDN and RF-RDN was superior to sacubitril at the 6- and 10-week time points ([Figure 8D](#)). Interestingly, sacubitril did not enhance the effects of RF-RDN, suggesting that some of the protective actions of sacubitril are related to neprilysin inhibition. Sacubitril treatment resulted in a >3-fold increase in BNP levels and significantly reduced renal neprilysin activity measured at the study endpoint ([Figures 8E and 8F](#)).

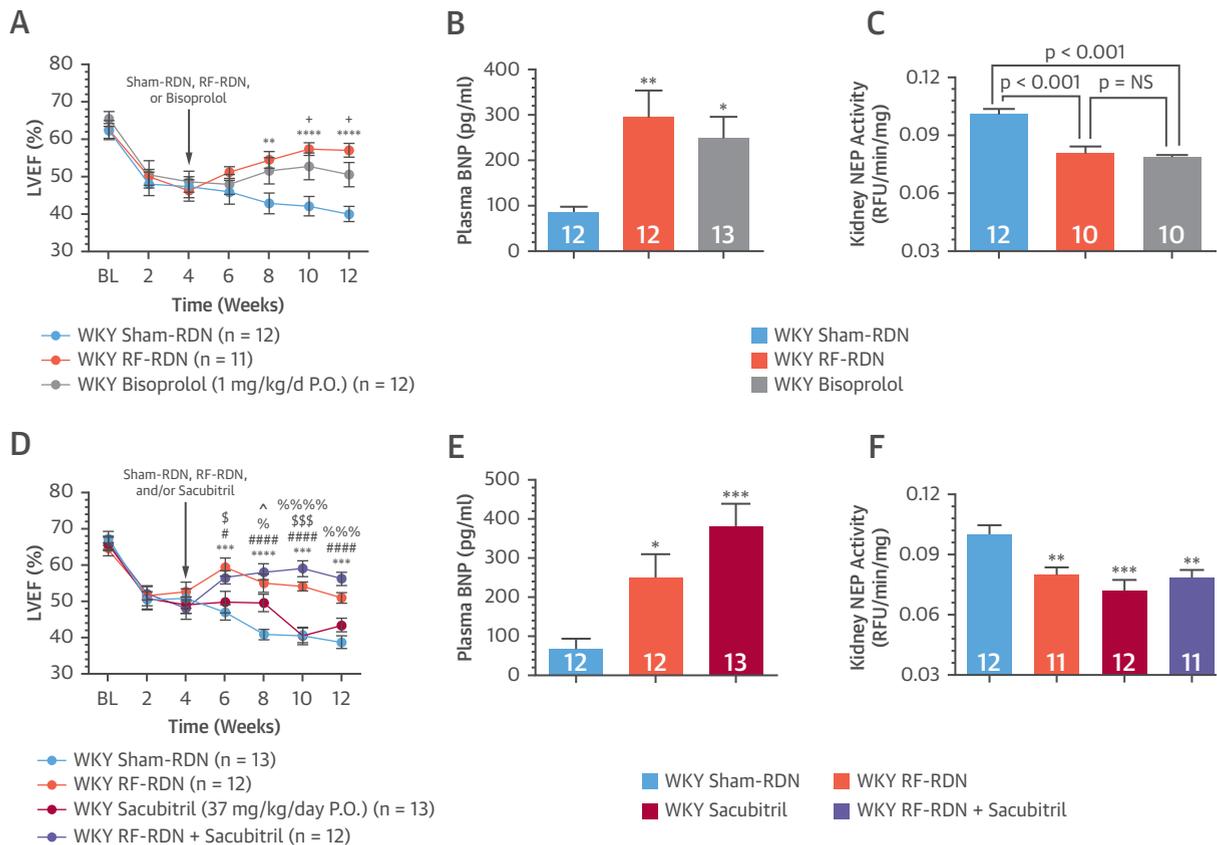
RF-RDN IMPROVES VASORELAXATION IN SHR AND WKY IN HF. We next investigated the effects of RF-RDN on peripheral vessel vasoreactivity in the setting of HF. At the 12-week endpoint, thoracic aortas were removed and tested in an isolated organ bath system. Sodium nitroprusside (SNP) and acetylcholine (ACh) were administered in increasing concentrations following phenylephrine-induced

contraction to test smooth muscle- and endothelial-dependent relaxation, respectively. Aortas from the RF-RDN-treated SHR exhibited significantly greater relaxation to SNP and ACh than did the sham-RDN group ([Figures 9A and 9B](#)). Similarly, aortas isolated from RF-RDN-treated WKY exhibited greater relaxation with both SNP and ACh than sham-RDN control aortas did ([Figures 9C and 9D](#)). Vascular compliance was also significantly ($p < 0.01$ vs. sham-RDN) improved in SHR treated with RF-RDN ([Online Figure 6A](#)), but not in WKY ([Online Figure 6B](#)).

BISOPROLOL IMPROVES VASCULAR RELAXATION TO A LESSER EXTENT THAN RF-RDN. At the 12-week endpoint, thoracic aortas were excised from WKY and tested for vascular reactivity. There were no significant differences in SNP-mediated relaxation between bisoprolol- and RF-RDN-treated rats ([Figure 9C](#)). Aortic vascular segments isolated from RF-RDN-treated rats exhibited superior relaxation to ACh ([Figure 9D](#)). Bisoprolol and RF-RDN-treated animals were superior to sham-RDN control animals in terms of SNP- and ACh-induced relaxation ([Figures 9C and 9D](#)).

SACUBITRIL AUGMENTS CIRCULATING ANGIOTENSIN II AND IMPAIRS VASCULAR RELAXATION. Angiotensin II is a substrate for neprilysin degradation. Animals

FIGURE 8 Cardiac Function, BNP levels, and NEP Activity in Bisoprolol- and Sacubitril-Treated WKY Following Ischemia Reperfusion Injury



(A) LVEF, (B) plasma BNP, and (C) kidney NEP activity in bisoprolol- and RF-RDN-treated WKY following ischemia reperfusion injury. (D) LVEF, (E) plasma BNP, and (F) kidney NEP activity in sacubitril- and RF-RDN-treated WKY following ischemia reperfusion injury. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, and **** $p < 0.0001$, RF-RDN versus sham-RDN. + $p < 0.05$, bisoprolol versus sham-RDN. ^ $p < 0.05$, sham-RDN versus sacubitril. # $p < 0.05$ and #### $p < 0.0001$, sham-RDN versus RF-RDN + sacubitril. \$ $p < 0.05$ and \$\$\$ $p < 0.001$, RF-RDN versus sacubitril. % $p < 0.05$, %% $p < 0.001$, and %%% $p < 0.0001$, sacubitril versus RF-RDN + sacubitril. P.O. = orally; other abbreviations as in Figures 1, 3, 4, and 7.

treated with the neprilysin inhibitor, sacubitril, had a >2-fold increase in plasma angiotensin II than did sham-RDN WKY (Online Figure 4D). This was accompanied by impaired vascular relaxation to both SNP and ACh compared with relaxation in sham-RDN control animals (Figures 9E and 9F). These data provide insight as to why neprilysin inhibitors, when used alone, are ineffective at reducing blood pressure and do not preserve cardiac function.

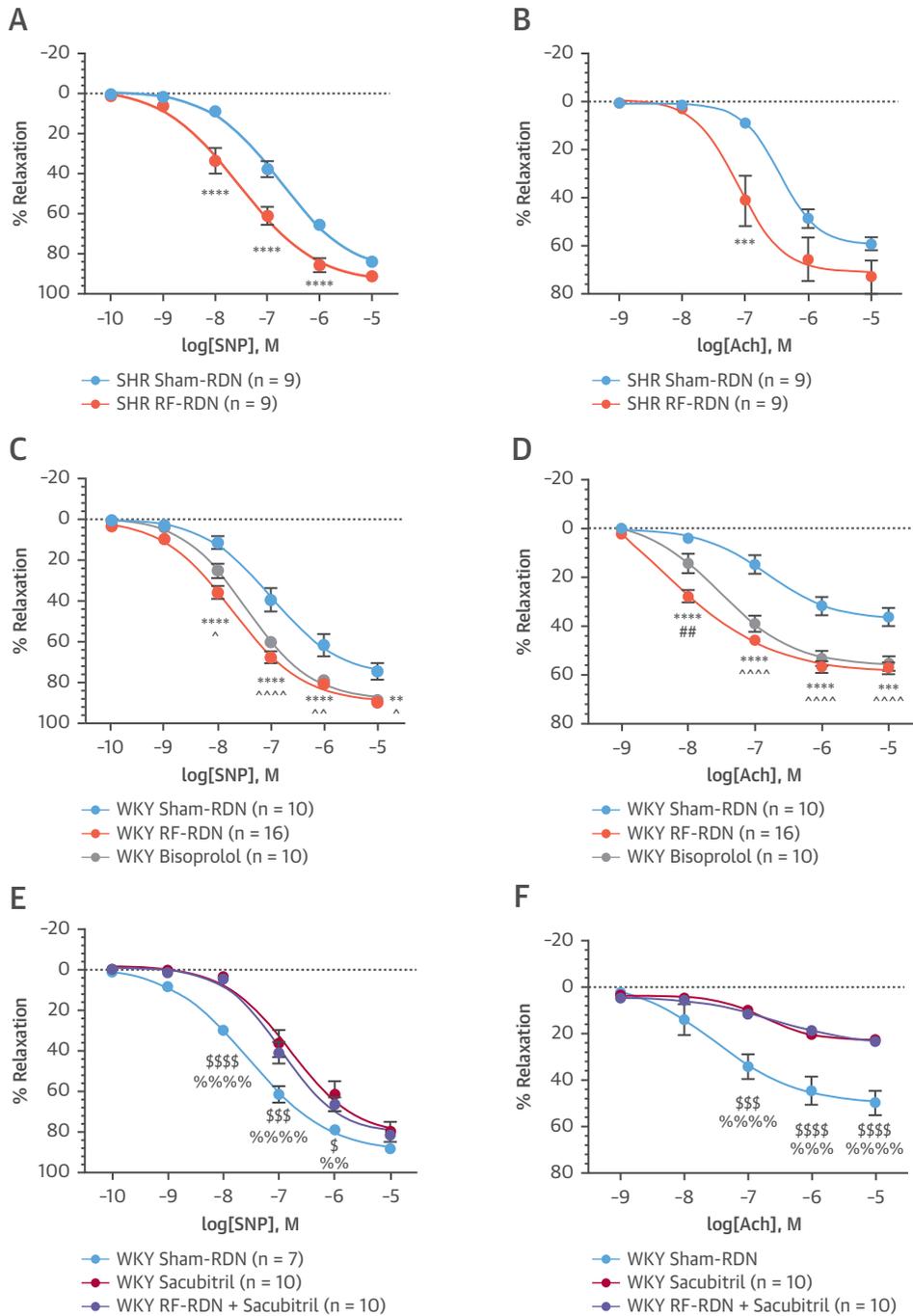
DISCUSSION

Modulation of the autonomic nervous system as a treatment for HF has been limited to pharmacologic therapies. In the current study, we examined the effects of RF-RDN on post-infarction remodeling in

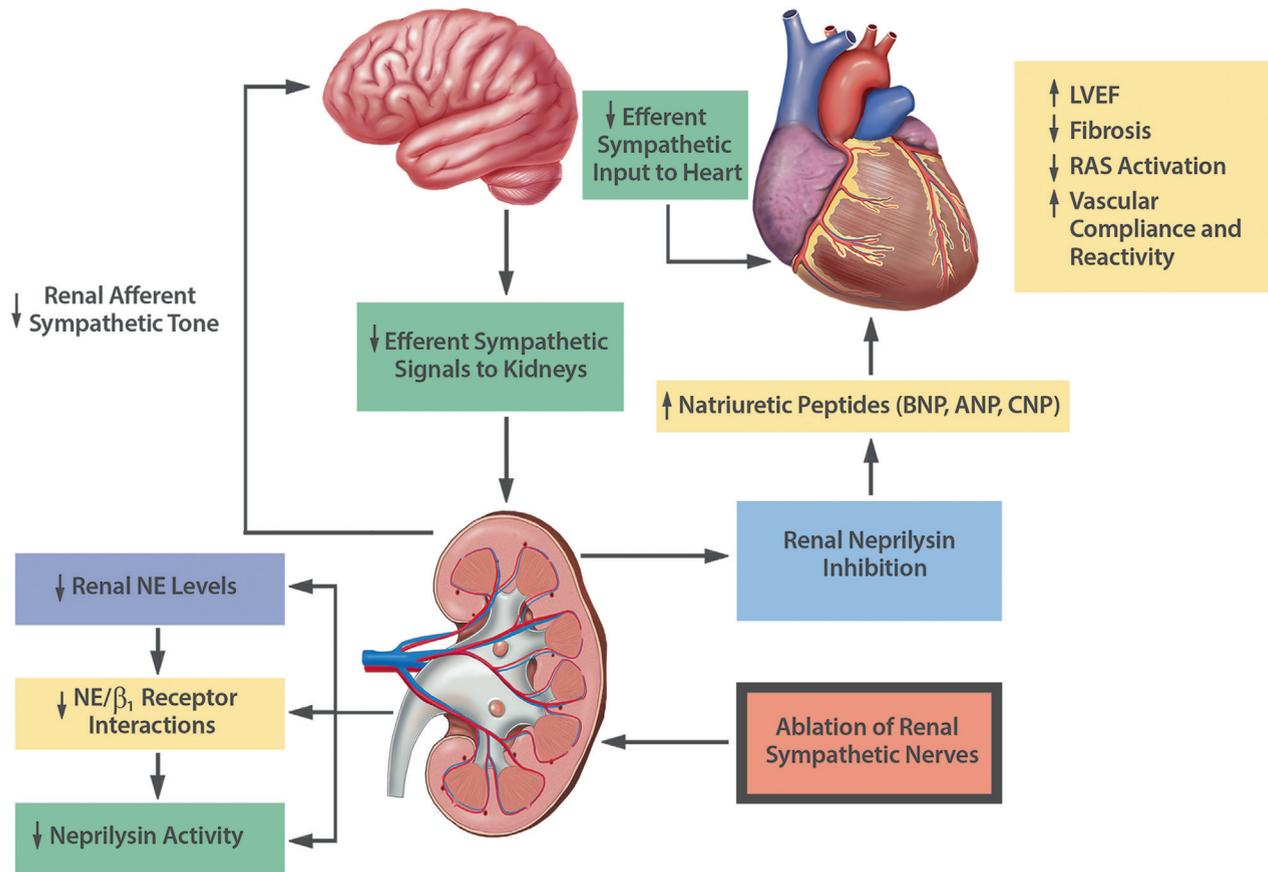
both the setting of hypertension (SHR) and normal blood pressure (WKY) (Central Illustration).

We observed significant preservation of LV systolic function in both hypertensive and normotensive rats following RF-RDN therapy at 4 weeks post-myocardial infarction. This improvement in LV function was accompanied by increases in plasma NP in rats treated with RF-RDN therapy. RF-RDN-induced augmentation of BNP peaked at the 6-week time point (2 weeks after RF-RDN) and was maintained above the sham-RDN control throughout the study. We did not observe any changes in LV and plasma N-terminal pro-BNP following RF-RDN, suggesting that the synthesis of BNP and other NP was not increased by RF-RDN. We did, however, observe significant inhibition of the activity of the NP degrading enzyme, neprilysin, in rats subjected to HF that received RF-RDN. Because

FIGURE 9 Vascular Relaxation in SHR and WKY Following Ischemia Reperfusion Injury



(A) Aortic relaxation to sodium nitroprusside (SNP) and **(B)** acetylcholine (ACh) in SHR. **(C)** Aortic relaxation to SNP and **(D)** ACh in WKY treated with sham-RDN, RF-RDN, or bisoprolol. **(E)** Aortic relaxation to SNP and **(F)** ACh in WKY treated with sham-RDN, RF-RDN, or bisoprolol. ** $p < 0.01$, *** $p < 0.001$, and **** $p < 0.0001$, RF-RDN versus sham-RDN. $\wedge p < 0.05$, $\wedge\wedge p < 0.01$, and $\wedge\wedge\wedge p < 0.0001$, bisoprolol versus sham-RDN. $\#\#\# p < 0.01$, RF-RDN versus bisoprolol. $\$ p < 0.05$, $\$\$\$ p < 0.001$, $\$\$\$\$ p < 0.0001$; sham-RDN versus RF-RDN + sacubitril. $\% p < 0.01$, $\%\%\% p < 0.001$, and $\%\%\%\% p < 0.0001$, sham-RDN versus sacubitril. M = mol/l; other abbreviations as in Figure 1.

CENTRAL ILLUSTRATION Renal Sympathetic Denervation Protects the Failing Heart

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Proposed mechanism of cardioprotection by radiofrequency renal denervation in the setting of heart failure. Radiofrequency ablation of the renal sympathetic nerves suppresses both efferent sympathetic signals to the kidney and afferent tone to the brain. Reduced sympathetics to the kidney results in renal neprilysin inhibition, augmentation of circulating natriuretic peptides, reduced cardiac fibrosis, and improved cardiac and vascular function. Additionally, repressed renal afferent tone modulates efferent sympathetic inputs on the heart, improving cardiac remodeling and function. ANP = atrial natriuretic peptide; BNP = B-type natriuretic peptide; CNP = C-type natriuretic peptide; LVEF = left ventricular ejection fraction; NE = norepinephrine; RAS = renin-angiotensin system.

total neprilysin protein levels were unchanged, the inhibition of activity was likely due to a post-translation modification of the protein.

In the 1980s, the heart was first recognized as an endocrine organ that secretes a family of hormones called NP (26). These peptides were originally shown to act on the vasculature and kidney to produce natriuresis, diuresis, and peripheral vasodilation to compensate for increased cardiac wall stress and volume (27). Ventricular dilation and myocyte stretch triggers the synthesis and release of NP, and therefore, circulating BNP levels are an established biomarker of HF severity (28-30). In addition to their ability to clear sodium and water and relax smooth muscle, NP act directly on the heart and elicit

multiple protective signaling cascades. NP are a target for HF therapeutics and inhibitors of NP degradation (i.e., neprilysin inhibitors) are at the forefront of chronic HF therapy (31-33). ANP, BNP, and CNP bind to NP receptors A, B, and C (27). NP receptors A and B are G-protein coupled receptors that stimulate guanylyl cyclase to generate cyclic guanosine monophosphate and activate protein kinase G (27). Increasing cyclic guanosine monophosphate and activating protein kinase G inhibit hypertrophy and increase intracellular calcium to improve contractility (34-36). Additionally, BNP reduces transforming growth factor beta-induced effects on cardiac fibroblasts (37), and BNP inhibits the expression of genes related to fibrosis (collagen type 1, connective tissue

growth factor, and metalloproteinase inhibitor 3), myofibroblast conversion, proliferation, and inflammation (COX2, interleukin 6, and tumor necrosis factor alpha) (37). In the current study we similarly observed reductions in gene transcription levels of transforming growth factor beta, collagen types 1 and 3, connective tissue growth factor, and interleukin 6 following RF-RDN therapy. Overall, we found that RF-RDN treatment reduced ventricular fibrosis, minimized infarct expansion, and improved LV function.

In the current study we also found that NP levels were elevated and renal neprilysin activity was inhibited in rats treated with the beta₁-specific antagonist, bisoprolol. These findings indicate that blockade of the sympathetically innervated renal beta₁-adrenergic receptor similarly regulates NP metabolism. Interestingly, endothelial-dependent vascular relaxation in RF-RDN-treated rats was superior to relaxation in bisoprolol-treated rats. One possibility for the superiority of RF-RDN on endothelial function, but not cardiac function when RF-RDN is compared with bisoprolol, is that the direct effect of RF-RDN is restricted to the kidney, whereas beta₁ receptors are located on numerous cell types throughout the body. Moreover, these findings suggest that RF-RDN can influence vascular endothelial function via alternative pathways beyond blockade of beta-adrenergic pathways and augmentation of circulating NP levels.

When compared with the neprilysin inhibitor, sacubitril, RF-RDN outperformed sacubitril in both cardiac and vascular function. In fact, endothelial- and smooth muscle-dependent relaxation in sacubitril-treated rats were impaired compared with relaxation in sham-RDN-treated control rats. This can potentially be explained by a nearly 3-fold increase in angiotensin II levels, because it is known that significant elevation in circulating levels of this hormone can cause extensive damage to the integrity of the vessel. As was previously reported in clinical trials (38,39), when neprilysin inhibitors are used alone as an antihypertensive agent, a major limitation is that these drugs also obstruct the degradation of angiotensin II. Elevated blood levels of angiotensin II can produce arterial vasoconstriction and promote cardiac remodeling. Benefits of NP modulation are likely offset by these and other actions of angiotensin II. Although RF-RDN inhibits neprilysin activity and augments NP, angiotensin II levels were significantly reduced in SHR with HF, as compared to levels in sham-RDN-treated animals, and were unchanged in WKY with HF. This is likely due to the interruption of sympathetic nerve traffic to the kidneys, which blocks neural activation of the RAS. Therefore, we believe that RF-RDN-induced inhibition of neprilysin provides greater clinical

benefits over a neprilysin inhibitor because of the simultaneous inhibition of RAS. Because angiotensin II is a substrate of neprilysin, clinical development of neprilysin inhibitors has involved the combined use of an angiotensin receptor blocker (24,40). This combination therapy has proven extremely efficacious in large clinical trials (31,32). RF-RDN has the potential to inhibit neprilysin in combination with dampening of the sympathetic nervous system and RAS. It is also important to note that the combination of RDN and sacubitril did not result in any additional benefit beyond RDN alone, suggesting that the protective actions of RDN are mediated in part via neprilysin inhibition. These data support our conclusion that the increased circulating levels of NP are a result of renal neprilysin inhibition.

There are several potential advantages to using RF-RDN as a treatment for HF instead of other sympatholytics. The most likely is that beta-blockers bind to the beta-adrenergic receptor and compete with endogenous ligands, epinephrine and NE, to antagonize their actions. In contrast, RF-RDN, by reducing sympathetic efferent nerve traffic, minimizes neurotransmitter release to the beta-adrenergic receptors and inhibits the sympathetic nervous system at a very proximal step in this pathological process. Another limitation of beta-blockers in HF is that they do not improve exercise capacity (41). Exercise capacity is related to peripheral vascular and endothelial function, and it is postulated that beta-blocker effects are limited to cardiac remodeling and function. We have shown that RF-RDN, compared with bisoprolol, improves vascular compliance and has significantly improved ACh-mediated vasorelaxation. In a previous study, we reported that RF-RDN improves endothelial NO synthase function and reduces oxidative stress in the setting of hypertension (19). Healthy redox balance and NO synthesis are critical for endothelial function, indicating another possible mechanism for superior vascular protection. Because RF-RDN improves vascular compliance and reactivity in HF, it is possible that RF-RDN therapy would have greater effects on exercise capacity than beta-blockers would in HF patients.

STUDY LIMITATIONS. Despite the beneficial effects of RF-RDN observed in the present study, there remain several possible limitations for the use of RF-RDN as a treatment for HF. Peripheral nerves have the potential of regrowth (42). In the present study, reductions in renal nerve viability were maintained over the course of the study, but we have not examined whether or when these nerves can regrow or regain function. In the early RDN clinical trials for hypertension, blood

pressure reductions were maintained up to 3-year follow-up (43). As the field remains in its infancy, we do not have long-term data to substantiate permanent denervation of the renal nerves.

CONCLUSIONS

We have shown that RF-RDN improves LV function in HF and that these cardioprotective actions are independent of changes in systolic and diastolic blood pressure. To date, there is no established relationship between the sympathetic nervous system and NP regulation in the kidney. Our results demonstrate that decreased sympathetic nerve activity to the kidney, via RF-RDN, or blockade of sympathetic nerve traffic to the kidney via β_1 -adrenergic receptor blockade, inhibits renal neprilysin activity and leads to prolonged increases in vasculoprotective and cardioprotective NP in the setting of heart failure. We conclude that RF-RDN is worthy of further exploration as a promising minimally invasive strategy for the treatment of cardiac injury and HF.

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PERSPECTIVES

COMPETENCY IN MEDICAL KNOWLEDGE: In patients with heart failure, activation of the sympathetic nervous system contributes to maladaptive cardiac remodeling, impaired ventricular function, peripheral vascular dysfunction, and adverse clinical outcomes. Renal sympathetic denervation inhibits the metabolism of protective natriuretic peptides.

TRANSLATIONAL OUTLOOK: Clinical trials of catheter-based renal sympathetic denervation in patients with heart failure are warranted.

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KEY WORDS fibrosis, heart failure, renal denervation, sympathetic nervous system, vascular function

APPENDIX For a supplemental Methods section as well as figures, please see the online version of this article.