Hypertension after kidney transplantation
Dobrowolski, L.C.

Citation for published version (APA):
CHAPTER 7

RENNAL SYMPATHETIC NERVE ACTIVITY AFTER CATHETER-BASED RENAL DENERVATION

Linn C. Dobrowolski*, Daan W. Eeftinck Schattenkerk*, C.T. Paul Krediet, Peter M. van Brussel, Liffert Vogt, Frederike J. Bemelman, Jim A. Reekers, Bert-Jan H. van den Born, Hein J. Verberne
* both authors contributed equally

Submitted
ABSTRACT

Objectives
We hypothesized that variation in the reported RDN efficacy might be explained by incomplete nerve disruption as assessed by renal ¹²³I-meta-iodobenzylguanidine (¹²³I-mIBG) scintigraphy.

Background
Catheter-based renal sympathetic denervation (RDN) has been considered a potential treatment for therapy resistant hypertension (RHT). However, in a randomized placebo-controlled trial, RDN did not lead to a substantial blood pressure (BP) reduction.

Methods
In 21 RHT patients (median age 60 years) we performed ¹²³I-mIBG scintigraphy before and 6 weeks after RDN. Additionally, we assessed changes in BP (24 h day, night and average), plasma- and urinary-catecholamines and plasma renin activity (PRA) before and after RDN. Planar scintigraphy was performed at 15 min and 4 h after ¹²³I-mIBG administration. The ratio of the mean renal (specific) counts vs. muscle (non-specific) counts represented ¹²³I-mIBG uptake. Renal ¹²³I-mIBG washout was calculated between 15 min and 4 h.

Results
After RDN office-based systolic BP decreased from 172 to 153 mmHg (p=0.036), while diastolic office BP (p=0.531), mean 24 h systolic and diastolic BP (p=0.602, p=0.369, respectively), PRA (p=0.409) and plasma catecholamines (p=0.324) did not significantly change post-RDN. Following RDN, ¹²³I-mIBG renal uptake at 15 min was 3.47 (IQR 2.26-5.53) compared to 3.08 (IQR 2.79-4.95) before RDN (p=0.289). Renal ¹²³I-mIBG washout did not change post-RDN (p=0.230). In addition there was no significant correlation between the number of denervations and the renal ¹²³I-mIBG parameters.

Conclusions
No changes were observed in renal ¹²³I-mIBG uptake or washout at 6 weeks post-RDN. These observations support incomplete renal denervation as a possible explanation for the lack of RDN efficacy.
INTRODUCTION

Reduction of sympathetic nerve activity by catheter based renal sympathetic denervation (RDN) has raised considerable attention as a new treatment modality for resistant hypertension (RHT). This interest was fuelled by the promising results of RDN in the initial open label studies Symplicity HTN-1 and HTN-2.\(^1\)\(^2\) However, the recent randomized sham-controlled Symplicity HTN-3 trial did not show a difference in blood pressure (BP) lowering efficacy between RDN and sham treatment.\(^3\) One of the potential causes for the lack of efficacy might be the failure of the RDN procedure to sufficiently ablate renal sympathetic nerves. Yet, a routine technique to measure the extent of renal denervation is lacking and potential causes of insufficient denervation remain hypothetical.

\(^{123}\)I-meta-iodobenzylguanidine (\(^{123}\)I-mIBG) scintigraphy offers the possibility to evaluate organ specific sympathetic nerve activity. mIBG is an analogue of the ‘false’ neurotransmitter guanetidine, a potent neuron blocking agent that acts selectively on sympathetic nerves. mIBG follows similar uptake mechanisms as norepinephrine: as such mIBG-uptake enables assessment of the intactness and density of the neural tissue. Radiolabelling of mIBG with \(^{123}\)Iodide enables scintigraphic assessment. \(^{123}\)I-mIBG organ uptake and washout reflect sympathetic activity.\(^5\)\(^6\) Previously, we validated this technique for visualizing renal sympathetic innervation by showing its ability to detect changes in sympathetic innervation during kidney allograft reinnervation.\(^7\)

Based on the inter-individual variation in BP response after RDN, we hypothesized that there is a wide variability in kidney sympathetic denervation following RDN. Secondly, we hypothesized that changes in renal sympathetic activity would relate to changes in BP and neurohormonal activity following RDN. Against this background, we examined changes in renal \(^{123}\)I-mIBG uptake and washout in RHT patients before and after RDN treatment.

METHODS

From July 2011 to December 2013, we performed a prospective observational study using \(^{123}\)I-mIBG scintigraphy as a parameter of renal sympathetic activity in patients with RHT undergoing RDN. Objectives were to compare measures of renal \(^{123}\)I-mIBG uptake (uptake at 15 min and washout between 15 min and 4 h) on planar and single photon emission computed tomography-CT (SPECT-CT) images, changes in office based BP and ambulatory BP measurements (ABPM) and neurohormonal activation before and 6 weeks after RDN.

Patients

In the present study, we enrolled 21 consecutive patients aged 40–70 years with a clinical indication for RSD because of therapy resistant hypertension defined as a mean daytime BP ≥ 150/100 mmHg despite the use of 3 or more anti-hypertensive drugs including...
or with intolerance to a diuretic. Secondary causes of hypertension (e.g., renal artery stenosis, pheochromocytoma, primary aldosteronism and hyper- or hypothyroidism) and abnormal renal artery anatomy, including the presence of accessory renal arteries, were ruled out prior to the intervention. Patients with renal insufficiency (estimated glomerular filtration rate (eGFR) > 45 mL/min/1.73 m²) or proteinuria (< 1 g/24 h) or having a pacemaker, implantable cardioverter-defibrillator (ICD), atrial fibrillation or type 1 diabetes mellitus were excluded. Antihypertensive treatment was performed according to international guidelines and included instructions on dietary sodium restriction, physical activity and instructions to remain compliant to antihypertensive medication. Six weeks prior to the first measurements patients were screened to assess eligibility for study participation. Patients were deemed eligible for study participation if they were at least 3 weeks on stable BP lowering medication prior to the first study visit. BP lowering medication was kept unchanged throughout the study until the final visit 6 weeks after RDN.

When fully informed and willing to participate, patients were asked to provide written informed consent. Six weeks hereafter, office BP and ABPM was measured. Patients were required to maintain the same antihypertensive drug regimen throughout study participation. All patients provided informed consent before inclusion in the study. This study was a part of a larger effort to assess the sympaticolytic potential of RDN with the predetermined idea to assess the effects of RDN on renal ¹²³I-mIBG uptake and washout.

For reference, we used data of 5 patients (aged 39-66 years) in whom ¹²³I-mIBG was performed of the kidney allograft after recent kidney transplantation (0.1 to 1.5 years after transplantation), whose detailed characteristics are described elsewhere. In summary, all these surgically denervated kidneys functioned well with creatinine clearance rates (calculated from 24 h urine collections) ranging from 54-128 ml/min. As a negative control we also included ¹²³I-mIBG data from a patient with complete renal denervation after autologous kidney transplantation for renal artery stenosis. Although ¹²³I-mIBG is primarily cleared via the kidneys, we have shown that both the cardiac as well as the renal ¹²³I-mIBG parameter are not influenced by kidney function.

Study protocol
The study protocol met the ethical guidelines of the Declaration of Helsinki (originally adopted by the 18th WMA General Assembly, Helsinki, Finland, June 1964 and last amended in Fortaleza, Brazil 2013) and was approved by the local ethics committee of the Academic Medical Center at the University of Amsterdam (number NL.36755.018.11). All patients gave oral and written informed consent.

Renal sympathetic denervation procedure
The renal denervation procedure was performed via the femoral artery approach by a single highly experienced interventional radiologist (JAR) with > 5 RDN procedures before this study was initiated. RDN was performed by use of radiofrequency energy
delivered by the Symplicity renal-denervation catheter (Medtronic Inc, Santa Rosa, California, USA). Prior to the procedure, midazolam 1.0 mg and metoclopramide 10 mg was given intravenously. After inserting a 6 F introducer in the right femoral artery, the guiding catheter was introduced in the aorta and an aortagram was made. The guiding catheter was advanced in the right and left renal artery in no pre-specified order. The denervation catheter was introduced in the renal artery via the delivery catheter. After nitroglycerine 0.2 mg and fentanyl 0.02 mg intravenously, catheter ablations were performed in a helical pattern with the goal of at least 4-6 ablations per renal artery to cover each short axis transaxial quadrant, according to the user’s instruction of the device. No peri-procedural complications occurred.

Blood pressure monitoring
At baseline and 6 weeks after RDN 24 h ABPM was performed using the Spacelabs 90217 ABPM monitoring device (Spacelabs Healthcare, Issaquah, Washington, USA). During day time between 06.00 am – 23.00 pm measurements were performed every 15 min and at night-time (i.e. 23.00 pm – 6.00 am) every 30 min. BP readings were accepted when the success rate of the measurements was minimally 70% per 24 h. Patients were blinded to their BP readings. Instructions were given to continue usual daily activities during 24 h of BP recording, but avoiding strenuous exercise. Office brachial BP using appropriate cuff-sizes was measured with a validated semi-automated oscillometric device (Omron 705it, Omron Healthcare Europe BV, Hoofddorp, The Netherlands), while seated and after 5 min rest in a quiet room, 3 times at 1 min intervals by a trained research assistant or physician. The mean of the last 2 measurements was recorded as representative of office brachial BP. No BP measurements were performed in the kidney transplant recipient group.

Blood and urine analysis
Plasma renin activity (PRA) (μg A1/L/h) was analysed using radioimmunoassays. Urine and plasma epinephrine, norepinephrine (NE), metanephrine and normetanephrine were analysed using liquid chromatography-mass spectrometry. Epinephrine and NE and were obtained in supine as well as after 5 min in standing position. The delta of supine minus standing position was calculated. Urinary sodium excretion (mmol/24 h), urine creatinine (μmol/L), was calculated from 24 h urine collections obtained before and 6 weeks post-RDN.

123I-mIBG scintigraphy
The protocol of the renal 123I-mIBG scintigraphy has been previously described. In summary, 2 h prior to the administration of 185 MBq (5 mCi; ± 10%) 123I-mIBG (AdreView™, GE Healthcare, Eindhoven, the Netherlands) patients received 100 mg potassium-iodide to block thyroid uptake of ‘free’ 123I. In addition subjects were given a single oral dose of furosemide retard 60 mg to promote the urinary excretion of 123I-mIBG. No specific instructions on fluid intake were given to enhance excretion of 123I-mIBG. Anterior and posterior planar semi-whole body images were performed at 15 min and 4 h after administration of 123I-mIBG. A vial with a reference amount of
radioactivity of $^{123}$I was included in the planar images. Additionally, at 4 h post-injection (p.i.), SPECT-CT (low dose) was performed. The CT-images were used for an adequate anatomical registration of $^{123}$I-mIBG uptake.

Since we recently showed that uptake at 15 min p.i. of $^{123}$I-mIBG and washout between 15 min and 4 h can detect renal sympathetic reinnervation over time after transplantation, we report in this study the $^{123}$I-mIBG uptake on the 15 min p.i. images and analysed the mean counts/pixel for calculation of washout between 15 min and 4 h.\(^7\)

$^{123}$I-mIBG imaging procedures

The planar images were acquired with a 20% energy window centred at 159 keV, using medium-energy collimators. Planar anterior and posterior planar semi-whole body acquisitions were used to create geometrical mean images.

$^{123}$I-mIBG image analysis

An investigator (LCD) analysed the geometric mean (GM) planar images (Hybrid Viewer\textsuperscript{TM}, Hermes Medical Solutions, Stockholm, Sweden) by manually drawing regions of interest (ROI) for kidneys, muscle (m. quadriceps femoris) and the $^{123}$I vial. A predefined and fixed ROI for the muscle (50 pixels) was used for all patients. We analysed the counts of the left kidney only since scatter or overlay of the liver with a high uptake of $^{123}$I-mIBG resulted in poor delineation of the right kidney. Mean counts per pixel per ROI (Figure 1) were used to calculate of $^{123}$I-mIBG uptake: specific (kidney) to non-specific uptake (muscle). Formulas to calculate uptake and washout were:

$$\text{Relative uptake} = \frac{\text{kidney (specific)} - \text{muscle (non-specific)}}{\text{muscle (non-specific)}}$$

$$\text{Washout} = \frac{\left( \frac{\text{uptake kidney 15 min}}{\text{uptake muscle 15min}} \right) - \left( \frac{\text{uptake kidney 4 h}}{\text{uptake muscle 4 h}} \right)}{\left( \frac{\text{uptake kidney 15 min}}{\text{uptake muscle 15min}} \right)} \times 100\%$$

The percentage uptake of the injected dosage of $^{123}$I-mIBG was calculated using the actual injected dose and mean counts per pixel in relation to the activity in $^{123}$I-vial. Washout (WO) in the left kidney was calculated from 15 min and 4 h images using skeletal muscle as reference.

A secondary analysis was focused on the SPEC-CT images. In this method the transverse CT images were used to optimize anatomical delineation of the kidney contours. The main advantage of this method is the availability of anatomical information obtained from the low dose CT, allowing for a superior delineation of kidneys and a subsequently a potential better estimation of the renal $^{123}$I-mIBG uptake. ROIs were drawn on the CT-images along the contours of kidney cortices, excluding the calyces. ROIs were then fused into volumes of interest (VOIs) and copied to the co-registered SPECT. Mean
counts/voxel expressed $^{123}$I-mIBG uptake. VOIs in muscle served as background activity. Based on the difference in $^{123}$I-mIBG uptake, we divided patients with a positive change in $^{123}$I-mIBG uptake, i.e. indicating an increase in $^{123}$I-mIBG uptake or washout and those with a negative change, i.e. a decrease in $^{123}$I-mIBG uptake or washout after RDN.

Statistical analysis
This study was part of a larger effort to study sympatholytic effects of RDN. The sample size has been described elsewhere. Data are presented as medians and interquartile ranges (IQR with 25 and 75 percentiles) and comparisons were performed by non-parametrical tests. P-values below 0.05 were considered statistically significant. All analyses were performed using IBM SPSS Statistics software for Windows version 21.0 (IBM Corp. Armonk, New York, USA).

RESULTS

Baseline characteristics
We studied 21 patients with therapy resistant hypertension (Table 1). The majority of patients were male (71% with a median 60 years) and were Caucasian (76%). Median body mass index was 28.0 kg/m$^2$ (24.8-30.5 kg/m$^2$). Diabetes mellitus was present in 33% and left ventricular hypertrophy, according to electrocardiography voltage criteria, was present in 29% of the patients. A history of a cardiovascular disease (i.e. coronary artery disease, angina pectoris, heart failure, stroke, peripheral arterial disease) was present in 48% of the study participants.
Renal $^{123}$I-mIBG uptake and washout in the left kidney

The planar derived mean relative uptake of $^{123}$I-mIBG of the left kidney at 15 min p.i. did not change significantly from pre RDN 3.08 (2.79-4.95) to post RDN 3.47 (2.26-5.53), $p=0.289$ (Table 2). Figure 2 represents pre vs. post RDN $^{123}$I-mIBG uptake at 15 min p.i. including recently transplanted kidneys as controls.

The percentage uptake of the injected dosage of $^{123}$I-mIBG in the left kidneys showed a non-significant decrease after RDN from 17.8\% to 15.4\% (delta -13\%, $p=0.881$). Washout rate between 15 min and 4 h p.i. was 41.5\% before and 42.7\% after RDN, $p=0.230$. The SPECT derived uptake at 4 h decreased non-significantly after RDN (1.41 to 1.07, $p=0.526$). None of the renal uptake or washout parameters were correlated with kidney function (data not shown).

Number of denervations and renal $^{123}$I-mIBG uptake and washout

No significant correlation was found between the number of denervations (left renal artery $4.3 \pm 0.6$, right renal artery $4.2 \pm 0.5$) and renal uptake of $^{123}$I-mIBG in the left kidney at either 15 min ($R=-0.27$, $p=0.243$), 4 h p.i. ($R=-0.37$, $p=0.103$) or $^{123}$I-mIBG washout ($R=0.05$, $p=0.837$).

Effect of RDN on blood pressure, PRA and catecholamines

Table 3 shows the effect of RDN on blood pressure and catecholamines. RDN resulted in a significant decrease in systolic office BP ($p=0.036$), without reducing diastolic BP ($p=0.531$). Systolic and diastolic daytime ABPM were not significantly different after denervation. Neither antihypertensive medication nor sodium intake, as inferred from urinary sodium excretion, were significantly different between pre vs. post-RDN (Table 2).

At baseline, plasma and urine catecholamine levels were within reference values. Plasma epinephrine and NE did not change ($p=0.780$ and $p=0.324$ respectively) nor did the 24 h urinary excretion of metanephrine ($p=0.51$) and normetanephrine ($p=0.91$) following RDN (Table 2).
Renal sympathetic nerve activity after renal denervation

except for the correlation between renal ${}_{123}^I$-mIBG uptake and office systolic BP ($p=0.018$), no correlations were found between any of the renal ${}_{123}^I$-mIBG uptake and washout parameters and blood pressure, PRA or catecholamines (Figure 3). Subgroup analyses revealed no changes in patients with a BP decrease and their ${}_{123}^I$-mIBG parameters (supplemental data).

**DISCUSSION**

In the present study we were unable to demonstrate that treatment with RDN results in significant changes in renal ${}_{123}^I$-mIBG uptake and washout. These data suggest that RDN does not significantly alter renal sympathetic tone and does not sufficiently denervate renal sympathetic nerves. This was further supported by the finding that ABPM and biochemical markers of sympathetic nerve activity remained unchanged after RDN, while the reduction in office BP was similar compared to Symplicity HTN-1 and HTN-2. The absence of consistent changes in ${}_{123}^I$-mIBG uptake and washout as well as the lack of a sustained BP decrease after RDN suggests that the present RDN technique fails to achieve adequate denervation of the kidneys. The degree of renal sympathetic nerve disruption required for inducing a sustained BP response remains unclear, but likely falls short with the current RDN technique. The lack of efficacy may be related to the number of ablations, since in a subset of patients of Symplicity HTN-3 a more profound BP decrease was observed in patients with more ablations, suggesting a relation between the quantity of ablations and the BP lowering effects. This effect, however, was also observed in patients receiving sham treatment. We found no association between the number of ablations and renal ${}_{123}^I$-mIBG uptake or washout, while the number of denervations in our study was similar to the Symplicity HTN-1 and

![Figure 2. Change in renal uptake of ${}_{123}^I$-mIBG after RDN](image-url)
HTN-2 trials that demonstrated a significant decrease in office BP.\textsuperscript{2,3} In a recent post-mortem study of a patient who received RDN it was shown that nerves in the (peri-) adventitial parts of the renal artery were unaffected, indicating that interruption of the nerve fibre continuity had not been successful.\textsuperscript{13} This suggests that the ablation pulse may not be sufficient to generate adequate denervation of renal sympathetic nerves.\textsuperscript{14} A previous study using NE spill-over to assess the effect of the nerve fibre continuity had not been successful.\textsuperscript{13} This suggests that the ablation pulse may not be sufficient to generate adequate denervation of renal sympathetic nerves.\textsuperscript{14} A previous study using NE spill-over to assess the effect of RDN on renal sympathetic activity in 10 patients with resistant hypertension showed that RDN reduced NE spill-over by 47% (95% CI 28-65%).\textsuperscript{15} In the present study we could not replicate these findings. Besides lack of procedural effectiveness, this discrepancy could also be explained by differences in population characteristics or technical shortcomings of \textsuperscript{123}I-mIBG scintigraphy. The patients in our study were however fully comparable to the populations studied in Symplicity HTN-1 and Symplicity HTN-2. Although, we used ABPM instead of office BP to include patients with resistant hypertension, baseline office BP in our study and the number of BP lowering drugs were comparable to that observed in Symplicity HTN-1 and Symplicity HTN-2. In addition, office BP was reduced to a similar extent with a decrease of 29 mmHg for systolic office BP following RDN and all other baseline parameters of our study population were similar to that of previous studies.\textsuperscript{2,3,4} In kidney transplant recipients we recently showed that uptake at 15 min p.i. of \textsuperscript{123}I-mIBG and washout is correlated with time after transplantation independent of kidney graft function.\textsuperscript{7} This suggests that renal \textsuperscript{123}I-mIBG scintigraphy can be used to assess differences in renal innervation. To assess whether our technique is also sufficient to assess changes in sympathetic innervation following RDN, we calculated the difference in renal \textsuperscript{123}I-mIBG-uptake after 15 min that could be detected using the observed standard deviation of our data. We calculated that we were able to demonstrate a difference between -1.21

### Table 2. Pre and post RDN differences in quantifications of \textsuperscript{123}I-mIBG uptake (n=21)

<table>
<thead>
<tr>
<th>Planar GM Images</th>
<th>PRE-RDN</th>
<th>POST-RDN</th>
<th>( p )-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uptake 15 min</td>
<td>3.08 [2.79-4.95]</td>
<td>3.47 [2.26-5.53]</td>
<td>0.289</td>
</tr>
<tr>
<td>Uptake 4 h</td>
<td>1.64 [1.44-1.98]</td>
<td>1.52 [1.12-2.27]</td>
<td>0.876</td>
</tr>
<tr>
<td>% Injected dose 15 min*</td>
<td>17.88 [17.88-21.75]</td>
<td>15.43 [13.73-22.13]</td>
<td>0.881</td>
</tr>
<tr>
<td>% Injected dose 4 h*</td>
<td>8.91 [8.91-13.52]</td>
<td>9.37 [7.20-12.35]</td>
<td>0.681</td>
</tr>
<tr>
<td>Washout 15 min-4 h (%)</td>
<td>41.53 [28.26-56.25]</td>
<td>42.69 [35.02-56.16]</td>
<td>0.230</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>SPECT-CT Images</th>
<th>Uptake CT 4 h</th>
<th>( p )-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uptake 4 h</td>
<td>1.41 [0.95-1.86]</td>
<td>1.07 [0.73-1.69]</td>
</tr>
</tbody>
</table>

Data are presented as medians with interquartile ranges (IQR 25-75%). Abbreviations: RDN = Renal denervation; GM = geometric mean images, with muscle as background; SPECT = single photon emission computed tomography. * data from n=20 patients since in one patient a \textsuperscript{123}I-vial was not included during the scintigraphy and therefore the percentage of injected dose \textsuperscript{123}I-mIBG could not be calculated.
Renal sympathetic nerve activity after renal denervation

to +1.21 in renal 123I-mIBG-uptake with 95% confidence at an alpha level of 0.05 and with 80% power. Using the baseline difference in renal 123I-mIBG uptake in the left kidney and after complete denervation in kidney allograft recipients as reference, we would be able to demonstrate a 44% difference in renal 123I-mIBG uptake assuming that background 123I-mIBG-uptake is similar. This suggests that our sample size was sufficient to detect a less than 50% reduction in renal sympathetic activity.

We previously showed that cardiac sympathetic activity did not change after RDN.12 This is also supported by the lack of change in neurohormonal activation following RDN in the present and in previous studies.16,17 Whether this is caused by insufficient denervation or results from a limited overall contribution of renal nerves in determining efferent sympathetic activity could not be assessed because quality parameters for successful RDN are lacking. In the present study we show that the lack of change in

<table>
<thead>
<tr>
<th>Table 3. Blood pressure, kidney function and catecholamines</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Blood pressure</strong></td>
</tr>
<tr>
<td>Office based Systolic (mmHg)</td>
</tr>
<tr>
<td>Office based Diastolic (mmHg)</td>
</tr>
<tr>
<td>ABPM Daytime Systolic (mmHg)</td>
</tr>
<tr>
<td>ABPM Daytime Diastolic (mmHg)</td>
</tr>
<tr>
<td>ABPM Night time Systolic (mmHg)</td>
</tr>
<tr>
<td>ABPM Night time Diastolic (mmHg)</td>
</tr>
<tr>
<td>ABPM Average Systolic (mmHg)</td>
</tr>
<tr>
<td>ABPM Average Diastolic (mmHg)</td>
</tr>
</tbody>
</table>

| **Antihypertensive drugs**                                  |
| Number of antihypertensive drugs                           | 4.6 ± 1.3           | 4.4 ± 1.4           | 0.157 |
| 3 classes, n (%)                                           | 5 (23.8)            | 7 (33.3)            |
| 4 or more classes, n (%)                                   | 16 (76.2)           | 14 (66.7)           |

| **Kidney function**                                        |
| Creatinine serum (μmol/L)                                  | 94.0 [76.5-107.5]   | 89.0 [73.5-113.5] | 0.369 |
| eGFR (ml/min/1.73m2)                                       | 60.7[48.5-101.9]    | 64.6 [48.0-99.9] | 0.218 |
| Proteinuria (g/L/24 h)                                     | 0.10 [0.07-0.20]    | 0.11 [0.07-0.26] | 0.722 |
| Sodium urine (mmol/24 h)                                   | 161 [102-203]       | 128 [90-161]      | 0.230 |

| **(Neuro) endocrine activity**                             |
| Plasma renin activity (µg/A1/L/h)                          | 1.70 [0.95-3.20]    | 1.0 [0.60-1.68] | 0.409 |
| Epinephrine supine, plasma (nmol/L)                       | 0.12 [0.05-0.23]    | 0.10 [0.05-0.17] | 0.780 |
| Norepinephrine supine, plasma (nmol/L)                    | 2.43 [1.32-3.78]    | 2.76 [1.49-4.02] | 0.324 |
| Epinephrine urine (nmol/24 h)                             | 27.5 [14.5-33.8]    | 26.0 [18.0-38.0] | 0.551 |
| Norepinephrine urine (nmol/24 h)                          | 268.5 [137.5-495.0] | 308.5 [237.5-479.3] | 0.245 |
| Metanephrine urine (nmol/24 h)                            | 0.78 [0.49-1.05]    | 0.68 [0.50-1.02] | 0.506 |
| Normetanephrine urine (nmol/24 h)                         | 2.13 [1.73-3.37]    | 2.53 [1.74-3.02] | 0.911 |
cardiac sympathetic activity may be caused by an inability of RDN to cause a sufficient decrease in afferent sympathetic nerve activity as $^{123}$I-mIBG-uptake was unchanged.

A few limitations of our study merit discussion. Firstly, it remains possible that the modulation of SNA induced by RDN lies below the detection level of $^{123}$I-mIBG. However, it may well be that sympathicolysis is achieved by RDN but that this does not influence BP, activity of the renin-angiotensin system and $^{123}$I-mIBG parameters. Radiotracer dilution NE spill-over for organ specific assessment of sympathetic nerve activity is an alternative to $^{123}$I-mIBG scintigraphy. Although this technique is considered the gold standard, its application is limited by its invasive nature. Moreover a widespread use of the technique is restricted by the poor availability of the required compounds. Secondly, $^{123}$I-mIBG is primarily cleared via the kidneys and therefore kidney function may have influenced our data. However we have shown that both cardiac and renal $^{123}$I-mIBG parameters are not influenced by kidney function. Finally, we were aware of the potential influence of antihypertensive medication (calcium blocking agents, beta blocking agents) that may alter sympathetic drive and thereby uptake of $^{123}$I-mIBG. In 2 patients, BP lowering medication had to be tapered because of hypotension post RDN. In the remaining patients however BP lowering medication and sodium excretion were unchanged during the study period. We therefore feel that changes in antihypertensive medication do not explain the lack of change in $^{123}$I-mIBG readouts.

In conclusion, we could not observe significant changes in functional kidney denervation as assessed with $^{123}$I-mIBG scintigraphy following RDN with the Symplicity Catheter System. Our data suggest that the lack of BP lowering efficacy in the sham-controlled Symplicity HTN-3 study may be related to lack of procedural effectiveness. In comparison to available clinical tools renal $^{123}$I-mIBG scintigraphy is minimally invasive and more widely available for clinical use. For future studies, renal $^{123}$I-mIBG scintigraphy may be used as a parameter to assess RDN effectiveness.

**CLINICAL COMPETENCIES**

A potential cause for the lack of efficacy of RDN might be the failure of RDN to sufficiently ablate renal sympathetic nerves. Yet, a routine technique to measure the extent of renal denervation is lacking and potential causes of insufficient denervation remain hypothetical. In this study we show that renal $^{123}$I-mIBG scintigraphy, a minimally invasive technique to measure sympathetic nerve activity, can be used as a parameter of nerve disruption efficacy. To our knowledge, renal $^{123}$I-mIBG scintigraphy has not been used for this purpose. Our study underlines the importance of evaluating procedural effectiveness and adds to the discussion whether fairly invasive tools need to have a clear read out of their efficacy.
Renal sympathetic nerve activity after renal denervation

Figure 3. Renal $^{123}$I-mIBG uptake with blood pressure and biochemistry
TRANSLATIONAL OUTLOOK

Our data suggest that RDN by means of the Symplicity catheter does not result in significant changes in functional kidney denervation as assessed with $^{123}$I-mIBG scintigraphy. This may explain the lack of BP lowering effect of this technique. Our results are relevant to further delineate the role of RDN in therapy resistant hypertension and create a better understanding of the lack of efficacy of the current RDN techniques. Most of the available tools, however, are invasive and not applicable for broad clinical use. In this study we showed that renal $^{123}$I-mIBG scintigraphy can be used as a parameter of nerve disruption efficacy. This technique is minimally invasive and is a measure of sympathetic nerve activity. Future RDN catheters could be evaluated for their potential to lower sympathetic activity using readouts such as renal $^{123}$I-mIBG scintigraphy.

ACKNOWLEDGEMENTS

CTPK received grants from the Dutch Kidney Foundation (IP-11.40 and KJPB12.29, Bussum, The Netherlands) and from ZonMW Clinical Fellowship (40007039712461), Zorg Onderzoek Nederland/Medische Wetenschappen (ZonMW, Den Haag, The Netherlands). This support is gratefully acknowledged.
REFERENCES