Renal Denervation Normalizes Arterial Pressure With No Effect on Glucose Metabolism or Renal Inflammation in Obese Hypertensive Mice

Ninitha Asirvatham-Jeyaraj, Jessica K. Fiege, Ruijun Han, Jason Foss, Christopher T. Banek, Brandon J. Burbach, Maria Razzoli, Alessandro Bartolomucci, Yoji Shimizu, Angela Panoskaltsis-Mortari, John W. Osborn

Abstract—Hypertension often occurs in concurrence with obesity and diabetes mellitus, commonly referred to as metabolic syndrome. Renal denervation (RDNx) lowers arterial pressure (AP) and improves glucose metabolism in drug-resistant hypertensive patients with high body mass index. In addition, RDNx has been shown to reduce renal inflammation in the mouse model of angiotensin II hypertension. The present study tested the hypothesis that RDNx reduces AP and renal inflammation and improves glucose metabolism in obesity-induced hypertension. Eight-week-old C57BL/6J mice were fed either a low-fat diet (10 kcal%) or a high-fat diet (45 kcal%) for 10 weeks. Body weight, food intake, fasting blood glucose, and glucose metabolism (glucose tolerance test) were measured. In a parallel study, radiotelemeters were implanted in mice for AP measurement. High fat–fed C57BL/6J mice exhibited an inflammatory and metabolic syndrome phenotype, including increased fat mass, increased AP, and hyperglycemia compared with low-fat diet mice. RDNx, but not Sham surgery, normalized AP in high-fat diet mice (115.8±1.5 mm Hg in sham versus 96.6±6.7 mm Hg in RDNx). RDNx had no significant effect on AP in low-fat diet mice. Also, RDNx had no significant effect on glucose metabolism or renal inflammation as measured by the number of CD8, CD4, and T helper cells or levels of inflammatory cytokines in the kidneys. These results indicate that although renal nerves play a role in obesity-induced hypertension, they do not contribute to impaired glucose metabolism or renal inflammation in this model. (Hypertension. 2016;68:929-936. DOI: 10.1161/HYPERTENSIONAHA.116.07993.) • Online Data Supplement

Key Words: cytokines ■ glucose ■ hypertension ■ metabolic syndrome ■ renal denervation ■ T cell

Obesity is often associated with hypertension and type 2 diabetes mellitus; a condition commonly referred to as metabolic syndrome. Obesity is also linked with inflammation in general, and renal inflammation, specifically. However, the extent to which obesity-associated inflammation is primary or secondary to hypertension and type 2 diabetes mellitus is not known.

It is now widely accepted that obesity is correlated with increased activity of sympathetic nervous system (SNA), with the growing consensus that SNA to the kidneys is a major contributor to the pathogenesis and maintenance of obesity-induced hypertension. This concept is supported by reports that renal nerve ablation prevents and reverses obesity-induced hypertension in experimental animals and improves glucose metabolism. Interestingly, catheter-based renal nerve ablation in drug-resistant hypertensive humans, many of whom are obese, has been reported to not only reduce arterial pressure (AP), but improve glucose metabolism as well. It has been hypothesized that this response is secondary to ablation of sympathoexcitatory renal sensory nerves and subsequent reduction of SNA to skeletal muscle, a key glucose regulatory tissue. Finally, recent studies suggest that renal nerve ablation may also reduce renal inflammation associated with hypertension. Angiotensin II (AngII)–induced hypertension in mice has been shown to be dependent on trafficking of T cells into the kidney, and this response is dependent on brain sites that regulate the activity of the SNA. Moreover, renal denervation (RDNx) ameliorates renal inflammation in mice with AngII-induced hypertension, as well as a model of experimental glomerulonephritis, suggesting an important role of renal nerves in trafficking of T cells and cytokines into the kidney.

The present study was designed to test the hypothesis that renal nerves serve as a nexus point linking obesity, hypertension, impaired glucose metabolism, and renal inflammation. Specifically, we hypothesized that trafficking of cytokine-producing T cells into the kidney, and subsequent inflammation, is dependent on renal nerves in obesity-induced hyperglycemia.
hypertension. Furthermore, we predicted that these inflammatory signals activate renal sensory nerves, resulting in global activation of the SNA.

We tested this hypothesis in C57BL/6J mice maintained on a high-fat diet (HFD) because this model has been reported to exhibit hypertension, hyperglycemia, and renal inflammation. Specifically, we measured the effects of RDNx on AP, glucose metabolism, and renal inflammation in obese, hypertensive C57BL/6J mice and lean normotensive controls.

Methods

Animals

See Figure 1 for details of the protocol timeline. Seven-week-old C57BL/6J mice weighing 20 to 25 g from Jackson laboratories (Bar Harbor, Maine) were maintained on a 12-hour light and 12-hour dark cycle in a temperature-controlled room with free access to normal chow diet and distilled water. A week later, mice were switched to a low-fat diet (LFD; 10 kcal% fat) or HFD (45 kcal% fat; Research Diets, New Brunswick, NJ) and assigned to enter either a cardiovascular or a metabolic protocol as described later. Separate protocols were necessary to avoid damage to the telemeters by EchoMRI. All experiments were performed in compliance with National Institute of Health Laboratory Animal Care and Use guidelines and Institutional Animal Care and Use Committee approval at the University of Minnesota.

Effect of RDNx on AP in Obesity-Induced Hypertension

See Figure 1A for cardiovascular Protocol timeline. Body weight was measured weekly. Ten to eleven weeks after switching to LFD or HFD, mice were anesthetized (1.5%–2% isoflurane) for implantation of radiotelemeters (HD-X11; Data Science International, St Paul, MN) using aseptic technique (Figure 1A). The protocol is described in Methods section in the online-only Data Supplement. Mean arterial blood pressure (MAP) and heart rate (HR) were measured continuously for 7 days post surgery. To assess the contribution of autonomic nerve activity to MAP and HR, a ganglionic blocker (hexamethonium; 10 mg/kg IP) was administered on control day 2 and postsurgery (sham/RDNx) day 5. After 17 weeks of HFD and LFD to match the metabolic protocol (described later), mice were euthanized, and RDNx was confirmed by analysis of tissue norepinephrine by high-performance liquid chromatography.

Effect of RDNx on Glucose Metabolism in Obesity-Induced Hypertension

See Figure 1B for the timeline of the metabolic protocol.

Body Weight and Composition

Body weight and food intake were measured weekly. Body composition was measured by placing the mice inside the EchoMRI chamber for 45 to 60 seconds (EchoMRI 3-in-1; Echo Medical Systems). Percent body fat was measured 2, 6, 10, and 17 weeks after switching the mice to a HFD or an LFD.

Glucose Metabolism

One week before RDNx or sham surgery, mice were fasted for 14 hours, the tail was cleaned with 70% ethanol, and 1 mm was snipped once from the tip using a sterile sharp scalpel. Fasting blood glucose was measured with a glucometer (Accu-chek Aviva). Three weeks after sham/RDNx, a glucose tolerance test (GTT) was conducted. Mice were fasted for 14 hours, and 2 g/kg of D-glucose solution (Sigma-Aldrich) in sterile saline was administered intraperitoneally. Blood samples were collected at 0, 15, 30, 60, 120, and 180 minutes post injection for glucose analysis.

At the end of the protocol, kidneys were harvested for confirmation of RDNx and for quantification of renal inflammation (see below). Kidneys were cut transversely (Figure 1C) and into 4 sections. One section was frozen in liquid nitrogen and stored at −80°C for norepinephrine analysis. Second and third sections were used for flow cytometry and cytokine analysis, respectively. Finally, 2- to 3-mm-thick section was used for hematoxylin and eosin staining.

Effect of RDNx on Renal Inflammation in Obesity-Induced Hypertension

Tissue harvested in the metabolic protocol was used to assess renal inflammation as follows.

Figure 1. Experimental protocol. A, In the cardiovascular protocol, mice on low-fat diet (LFD) or high-fat diet (HFD) underwent telemetry surgery. After 12 weeks of diet and a 3-day control (C) recording of mean arterial pressure (MAP) and heart rate (HR), mice in LFD-Sham (n=7), LFD-RDNx (n=5), HFD-Sham (n=6), and HFD-RDNx (n=5) groups underwent renal denervation (RDNx) or sham surgery. At 17 weeks of diet, kidneys were collected for norepinephrine analysis. B, In the metabolic protocol, body weight (weekly), food intake (weekly), and body composition (monthly) were measured in LFD-Sham, LFD-RDNx, HFD-Sham, and HFD-RDNx (n=9/group) groups. Fasting glucose was measured 1 week before RDNx/Sham surgery; a glucose tolerance test (GTT) and indirect calorimetry were conducted 3 and 4 weeks after RDNx/Sham, respectively. Tissues were collected at the end of the protocol for norepinephrine (NE) analysis and for inflammatory profile. C, Diagram of the kidney sections used for norepinephrine analysis, flow cytometry, cytokine analysis, and histopathology.
Flow Cytometry
Mice were euthanized, and the rostral part (as shown in Figure 1C) of both kidneys were cut into small pieces (2–3 mm) and incubated in collagenase solution (100 U/mL collagenase in Roswell Park Memorial Institute buffer with 2% calf serum, 2 mmol/L MgCl₂, and 2 mmol/L CaCl₂) for 45 minutes at 37°C. Samples were homogenized gently with MACS C-tubes and poured through a 70-µm filter. A 44%/67% percoll was used to purify the lymphocytes from the kidney. The leukocyte interface was transferred to a new tube and washed with fluorescence-activated cell sorter buffer, and a single cell suspension was obtained by mashing through a 70-µm filter. A 44%/67% percoll was used to purify the lymphocytes from the kidney. The leukocyte interface was transferred to a new tube and washed with fluorescence-activated cell sorter buffer to prepare for cell staining. The single cell kidney suspension was then stained with fluorochrome-labeled antibodies: anti-CD8 (Clone 53–6.7; Tonbo Biosciences, CA), anti-CD4 (Clone RM4-5; Biolegend, CA), anti-FoxP3 (Clone FJK-16S; eBioscience, CA), anti-Helios (Clone 22F6; Biolegend, CA), anti-T-bet (Clone 4B10; Biolegend, CA), and anti-CD44 (Clone IM7; Biolegend, CA). Spleen and mesenteric lymph nodes were harvested described earlier. Intracellular staining with anti-FoxP3 was performed using the FoxP3 kit as per the manufacturer’s directions (eBioscience). Events collected with LSRFortessa flow cytometer (BD Pharmingen, CA) were analyzed by FlowJo software (Treestar, San Carlos, CA).

Renal Histopathology
Hematoxylin and eosin staining was used to determine structural changes in the kidney and score for infiltration of mononuclear cells. Detail is presented in the online-only Data Supplement.

Renal Cytokine Analysis
Multiplex kits (R&D Systems, Minneapolis, MN) were used on the Luminex 200 platform (with Bioplex protocol) to determine renal cytokine levels. Renal tissue was collected and homogenized in PBS with protease inhibitors. Analysis of renal interleukin (IL) 1β, IL-2, IL-6, IL-17, IL-10, tumor necrosis factor-α, and interferon-γ were performed following the protocol provided by the manufacturer and expressed as renal cytokine per milligram of renal protein. Protein levels were quantified by Bradford assay.

Statistical Analysis
An unpaired t test was used for comparisons between 2 groups. When >2 groups were analyzed (ie, cardiovascular and metabolic parameters), a 2-way repeated measures in 1 dimension (time) analysis of variance (ANOVA) followed by post hoc Bonferroni’s multiple comparison between all groups (GraphPad Prism 6, La Jolla, CA) was used. Two-tailed analysis was performed for all tests. A P value of <0.05 was considered statistically significant. Results are presented as means±standard error (means±SE).

Results
Effect of HFD on Caloric Intake and Body Composition
Figure 2 shows caloric (Figure 2A) and food (Figure 2B) intake throughout the protocol for the 4 experimental groups: LFD-Sham, LFD-RDNx, HFD-Sham, and HFD-RDNx. Although food intake between groups was similar (Figure 2B), caloric intake was significantly higher in HFD compared with LFD mice (Figure 2A). Body weight (Figure 2C) and fat mass (Figure 2D) were higher in HFD compared with lean normotensive LFD mice. The rate of rise of body weight and fat mass plateaued after RDNx and Sham surgeries, but the difference between HFD and LFD groups remained. Finally, RDNx had no significant effect on these variables compared with sham controls for both HFD and LFD groups.

Effect of RDNx on AP, Heart Rate, and Responses to Ganglionic Blockade in Obese Hypertensive and Lean Normotensive Mice
Body weight in mice instrumented with telemeters was similar throughout the entire course of the cardiovascular protocol to that observed in noninstrumented mice in the metabolic protocol (Figure S1 in the online-only Data Supplement). As shown in Figure 3A, the 3-day control average of MAP was higher in HFD (116±2 mm Hg) compared with LFD (103±5 mm Hg) mice. The response of MAP to Sham and RDNx was calculated as the difference on each day post surgery to the 3-day

Figure 2. Metabolic protocol: effect of high-fat diet (HFD) on food and caloric intake, body weight, and percent body fat. A, Calorie intake (kilocalories/week). Letters a, b, c, and d represent the time at which fasting glucose, Sham/RDNx, glucose tolerance test, and indirect calorimetry were conducted, respectively. *P<0.05 low-fat diet (LFD) vs HFD. Two-way repeated measures analysis of variance (ANOVA), Bonferroni’s multiple comparison post hoc test. B, Food intake (grams/7 days). Two-way repeated measures ANOVA, Bonferroni’s multiple comparison post hoc test. C, Body weight (g). *P<0.05 LFD vs HFD. Unpaired t test. D, Percent body fat. *P<0.05 LFD vs HFD. Unpaired t test. RDNx indicates renal denervation.
control MAP (ΔMAP; Figure 3B). RDNx normalized MAP in hypertensive HFD mice, with MAP falling 19±8 mm Hg from 116±2.0 mm Hg before RDNx to 97±7 mm Hg 7 days later. In contrast, Sham surgery had no significant effect on MAP in HFD mice over the 7-day period. Finally, RDNx and Sham surgery had no significant effect on MAP in LFD mice.

We have previously validated the acute depressor response to ganglionic blockade as an indirect measure of sympathetic pressor activity.22 As shown in Figure 3C, the depressor response to hexamethonium was greater in hypertensive HFD mice compared with LFD controls. Assessment of sympathetic pressor activity was repeated on day 5 after Sham/RDNx in LFD and HFD mice (Figure 3D). Sympathetic pressor activity remained higher in HFD-Sham compared with LFD-Sham mice, but RDNx abolished this difference, in that LFD-RDNx and HFD-RDNx groups were nearly identical.

HR was elevated in hypertensive HFD compared with normotensive LFD mice (Figure 4A); however, the response
of HR to RDNx and Sham was similar in the 4 groups (Figure 4B). In contrast to the response of MAP to ganglionic blockade, which is mediated by the loss of sympathetic tone exclusively, the HR response is dependent on sympatho-vagal balance to the heart. Ganglionic blockade had no significant effect on HR in LFD mice before Sham/RDNx, suggesting equally balanced sympatho-vagal tone (Figure 4C). In contrast, HR fell in HFD mice, indicating a shift in cardiac sympatho-vagal activity. More importantly, RDNx abolished the difference in cardiac sympatho-vagal activity between LFD and HFD mice 5 days later (Figure 4D).

Finally, successful denervation of the kidneys was confirmed, in that renal norepinephrine content was ≈88% lower in RDNx compared with Sham mice (Figure S2).

**Effect of RDNx on Glucose Metabolism**

Fasting blood glucose was significantly elevated in HFD compared with LFD mice before Sham/RDNx (Figure 5A). Further evidence of impaired glucose metabolism in HFD mice was evident from the GTT conducted 3 weeks after RDNx or Sham surgery. HFD-fed mice exhibited significantly higher blood glucose levels than LFD-fed mice over the 180-minute period after glucose administration (Figure 5B and 5C). However, RDNx had no significant effect on the GTT in either HFD or LFD groups.

Successful denervation was confirmed, in that renal norepinephrine was ≈78% lower in RDNx compared with Sham mice (Figure S3).

**Effect of RDNx on Renal Inflammation in HFD and LFD Mice**

One approach we used to assess the effect of a HFD and RDNx on renal inflammation was to quantify the trafficking of T cells into the kidneys. We also assessed the number of T cells in the spleen and mesenteric lymph node as measures of systemic immune status. The gating parameters used to quantify populations of CD4, CD8, T helper 1 cell, and T regulatory cells are described in the supplement (Figure S4).

HFD compared with LFD mice exhibited generalized inflammation, with significant elevation of splenic CD8, CD4, T helper 1 cell, and T regulatory cells. Note that RDNx had no significant effect on splenic (Figure 6A) or mesenteric lymph node (Figure 6B) T cells. In contrast to our hypothesis, with the exception of a higher number of T regulatory cells in the HFD-RDNx compared with LFD-Sham, we did not observe any effect of either HFD or RDNx on the number of renal T cells (Figure 6C). Finally, protein levels of renal proinflammatory cytokines IL1β, IL2, IL6, IL17, tumor necrosis factor-α, and interferon-γ, as well as the anti-inflammatory cytokine IL-10 (Figure 6D), showed no differences between HFD and...
LFD groups. Similarly, renal cytokines were not different between RDNx and Sham groups.

Hematoxylin and eosin staining was used to assess renal pathology. In contrast to previous report in which HFD was associated with renal pathology,4 we observed only minor structural changes in the kidney, indicative of fatty degeneration compared with LFD (Figure S5A). Indeed, the renal inflammation score (single-blinded score based on inflammatory mononuclear cell infiltration) showed no significant difference between HFD versus LFD groups, and RDNx had no impact on this measure (Figure S5B).

**Discussion**

Obesity-induced hypertension has been reported to be associated with increased SNA,23,24 impaired glucose metabolism,25 and renal inflammation.4 Moreover, it has been reported that RDNx reverses preclinical models of obesity-induced hypertension,9,11 improves glucose metabolism in hypertensive humans10 and an animal model of type 2 diabetes mellitus,10 and prevents trafficking of T cells into the kidney in a murine model of AngII-induced hypertension.17 Collectively, these reports led us to the hypothesis that renal nerves serve as a mechanistic nexus point between obesity, hypertension, impaired glucose metabolism, and renal inflammation. We tested this hypothesis by measuring the effects of RDNx on AP, glucose metabolism, and renal inflammation in C57BL/6J mice fed a HFD. We found that although RDNx reversed hypertension caused by high fat feeding, it had no significant effect on this measure (Figure S5B).

Obesity-induced hypertension is associated with increased SNA to the kidneys,1,5,6 This idea is supported by direct measurement of renal SNA in rabbits fed a HFD26 and reports that RDNx prevents and reverses obesity-induced hypertension in experimental animals.7–9 The 3 classical explanations for RDNx prevents and reverses obesity-induced hypertension caused by high fat feeding, it had no significant effect on levels observed in lean normotensive controls. Taken together, these findings suggest that RDNx reverses obesity-induced hypertension secondary to a reduction in sympathetic pressor activity.

The simplest explanation for these findings is that ingestion of a HFD can increase SNA to the kidneys specifically, resulting in increased renal vascular resistance and hypertension. This is consistent with the acute reversal of hypertension by ganglionic blockade, as well as the chronic reversal by RDNx. Although we cannot rule out the effects of RDNx on renin release or renal sodium excretion, these may not completely explain the responses to acute ganglionic blockade because these mechanisms operate on a longer time scale than neural control of renal vascular resistance. Our findings are also consistent with studies in which renal SNA was directly recorded in rabbits28 and rats29 consuming a HFD.

It is important to note that RDNx had no significant effect on long-term regulation of AP or measures of sympathetic pressor activity and cardiac sympatho-vagal balance in lean normotensive mice. Similarly, the responses of obese mice to RDNx were not secondary to changes in food intake or body weight because there were no differences between Sham or RDNx groups for these variables between groups.

**Effect of RDNx on Glucose Metabolism in Obese C57BL/6J Mice**

Sympathetic over activity is linked to hyperglycemia and insulin resistance in patients with drug-resistant hypertension.31,32 In addition, RDNx has been reported to improve glucose metabolism in drug-resistant hypertensive patients.12 This unexpected finding has been hypothesized to result from ablation of sympathoexcitatory renal afferent nerves and a reduction in SNA to insulin sensitive tissues.33,34 Based on these studies and our observation that RDNx abolished the increase in sympathetic pressor activity in HFD mice, we predicted that RDNx would also improve glucose metabolism in this model.

Obese mice were characterized by elevated fasting glucose and impaired glucose tolerance. Importantly, RDNx had no significant effect on fasting glucose or the GTT metabolism in any of the experimental groups in spite of improved obesity-induced hypertension. This suggests that RDNx did not alter SNA to vascular beds important for glucose homeostasis such as liver, pancreas, adipose tissue, and muscle. One caveat to our study is that we did not measure the effect of RDNx on plasma insulin or insulin sensitivity as has been reported in clinical studies demonstrating that RDNx improved glucose metabolism.11,12 Previous studies have shown that high fat–induced hyperglycemia in mice is associated with hyperinsulinemia and increased insulin resistance as measured by the homeostatic model assessment for insulin resistance (HOMA-IR).35,36 Although we did not measure insulin resistance in the present study, because RDNx had no effect on the GTT in either LFD or HFD mice, we hypothesize that RDNx did not affect insulin resistance. However, this hypothesis remains to be tested.
Effect of RDNx on Renal Inflammation in Hypertensive Obese C57BL/6J Mice

We tested our hypothesis in obese C57BL/6J mice in this study because it was recently reported that HFD causes renal inflammation and hypertension in this strain. Moreover, our hypothesis was based on reports that (1) renal nerves mediate trafficking of T cells into the kidney, and (2) subsets of T cells (CD4 and CD8) contribute to renal inflammation in AngII hypertensive mice, and these responses are prevented by RDNx.

Contrary to previous reports, we did not find any differences between HFD and LFD mice in the number of CD8 or CD4 cells in the kidneys or renal inflammatory cytokines, such as IL1β, IL2, IL6, IL17, tumor necrosis factor-α, and interferon-γ. Moreover, RDNx had no significant effect on either the number of T cells or levels of renal cytokines in lean or obese mice. We conclude that the renal inflammatory cells do not play a role in the pathogenesis of hypertension in obese C57BL/6J mice and that the antihypertensive response to RDNx occurs independent of renal inflammation.

The reasons for the discrepancies between our findings and studies that led to our hypothesis remain to be determined but there are several possibilities. First, although feeding a HFD to C57BL/6J mice has been shown to result in structural and functional changes in the kidneys, this previous study used 60% fat diet in contrast to the 45% fat diet in the our study. Second, the emerging concept that renal nerves traffic T cells into the kidney is predominantly based on the AngII-induced model in the mouse. Our results suggest that this mechanism does not translate to obesity-induced hypertension because the antihypertensive response to RDNx occurred independent of differences in renal T cells or cytokines. It should be noted that the AngII-induced increase in AP is on the order of 40 to 50 mm Hg. In contrast, there is only a 10 to 15 mm Hg increase in obesity-induced hypertension. Thus, the difference in the magnitude of the hypertension may be a factor in the interaction of renal nerves and renal inflammation. However, recently, Xiao et al. investigated the contribution of pressure-induced renal inflammation in AngII-induced hypertension in mice with unilateral RDNx. Their study suggested a role for renal nerve–dependent (i.e., pressure-dependent) renal inflammation in this model.

It should be noted that we did not investigate the role of renal macrophages in our study. Deji et al. found that a 60% HFD increased inflammatory macrophages in the kidney of C57BL/6J mice. Xiao et al. have also reported increased macrophages in the kidneys of AngII-induced hypertensive mice that were reduced by RDNx. Nonetheless, we did not observe any differences between HFD and LFD mice in the number of CD8 or CD4 cells in the kidneys or renal inflammatory cytokines, such as IL1β, IL2, IL6, IL17, IL10, tumor necrosis factor-α, and interferon-γ. Finally, RDNx had no significant effect on either the T cells and cytokines in the kidney, renal cytokines, or renal histopathology in lean or obese mice.

**Perspectives**

The antihypertensive effect of RDNx in obesity-induced hypertension in C57BL/6J mice occurred independent of effect on inflammatory mediators with no improvement in glucose metabolism. The results of this study provide important new information regarding the clinical benefit of renal nerve ablation in the treatment of hypertension and associated metabolic diseases.

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**Disclosures**

A. Panoskalis-Mortari has a family member who works for BioTechnie that owns R&D Systems. The other authors report no conflicts.

**References**


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**Novelty and Significance**

**What Is New?**

- Renal denervation lowers mean arterial pressure in hypertensive obese C57BL/6J mice by normalizing sympathetic pressor activity.
- Despite the normalization of sympathetic pressor activity in obese hypertensive mice, renal denervation had no impact on glucose metabolism.
- The effect of renal denervation on arterial pressure in obesity-induced hypertension occurred independent of indicators of renal inflammation.

**What Is Relevant?**

- The results of this study provide further mechanistic insight to the use of renal nerve ablation for the treatment of human hypertension and associated metabolic syndromes.

**Summary**

Renal nerves in obese hypertensive mice affect blood pressure but do not control changes in glucose metabolism and renal inflammation.
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RENNAL DENERVATION NORMALIZES ARTERIAL PRESSURE WITH NO EFFECT ON GLUCOSE METABOLISM OR RENAL INFLAMMATION IN OBESE HYPERTENSIVE MICE

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Running title: Renal denervation, metabolic syndrome, T cells, and hypertension

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Methods

Radio telemetry implantation and renal denervation: The transmitter catheter was placed in the left carotid artery and advanced 5-6 mm to place the tip at the junction of the carotid artery and ascending aorta. A subcutaneous tunnel was made to place the transmitter body on the left side of the mouse. The arterial pressure signal was sampled for 10 seconds every minute.

After 7 days of convalescence and 3 days of control measurements, mice were randomly assigned to undergo renal denervation (RDNx) or sham surgery. Atropine (2mg/kg, IP), an analgesic (ketoprofen 2.5mg/kg, SC), and an antibiotic (gentamicin 0.2mg/kg, IM) were administered pre-operatively. For RDNx, the renal artery and vein were exposed via a midline abdominal incision and all visible renal nerves were sectioned followed by application of a 10% phenol solution on the vessels to destroy remaining nerve fibers. The same procedure was repeated on the other kidney. Sham surgery consisted of exposing the renal artery without nerve ablation. Following surgery, 5-0 silk was used to close the incision. Ketoprofen (2.5mg/kg, SC), and an antibiotic (amoxicillin, 0.5mg/kg, in drinking water) were administered for 3 days postoperatively and animals were kept on heating pads.

Renal histopathology: Optimal cutting temperature (OCT) embedded 2-3 mm thick kidney sections were frozen using liquid nitrogen. Six-micron thick sections were cut with a cryostat and fixed using acetone. Sections were stained with hematoxylin and eosin and examined using a bright field microscope by a pathologist blinded to treatment. Sections were scored 0-4 for leukocyte infiltration and inflammation. 0 indicated a normal kidney and 4 indicated a kidney with extensive infiltration of inflammatory cells.
Figure S1: Effect of HFD on body weight in A) cardiovascular protocol and B) metabolic protocol (g). Letter b represents the time at which Sham/RDNx was performed. *p<0.05 LFD vs. HFD.
Figure S2: Effects of RDNX on renal norepinephrine levels compared to sham in the left and right of C57Bl6J mice in the Cardiovascular Protocol. LFD-Sham (n=7), LFD-RDNx (n=5), HFD-Sham (n=6), HFD-RDNx (n=5). *p<0.05.
Figure S3: Effects of RDNX on renal norepinephrine levels in the left and right of C57Bl6J mice in the Metabolic Protocol. (N=9/group) *p<0.05.
**Figure S4**: Flowcytometry gating. A representative figure of kidney tissue flowcytometry follows gating for live cells. At the end of the protocol tissues were processed and fluorescent dyes were used to gate for: Live cells, singlets (single cells), CD4, CD8, T helper 1 (Th1) and T regulatory (Treg) cells.
**Figure S5:** Effect of diet and renal denervation on renal degeneration and renal inflammation score. A) Panels with hematoxylin and eosin stained kidney. Fatty degeneration of the kidney is indicated as * on the high fat sham (HFD-Sham) panel (200X magnification, bar represents 25µm). B) HFD mediated changes in renal inflammation in the sham and RDNx groups. Scores 0 (low) to 4 (high) was assigned by single blinded method to access the mononuclear cell infiltration. Groups: LFD-Sham (n=6), LFD-RDNx (n=6), HFD-Sham (n=6), HFD-RDNx (n=4).