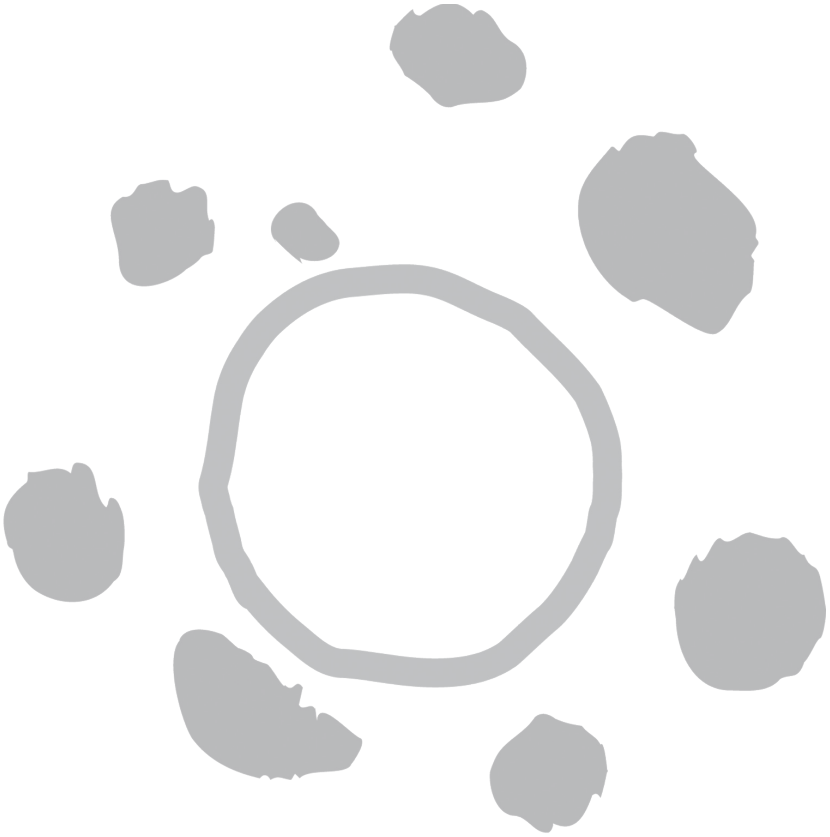
The dreams of renal denervation

A translational approach

Willemien Verloop



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The dreams of renal denervation
A translational approach

Dromen van renale denervatie
Een translationele benadering

(met een samenvatting in het Nederlands)

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Wilhelmina Liberta Verloop

geboren op 26 maart 1985

te Miri, Maleisië

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Voor oma Berg
Dank voor je inspiratie en aanmoediging

Contents

Abbreviations

Abbreviations

ABPM	Ambulatory Blood ressure Measurement
ACEi	Angiotensin Converting Enzyme Inhibitor
AE	Adverse Event
AIx	Augmentation Index
AIx@75bpm	Augmentation Index Corrected for 75 Beats Per Minute
alpha-SMA	Alpha Smooth Muscle Actin
APV	Average Peak Flow Velocity
ARB	Angiotensin Receptor Blocker
BMI	Body Mass Index
BMR	Baseline Microvascular Resistance
BP	Blood Pressure
BPM	Beats Per Minute
BSA	Body Surface Area
CAD	Coronary Artery Disease
CCT	Captopril Challenge Test
CGRP	Calcitonin Gene-Related Peptide
CKD	Chronic Kidney Disease
CKD-EPI	Chronic Kidney Disease Epidemiology Collaboration formula
CNS	Central Nervous System
CT	Computed Tomography
CTa	Computed Tomography Angiography
CVD	Cardiovascular Disease
CVZ	College Voor Zorgverzekeringen
DBP	Diastolic Blood Pressure
DD	Diastolic Dysfunction
DDD	Daily Defined Dosage
DM	Diabetes Mellitus
DU	Daily Unit
ECG	Electrocardiogram
EEL	External Elastic Lamina
eGFR	Estimated Glomerular Filtration Rate
EOD	End Organ Damage
ESC	European Society of Cardiology
ESH	European Society of Hypertension
GFR	Glomerular Filtration Rate
GTT	Glucose tolerance test
HDL	High Density Lipoprotein
HF	High frequency
HF	Heart Failure
HFPEF	Heart failure with a preserved ejection fraction
HFREF	Heart Failure with a Reduced Ejection Fraction
HMR	Hyperemic Microvacular Resistance
HOMA-IR	Homeostasis Model of Assessment-Insulin Resistance
HR	Heart Rate
HRV	Heart Rate Variability
IEL	internal Elastic Lamina
IR	Insulin Resistance

IS	Insulin Sensitivity
ISO	Isoproterenol
LF	Low Frequency
LV	Left Ventricular
LVEF	Left Ventricular Ejection Fraction
LVH	Left Ventricular Hypertrophy
LVM	Left Ventricular Mass
MAP	Mean Arterial Pressure
METC	Medisch Ethische Commissie
MetS	Metabolic Syndrome
MIBG	MetalodoBenzylGuanidine
MRa	Magnetic Resonance Angiography
MRI	Magnetic Resonance Imaging
MSNA	Muscle Sympathetic Nerve Activity
MST	Masson's Trichome
NE	Norepinephrine
NYHA	New York Heart Association
OCT	Optical Coherence Tomography
PAC	Plasma Aldosterone Concentration
PGP 9.5	Protein Gene Product 9.5
PRA	Plasma Renin Activity
pRDN	Percutaneous Renal Denervation
PRISMA	Rreferred Reporting Items for Systematic Reviews and Meta-Analyses
PWA	Pulse Wave Analysis
PWV	Pulse Wave Velocity
RBF	Renal Blood Flow
RDN	Renal Denervation
RF	Radiofrequency
RFVR	Renal Flow Velocity Reserve
RRR	Renal Resistance Reserve
SAE	Serious Adverse Event
SBP	Systolic Blood Pressure
SBPM	Self-monitored Blood Pressure Measurement
SD	Standard Deviation
SIisOGTT	Simple Index Assessing Insulin Sensitivity Oral Glucose Tolerance Test
SNA	Sympathetic nervous activity
SNS	Sympathetic nervous system
TH	tyrosine hydroxylase
UMC	University Medical Centre
VMA	Vanillylmandelic Acid
WCE	White Coat Effect
WHO	World Health Organization

Introduction

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Renal denervation: a new treatment option in resistant arterial hypertension

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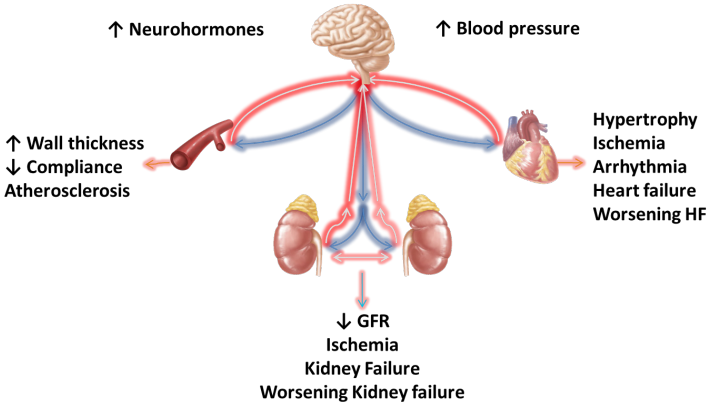
The sympathetic nervous system

In the body, many processes are carried out without raising conscious sensations. These processes zoccur independent of the will, without thought, and are more or less automatic in their accomplishment.[1] The activities of these processes are controlled by a special system of nerve cells and nerve fibers: the autonomic nervous system.[1] The autonomic nervous system is divided into the sympathetic nervous system (SNS) and the parasympathetic nervous system (PSNS).[2]

A specific function of the sympathetic nervous system is to mobilize the human body in acute, dangerous, and/or stressful situations by inducing the fight-or-flight response.[3] However, the SNS is constantly active at a basic level to maintain homeostasis.[3]

During the fight-or-flight response the SNS achieves numeral actions among which the production of cortisol by the adrenal glands, the suppression of the immune system, the constriction of blood vessels, the increase in heart rate and fortification of the contraction, and the production of renin by the kidneys.[4] This response acts primarily on cardiovascular functions in a direct and an indirect way. The direct way is through sympathetic afferent and efferent nerve fibers, the indirect way is through production of adrenaline and norepinephrine (NE) in the adrenal medulla.[4] In addition to the fight-or-flight response, the sympathetic tone is chronically elevated in a number of cardiovascular diseases such as primary hypertension, heart failure, chronic kidney disease, ischemic stroke, myocardial infarction, diabetes, obstructive sleep apnea, and obesity.[5] The kidneys and especially the renal sympathetic nerves contribute to a state of elevated sympathetic activity.[5] Renal sympathetic nerves induce hypertension by increasing renin secretion and tubular reabsorption of sodium, and by decreasing renal blood flow.[6]

Figure 1



Anatomy of the sympathetic nervous system

The SNS comprises of both efferent and afferent nerves. The efferent sympathetic fibers leave the central nervous system and all end in sympathetic ganglia known as preganglionic fibers.[7] The sympathetic nerves going to the kidney originate from the 10th thoracic through the 2nd lumbar spinal cord segments.[7] They are assembled in the renal plexus that is located around the renal artery. The afferent fibers provide a feedback loop to connect the different organs with the central nervous system. Afferent nerve fibers enter the spinal cord at the 10th through the 12th thoracic spinal cord segments.[7]

Resistant hypertension

The prevalence of hypertension is estimated to be 40% of the adult population worldwide.[8, 9] Hypertension can be classified as either primary or secondary hypertension.[10] Primary hypertension indicates that no specific cause can be found to explain why a patient has an increased blood pressure (BP).[10] Secondary

hypertension indicates that the increased BP is the result of an underlying condition.[10] Approximately 90-95% of the patients with hypertension are thought to have primary hypertension.[10]

A large proportion of patients that have hypertension are not aware of their diseased state and are also not adequately treated.[9, 11] Only 34-44% percent of the subjects with hypertension are aware of the elevated BP levels.[9, 11] Next, only 56-61% of the patients who are aware of their elevated BP receive antihypertensive treatment.[11] Subsequently, only 42-48% of the treated patients have a well-controlled BP.[9, 11]

Despite a broad availability of antihypertensive drugs, a number of patients still have an uncontrolled blood pressure, despite adherence to multiple antihypertensive drugs.[12] These patients can be classified as having resistant hypertension. Resistant hypertension is defined as blood pressure that remains above goal despite the concurrent use of three antihypertensive agents of different classes, one of which should be a diuretic.[12] Patients whose blood pressure is controlled with four or more medications are also considered to have resistant hypertension.[12]

As mentioned above, the SNS and in particular the renal sympathetic nerves are involved in the pathophysiology of (resistant) hypertension. The mechanisms of increased sympathetic activity in hypertension involve alteration in baroreflex and chemoreflex pathways.[13] Arterial baroreceptors are reset to a higher pressure in hypertensive patients, and this peripheral resetting reverts to normal when arterial pressure is normalized. The aortic baroreflex is centrally resetted in hypertensive patients, resulting in suppression of sympathetic inhibition after activation of aortic baroreceptor nerves. This baroreflex resetting seems to be mediated, at least partly, by a central action of angiotensin II.[14]

In addition, kidney injury can cause increased sympathetic activity through a cascade of actions: renal blood flow and glomerular filtration rate decreases by renal vasoconstriction. As a result the release of renin by the juxtaglomerular cells is stimulated and Angiotensin II is produced. This process is further amplified by direct activation of the RAS by kidney injury. In conclusion, increased renal sympathetic activity can also directly increase renal tubular sodium reabsorption.[15]

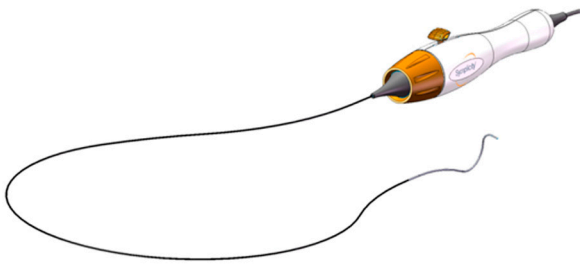
Based on the involvement of the SNS in resistant hypertension, renal denervation (RDN) has been developed as a new treatment option for patients with resistant hypertension.

Percutaneous denervation of the renal arteries

The concept of renal denervation, and thereby lowering central SNS activity in order to treat hypertension, is not new. Based on the findings that surgical nephrectomy in humans resulted in a reduction of MSNA[16], the concept arose, that surgical denervation would be an effective treatment for patients with hypertension. Indeed surgical denervation (e.g. splanchnicectomy) has been shown to be effective in lowering blood pressure.[17] However, these methods were associated with high perioperative morbidity, long-term complications, and even mortality.[18]

In this context, a percutaneous, catheter-based approach has been developed using radiofrequency (RF) energy to disrupt renal sympathetic nerves without affecting other abdominal, pelvic, or lower extremity innervations.[19] The catheter is introduced into the renal arteries via a femoral access. An example of a frequently used catheter is displayed in figure 2. A bilateral treatment of the renal arteries is performed using radiofrequency (RF) energy. The ablations in the renal artery are conducted from distal towards proximal in a circumferentially rotating manner with 5 mm spacing between each ablation treatment.

Figure 2



Pre-clinical studies

Before RDN was conducted in a clinical setting, the treatment has been studied in a preclinical study.[20] Rippy et al. performed RDN in a swine model to characterize vascular safety and healing response 6 months after denervation.[20] They showed that RDN was effective, reducing norepinephrine (NE) levels by 80-90%, without adverse effects on the vessel wall.[20] Surprisingly, this was the only industry-sponsored preclinical study that was published before RDN took a wide dive into clinical studies. In 2012 Steigerwald et al. published an investigator-driven preclinical study in 14 pigs to characterize the morphological changes of the renal arteries up to 10 days after RDN using the Symplicity catheter.[21] The authors showed that RDN can lead to circumscribed transmural injury within the arterial wall affecting autonomic nerve fascicles. Secondly, the authors performed optical coherence tomography and observed acute loss of endothelialization resulting in thrombus formation. Yet, this thrombus formation did not affect kidney perfusion. A few other studies investigated the morphological changes as a secondary endpoint, but did not show any remarkable negative effects on the vasculature.[22, 23]

Clinical studies

The clinical efficacy of RDN as a treatment strategy for patients with resistant hypertension has mainly been evaluated by the Symplicity HTN-1[19], HTN-2[24], and HTN-3 trials.[25] In these studies, RDN showed to be safe, illustrated by a lack of vascular or renal injury up to three years after treatment.[26, 27] Furthermore, the first studies showed impressive reductions in office BP (approximately 30 mmHg reduction in systolic blood pressure) up to three years after RDN.[28, 29]

Proof of principle study, HTN-1

In 2009, Krum et al., performed a multicenter proof-of-principle study to assess safety effectiveness of RDN. Authors enrolled 50 patients with resistant hypertension, of which 45 underwent RDN. In the treated patients, bilateral RDN reduced office systolic and diastolic BP by -14/-10, -21/-10, -22/-11, -24/-11, and -27/-17 mm Hg at 1, 3, 6, 9, and 12 months follow-up. The effectiveness of RDN was assessed by norepinephrine spillover in a subgroup of 10 patients, showing a mean reduction of 47% NE levels. One intraprocedural renal artery dissection occurred before delivery of energy. No other renovascular complications were reported. In December 2013, the Symplicity HTN-1 Investigators presented the final 3-year report of the Symplicity HTN-1 study.[30] Three years after RDN, a mean reduction of 32/14 mm Hg in office BP was established in 88 patients. Periprocedural safety was monitored in 153 patients undergoing RDN. In this population, 3 groin pseudoaneurysms and 1 renal artery dissection were noted. Based on the HTN-1 study, RDN seemed safe and effective, also after long-term follow-up.

Randomized controlled trial, HTN-2

The randomized controlled Symplicity HTN-2 trial was conducted to confirm the findings of the proof-of-principle study. In this trial, 106 patients were randomly allocated in a one-to-one ratio to undergo renal denervation on top of previous medical treatment (n=52) or to maintain previous medical treatment alone (control group, n=54).[24] After 6 months follow-up, office BP reduced by 32/12 mm Hg (P<0,0001), whereas BP did not change in the control group.[24] Home and ambulatory BP followed a similar pattern in a subpopulation; reductions were 20/12 mm Hg and 11/7 mm Hg respectively with RDN, while no reductions were observed in the control group. Furthermore, a cross-over design was included, showing a similar reduction in BP in the patients treated after 6 months.[24] The 3-year follow-up report showed a sustained reduction in the initial RDN group (mean reduction of 33 mmHg in 40 subjects; P<0.01) and the crossed-over group (mean reduction of 34 mmHg in 30 patients; P<0.01).[24] Although the investigators noted no substantial adverse events, the trial had several limitations (no sham, no exclusion of white coat hypertension). Furthermore, a small subgroup of patients (n=5) did not respond to treatment.

Sham-controlled double blind trial, HTN-3

The HTN-3 trial was the first randomized, sham-controlled, double blind trial.[25] Patients with severe resistant hypertension were randomly assigned in a 2:1 ratio to undergo RDN or a sham procedure. This trial failed to show a significant reduction of systolic blood pressure, as assessed with both office and ambulatory blood pressure measurements, in patients with resistant hypertension 6 months after renal-artery denervation as compared with a sham control.[25] This was due to both a less impressive office BP reduction in the treated group (-14±24 mmHg), as well as a pronounced BP decrease in the sham control group (-12±26 mmHg; P=0.26). This trial tempered the enormous enthusiasm of RDN and led to the consensus that more research is needed in the field of RDN.

DREAM or reality?

Next to the blood pressure reduction induced by renal denervation, some small studies have reported that RDN can positively affect other conditions characterized by sympathetic overactivity. These conditions include heart failure, sleep apnea, insulin resistance, and metabolic changes in polycystic ovary syndrome.[31, 32] Obviously, these preliminary results have to be confirmed in larger studies. With the negative results of the HTN-3 in mind, one should be careful not to overrate the effects of RDN upfront. In this thesis the effects of RDN on some of these conditions are further investigated.

Outline of the thesis

The first part of the thesis concerns pathophysiological changes after renal denervation, as potential explanations for the observed BP-reduction. **Chapter 2** discusses the changes in renal hemodynamics after RDN in a porcine model. Secondly, this chapter describes the effects of RDN on vascular damage and nerve damage. **Chapter 3** describes the change in renal hemodynamics after RDN in hypertensive patients. **Chapter 4** describes the role of reactive oxygen species in hypertension and how they can be altered after treatment by RDN.

The second part of the thesis concerns the selection of patients eligible for renal denervation. Not all patients with an uncontrolled BP have resistant hypertension. Secondary causes or non-compliance are also ground for uncontrolled hypertension. **Chapter 5** provides evidence that patients should be thoroughly screened before they can be categorized as “eligible for RDN”. Since a wide variation in effect is observed after RDN, we aimed to identify predictors to determine on forehand which patients will benefit the most from RDN in **chapter 6**. **Chapter 7** describes the prevalence of multiple renal arteries in patients referred for RDN. Secondly this chapter analyzed whether patients with multiple renal arteries respond different to RDN compared to patients without multiple renal arteries.

The third part of the thesis concerns the effect of RDN in hypertensive patients. **Chapter 8** describes the effect of RDN in the first 11 patients treated with RDN in the Netherlands. **Chapter 9** describes whether RDN leads to a reduction in muscle sympathetic nerve activity 6 months after treatment. **Chapter 10** describes the effect of RDN on end organ damage in hypertensive patients one year after treatment.

The fourth part of the thesis describes the indications of RDN beyond resistant hypertension. In the prospective DREAMS study, the influence of RDN on metabolic changes is evaluated. **Chapter 11** describes the effect of RDN on insulin sensitivity, BP, and sympathetic activity in a population of metabolic syndrome patients. **Chapter 12** evaluates whether RDN has an effect on muscle sympathetic nerve activity and heart rate variability in metabolic patients. **Chapter 13** is a systematic review that describes the relation between the SNS and heart failure with preserved ejection fraction (HFPEF).

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Part 1. Pathophysiology in a translational setting

The effects of renal denervation on renal hemodynamics and renal vasculature in a porcine model

Submitted

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Abstract

Rationale Recently, the efficacy of renal denervation (RDN) has been debated. It is discussed whether RDN is able to adequately target the renal nerves.
Objective We aimed to investigate how effective RDN was by means of functional hemodynamic measurements and nerve damage on histology.

Methods and results We performed hemodynamic measurements in both renal arteries of healthy pigs using a Doppler flow and pressure wire. Subsequently unilateral denervation was performed, followed by repeated bilateral hemodynamic measurements. Pigs were terminated directly after RDN or were followed for 3 weeks or 3 months after the procedure. After termination, both treated and control arteries were prepared for histology to evaluate vascular damage and nerve damage. Directly after RDN, aortic pressure increased numerical. Renal blood flow tended to increase, while microvascular resistance remained stable. During 3-12 weeks follow-up, renal resistance reserve increased from 1.74 (1.28) to 1.88 (1.17) (P=0.02) and all other initial hemodynamic effects disappeared. Vascular histopathology showed radiofrequency (RF)-related lesions up to 2 mm deep and in the form of a diverging spot. Most nerves around the treated arteries were located outside the lesion areas. In the nerves within a RF-lesion weaker immunohistochemical staining was observed compared to control arteries.

Conclusion The present study demonstrated limited vascular and nerve damage after RDN. Moreover, RDN did not result in a long-term improvement in renal hemodynamics.
The observations from the present study may indicate that the used RF-catheter was not adequate enough to sufficiently target the renal nerves. Potentially, this may explain the significant rate of non-responders after RDN.

Introduction

Chronic (hyper) activation of the sympathetic nervous system (SNS) is causative in the pathophysiology of hypertension.[1] Renal sympathetic nerves are part of this hyperactive system.[2] To investigate whether disruption of the renal nerves is effective in the treatment of hypertension, surgical renal denervation (RDN) has been studied upon. RDN appeared effective in preclinical studies and also in clinical studies that performed non-selective procedures such as radical surgical sympathectomy.[3, 4] Recently, a percutaneous approach was developed using radiofrequency (RF) energy to target the renal nerves.[3] The initial clinical studies were very promising.[5-7]. However, the only sham controlled randomized Symplicity HTN-3 trial showed disappointing results.[8] Moreover, a recent case-report demonstrated that the RF-energy induced damage to and around the vessel wall with limited penetration, leaving a large part of the nerves unaffected.[9] In this light, the aim of the current study was to investigate the level of renal nerve damage after RDN using histological techniques in a porcine model. Next to histological changes, the effect of RDN may also be measured by functional tests using hemodynamic measurements. Beforehand, we hypothesized that a reduction of resistance of the microvascular bed following RDN may lead to an improved renal blood flow. This improved renal perfusion may consequently reduce blood pressure (BP) in a clinical setting. To investigate this topic, intravascular hemodynamic measurements were performed before and directly after RDN.

Methods

Animals and study design

Thirteen female Dalland Landrace pigs (weight, 60-75 kg) received care in accordance with the *Guide for the Care and Use of Laboratory Pigs* prepared by the Institute of Laboratory Animal Resources. Experiments were approved by the Animal Experimentation Committee of the Medicine Faculty of the University of Utrecht, the Netherlands.

We intubated and anesthetized the animals using standard procedures. Using a sheath introducer in the femoral artery, a 6Fr RDC or IMA guiding catheter was introduced in the renal arteries for selective angiography. Hereafter, we performed hemodynamic measurements in both renal arteries. Subsequently, we treated one renal artery by RDN, followed by repeated hemodynamic measurements in both renal arteries. We terminated the first group of pigs (n= 3) directly after renal denervation. We terminated a second group of pigs (n=5) 3 weeks after RDN and we terminated a third group of pigs (n=5) 3 months after treatment. After termination, we processed both the treated and control renal arteries for histology.

Hemodynamic measurements

We performed hemodynamic measurements in 10 pigs, using a 0,014-inch Combowire (Volcano Corporation, San Diego, CA, USA). This wire has a Doppler crystal located on the distal tip and a pressure sensor 3 cm proximal to the tip.[10] Pressure and flow velocity signals, combined with aortic pressure and ECG signals were recorded using the ComboMap system (Volcano Corporation). We recorded velocity and pressure signals during baseline and hyperemic conditions. Hyperemia was induced by an intra-arterial bolus of 20 mg papaverine. We assessed the hemodynamic measurements in both renal arteries prior to renal denervation and directly thereafter. After the predefined follow-up period of 3 weeks (n=5) or 3 months (n=5), we repeated the pressure and flow velocity measurements.

The data sets of the hemodynamic measurements were analyzed using AMC Studymanager, a custom software package (written in Delphi version 6.0, Borland Software Corporation and Delphi Version 2010, Embarcadero, San Francisco, CA, USA).[10] We calculated the average peak velocity (APV, expressed in cm/s) as the mean of four beats at baseline conditions (bAPV) and the mean of three successive beats at maximal hyperemia (hAPV). Pressure and flow velocity derived parameters were: aortic pressure (Paorta), baseline microvascular resistance (BMR), hyperemic microvascular resistance (HMR), renal

flow velocity reserve (RFVR), and renal resistance reserve (RRR). The definitions of the evaluated parameters are shown in table 1.

Table 1: Definitions of the parameters used

Baseline microvascular resistance (BMR)	Paorta / bAPV
Hyperemic microvascular resistance (HMR)	Paorta / hAPV
Renal flow velocity reserve (RFVR)	hAPV / bAPV
Renal resistance reserve (RRR)	BMR / HMR

APV indicates average peak flow velocity; Paorta, aortic pressure; b, baseline; h, hyperemia

Renal Denervation

We used the Symplicity flex device (Medtronic, Minneapolis, USA) to perform RDN. We performed a unilateral treatment of the renal arteries with at least 6 treatment points. The treatment points were made in a circumferential way with a minimum of 5 mm distance in between.[11] After the procedure a control angiography was performed. The non-treated renal artery served as control vessel.

Preparation for histology

We isolated the renal arteries and created a 2 to 3 cm wide renal stump. The renal stumps were dehydrated in ascending concentrations of alcohol and embedded in paraffin. Transverse sections of the renal artery were made after every 5 mm from its origin up to just distal to the main bifurcation of the renal artery resulting in 3 to 5 sectioning levels, dependent on the vessel length. On each level 5µm serial sections were cut and per artery all sectioning levels were mounted on a single glass slide.

Histological examination for arterial damage

The detailed methods description is given in Appendix A. In brief, we examined the Masson's trichrome (MST) and Haematoxylin Eosin (HE) stained sections for the location, type, and extent of vascular damage, neural damage and inflammation. Damage to the vessel wall within the lesional area and inflammation were assessed using scoring systems listed in the supplemental data (Appendix A, table 2).[12, 13] To determine whether loss of myofibroblasts was part of the vascular damage induced by RDN, we stained the slides with an antibody directed against alpha smooth muscle actin (alpha-SMA, see appendix A for the protocol) and compared treated arteries with control arteries.

Histological examination of nerve damage

After pretreatment the sections were immunohistochemically stained for the general neural marker protein gene product 9.5 (PGP9.5), the glial marker S-100, and for tyrosine hydroxylase (TH) and calcitonin gene-related peptide (CGRP) which are markers for sympathetic and a subpopulation of afferent nerve fibers respectively (appendix A).

We examined the MST and HE stained sections for signs of neural degeneration such as pyknotic Schwann cells, digestion chambers, inflammation and/or peri-/endoneurial fibrosis.[14] We examined sections immunostained for PGP 9.5 for total nerve number. We examined sections immunostained for S-100 for the evaluation of Schwann cells within nerve fascicles. The intensity and distribution of TH staining was semi-quantitatively determined using a scoring system (appendix A, table 4).[14]

Statistical analysis

The variables about the hemodynamic measurements were reported as median (range), or as proportion when appropriate. We used the Wilcoxon signed rank test for paired sample analyses and the Mann-Whitney U test for non-paired sample analyses. The relation between change in hemodynamics (dependent variable) and histological parameters (independent variables) were analyzed using linear regression models. A two sided *P* value of <0.05 was considered statistically significant. All analyses were performed with the SPSS statistical package version 20 (IBM SPSS Data Collection, Chicago, IL).

Results

All pigs had a renal anatomy eligible for treatment, particularly having a diameter of at least 4mm and a vessel length of at least 20mm.[15] All 13 pigs were treated unilaterally with 6-7 ablations per pig. Three pigs were terminated directly after RDN. The remaining 10 pigs were alive and healthy after a follow-up of respectively 3 weeks (n=5) and 3 months (n=5). Directly after RDN, we observed no macroscopic vascular complications like dissection or significant stenosis on angiograms, nor did we observe such complications at angiography during follow-up.

Renal hemodynamic changes

Hemodynamic measurements were successful in 8 animals with follow-up; in 2 animals we had a technical failure of the measurements. Table 2 presents the hemodynamic changes directly after RDN and at follow-up. Directly after RDN resting average peak flow velocity (bAPV) increased by 29 ±67% (P=0.01) and hyperemic APV (hAPV) increased by 39±54% (P=0.04). The increase in APV was accompanied by a numerical increase in aortic pressure (table 2). Baseline microvascular resistance (BMR) and hyperemic resistance (HMR) tended to decrease by 12±35% (P=0.18) and 14±28% (P=0.13), respectively. Renal flow velocity reserve (RFVR) and renal resistance reserve (RRR) did not chance directly after RDN.

During follow-up, RRR increased significantly in the treated arteries (mean increase compared to baseline 0.18±0.16; P=0.02). This was accompanied by a trend towards increase in BMR (P=0.09). Other indices at follow-up were not significantly different from baseline measurements in both the treated and control arteries (table 2).

Both before and directly after RDN, no significant differences in hemodynamic variables were observed between the treated and control arteries (table 2). At follow-up a significant lower HMR was observed in the control arteries (P=0.04, table 2). All other hemodynamic measurements showed no significant differences between treated and control arteries.

Table 2: Change in renal hemodynamic parameters directly after renal denervation and after termination

All					
	Baseline	Directly after RDN	P-value*	Termination	P-value†
Aortic pressure, mmHg	86 ± 18	92 ± 19	0.26	87 ± 22	0.70
Resting heart rate, bpm	72 ± 22	72 ± 13	0.99	70 ± 9	0.80
Resting APV, cm/sec	25 (19)	30 (43)	0.01	22 (19)	0.35
Hyperemic APV, cm/sec	43 (61)	47 (66)	0.04	39 (39)	0.61
Resting MR, mmHg/cm/sec	3.5 (6.8)	3.2 (3.5)	0.18	3.9 (4.7)	0.44
Hyperemic MR, mmHg/cm/sec	2.0 (0.8)	1.9 (1.5)	0.13	2.2 (2.4)	0.92
Renal flow velocity reserve	1.7 (1.3)	1.7 (1.6)	0.38	1.9 (1.1)	0.23
Renal resistance reserve	1.75 (1.61)	1.70 (2.08)	0.72	1.84 (1.34)	0.12
Treated					
	Baseline	Directly after RDN	P-value*	Termination	P-value†
Aortic pressure, mmHg	87 ± 18	89 ± 20	0.67	91 ± 23	0.50
Resting heart rate, bpm	72 ± 17	69 ± 12	0.69	69 ± 9	0.72
Resting APV, cm/sec	29 (39)	35 (40)	0.11	20 (20)	0.12
Hyperemic APV, cm/sec	49 (61)	64 (59)	0.11	39 (44)	0.21
Resting MR, mmHg/cm/sec	3.3 (5.6)	2.7 (2.6)	0.44	4.3 (4.7)	0.09
Hyperemic MR, mmHg/cm/sec	1.9 (3.3)	1.5 (1.5)	0.26	2.2 (2.4)	0.33
Renal flow velocity reserve	1.7 (1.1)	1.6 (1.2)	0.37	1.9 (2.4)	0.09
Renal resistance reserve	1.74 (1.28)	1.70 (1.17)	0.86	1.88 (1.17)	0.02

	Non-treated				
	Baseline	Directly after RDN	P-value*	Termination	P-value†
Aortic pressure, mmHg	84 ± 20	94 ± 20	0.32	83 ± 22	0.87
Resting heart rate, bpm	72 ± 28	74 ± 14	0.81	71 ± 9	0.97
Resting APV, cm/sec	24 (17)	26 (21)	0.21	25 (21)	0.44
Hyperemic APV, cm/sec	45 (29)	41 (66)	0.31	51 (46)	0.17
Resting MR, mmHg/cm/sec	3.4 (6.8)	3.1 (3.6)	0.77	3.2 (3.6)	0.95
Hyperemic MR, mmHg/cm/sec	2.1 (3.0)	2.2 (1.9)	0.95	1.7 (1.0)	0.68
Renal flow velocity reserve	1.7 (1.3)	1.7 (1.6)	0.59	1.9 (0.8)	0.68
Renal resistance reserve	1.75 (1.49)	1.80 (2.08)	0.86	1.87 (0.95)	0.59

**Directly after measures compared to baseline measures, †Termination measures compared to baseline measures. RDN: renal denervation, APV: average peak velocity, MR: microvascular resistance.*

Arterial damage

Histology was performed in eleven pigs. In two pigs histology failed due to technical issues. The geometrical changes are shown in table 4, the vascular changes after RDN are shown in figures 1-4. Directly after RDN we observed paralysis of the media expressed as a numerical increased diameter of the lumen between treated and control vessels (P=0.11). At three weeks follow-up contraction of the media was observed, expressed as a decreased ratio between circumference of the lumen and media (P=0.08). At three months follow-up the ratio's between the circumference of the lumen and the media were similar in the treated and control arteries (P=0.66).

Ten out of eleven treated arteries that were evaluated showed vascular lesions, one treated artery (follow-up duration 3 months) showed no vascular lesions. The RF-energy induced lesions up to 1,1 mm deep directly after RDN, up to 2,2 mm deep after 3 weeks follow-up, and up to 1,9 mm deep after 3 months follow-up. All lesions were in the form of a diverging spot. The supplemental data shows the grading of vascular damage (appendix B).

In the pigs that were terminated acutely, we observed in both treated and control arteries a scattered presence of inflammation cells. Immunostaining for alpha-SMA (figure 1A) showed a diffuse and less intense staining within the lesions of the treated arteries compared to the control arteries.

At 3 weeks follow-up, we observed a massive inflammation response in the treated arteries (figure 3), compared to a scattered presence in the control arteries. Immunostaining for alpha-SMA (figure 1B) showed an increased staining within the lesion of treated arteries compared to control arteries. This suggests that in response to damage, myofibroblast proliferation is increased at the sites affected by RDN to heal the injured sites by deposition of collagen.

At 3 months follow-up, we observed scarring of the intima, media, and adventitia in two pigs (figure 1C). The media and adventitia could not be differentiated from each other since the external elastic lamina was no longer visible. Immunostaining for alpha-SMA (figure 1C) showed slightly increased labeling in the media within the lesion of treated arteries compared to control arteries. In one pig we observed no clear vascular lesions three months after RDN.

Table 3: Geometrical changes after renal denervation

	Diameter lumen (μm)			Ratio between the circumference of the lumen and the media			Percentage of intact media (%)	Diameter of intact media	Diameter of media within lesion
	treated artery	control artery	P-value	treated artery	control artery	P-value			
Acute	1459 (804)	841 (85)	0.11	0.70 (13)	0.51 (0.04)	0.11	57 (40)	509 (57)	270 (127)
Three weeks	1088 (736)	1143 (568)	0.23	0.50 (0.25)	0.55 (0.17)	0.08	67 (12.5)	574 (351)	695 (466)
Three months	1081 (618)	989 (784)	0.31	0.50 (0.18)	0.50 (0.19)	0.66	67 (25)	560 (198)	649 (301)

Immunohistochemical staining of nerve fibers

Figures 4-6 display the immunohistochemical staining of the nerve fibers. All nerves contained TH-positive nerve fibers (figure 5).

Directly after RDN, we observed no nerve damage with MST, PGP9.5, S100, and TH staining (figure 4, 5). In the pigs with follow-up 55±25 nerves per pig were observed, only 8±7 of these nerves were within a lesion area. We analyzed the nerve fibers within the lesions area for possible damage.

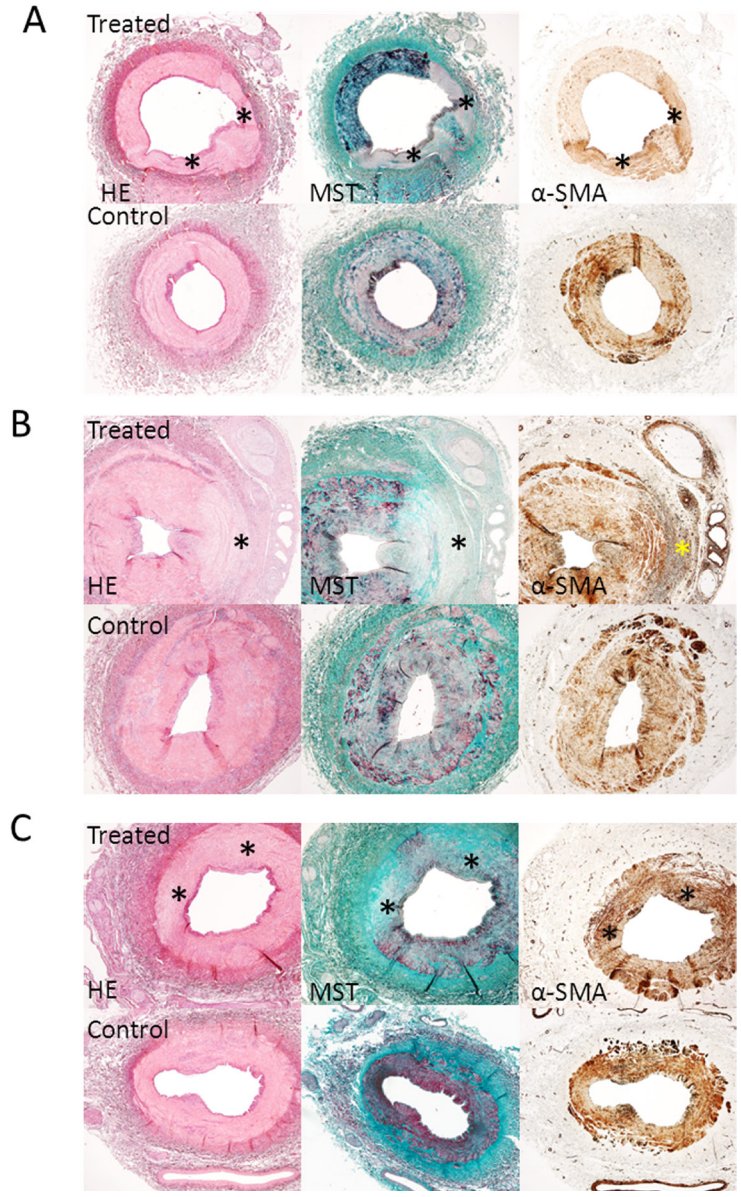
After three weeks follow-up MST staining showed neural degeneration (figure 4, 6) of nerve fascicles with moderate to marked swelling of endoneurial tissue, proliferating Schwann cells, and severe inflammation (figure 4). PGP9.5 and S100 staining was slightly weaker in the nerves (figure 4) of the treated arteries. TH staining intensity was weak or even absent in the treated arteries (figure 4, 6).

After three months we only observed two bundles of nerve fibers in lesion areas. In these nerve fibers we observed peri-/endoneural fibrosis and inflammatory cells in the perineurium/epineurium (figure 4). Moreover, small PGP9.5, S100, and TH positive nerve fascicles were observed around a big nerve bundle in the treated arteries (figure 4). All other nerves bundles of treated arteries stained similar as control arteries, that showed normal morphology (figure 6).

Relation between hemodynamic and histological findings

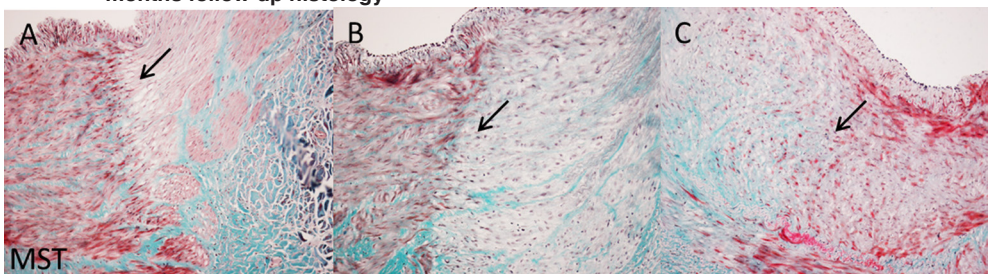
We observed a relation between a more impaired adventitia and a reduction in the renal resistance reserve (β: -0.33; P=0.05) after three weeks follow-up in a univariate linear regression model.

Figure 1: Acute, 3 weeks, and 3 months follow-up histology results



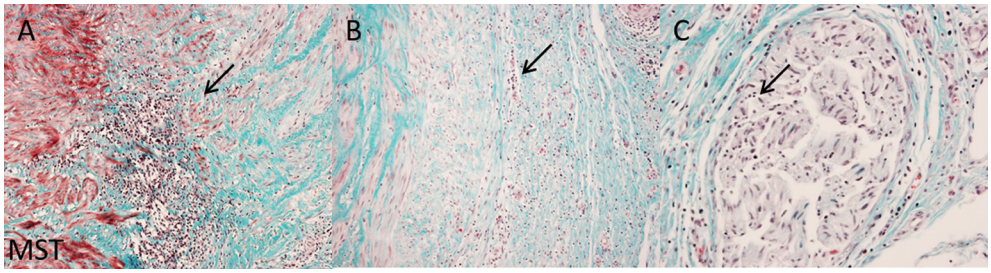
Acute (A), 3 weeks (B) and 3 months (C) histology results (2x magnification) showing treated vessels with lesions and control vessels. Serial sections were stained with Haematoxylin Eosin (HE), Masson's trichome staining (MST) and alpha-smooth muscle actin (α-SMA immunostaining).
**=lesion area.*
A. HE and MST staining showing a treated vessel with two lesions immediately after denervation. The lesions have a pale color and the media is most affected. MST shows no increased collagen deposition at the site of the lesion (no increased presence of blue fibers). α-SMA staining shows a diffuse increased medial staining at the site of the lesion in treated vessels.
B HE and MST staining at three weeks follow-up. The media and adventitia of treated vessels are most affected by denervation. MST staining shows increased medial collagen deposition (blue fibers). α-SMA staining shows increased medial, adventitial and perineural staining at the site of the lesion (dark brown).
C. HE and MST staining showing a treated vessel with two lesions 3 months after denervation. MST staining shows transmural collagen deposition at the site of the lesion and the adventitia is most affected . α-SMA staining shows a slightly increased medial staining (dark brown) at the site of the lesion.

Figure 2: Tenfold magnification of Masson's trichome staining of acute, 3 weeks, and 3 months follow-up histology



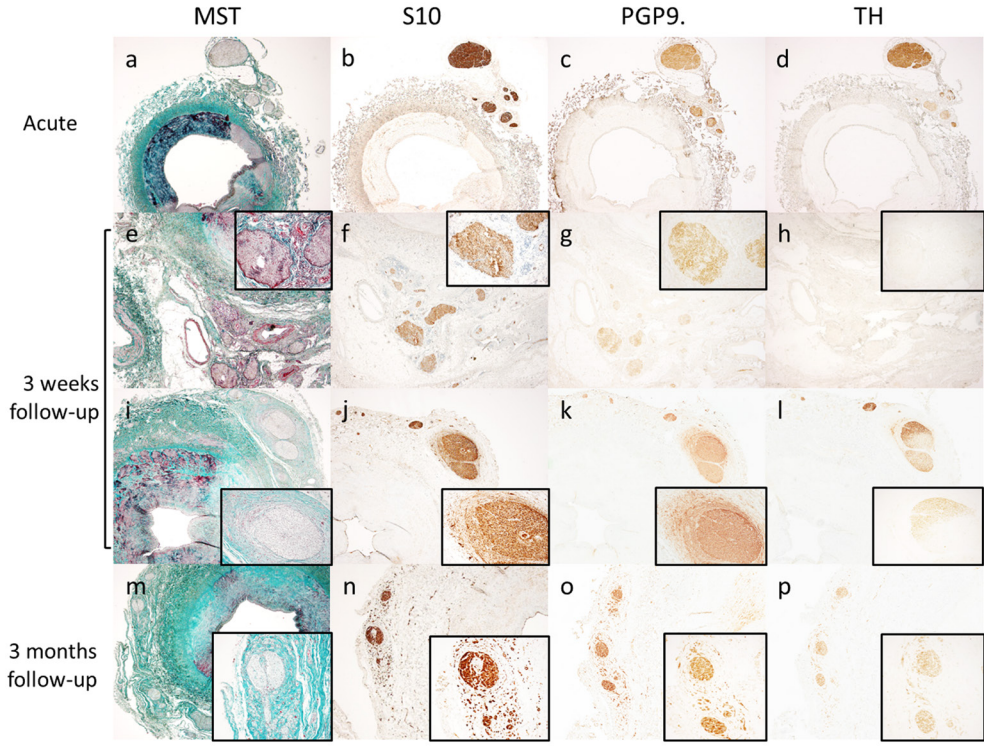
Acute (A), 3 weeks (B), and 3 months (C) histology results (10x magnification) showing the border zone of lesions in treated vessels. Sections were stained with MST staining. The arrows indicate the border zone.
A. Border zone of lesion immediately after denervation. The border zone is characterized by a red to pink color transition. At the border zone cell depletion is present (white holes).
B. Border zone of lesion three weeks after denervation. The border zone is characterized by a red to light green color transition. At the site of de border zone inflammatory cells are present (tiny dark blue/black spots).
C. Border zone of lesion 3 months after denervation. The border zone is characterized by blue to red color transition. At the border zone increased collagen deposition is present (blue fibers) and the collagen fibers are intertwined the adjacent healthy muscle tissue of the media (red).

Figure 3: Massive inflammatory response after 3 weeks follow-up



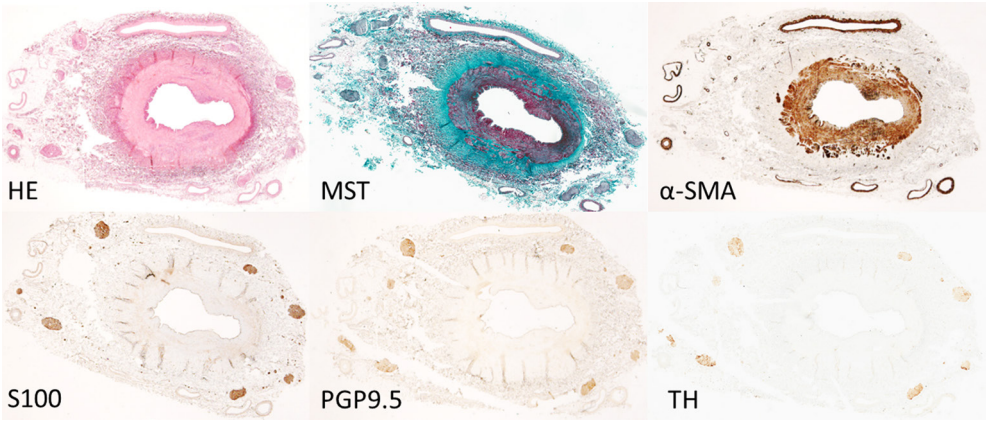
3 week follow-up histology showing details of the massive inflammatory response in lesions of treated vessels. Sections were stained with MST staining. Arrows indicate inflammatory cells. Inflammatory cells are tiny dark blue/ black spots.
A. A 10x magnification showing a massive inflammatory response of the border zone at the media to adventitia transition.
B. A 10x magnification showing a massive inflammatory response of the adventitia.
C. A 20x magnification showing inflammatory cells within a nerve.

Figure 4: Immunohistochemical staining of nerves of treated arteries



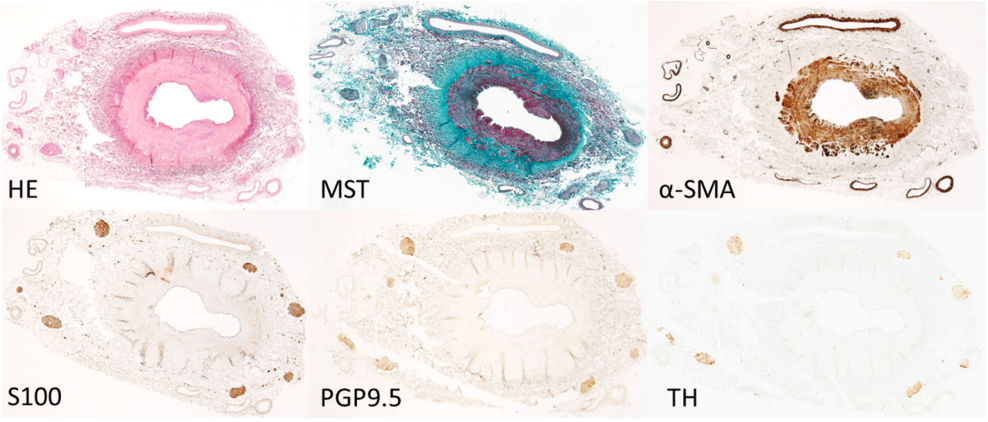
Acute (a-d), 3 weeks (e-l) and 3 months (m-p) histology and immunohistochemical staining results of nerves within the lesion area of treated vessels. The rectangular boxes are a 10x magnification of the affected nerves. a-m shows a 2x magnification and n-p a 5x magnification. Immediately after denervation no signs of nerve damage and a scattered presence of inflammatory cells were observed (a). S100 (b), PGP9.5 (c), and TH (d) showed similar staining patterns and the staining intensity was similar to controls. 3 weeks after denervation neural degeneration and inflammation of nerves and perineural tissue (f) was observed. S100 staining intensity of affected nerves was similar to control (f, j) PGP9.5 staining was slightly lower in intensity (g, k)and TH (h, l)staining was weak or absent compared to control. Scattered presence of S100 (j)and PGP9.5 (k) positive neuron cell bodies was observed around a part of the affected nerves. 3 months after denervation the majority of nerves were embedded in thick sheets of fibrotic tissue(m) and there was scattered presence of inflammatory cells (m). S100, PGP9.5, and TH showed similar staining patterns and the staining intensity was similar to controls. Around affected nerves small S100 (n), PGP9.5 (o) and TH (p) positive nerve bundles were present and they were embedded in thick sheet of fibrotic tissue (m).

Figure 5: Histology and imunnostaining of control arteries



Histology results and immunostaining (0.05 magnification) of a control vessel. Serial sections were stained for HE, MST, α-SMA, S100, PGP9.5 and TH. Sections show no signs of vessel damage (HE, MST), they have minimal inflammation (HE, MST) and have no increased areas of α-SMA at the media and no staining outside the media, except the arterioles. The nerves show similar staining patterns for structural (S100, PGP9.5) and functional (TH) nerve components.

Figure 6: Nerve damage outside the lesion area



3 weeks histology results showing 0.05 magnification (1-6) of a treated vessel with nerve damage outside the lesion area. A 20 x magnification (a-e) zooms in on the affected nerve that is indicated with an arrow in picture 1-6. Serial sections were stained with HE, MST, α-SMA, S100, PGP9.5 and TH. The perineurial tissue and nerves located at the opposite site of the lesion were affected by a massive inflammatory response (1,a and 2,b), increased proliferation of myofibroblasts (3,c), a reduction in neural tissue (4,d;5,e) and loss of neurotransmitter production of the affected nerves (6).

Discussion

The present study showed that RDN induced a variety of lesions in and around the arterial wall and a limited penetration up to 2 mm deep. Moreover, a large part of the renal nerves were remote to the vascular lumen and therefore not reached by the RF-energy. Directly after RDN we observed a trend towards an improvement in renal hemodynamics. However, most of the initial effects disappeared at follow-up. Remarkably, a reduction in RRR in the treated arteries at follow-up was related to more severe adventitial damage.

Hemodynamic changes

This study was not the first evaluating hemodynamic changes after RDN in a porcine model. Yet, we could only partially confirm the results from the previously performed study.[16] Both previous and current studies showed an improvement in hemodynamics directly after treatment. However, we observed a nullification of most hemodynamic changes after follow-up in contrast to the previous study.[16] Moreover, the increase in APV directly after RDN may also be explained by the (non-significant) increase in aortic pressure since we did not observe a reduction in MR. At follow-up, we did observe an increase of the RRR in the treated arteries. This increased RRR resulted in a lower APV, while we anticipated the reverse. A possible explanation could be the absence of sympathetic overactivity.

Surprisingly, we observed non-significant changes in the hemodynamics in the control arteries, especially at follow-up. Potentially, RDN induced some changes in the kidneys itself, which consequently induced a systemic reflex from the afferent renal nerves. Such a systemic reflex would explain the hemodynamic changes on the contralateral side. Given the limited nerve damage, it is not unlikely that the hemodynamic response in the treated side is also explained by this systemic reflex through the efferent nerves of the treated side.

Histology

Overall, the effects of RDN on the vasculature showed a wide variety and we observed lesions up to 2.2 mm deep, which is less deep than previously reported.[12] Moreover, one of the most surprising observations was that only a minority of the nerves around the treated arteries were captured within the vascular lesions. This emphasizes the fact that RDN is a more or less “black box” procedure as the nerves cannot be visualized.

Regardless from follow-up duration and treatment or no treatment, we found that all nerves along the renal arteries contained many sympathetic fibers. This finding is in line with the available literature.[17]

Directly after RDN we observed no nerve damage in contrast to a previous study that showed reduced staining intensity of neurofilament protein and vacuolic appearance in a small number of nerve fascicles.[13] However, this may be explained by the fact that we observed a limited number of nerves within the lesions. We did observe minimal morphological changes in some nerves of the treated arteries (outside the lesion area). However, we also observed these changes in nerves of control arteries.

At three weeks follow-up the most prominent lesions were observed and the media, EEL, and adventitia were most affected by RDN. The massive inflammatory response represented most of the arterial and neural damage. Similar to Steigerwald et al., we observed neural inflammation, degeneration, and reduced marker expression at three weeks follow-up.[13] The weaker staining of PGP 9.5, S100, and TH may be explained by a loss of function from the affected nerves.

At three months follow-up, we only observed clear vascular damage in two out of three treated arteries. Since we only had a limited number or arteries with three months follow-up and vascular damage these results should be interpreted as explorative. In the arteries with vascular lesions, the EEL and adventitia were most affected by RDN. The inflammatory response had ceased and the entire lesion was scar tissue. Most nerves visualized where outside a vascular lesion area. Although the majority of the nerves had a thickened epi- and perineurium, the nerves were devoid of neural degeneration. These results were

in accordance with the study of Rippy et al.[12] Although speculative, this may implicate that nerves are able to recover.

Limitations and strengths

Although we invested much effort to conduct the study as properly as possible, some limitations should be mentioned. The first limitation is the lack in follow-up regarding blood pressure and renal function. However, one would not expect BP effects in healthy pigs using RDN. Also, this was not the primary goal of the study; we mainly intended to extend our knowledge about the influence of RDN on vascular anatomy and renal hemodynamics.

One can wonder whether current results in young healthy porcine arteries can be translated to RDN in renal arteries of patients with long-lasting (resistant) hypertension. It is likely that the arteries of hypertensive patients have an increased intima and/or media thickness and therefore show a different histopathologic pattern. Secondly, it may be that hemodynamics in patients do respond differently to RDN since patients are subject to an increased sympathetic activity.

Finally, the number of pigs used for the current analysis was limited and most likely underpowered to detect any significant differences in hemodynamics between treated and control arteries.

Strengths of the current study were the comprehensive work-up and different follow-up durations used. The histological analyses of the vascular damage and nerve damage were extensive and were performed by an investigator that was blinded for the treatment site of RDN.

Clinical implications

The current results may have some clinical implications. First of all, RDN resulted in only a limited penetration of the artery wall and the majority of the nerves around the arteries were not targeted by the RF-energy. It was recently shown that the mean distance from lumen to nerve is 3.12±0.54 mm in a human cadaver study.[18] The combination of the limited penetration and the location of nerves outside the lesion area may explain why a significant proportion of patients are 'non-responder' after RDN in 'real-life' cohorts.[19] Potentially, the absence of targeted nerves played a role in the negative HTN-3 trial.[8]

Surprising was the observation of a univariate relation between decreased RRR and more damaged adventitial tissue. This may implicate that deep lesions (e.g., up to the adventitia) are needed to establish these hemodynamic changes. In the development of new devices for RDN it should be kept in mind that deeper lesions are needed.

In conclusion, the present study demonstrated limited vascular and nerve damage after RDN. Moreover, RDN did not result in a long-term improvement in renal hemodynamics.

The observations from the present study may indicate that the used RF catheter was not adequate to sufficiently target the renal nerves. The finding that a decreased RRR was related to more adventitial damage suggests that deeper vascular lesions are needed to induce hemodynamic improvements after RDN.

Acknowledgements

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Appendix A, methods extended

Histological staining protocols

Prior to all stainings, the isolated renal stumps were embedded in paraffin. The renal stumps were sectioned every 5mm, producing 3 to 5 sectioning levels, depending on the length of the artery. Per artery all sections levels were captured on a single glass slide. The sections were cut approximately 5µm serially.

Mayer's Haematoxilyn-Eosin staining

Slides were deparaffinized and incubated with Mayer's Haematoxylin for 15 minutes. Then slides were washed in running tap water for 15 minutes. Subsequently, slides were three times quickly rinsed in 80% ethanol. Then slides were incubated with eosin for 30 seconds, followed by differentiation in 96% ethanol. Slides were dehydrated with 100% ethanol (2 x 1 minute) and xylene (2 x 1 minute).

Masson's Trichrome staining

Slides were deparaffinized and refixated in Bouin's fluid for 1 hour at 56°C. The nuclei were stained with Weigert's iron haematoxylin for 10 minutes. Then the slides were washed with running tapwater for 10 minutes and rinsed with distilled water. Subsequently, slides were incubated with solution A (0.5g Acid fuchsine, 0.5g Xylidine ponceau and 1 ml glacial acetic acid in 99ml distilled water) for 10 minutes and rinsed with distilled water. Then slides were incubated with solution B (1g phosphotungstic acid in 100 ml distilled water) for 5 minutes and rinsed with distilled water. Finally slides were incubated with solution C (2g light green SF yellowfish and 2ml glacial acetic acid in 100ml distilled water) for 10 minutes and dehydrated.

Immunostaining protocols

Slides were deparaffinized and pretreated using heat activated antigen retrieval in sodium-citrate buffer (0.01M, pH 6.0). Endogenous peroxidase activity was blocked using 3% hydrogen peroxidase in distilled water for 5 minutes. Slides were subsequently incubated with a protein block, primary and secondary antibody according to table 1. Detection of primary antibody binding for Alpha-SMA, PGP9.5, TH and S100 was performed by incubation with Sigma Fast DAB for 10 minutes. Detection of CGRP primary antibody binding was performed using ABC elite for 30 minutes at room temperature (RT) followed by incubation with Sigma Fast DAB for 10 minutes.

Table 1: Antibodies

Block	Primary antibody (Species, dilution)	Incubation time primary antibody	Secondary antibody	Incubation time secondary antibody
None	Alpha-SMA (1:3200)	32 minutes at RT	Ready-to-use poly HRP anti mouse/rabbit IgG	16 minutes at RT
Ultra V	PGP9.5 (rabbit, 1:1000)	2 hours at RT	Ready-to-use poly HRP anti mouse/rabbit/rat IgG	30 minutes at RT
None	S100 (1:2000)	32 minutes at RT	Ready-to-use poly HRP anti mouse/rabbit IgG	16 minutes at RT
Ultra V	TH (rabbit, 1:400)	2 hours at RT	Ready-to-use poly HRP anti mouse/rabbit/rat IgG	30 minutes at RT
Ultra V	CGRP (rabbit, 1:4000)	1 hour at RT	Goat anti rabbit biotinylated antibody (1:250)	1 hour at RT

Histopathological grading scales

Table 2: Histologic vascular injury grading scale

0	no injury/disruption/hyperplasia
1	minimal (injury/disruption hyperplasia of 10% or less)
2	mild (injury/disruption/hyperplasia of 11-25%)
3	moderate (injury/disruption/hyperplasia of 26-50%)
4	marked (injury/disruption/hyperplasia of 51-75%)
5	severe (injury/disruption/hyperplasia of 76-100%)

Injury and/or disruption of the intima (IH), internal elastic lamina (IEL), media, external elastic lamina (EEL), and adventitia: Grades 0-5

Table 3: Inflammation of vascular and perivascular tissue: Grades 0-3

0	no inflammation
1	presence of scattered inflammation cells
2	modest inflammatory reaction comprising less than 25% of the vessel circumference
3	massive inflammatory reaction comprising more than 25% of the vessel circumference

Table 4: Tyrosine Hydroxylase staining: Grades 0-3

0	no reaction
1	patchy/very weak reaction
2	weak reaction
3	strong reaction

Appendix B. results histology extended

Arterial damage

Table 1: Grading of arterial damage

	Acute termination	Three weeks follow-up	Three months follow-up
Intima	Grade 3	Grade 2	Grade 1
Internal elastic lamina	Grade 3	Grade 2	Grade 1
Media	Grade 4	Grade 3	Grade 2
External elastic lamina	Grade 4	Grade 3	Grade 3
Adventitia	Grade 2	Grade 3	Grade 3
Inflammation	Grade 1	Grade 3	Grade 1
Inflammation control artery	Grade 1	Grade 1	Grade 1

Pigs that were terminated directly after RDN: Damage was observed as thrombus formation at the site of the lesion, stretched fibers of the IEL and EEL, retraction of the media, cell depletion at the borders of the lesion e.g. 'border zone' (figure 3B), and coagulation of the adventitia. Immunostaining for alpha-SMA (figure 2A) showed a diffuse and less intense staining within the lesions of the treated arteries compared to the control arteries.

At 3 weeks follow-up, the damage was observed as intimal hyperplasia, intimal and medial fibrosis, and adventitial inflammation. At the border zone invasion of leukocytes and deposition of collagen was present (figure 3B, 4). Immunostaining for alpha-SMA (figure 2B) showed an increased staining within the lesion of treated arteries compared to control arteries.

At 3 months follow-up, we observed vascular injury in one of the two treated arteries. Damage was observed as scarring of the intima, media, and adventitia. The media and adventitia could not be differentiated from each other since the external elastic lamina was no longer visible. The border zone was less pronounced because the collagen fibers of the scar tissue were intertwined with the adjacent healthy tissue (figure 3C). Immunostaining for alpha-SMA (figure 2C) showed a slightly increased labeling in the media within the lesion of treated arteries compared to control arteries.

Immunohistochemical staining of nerve fibers

All nerves contained TH-positive nerve fibers (figure 6).

Masson's trichome staining in the pigs that were terminated directly showed no nerve damage. PGP9.5, S100, and TH staining showed similar intensity (grade 3) for treated (figure 5) and control arteries (figure 6).

Masson's trichome staining of the treated arteries of the pigs with three weeks follow-up showed neural degeneration (figure 5, 7) of nerve fascicles with moderate to marked swelling of endoneurial tissue, proliferating Schwann cells, and severe inflammation (figure 4). PGP9.5 and S100 staining was slightly weaker in the nerves (figure 5) of the treated arteries compared to the control arteries. TH staining intensity was weak or even absent in the treated arteries (grade 1, figure 5, 7), whereas in the control arteries staining intensity was strong (grade 3, figure 6). A scattered presence of PGP9.5 and S100 positive neuron cell bodies was observed around damaged nerve fibers of the treated arteries (figure 5).

Masson's trichome staining of the treated arteries of the pigs with three months follow-up showed peri-/endoneurial fibrosis and occasional inflammatory cells in the perineurium/epineurium (figure 5). The degree of neural degeneration was similar in the treated and control arteries. The majority of nerves of the treated arteries had a thickened perineurium. Immunostaining for TH (grade 2), PGP9.5, and S100 was similar for both treated (figure 5) and control arteries (figure 6). In the treated artery that contained vascular damage, small PGP9.5, S100, and TH positive nerve fascicles were observed around a big nerve bundle (figure 5). They were all surrounded by thick sheets of fibrotic epi- and perineurium. In control arteries the nerves showed normal morphology (figure 7).

The effect of renal denervation on renal haemodynamics in a hypertensive population

Submitted

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Abstract

Aims

We evaluated the effects of renal denervation (RDN) on renal hemodynamics to gain more insight in the mechanism behind RDN. We hypothesized that sympathetic denervation of the kidney would achieve a decreased microvascular resistance (MR) leading to an increased renal blood flow.

Methods and results

A consecutive cohort of 15 hypertensive patients scheduled to undergo RDN gave informed consent for study participation. We performed haemodynamic measurements in both renal arteries using a Doppler flow and pressure wire directly before and after denervation. Blood pressure (BP) was measured at baseline and at 6 months follow-up.

Mean age was 58±12 years, 67% was male. Baseline microvascular resistance (BMR) tended to decrease directly after RDN (P=0.12), whereas there was no overall change in hyperaemic microvascular resistance (HMR). Consequently, there was a significant decrease in the arteriolar resistance (AR) immediately following RDN (P=0.01).

Office BP showed a non-significant reduction of 6±16/4±14 mm Hg six months after RDN (P=0.07/0.13). Changes in HMR and AR were related to a change in SBP in the bivariable analyses that was corrected for baseline systolic BP (HMR: β:6.851; P=0.03. AR: β:-20.382; P<0.01).

Conclusions

Changes in HMR and AR after RDN were associated with changes in systolic BP during follow-up, suggesting that assessment of renal hemodynamic measures may be used as per-procedural markers of a successful RDN.

Background

Hypertension is one of the most prevalent cardiovascular risk factors. Globally, 34% of adults have hypertension and this number is rising.[1, 2] The sympathetic nervous system (SNS) plays an important role in the development and progression of systemic hypertension.[3] On this basis, a percutaneous, catheter–based approach has been developed using radiofrequency (RF) energy to disrupt renal sympathetic nerves.[4] The safety of this renal denervation (RDN) has been reported in a number of studies, and initially, RDN seemed to be a promising therapy for resistant hypertension.[5, 6] However, the sham-controlled Symplicity HTN-3 trial was unable to document a difference in blood pressure reduction by RDN compared with individuals undergoing a sham RDN-procedure.[7] These disappointing results from the HTN-3 trial illustrate the fact that we lack understanding of the physiological changes in the kidney induced by RDN that are associated with a blood pressure (BP) reduction. Consequently we also lack methods to identify effective application of the RF-energy per-procedurally. Schlaich et al. showed that RDN leads to a decreased activity of the SNS by performing measurements of the muscle sympathetic nerve activity (MSNA).[8] However, it remains unclear how this decreased activity has its precise effects on intrarenal processes. Nonetheless, it is documented that sympathetic hyperactivity is accompanied by an impaired renal blood flow and that hypertension in patients with end-stage kidney disease (ESKD) is caused by an increased peripheral resistance.[9] In these cases, nephrectomy resulted in a significant reduction of blood pressure (BP) and peripheral resistance. These findings suggest a direct relationship between the renal response to a reduction in sympathetic nerve activity and alterations in BP during follow-up, and imply that intra-renal assessment of the acute effect of RDN may allow to identify markers of effective RDN. In the present study we aimed to investigate the changes in renal artery physiology associated with RDN, and their association with blood pressure reduction during clinical follow-up in a clinical cohort of patients with resistant hypertension. We hypothesized that RDN might decrease MR and consequently increase renal blood flow.

Methods

The present study was designed as a cohort study (NCT01848314). The local ethics committee of the UMC Utrecht approved the study in accordance with the Declaration of Helsinki. A consecutive cohort of patients scheduled for RDN between January 2013 and March 2014 were asked to participate in the current study. Written informed consent was obtained from all participants.

Study population

Patients with resistant hypertension (defined as a SBP ≥160 mmHg, despite use of ≥3 antihypertensive drugs) as well as patients fulfilling the same BP criteria, but without optimal pharmacological treatment due to recorded intolerance for antihypertensive drugs were eligible for enrolment. Since the study was set-up as an exploratory study, no power calculation was performed on beforehand. Major exclusion criteria were: an estimated glomerular filtration rate (eGFR) <30 mL/min/1.73m^2, secondary causes of hypertension, and a history of renal artery stenting or severe co-morbidity.

Blood pressure monitoring, antihypertensive drugs and renal function

Measurements of BP and renal function were assessed as standard care. Detailed information on drug use and medical history was collected and physical examination was performed. Blood pressure was assessed three times in sitting position using a non-invasive device. The mean BP level from these 3 recordings was used for analysis. Six months after RDN, patients visited the outpatient clinic. Blood pressure was measured again in the same way as before treatment. Renal function was estimated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula.[10]

We calculated the difference between the means of BP at baseline and at 6 months and used this difference for the present analysis. A negative number represented BP-reduction 6 months after RDN. We

converted prescribed dosages of antihypertensive drugs to daily defined doses (DDD) using conversion factors as provided by the World Health Organization (WHO) Drug Classification (http://www.whocc.no/atcddd/). Using DDD's and total prescribed dosages; daily use (DU) of all antihypertensive drugs was calculated.

Hemodynamic measurements

Renal angiography was performed using standard procedures. After exclusion of renal stenosis, a 0,014-inch dual sensor-equipped guide wire (Combowire, Volcano Corporation, San Diego, CA, USA) was introduced in the renal artery to obtain simultaneous recording of distal pressure (Pd) and flow velocity.[11] Aortic pressure (Pa) was measured simultaneously through the guiding catheter. Pressure and flow velocity signals, combined with aortic pressure and ECG signals were recorded using a dedicated console (ComboMap, Volcano Corporation, San Diego, CA). The wire was positioned in the left and right renal artery, and velocity and pressure signals were recorded during baseline conditions, as well as during hyperaemia induced by a bolus of 30 mg papaverine in the renal artery[12] The measurement protocol was assessed prior to renal denervation and directly thereafter.

Analysis of pressure-flow velocity-derived indices

Data sets were analysed using a custom software package (written in Delphi versus 6.0, Borland Software Corporation and Delphi Versus 2010, Embarcadero, San Francisco, CA, USA). Renal Flow Velocity Reserve (RFVR) was calculated as RFVR = hyperaemic APV (hAPV)/baseline APV (bAPV)[11], where APV is the average peak flow velocity in cm/s. The bAPV was calculated as the mean of seven beats during baseline conditions, hAPV as the mean of three beats during maximal hyperaemia. Microvascular resistance was calculated as MR =Pd /APV, and was calculated both during baseline (baseline microvascular resistance (BMR)) and hyperaemic conditions (hyperaemic microvascular resistance (HMR)). Arteriolar resistance (AR) was defined as the difference between the renal resistance indices measured at baseline conditions and during maximal hyperaemia. The AR was calculated as BMR-HMR.[13] The definitions of the evaluated parameters are shown in table 1.

Table 1: Definitions of the parameters used

Baseline microvascular resistance (BMR)	Pd / bAPV
Hyperaemic microvascular resistance (HMR)	Pd / hAPV
Renal flow velocity reserve (RFVR)	hAPV / bAPV
Arteriolar reserve (AR)	BMR - HMR

APV indicates average peak flow velocity; Pd, distal pressure; b, baseline; h, hyperaemia.

Renal denervation

Patients were treated using the Symplicity Flex device (Medtronic, Minneapolis, USA). A bilateral treatment of the arteries was performed using series of 2-minute radio frequency energy deliveries along each renal artery.[14] The treatment points were made in a circumferential way with a minimum of 5 mm distance in between.

Statistical analysis

All variables were reported as mean ± standard deviation (SD), median (range), or proportion when appropriate. We used the paired student t-test or Wilcoxon signed rank test when appropriate for a paired samples analysis. We used the unpaired t-test or Mann Whitney U test for non-paired sample analysis when appropriate.

We analysed the relations between change in haemodynamics (independent variable) and change in BP 6 months after RDN (dependent variable) with linear regression models. Bivariable linear regression models were used to adjust for baseline SBP. A two sided *P* value of <0.05 was considered statistically

significant. All analyses were performed with the SPSS statistical package version 20 (IBM SPSS Data Collection, Chicago, USA).

Results

Patient characteristics

Fifteen patients gave informed consent to participate in the study. All patients underwent RDN in combination with hemodynamic measurements. The baseline characteristics are shown in table 2. Mean age of the population was 58±12 years old and the majority of the population was male. Eleven of the included patients (73%) used three or more antihypertensive drugs, four patients (27%) had (recorded) intolerance to multiple groups of antihypertensives.

Table 2: Baseline characteristics

	All patients n= 15
Age (yrs)	58 ± 12
Sex (male/female)	10/5
Body-mass index (kg/m^2)	27.2 ± 5.4
eGFR† (mL/min/1.73m^2)	82 ± 17
Office SBP (mm Hg)	186 ± 30
Office DBP (mm Hg)	105 ± 17
Mean 24-hour SBP* (mm Hg)	168 ± 10
Mean 24-hour DBP* (mm Hg)	100 ± 13
Mean 24-hour HR* (bpm)	71 ± 13
Comorbidity	
Hypercholesterolemia	6 (43%)
Diabetes Mellitus Type II	4 (27%)
TIA/stroke	0 (0%)
CAD	2 (13%)
Antihypertensive medication	
daily use of antihypertensive drugs	2.8 (7.7)
Nr of antihypertensive drugs	3.0 (6.0)
ACEi/ARB/Renin inhibitor	11 (73%)
ß-Blocker	8 (53%)
Calcium-channel blocker	9 (60%)
α-Blocker	2 (13%)
Diuretic	10 (67%)
Central Acting	0 (0%)

Continuous variables are displayed as a mean ± SD or median (range).Categorical variables are displayed as a number (percentage). Yrs indicates: years, TIA transient ischemic attack, CAD: coronary artery disease, ACEi: angiotensin-converting enzyme inhibitor, ARB: angiotensin II receptor blocker, SBP: systolic blood pressure, DBP: diastolic blood pressure, PP: pulse pressure, HR: heart rate, bpm: beats per minute, eGFR: estimated glomerular filtration rate.
†Calculated on the basis of the Chronic Kidney Disease Epidemiology Collaboration formula.
** Determined using ABPM.*

Blood pressure and renal function after renal denervation

Mean office BP decreased numerically six months after RDN, although formal statistical significance was not met (from 184±29/106±17 mm Hg at baseline to 179±24/102±16 mm Hg at follow-up; P=0.07/0.13). Renal function remained stable during follow-up (from 86 (56) mL/min/1.73m^2 at baseline to 92 (61) mL/min/1.73m^2 at follow-up; P=0.42). Medication also remained stable during follow-up (from 3 (8) DU at baseline to 5 (6) DU at 12 months follow-up; P=0.95).

Hemodynamic effects of renal denervation

The changes in renal haemodynamics before and directly after RDN are shown in table 3. Mean aortic pressure and heart rate during baseline conditions decreased by respectively 10±14 mmHg (P<0.01) and 7±13 bpm (P<0.01) due to the sedation and analgesia started during RDN. Despite this drop in blood

pressure, no significant change in baseline or hyperaemic flow velocity was observed directly after RDN (table 3), and thus RFVR remained equivalent. Baseline microvascular resistance tended to decrease directly after RDN (P=0.12), whereas there was no overall change in HMR. Consequently, there was a significant decrease in the AR directly after RDN (P=0.02).

Table 3: Change in renal hemodynamic parameters directly after renal denervation

	Baseline	Directly after RDN	P-value
Resting aortic pressure, mmHg	117 (93-129)	104 (92-120)	0.001
Resting heart rate, beats per minute	70 (64-77)	64 (59-68)	0.005
Baseline APV, cm/sec	38 (29-52)	40 (30-53)	0.74
Hyperaemic APV, cm/sec	59 (44-76)	63 (42-80)	0.77
Baseline MR, mmHg/cm/sec	3.0 (2.5-3.5)	2.5 (2.2-3.4)	0.12
Hyperaemic MR, mmHg/cm/sec	1.8 (1.5-2.3)	1.7 (1.5-2.1)	0.71
Renal flow velocity reserve	1.55 (1.39-1.70)	1.44 (1.24-1.67)	0.28
Arteriolar resistance	1.11 (0.76-1.40)	0.82 (0.52-1.12)	0.02

Continuous variables are displayed as median (range). The Wilcoxon signed rank test is used for analysis of paired samples analysis. APV indicates average peak flow velocity, MR indicates microvascular resistance.

Table 4 shows the results of the univariable and bivariable linear regression model. The univariable linear regression model showed a linear relation between change in HMR and change in SBP during follow-up (β:7.738 mmHg in SBP per mmHg/cm/sec change in HMR; P=0.03, table 4 and figure 1), where a reduction in SBP at follow-up was associated with a decrease in HMR directly after the procedure. Also, inverse relations between a change in RFVR or AR and change in SBP and were observed in the univariable analysis (table 4, figure 1). In the bivariable linear regression model, corrected for baseline SBP, the changes in HMR and AR were slightly attenuated, but remained significantly associated with a change in SBP (β:6.851 mmHg in SBP per mmHg/cm/sec change in HMR; P=0.03 and β:-20.382 per unit increase in AR; P<0.01, respectively).

Figure 1: Change in blood pressure plotted against change in renal hemodynamic parameters

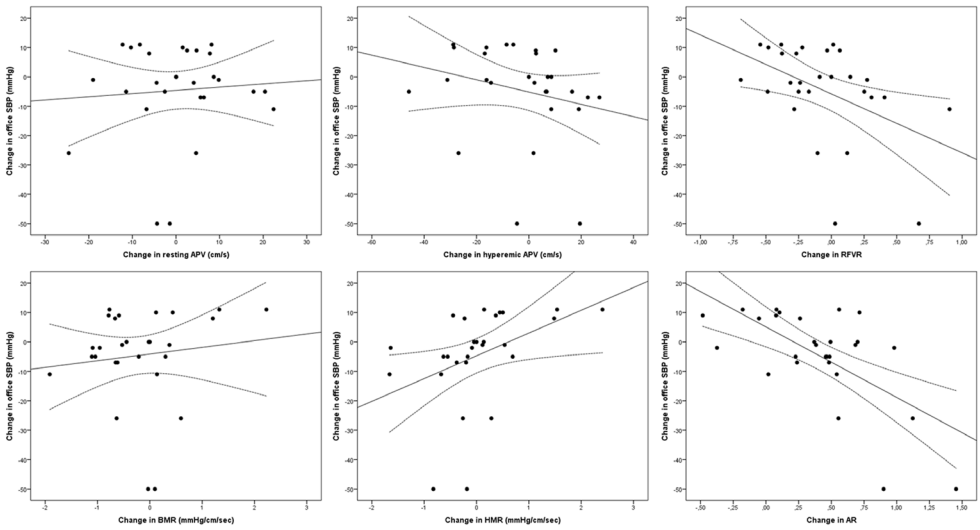

Table 4: The relation between change in blood pressure and change in renal hemodynamic parameters

	Univariable model			Bivariable model†		
	β	95%CI	p	β	95%CI	p
Change in systolic blood pressure						
Delta resting APV	0.110	-0.486 – 0.706	0.71			
Delta hyperaemic APV	-0.210	-0.557 – 0.137	0.23			
Delta resting MR (BMR)	2.275	-5.286 – 9.837	0.54			
Delta hyperaemic MR (HMR)	7.738	0.764 – 14.712	0.03	6.851	0.731 – 12.971	0.03
Delta renal flow velocity reserve	-20.088	-36.040 – -4.135	0.02	-13.538	-29.497 – 2.421	0.09
Delta arteriolar resistance	-23.991	-35.631 – -12.352	<0.01	-20.382	-31.278 – -9.486	<0.01

ß indicates regression coefficient, 95%CI: 95% confidence interval SBP: systolic blood pressure.
†Adjustment for baseline SBP.

Discussion

In the present study we observed a numerical decrease in baseline microvascular resistance and a significant reduction in arteriolar resistance. Moreover, we observed that acute changes in HMR and AR following RDN were significantly associated with the change in systolic BP (SBP) during follow-up, independent of the magnitude of baseline SBP. These findings suggest that invasive assessment of the immediate physiological response to renal denervation can provide markers of a successful renal denervation procedure, or an adequate patient response to RDN.

We observed that resting and hyperaemic flow velocity in the renal artery remained unchanged after renal denervation. There was a numerical decrease in microvascular resistance during resting conditions immediately following renal denervation, while no overall difference in microvascular resistance in hyperaemic conditions was observed. These characteristics led to a significant reduction in the reserve vasodilatory capacity of the renal microcirculation (AR) in the absence of changes in renal flow or RFVR. It is important to note that these characteristics were documented in the presence of a significant driving pressure reduction between baseline and post-RDN measurements due to the instalment of anaesthesia and potent analgesia for the RDN procedure, which complicates the appropriate interpretation of our findings.

In resting, autoregulated, conditions, stable renal flow is maintained at a range of perfusion pressures by adaptive dilation and constriction of the renal resistance vessels. In contrast, when autoregulation of the resistance vessels is abolished by a potent vasodilator, such as papaverine, resistance vessels are pressure-distensible, and therefore change vascular tone with changing perfusion pressure. Hence, in resting conditions, a decrease in driving pressure is counteracted by microvascular adaptation, meaning that flow remains stable due to an adaptive physiological decrease in microvascular resistance. The documented decrease in BMR is therefore most likely a direct result of the drop in perfusion pressure during the denervation procedure. In contrast, at maximal vasodilation, a drop in perfusion pressure cannot be counteracted by alterations in microvascular resistance but decreases resistance vessel diameter, and is therefore associated with an increase in HMR and a consequent decrease in hAPV. The documented equivalence of both hAPV and HMR in the present study are therefore counterintuitive, and suggest that RDN results in an acute reduction minimal tone of the microvasculature, maintaining equivalent minimal microvascular resistance and thereby maintaining hAPV despite a substantial drop in perfusion pressure. These observations are also in accordance with our findings regarding the relationship between renal physiology and the SBP alterations at follow-up. The documented decrease in BMR, attributable to the per-procedural drop in BP, was indeed not associated with alteration of SBP. In contrast, despite no overall differences in HMR pre- and post-RDN, HMR was significantly associated with a change in BP at follow-up; a decrease in HMR was largely associated with a decrease in SBP during follow-up, whereas and increase in HMR was associated with no change or an increase in SBP, independent of baseline SBP. The highly variable response of HMR towards RDN, increasing in some and decreasing in others,

explains the absence of a reduction in HMR induced by RDN across the study population. Nonetheless, the magnitude of the change in HMR is directly associated with the SBP response during follow-up, and may be a physiological marker of effective application of RDN or an adequate patient-response to renal denervation. The wide variability in HMR reduction may provide physiological insight into the variable inter-patient effectiveness of RDN identified in previous studies focusing on SBP changes induced by RDN, and warrants further study. The observations that a change in AR was also related to a change in BP at follow-up can be explained by the change in HMR.

The observations in the present study are also in accordance with previous preclinical studies. Osborn et al. showed that stimulation of the renal nerves resulted in an increased renal vascular resistance and decreased renal blood flow in dogs.[15] DiBona et al. showed that RDN increased renal blood flow variability in spontaneously hypertensive rats and congestive heart failure rats.[16] Tsioufis et al. showed a significant increase in renal blood flow in healthy pigs that persisted up to 1 month.[17]

Limitations

Some limitations should be mentioned. Most importantly, the present study should be considered hypothesis-generating in the light of the small sample size. A priori, the small sample size limits the potential to detect differences between groups. Hence, the small sample may explain a lack of statistical significance in the present study. Secondly, the influence of anaesthetics and analgesics on renal blood flow and microvascular resistance is unknown. Furthermore, it would be of value to repeat hemodynamic renal measurements during follow-up. In the present study, we did not assess measures of sympathetic activity and therefore we cannot conclude whether RDN has established a reduction in sympathetic activity.

In contrast to most studies investigating coronary haemodynamics we used papaverine intra-arterially to induce hyperaemia. However, adenosine is a potent coronary vasodilator, whereas it constricts renal arteriole.[18] When given in the renal arteries, papaverine has been shown effective and safe without electrocardiographic effects.[19]

In perspective

In the light of the recent HTN-3 trial the discussion about the role of RDN in clinical practice has emerged. At present, RDN is a more or less black box procedure and we lack any periprocedural markers to observe if the energy is successfully delivered. The observations that changes in HMR and AR were related to a changed SBP at follow-up suggest that these variables can serve as periprocedural markers in the future. However, this is only an explorative study. Larger trials should confirm this preliminary finding. Moreover, these trials should consider adding measurements of systemic sympathetic activity as well. Such measures offer the possibility to investigate whether a changed MR is associated with a reduced sympathetic activity.

In conclusion, we observed that changes in HMR and AR immediately following RDN were associated with the change in SBP during follow-up, independent of the magnitude of baseline SBP. These findings suggest that assessment of renal hemodynamic measures may be used as per-procedural markers of a successful RDN.

Impact on daily practice

The observations from the present study may give more insight in the physiological changes after renal denervation (RDN). We demonstrated that RDN induces a hyperaemic state leading to a significant reduction of the AR. Moreover, the present study suggests that assessment of microvascular resistance may be used as a periprocedural marker during RDN. This may help in the application of RDN since the procedure is a more or less black procedure at present.

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Ischemia and reactive oxygen species in sympathetic hyperactivity states: a vicious cycle that can be interrupted by renal denervation?

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Abstract

Renal denervation has developed as a new treatment strategy for patients suffering from resistant hypertension. The success of this therapy is due to the fact that sympathetic hyperactivity is involved in the pathogenesis of elevated blood pressure. However, not only the sympathetic nervous system (SNS) but also the renin angiotensin system (RAS) is known to be involved in hypertension. In addition, RAS is involved in other sympathetic hyperactivity states, such as heart failure, chronic kidney disease, insulin resistance and obstructive sleep apnea. Moreover, renal denervation has a beneficial effect on patients suffering from these disease states. Recent research suggested that the production of reactive oxygen species (ROS) is elevated in sympathetic hyperactivity states and that ROS are able to activate the SNS and local tissue renin angiotensin system. Therefore, this review discusses the possibility of ROS as a common trigger of SNS and RAS activity in sympathetic hyperactivity states and the effect of renal denervation on this ROS production.

Introduction

Since 2009, percutaneous renal denervation (RDN) has developed as a new and safe treatment strategy for hypertension.[1] Its first clinical evidence has been evaluated in the Symplicity HTN-1 study and HTN-2 trial.[2, 3] In the HTN-2 trial a BP reduction of 33/11 mmHg was established 6 months after treatment[3], and this result was confirmed by other single center studies throughout the world.[4-10] Its mode of action is based on the reduction of sympathetic nervous system (SNS) activity via catheter-based radiofrequent ablation of the renal sympathetic nerves located around the renal artery.[11] SNS activity is - besides the renin-angiotensin-aldosterone system (RAAS) - known to be increased in hypertension and hypertension related disease states, such as chronic kidney disease (CKD)[12, 13], heart failure (HF)[14, 15], insulin resistance, and obstructive sleep apnea (OSA)[16, 17]. Moreover, in 50% of all cases of high blood pressure (BP) renal noradrenaline spillover measurements, representing the sympathetic outflow of the kidneys, were elevated two to three times.[18] In addition, two commonly applied non-pharmacological therapies for hypertension, aerobic exercise training and calorie restriction, inhibit the SNS and have shown to lower BP.[19, 20]

Patients treated with RDN in the above mentioned studies suffer from 'therapy resistant hypertension', i.e. patients with hypertension without a secondary cause that fail to meet therapeutic targets despite taking multi-drug therapies at the highest tolerated doses.[21] In contrast to RDN, the antihypertensive drugs mainly focused on RAAS inhibition, natriuresis and dilatation of the peripheral vasculature. The fact that both RAAS inhibition and reduction of sympathetic hyperactivity lower BP and that RAAS inhibition solely is not sufficient in a large fraction of patients[21], suggests that both mechanisms are involved in the pathogenesis of hypertension and hypertension related disease states.

In the current manuscript the question is raised whether SNS activity and RAAS are linked to each other and whether they could have a common initiator. Based on current available literature reactive oxygen species (ROS) appear to be important mediators of increased SNS activity and local tissue renin angiotensin system (RAS) activation in hypertension and other hypertension related diseases.[22••] Consequently, we discuss whether ROS production could be affected by RDN in states of sympathetic hyperactivity.

Sympathetic nerve activity and its role in essential hypertension

The SNS mobilizes the human body in acute dangerous and/or stressful situations by inducing the so called 'fight-or-flight' response.[23] In the brain, danger or stress is documented by the amygdala and the fight -or flight response is initiated by the hypothalamus. The hypothalamic paraventricular nucleus together with the rostral ventrolateral medulla (RVLM) and the nucleus of the solitary tract (NTS) form an extensive neural network, that initiates, maintains and regulates SNS activity (see Fig. 1).[24] Kumagai and co-workers demonstrated the importance of the RVLM in determining sympathetic efferent outflow and blood pressure.[25•] They concluded that neurons of the RVLM are the primary cause of experimental and essential hypertension and suggested these will also be involved in other cardiovascular diseases. In addition, it was found that neuron activity of the RVLM is potentiated by (local) Angiotensin II (AngII) and inhibited by AngII receptor blockers.[25]

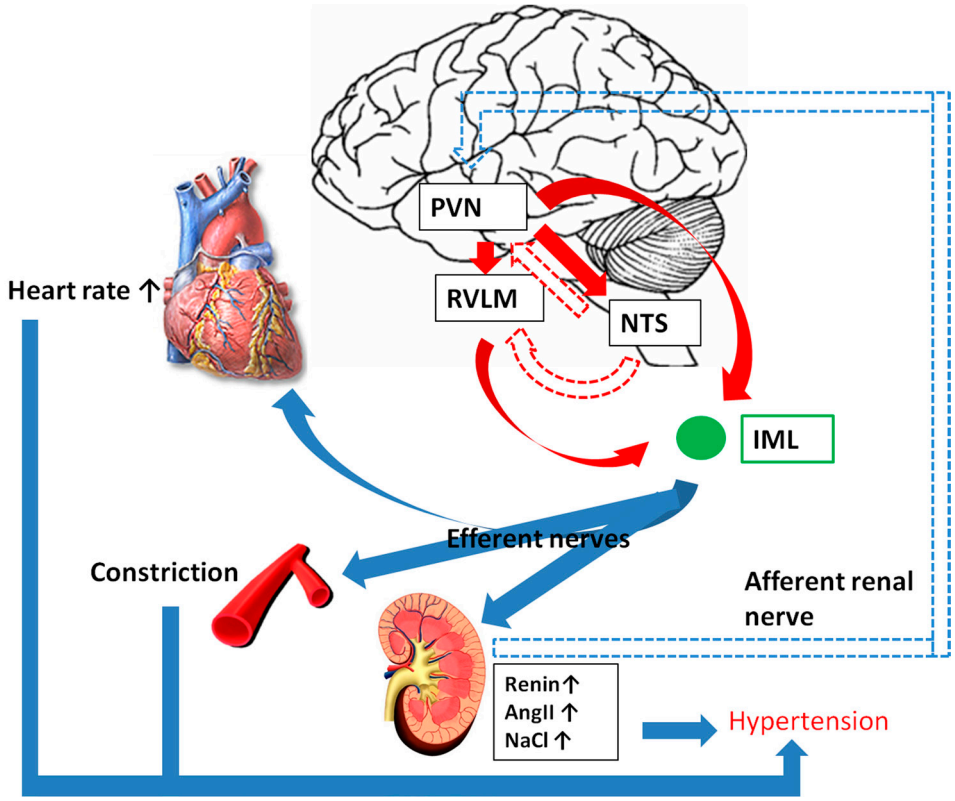
The sympathetic efferent nerve fibers present in the intermediolateral (IML) cell column of the spinal cord are a functionally specific group of fibers. They separately innervate and control the function of blood vessels, juxtaglomerular apparatus, and renal tubules and exert an effect on the highly innervated organs such as the heart, blood vessels and kidneys (see Fig. 1). In the heart they can induce hypertrophy, arrhythmias, ischemia, remodelling and apoptosis. In the vessels high SNS activity can lead to medial hyperplasia, reduced arterial compliance and endothelial dysfunction.[26] Upon stimulation, vasoconstriction leads to a reduction of renal blood flow and glomerular filtration rate, increased renin release leading to production of AngII, and sodium reabsorption is increased.[27] AngII is a central mediator of blood pressure, i.e. it causes directly vasoconstriction, has trophic effects, stimulates SNS vasomotor center in the brain

stem, regulates water and chloride reabsorption[28], and it stimulates aldosteron production in the adrenal glands.[29] Aldosterone is a steroid that increases sodium resorption and potassium secretion in the kidneys. In the brain it stimulates SNS activity by upregulation of brain renin-angiotensin system (RAS) components and induction of oxidative stress in the hypothalamus.[30·] Together, these changes lead to elevated BP.

The afferent renal nerves further augment and/or initiate these changes through communication between the kidneys and integral structures of the CNS where they have the capacity to modulate posterior hypothalamic activity and subsequent widespread efferent sympathetic outflow.[31] In humans, SNS activity was shown to contribute to left ventricular (LV) hypertrophy and chronic kidney disease (CKD).[13, 14, 32]

Pharmacological strategies to treat essential hypertension, such as sympatholytic drugs, ß-blockers, angiotensin-converting enzyme inhibitors, AngII receptor blockers and diuretics, only act on the consequences of renal efferent sympathetic stimulation. As suggested by animal studies, it is likely that in hypertensive patients who underwent RDN, also afferent nerve fibers are ablated. Therefore, it can be hypothesized that RDN leads to reduced activity of the hypothalamus, RVLM, renal efferent nerves and subsequent reduced BP.[31, 32] Furthermore, besides effects on BP, this strategy appears to have beneficial effects on the integrity of the heart and kidneys.[33-35··]

Figure 1: Schematic overview of the central and peripheral sympathetic nervous system and effects on target organs



Danger or stress is recognized by the amygdala and transferred to the hypothalamic paraventricular nucleus (PVN) that, together with the rostroventrolateral medulla (RVLM) and the nucleus solitary tract (NTS), initiates a response of sympathetic hyperactivity. Subsequently, this network activates the peripheral sympathetic nerves of the heart, kidneys and arterioles via the intermediolateral (IML) cell column of the spinal cord, and an increase in blood pressure is induced. The blue dotted line represents the renal afferent nerve fiber and the red dotted lines represent brain afferent nerve fibers

Reactive oxygen species and antioxidants: molecular mechanism and involvement in hypertension

ROS are products of normal cellular metabolism and arise during the reduction of oxygen into unstable free radicals such as superoxide ($O_2^{\bullet -}$) and non-free radicals such as hydrogen peroxide (H_2O_2).[36••] They increase during numerous pathophysiological processes of hypertension such as sympathetic hyperactivity, upregulation of the RAAS, abnormal G-protein coupled receptor signalling, and inflammation.[37-39] ROS are generated by many enzymes of which NADPH oxidase appears to be essential in hypertension.[40]

ROS are injurious and through acting as second messengers they induce alterations in intracellular signalling that -at vascular level- eventually lead to endothelial dysfunction, increased contraction, reduced vasodilatation, and structural remodelling responsible for increased peripheral resistance and hypertension.[41-44] To protect against oxidative damage, biological systems produce enzymatic and nonenzymatic antioxidants. In the cardiovascular system, superoxide dismutase (SOD)[45], catalase[46] and glutathione oxidase/reductase are important enzymatic antioxidants, whereas vitamin E, vitamin C, and glutathione[47] are major nonenzymatic antioxidants.

A link between oxidative stress and hypertension has been demonstrated in several animal models of hypertension and AngII is well recognized as a potent inducer of both.[48] Interestingly, markers of oxidative stress were increased in experimental hypertension and nitric oxide (NO) and antioxidant enzymes were decreased.[49••] Markers of experimental hypertension are, among others, plasma and urine thiobarbituric acid reactive substances, F-isoprostanes and H_2O_2 and tissue $\bullet O_2^-$ levels.

In humans increased ROS production has been reported for several forms of hypertension, i.e. essential hypertension, renovascular hypertension, salt-sensitive hypertension, cyclosporine-induced hypertension, malignant hypertension and pre-eclampsia.[50, 51] In most of these studies increased ROS production is based on increased plasma levels of the biomarkers thiobarbituric acid-reactive substances and 8-epi-prostanes.[52, 53]

Furthermore, plasma H_2O_2, asymmetric dimethylarginine (ADMA) (endothelial NOS inhibitor) and the lipid peroxidation product of linoleic acid, 13-hydroxyotadecadienoic acid (HODE) have shown to be appropriate biomarkers of oxidative stress in hypertensive patients.[54-57] Also a decreased content of antioxidants, both enzymatic as well as nonenzymatic, was present and SOD correlated inversely with BP.[58] Serum derivates of reactive oxygen metabolites (ROM) are proposed as useful markers for detection of endothelial damage, predicting cardiovascular disease and for cardiovascular events in patients with coronary artery disease (CAD).[59, 60] ROMs can be measured in blood serum with a commercially available kit [62, 63].

Bilirubin is a molecule with anti-oxidant capacity produced by the human body itself. Serum bilirubin plasma levels have been shown to negatively correlate with cardiovascular disease (CVD) and with CVD related diseases and risk factors such as hypertension, diabetes mellitus, obesity and metabolic syndrome.[61] Not only serum bilirubin but also other components of the heme catabolic pathway, such as heme oxygenase, showed to have protective effects and to be suitable for diagnostic purposes.[61]

Since ROS are elevated and antioxidants are decreased in hypertension and CVD, and antioxidant therapy showed to have a curative effect on them in animal studies, large clinical trials using antioxidants as treatment strategy had been performed. However, these trials failed to show beneficial effects on hypertension and cardiovascular endpoints.[62, 63] To date, strategies that do lower ROS production are glucose-6-phosphate (G6PD) (source of NADPH) inhibitors[64], antioxidant rich diet[65], exercise[66], and possible classical antihypertensive agents of which the AngII receptor blocker, candesartan, showed to have antioxidant capacity independently of inhibiting the angiotensin II receptor type 1 (ATI).[67]

The involvement of kidney ischemia and reactive oxygen species in sympathetic hyperactivity and hypertension

In CKD, kidney ischemia could be the central mechanism that stimulates RAS and subsequently leads to

sympathetic hyperactivity.[36••] In rats, a small locus of ischemia, caused by intrarenal injection of phenol, leads to hypertension in association with increased central sympathetic activity and without affecting glomerular filtration rate.[68] In CKD, agents that interfere with the RAS have been shown to be able to lower SNS activity with 20-25%.[36••] However, it is still unclear whether kidney ischemia directly leads to sympathetic hyperactivity or indirectly via AngII. In patients with essential hypertension without kidney disease, agents that interfere with the RAS did not show major effects on reducing MSNA activity.[36••] These results suggest that in patients with essential hypertension, sympathetic hyperactivity is not indirectly regulated via the RAS but directly via local kidney ischemia.

As stated above, experimental models and human studies suggested that local kidney ischemia could induce hypertension via increased SNS activity.[36, 68••] Local hypoxia-ischemia in the heart, liver, pancreas and kidney is associated with increased reactive oxygen species (ROS) and activation of the ROS-tissue(t) RAS axis.[22••] In transgenic male mice, designed to investigate the sex-dependent severity of hypertension by overexpressing kidney androgen regulated protein (KAP) in the proximal tubule, ROS were involved in pathological signalling leading to hypertension by increasing central sympathetic activity and activating RAAS.[69] In this model, the relationship between oxidative stress and sympathetic overactivity was derived from the observation that Tempol, a membrane permeative superoxide dismutase mimetic, reduced norepinephrine plasma levels and simultaneous administration of Tempol and the anti-oxidant N-actetyl cysteine produced even stronger effects on epinephrine and norepinephrine plasma levels. Since the nicotanimide adenine dinucleotide phosphate (NADPH) oxidase inhibitor apocynin also reduced epinephrine and norepinephrine, one of the sources for ROS production could be NADPH oxidase.

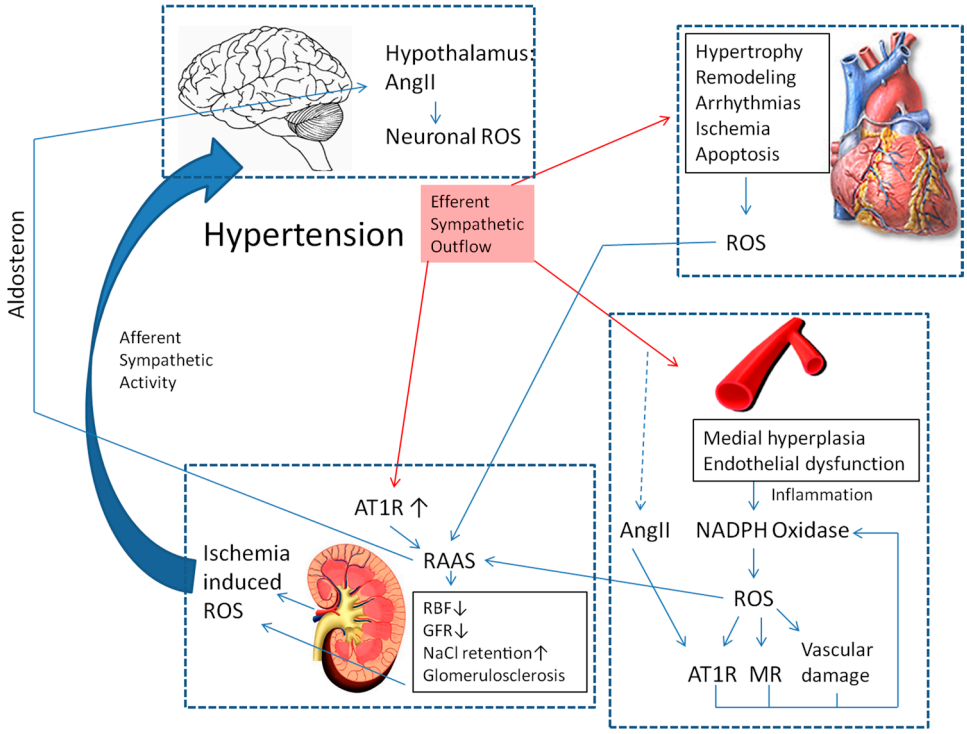
Neuronal, renal, vascular and cardiac ROS have been implicated to play a role in the pathogenesis of hypertension in several studies.[70•] Whether these systems act collectively or independently in disease conditions is unclear. Interestingly, neuronal ROS produced in the hypothalamus are implicated in norepinephrine secretion and peripheral sympathetic nerve activity of phenol-induced renal injury and Dahl salt-sensitive hypertensive rat models.[69, 71] With the phenol renal injury model, it was found that during conditions of local kidney ischemia, sympathetic hyperactivity was mediated by afferent pathways that activated brain regions involved in noradrenergic control of blood pressure via the production of local ROS in the brain.[69] This local production of ROS could in turn be a consequence of local AngII production in the brain, since losartan, an ATI receptor antagonist completely reversed the effects of phenol-renal injury on blood pressure and SNS activity. This was confirmed by other studies showing AngII to activate the central nervous system via increased superoxide generation.[72-74] In addition, NO is known to exert a tonic inhibition on central SNS activity, and this effect can be abolished by ROS through oxidation/inactivation. In brain nuclei regulating the noradrenergic control of BP of the phenol-renal injury model, the abundance of nuclear NO species was decreased. Furthermore, the two representative brain stem sites that set the sympathetic vasomotor tone, RVLM and NTS, showed increased ROS production upon AngII stimulation in spontaneously hypertensive rats (SHR) or stroke prone SHRs.[75]

The beneficial modulating effects of renal denervation on molecular level

To understand the observed detrimental effects of an increased sympathetic tone on BP, kidneys, heart, and blood vessels, the molecular changes induced by this activity were measured in rat or rabbit models of sympathetic hyperactivity by comparison with renal denervation conditions. Overall it was found that renal denervation had beneficial effects on the changes induced by sympathetic hyperactivity through normalization of gene or protein expression levels.

Clayton et al. investigated the effect of excessive sympathetic drive on AngII receptor type I (ATI) and II (ATII) expression in the renal cortex of rabbits with chronic systolic heart failure (HF).[76] They hypothesized that excessive renal sympathetic nerve activity decreases renal blood flow in HF and is associated with altered AT expression. In surgically denervated HF rabbits the renal blood flow and vascular resistance were not changed.[76] However, ATI expression was increased by 67% and ATII expression was decreased

Figure 2: Reactive oxygen species as initiators and maintainers of sympathetic hyperactivity and hypertension



AT1R Angiotensin receptor type 1; MR mineral corticoid receptor; RBF renal blood flow; GFR glomerular filtration rate; RAAS renin-angiotensinaldosterone system

by 87% in rabbits with HF.[76] The kidneys of the denervated rabbits with HF showed near to normal expression levels of these receptors suggesting a role for renal sympathetic activity in modulating AT expression.[76]

Satoh et al. found ß-agonists to directly stimulate NADP(H) oxidase in endothelial cells of the kidney and to cause glomerular injury through the production of ROS in rat models of kidney disease.[77-79] Furthermore, they showed that a reduction of oxidative stress was effective in ameliorating renal tissue injury.[79] Subsequently, they investigated whether renal denervation protects kidney function through an anti-oxidative effect in Dahl salt-sensitive hypertensive rats.[80] They found reduced oxidative stress and suppressed NADPH oxidase activity in isolated glomeruli of denervated rats compared to sham-operated controls.[80] Glomerular injury, determined by urinary albumin excretion and glomerular sclerosis index, was lower in denervated rats. Torp et al. investigated the molecular mechanism connecting HF sympathetic nerve activity to sodium retention in a rat model of HF.[81] In previous studies they found that rats with HF display increased expression of a sodium transporter, the Na-K-2Cl cotransporter (NKCC2) in the medullary thick ascending loop of Henle (mTAL).[82] Subsequently, they investigated the effect of renal sympathetic nerve activity (RSNA) on NKCC2 expression in mTAL of rats with HF.[81] They found increased mTAL NKCC2 expression in HF rats which was abolished by denervation. These results suggest that RSNA increases sodium retention by modulating NKCC2 expression in mTAL.

Potential effects of RDN on ROS in man

Kidney ischemia is causally involved in hypertension through SNS hyperactivity as stated above. In patients with CKD, HF, metabolic syndrome or insulin resistance, sympathetic hyperactivity could be reinforced by production of ROS in various organs since kidney ischemia, cardiac remodeling and/or damage, hyperlipidemia, hyperglycemia and hyperinsulinemia result in increased ROS production.[17] These findings link ROS not only to essential hypertension but also to other disease states (see Fig. 2). Consequently, interruption of this vicious cycle by RDN would be a powerful tool in shutting down this excessive production of ROS.

Future experimental and subsequently clinical studies should evaluate potential changes in ROS production after renal denervation. Hereby, a biological marker or minimal invasive readout method of local ROS production is desirable for investigating the relation between ROS production and SNS hyperactivity in patients suffering from cardiovascular-(related) disease before and after treatment with RDN.

Summary

In conclusion, renal denervation is a promising novel minimally invasive therapeutic option for the modulation of sympathetic activity in disease states in which the SNS plays an important role. Animal studies have shown that in a hypertension-state, sympathetic outflow from the brain directly induces changes in the kidney causing elevated blood pressure. This sympathetic outflow from the brain is in turn activated by afferent signals deriving from the kidney, potentially leading to a vicious circle. Local kidney ischemia leading to production of ROS may play a central role in this cascade of events. Future studies should define the exact response of ROS after RDN in the treatment of the different SNS related disease states.

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• Of importance
•• Of major importance

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Part 2. Selection of patients for renal denervation

Eligibility for percutaneous renal denervation: the importance of a systematic screening

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Abstract

Objective

Percutaneous Renal Denervation (RDN) is a new and promising therapy for resistant hypertension. Among patients suspected of having resistant hypertension, the actual presence of this condition needs to be well established; pseudoresistant hypertension and significant white coat effect (WCE) should be excluded. This analysis presents the results of a standardized screening program for patients referred for RDN.

Methods

All patients referred to our centre for RDN underwent a standardized stepwise screening and were subsequently discussed in a multidisciplinary team. The screening included a 24-hour blood pressure measurement (ABPM), collection of plasma, urine and saliva, and finally imaging of the renal arteries.

Results

From August 2010 till October 2012, 181 patients were referred for RDN. Mean blood pressure (BP) was 182/100 mmHg, median use was 3 antihypertensives. Ultimately, 121 patients (67%) were excluded from RDN. Main reasons for exclusion were BP-related. 23 Patients (19%) had an office SBP <160 mmHg and 26 patients (22%) showed a WCE. 14 Patients (12%) had a so far undetected underlying cause of hypertension, the majority being primary aldosteronism (n=11). Nine patients had an ineligible renal anatomy.

Conclusions

A high percentage of patients were excluded from treatment with RDN due to secondary causes of hypertension, WCE, or a BP below the currently advised thresholds. Treatment of these excluded patients would lead to inappropriate use of RDN, leading most likely to little benefit for the patients and a burden to health care. Therefore it is recommended to use a standardized screening before treatment with RDN.

Introduction

Globally, 34% of the adult population have hypertension and this prevalence is still rising.[1] Hypertension is listed in the top three of modifiable factors that impact the occurrence of disease burden globally.[1] It is well established that lowering blood pressure (BP) reduces cardiovascular risk.[2] Despite a broad availability of effective pharmaceutical agents, only 32% of treated men and 37% of treated women reach treatment goals.[3]

Increased activation of the sympathetic nervous system (SNS) is identified as an important factor in the development and progression of hypertension.[4] In this context, a percutaneous, catheter-based approach has been developed to disrupt the renal sympathetic nerves, using radiofrequency energy.[5] The first clinical studies in a relatively small number of patients showed that this catheter-based technique is efficacious. Office systolic BP / diastolic BP (SBP/DBP) values after bilateral percutaneous renal denervation (RDN) were reduced by -14/-10 to -27/-17 mmHg from 1 to 12 months of follow-up. Furthermore, the approach seems safe.[6-8]

According to the recently published ESH position paper, RDN is currently only indicated for patients with resistant hypertension.[9] The American Heart Association (AHA) defined resistant hypertension in 2008 as a blood pressure (BP) that remains above treatment goals despite the concurrent use of medication from three different antihypertensive classes, one ideally being a diuretic, with all agents prescribed at doses that provide optimal benefit.[10] Several reports provided insight into this prevalence; however, numbers vary from 1.9% to 30% of all patients that use ≥3 medications for hypertension.[11-15]

The potential success of RDN as an adequate treatment option for hypertension depends on the ability to select patients most likely to benefit. Among patients suspected of having resistant hypertension, the actual presence of this condition needs to be well established. Individuals with white coat hypertension, with a BP that may still be manageable with improved standard care, and those with secondary forms of hypertension need to be excluded. Prevalence estimates of secondary causes in hypertensive patients ranging from 10 to 15% have been described.[16-18] Secondary forms of hypertension are more prevalent in patients suspected of having resistant hypertension.[16-18]

In the current analysis, the results of our standardized stepwise screening of all patients referred to our tertiary centre for treatment with RDN are evaluated.

Methods

Study population

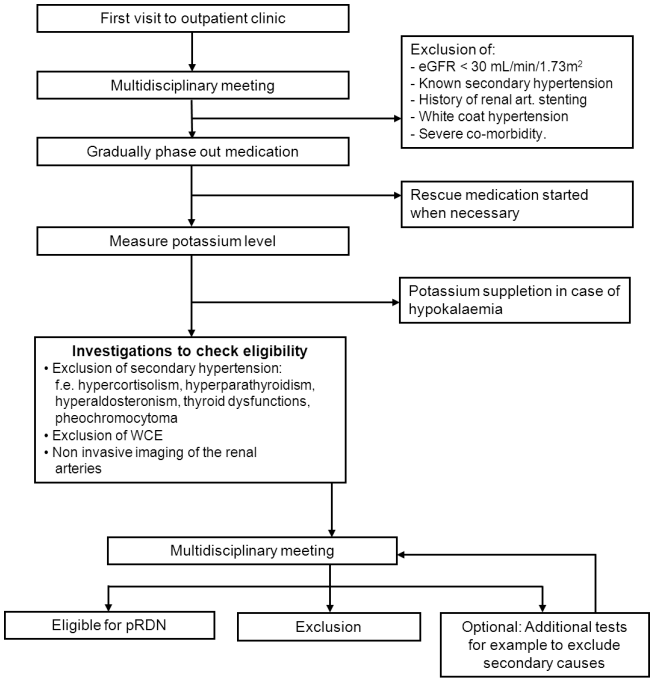
Between August 2010 and October 2012 all patients referred to the University Medical Centre (UMC) Utrecht (European society of hypertension (ESH) excellence centre) for treatment with RDN were screened using a standardized protocol. Primarily, patients with resistant hypertension were considered eligible for RDN. This condition was defined as an office systolic blood pressure (SBP) of ≥160 mmHg, despite the use of ≥3 antihypertensive drugs, preferably including a diuretic. Secondly, patients fulfilling the same BP criteria, but without optimal pharmacological treatment due to recorded intolerance for antihypertensive drugs, were accepted. Major contraindications for RDN were: an eGFR<30 mL/min/1.73m^2, known secondary causes of hypertension, a history of renal artery stenting, and severe co-morbidity (defined as any serious medical condition which, in the opinion of the physician, may adversely affect the safety of the patient or the effectiveness of the procedure).

Standardized screening

The departments of Nephrology, Cardiology, Vascular Medicine and Radiology collaborated closely and developed a standardized stepwise protocol. The aims of this work-up were to confirm the diagnosis of resistant hypertension, to exclude secondary forms of hypertension (including sleep apnoea), to exclude significant white coat effect (WCE, defined as difference between office BP and daytime ambulatory BP >20 mmHg SBP and/or >10 mmHg DBP[19], leading to an ambulatory SBP <140 mmHg), and finally to

determine whether the anatomy of the renal arteries was suitable for RDN.
All referral letters were checked before invitation to the outpatient clinic. Patients with a renal artery stent were excluded on forehand. After the first visit at the outpatient department, all patients were discussed in a multidisciplinary meeting to decide whether a patient was a potential candidate for RDN and whether the patient can undergo the screening. A patient could be excluded from further work-up due to co-morbidity or an office BP below treatment criteria. The standardized work-up consisted of a stepwise program for every patient (figure 1 and the online supplement 1). All patients were advised to reduce salt intake and to reduce weight when applicable.
Subsequently after completion of the work-up, patients were discussed for the second time in the multidisciplinary meeting. Additional tests, for example to exclude secondary causes of hypertension, were performed whenever this was deemed necessary. The final decision whether RDN was indicated was unanimously made by the multidisciplinary team. This team consists of two hypertension specialists (vascular internist and nephrologist), an interventional cardiologist, and an interventional radiologist. All departments were represented every meeting.

Figure 1: Flowchart of the stepwise screening protocol



ABPM, ambulatory blood pressure measurement; HTN, hypertension; WCE, white-coat effect.

Data analyses

Results are expressed as means with standard deviations or as absolute numbers and percentages unless otherwise stated. All analyses were performed with the SPSS statistical package version 20 (IBM SPSS Data Collection, Chicago, IL).

Results

From August 2010 till October 2012, 181 patients were referred to the UMC Utrecht for RDN. The majority of patients were referred by a cardiologist (39%) or hypertension specialist (33%). Table 1 shows the characteristics of the patients. The majority (52%) of the patients was female; mean age of the screened patients was 60±12 years. At the first visit to the outpatient clinic, mean SBP was 182±30 mmHg and mean diastolic BP was 100±15 mmHg. Patients used a median number of 3 (range: 0-8) blood pressure-lowering drugs. Twenty-four per cent of patients used an aldosterone antagonist at moment of referral. A substantial group had used aldosterone antagonists in the past, but stopped due to side effects (i.e., hyperkalaemia or gynaecomastia).

Table 1: Characteristics of referred patients

	All referred patients (n=181)	Patients suitable for RDN (n=60)	Patient not suitable for RDN (n=121)
Gender (female, No. (%))	94 (52%)	34 (57%)	60 (50%)
Age (years)	60 (±12)	58 (± 12)	61 (± 12)
Renal function			
Mean eGFR* (mL/min/1.73m^2)	74 (± 19)	73 (± 18)	74 (± 20)
eGFR* > 60 (mL/min/1.73m^2)	128 (75%)	43 (72%)	85 (77%)
eGFR* 45-60 (mL/min/1.73m^2)	27 (16%)	10 (17%)	17 (15%)
eGFR* 30-45 (mL/min/1.73m^2)	16 (9%)	7 (12%)	9 (8%)
Body-mass index (kg/m^2)	28.8(± 5.0)	28.4(± 5.1)	29.1(± 5.0)
Co-morbidity			
Hypercholesterolemia	99 (55%)	34 (57%)	65 (55%)
Diabetes Mellitus Type II	32 (18%)	10 (17%)	22 (18%)
Cardiovascular diseases	58 (32%)	16 (27%)	42 (35%)
TIA/stroke	21 (12%)	4 (7%)	17 (14%)
CAD	34 (19%)	9 (15%)	25 (21%)
PAD	42 (23%)	16 (27%)	26 (22%)
Smoking status			
Current smoking	25 (16%)	11 (19%)	14 (14%)
Smoked in the past	44 (28%)	13 (23%)	31 (32%)
Recently stopped smoking	3 (2%)	2 (4%)	1 (1%)
Never smoked	83 (54%)	31 (54%)	52 (53%)
Office blood pressure			
Systolic BP (mmHg)	182 (± 30)	198 (± 26)	175 (± 29)
Diastolic BP (mmHg)	100 (± 15)	108 (± 13)	96 (± 14)
Heart rate (bpm)	75 (± 14)	76 (± 12)	74 (± 15)
Antihypertensive medication			
Number of antihypertensives	3 (0-8)	4 (0-8)	3 (0-8)
Patients on ≥ 3 antihypertensives	126 (70%)	45 (75%)	73 (67%)
Current use of an aldosteron-receptor blocker	43 (24%)	19 (31%)	24 (20%)

**Calculated on the basis of Modification of Diet in Renal Disease Study criteria. Continuous variables are displayed as a mean (SD), except the number of antihypertensives, which is displayed as median (range). Categorical variables are displayed as a number (percentage). TIA: Transient Ischaemic Attack, CAD: Coronary Artery Distease, PAD: Peripheral Arterial Disease.*

Of all referred patients, 121 (67%) were excluded from treatment with RDN. They were slightly older and had a lower office BP compared to the patients judged eligible for RDN. In addition, co-morbidity was more prevalent in the group considered not eligible. In some patients, reason for exclusion was multi-causal. However, only the primary reasons are shown in table 2. For example, a patient with a WCE and co-morbidity is formally excluded because of WCE. Out of the 121, 23 patients (19%) were excluded because of an office based SBP <160 mmHg. Twenty six patients (22%) were excluded based on an ABPM during antihypertensive treatment <140 mm Hg or ABPM <150 during the medication free interval. This last category of patients did have a clear WCE. Part of the patients appeared to be normotensive by ABPM; the majority of the excluded patients had white coat resistant hypertension (ambulatory SBP

130-140 mmHg). The patients that did not meet the BP criteria were excluded in this early phase from the remaining work-up.

Hundred patients (55%) continued with the full stepwise protocol after the first screening phase. After completing the program in these patients, fourteen cases (12%) were diagnosed with secondary hypertension, the majority being primary aldosteronism (11 patients, 9%). Antihypertensive treatment was successfully adjusted in 15 patients (i.e. BP <160 mmHg). Therefore, these patients were excluded from further screening. If a patient was not using a diuretic, this was added, leading to an improved regulation in 7 patients. In 2 patients a fixed combination drug was prescribed with good result. Other adjustments were addition of an alpha-blocker, renin inhibitor, or increase of prescribed dosage. Ten patients were excluded from treatment with RDN because of severe co-morbidity. For example presence of a malignancy, vascular dementia, or severe heart failure were reasons to discontinue the screening.

In some cases (n=8) the multidisciplinary team decided to exclude patients, since they had proven either not to be compliant to prescribed medication or repeatedly did not show up for their visits to the outpatient clinic. Because the current program involves an extensive work-up and follow-up, full expected compliance of patients is required. Finally, although referred, not all patients (n=12) or referring doctors (n=1) in the end supported treatment with RDN.

In total 60 patients did meet the inclusion criteria for treatment with RDN and all were treated. 20 (33%) of them had multiple renal arteries: 17 patients (28%) had multiple arteries at one side, 3 patients (5%) had dual arteries at both sides.

Table 2: Reasons for excluding patients (n=121) from treatment with RDN

Blood Pressure:	**49 (41%)**
Office SBP <160 mmHg	23 (19%)
Mean 24-h ambulatory SBP <150 mmHg without antihypertensive treatment or SBP <140 mmHg during antihypertensive treatment	26 (22%)
Secondary cause of hypertension	**14 (12%)**
Primary aldosteronism	11 (9%)
Primary hyperparathyroidism	1 (1%)
Pseudo-hyperaldosteronism	1 (1%)
Coarctatio aortae	1 (1%)
Co-morbidity	**10 (8%)**
Renal artery anatomy is ineligible for treatment with RDN	**9 (7%)**
History of renal artery stenting	3 (3%)
Renal artery stenosis	6 (4%)
Options for pharmaceutical treatment of hypertension	**15 (12%)**
Other reasons	**24 (20%)**
Patient did not want to be treated with RSD	12 (10%)
Referring physician did not want his patient to be treated	1 (1%)
Patient is expected not to be compliant	8 (7%)
Severe claustrophobia	1 (1%)
Adequate regulation of BP after lifestyle adjustments	2 (1%)

Results are displayed as number (percentage)

Discussion

Out of all 181 patients referred to the UMC Utrecht, only 33% was eligible to undergo RDN. The main reasons for exclusion were an office SBP <160 mmHg, pseudoresistant hypertension due to a WCE and a secondary cause of hypertension.

The developed screening program has three aims. First aim of the program is to confirm the diagnosis of hypertension. More specifically, the office SBP has to be ≥160 mmHg under at least three antihypertensives (or confirmed intolerance to medication) to be treated with RDN. In addition a 24-hour ABPM was

performed, since one-third of patients with suspected resistant hypertension has in fact a WCE.[20] An ABPM offers a large number of BP measurements during both day- and nighttime. This results in a more precise assessment of BP than can be obtained from single measurements.[21] ABPM is also recommended in the work-up before RDN in the ESH position paper on RDN.[9] This simple, inexpensive test excludes a considerable number of patients from further, more expensive, screening. This may increase the cost-effectiveness of the screening process.

The second aim of the screening is to exclude secondary forms of hypertension. In particular among patients suspected of resistant hypertension, secondary forms have shown to be more prevalent.[16-18] Various forms of secondary hypertension are unlikely to respond to RDN. For example, hypertension due to primary aldosteronism is volume dependent and is characterized by a decreased sympathetic activity.[22] Based on the working mechanism of RDN, it is unlikely that these patients will respond to this treatment. The current screening is a stepwise work-up that allows exclusion of a patient from further screening in an early phase. This resulted in a fully completed screening in only 93 patients. In these 93 patients, 10 patients were diagnosed with a secondary form of hypertension. Thirty-four per cent of this preselected population was diagnosed with resistant hypertension. This is in line with previous studies.[11-15]

The majority of patients with an identified secondary cause had been diagnosed with primary aldosteronism (77% of all secondary causes). It is remarkable that all referred patients had an extensive history of hypertension and the majority had already been screened in some way for secondary causes before referral. The guidelines of the Endocrine Society recommend screening for primary aldosteronism in particular in all patients with resistant hypertension.[23] The aldosterone-renin-ratio is currently the most reliable manner to screen for primary aldosteronism.[24-26] Washout of all interfering medication is preferred and patients should have an unrestricted dietary salt intake before testing.[23] Temporary treatment with antihypertensives, for example diltiazem or doxazosin, with neutral effects on plasma renin and aldosterone levels can be used in severe hypertension. The standardized scheme of treatment tapering is given in the online supplement 2.

The prevalence of pheochromocytoma is about 0.2 per cent of patients with hypertension.[27, 28] In the screened patient cohort, pheochromocytoma was not diagnosed. Four false-positive cases with elevated metanephrines levels in 24-h urine were obtained during the screening. However, all patients had normal levels at repeated investigation. Since the majority of the patients diagnosed with a secondary cause of hypertension were diagnosed with primary aldosteronism in our cohort, it is open for debate whether patients should only be screened for this secondary cause of hypertension. More extensive screening for rare causes, i.e., pheochromocytoma, might be performed only in those patients suspected for such specific disease.

According to the position paper on RDN it is recommended to obtain renal artery imaging to assess renal artery anatomy before treatment with RDN.[9] As stated, this is the third aim of the screening program. Three of 181 referred patients did have a history of renal artery stenting and were therefore not suitable for treatment and were excluded based on the referral letter. Four patients were not eligible due to a significant renal artery stenosis, as shown by magnetic resonance angiography (MRa).

Multiple non-invasive techniques are available to obtain imaging of the renal arteries. In the current work-up, MRa was chosen because of excellent vascular imaging without radiation exposure. In addition, MRa uses a gadolinium-like contrast agent. This technique can be applied safely in patients with kidney failure (eGFR>30 ml/min per 1.73m^2).[29] When MRa was contraindicated, computed tomography angiography (CTa) was performed. CTa has both radiation and contrast agent exposure but is also an accurate non-invasive imaging technique.[30] Doppler Duplex ultrasonography is another alternative that provides functional, as well as some anatomical, information. This technique is relatively inexpensive but it is time consuming and operator dependent, especially in obese patients.[10]

We are among the first to provide an overview of screening results of patients referred for RDN in clinical practice. Azizi et al. performed a retrospective analysis.[31] They applied the in- and exclusion criteria of

the ESH position paper[9] to a cohort of patients referred to their tertiary care hypertension department and concluded that only 1.5% of this population would be fully eligible for treatment with RDN.[31] This is clearly in contrast with our results, but is explained by the different patient population. Our population comprised of patients specifically referred for treatment with RDN. The discrepancy between prevalence of secondary causes for hypertension in our population compared to the population of Azzizi et al. may be explained by our selected population. [31] Most of the patients were previously screened by their referring physician. Although exclusion rates in the population of Azzizi may differ, the overall conclusion is comparable; a substantial number of patients are excluded from treatment. Furthermore the number of excluded patients in the Symplicity HTN-2 trial is comparable with our current data, although, we did not exclude patients with a dual renal artery system.[7] Surprisingly, in the HTN-2 trial no patients were excluded due to a secondary from of hypertension.[7] Other papers with recommendations for proper patient selection were published.[9, 32, 33],

One of the aims of this article is to give some recommendations for screening and selection of patients candidate for RDN. Multiple additional papers with recommendations for proper patient selection were published.[9, 32-34] However, these papers only give general recommendations and are not based on actual patient data. In contrast to the position paper, a slightly different patient selection and modified exclusion criteria were applied by us. The position paper states that RDN is currently only indicated for patients with resistant essential hypertension.[9] However we decided to treat some patients (n=10) with documented intolerance for antihypertensive drugs. Most of these patients experienced serious side effects like angioedema from ACE-inhibitors, gout from diuretics, or asthma from beta-blockers. These patients often pose dilemmas to the treating physician and especially for these patients RDN can be of potential benefit. This approach is partly supported by the German Consensus Document, arguing patients intolerant for the combination of 3 antihypertensive drugs are also eligible for treatment.[33] The Swiss Consensus Document is more conservative and states that patients should at least use 4 different antihypertensive drugs and that both a diuretic and a mineralocorticoid receptor antagonist have been tried.[32] The French Consensus Document gives a more general approach to patients suspected of resistant hypertension without discussing specific details on in- exclusion criteria for RDN. Authors advice addition of aldosterone antagonist and other pharmacological groups, or to prescribe a fixed combination agent.[34]

With respect to renal function, a cut-off value of eGFR<30 ml/min per 1.73m^2 rather than eGFR<45 ml/min per 1.73m^2 was applied (as also proposed in the position paper and the German consensus document). Patients with renal failure have an increased sympathetic activity compared with hypertensive patients without renal failure.[35, 36] Therefore, RDN could be especially beneficial for these patients.

Patients with multiple main renal arteries were not excluded. This is a pragmatic approach, since multiple renal arteries are not exceptional among treated patients (33%). In most cases, accessory branches are ≥4 mm in diameter. In general this is considered as a minimum diameter for safety issues (i.e. to prevent potential occlusive spasms). Therefore it is considered safe to include these patients and treat all vessels of sufficient size.

Poor adherence to antihypertensive drugs is a major cause of uncontrollable hypertension37, but is essentially different from true resistant hypertension. In order to state that an antihypertensive drug regimen has failed, it is a prerequisite that the antihypertensive medication has been taken correctly. This difference is relevant, since non-compliant patients should not be subject to an extensive evaluation.10 Determination of ACE in serum can be helpful in patients using RAS inhibition. In the current cohort, 8 patients were excluded since they had proven either non-compliance with prescribed medication, or they did repeatedly not show up for their visits to the outpatient clinic.

Conclusions

This is the first report reviewing the results of a clinical screening program of patients referred for RDN. In this cohort of patients suspected for resistant hypertension, a relevant number of patients appeared not to have resistant hypertension. The number of secondary causes of hypertension and the presence of significant white coat effect was surprisingly high. Treatment of these excluded patients would lead to inappropriate use of RDN, a burden for health care, and a less beneficial effect of RDN. To prevent inappropriate use of RDN, we recommend screening of all patients with the use of a standardized screening before treatment with RDN, even when previous screening was applied in the past. The first step should be an ABPM. Preferably all patients are evaluated in a multi-disciplinary setting.

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Appendix A: Online supplement 1: stepwise program

	Online supplement 1: stepwise program	
1	24-hour BP measurement (ABPM)	An initial 24-h ambulatory BP measurement (ABPM) is performed, unless a recent (<1 year) ABPM is available. This ABPM is used to exclude a WCE. Patients with a mean 24-h ambulatory SBP <140 mmHg are excluded for further work-up in the protocol and treatment with pRDN.
	Laboratory testing	When no recent measurements about kidney function are available, creatinine in plasma is determined for estimation of the eGFR.
2	Eppworth Sleepiness scale	The eppworth sleepiness scale and medical history give information about sleep apnea. When sleep apnea is suspected, the patient is referred to the pulmonologist for further diagnosis.
2	Tapering of medication	Antihypertensives are gradually tapered and temporarily stopped, when this is considered to be safe. This medication free interval is incorporated in the protocol, since most antihypertensive drugs interfere with tests of the protocol (e.g. aldosterone-renin-ratio and metanephrines). During the medication free interval, patients are contacted by telephone regularly by trained nurses or a physician. Also, patients are informed to contact when they develop symptoms. If necessary, escape medication, using alpha-blocker (doxazosin) or calcium antagonist (dialtiazem), is prescribed. These drugs do not interfere with the laboratory investigations.(Funder et al 2008)
3	Measurement of potassium level	One week before the investigations potassium level is measured. In case of hypokalaemia, supplementation is prescribed to prevent hypokalaemia during the measurement of the aldosterone-renin-ratio. Three days before the investigations a constant intake of salt is advised.
4	ABPM	A second 24-h ABPM is performed under untreated conditions. Patients with a mean 24 hour ambulatory SBP <150 mmHg, without the use of antihypertensive drugs, are excluded from treatment with pRDN. This additional ABPM is assumed to give more insight in compliance of the patients and can be used to monitor the effect of pRDN.
5	24-h urine collection	24-h urine is collected for determination of sodium, potassium, creatinin, protein, albumin, metanephrines, catecholamines and cortisol.
6	Plasma	Blood samples are obtained after overnight fasting for determination ofcreatinin, sodium, potassium, calcium, thyroid stimulating hormone, lipid profile, , glucose, insulin, and haemoglobinare collected for a cardiovascular risk determination.
7	Aldosterone-renin-ratio	Aldosterone and plasma renin concentration are measured in the morning in sitting position (15 minutes) after patientshave been out of bed for at least 2 hours.
8	Saliva	A sample of saliva is collected at 11.00 p.m. to determine cortisol level.
9	Imaging	The final investigation in the protocol is to evaluate whether renal anatomy is eligible for pRDN. Preferably, a MRaof the renal arteries is used. When MRa is contra-indicated, a CTa is performed.
10	Multidisciplinary meeting	All results from the standardized screening are discussed in the multidisciplinary meeting. Additional tests, for example to exclude secondary causes of hypertension, are performed whenever this is necessary. The final decision whether pRDN is indicated is unanimously made by the multidisciplinary team. This team comprises a vascular internist, a nephrologist, a cardiologist and a radiologist.

Appendix B: Online supplement 2: Scheme of tapering medication

Online supplement 2: Scheme of tapering medication		
4 weeks before laboratory testing	Stop: diuretica (including aldactone) and aliskiren	
	Gradually reduced: Bèta blockers and central working antihypertensive drugs are reduced in two weeks:	
	Day 1: 100%	Day 8: 0%
	Day 2: 50%	Day 9: 25%
	Day 3: 50%	Day 10: 0%
	Day 4: 50%	Day 11: 25%
	Day 5: 50%	Day 12: 0%
	Day 6: 0%	Day 13: 25%
	Day 7: 50%	Day 14: 0%
2 weeks before laboratory testing	Stop: ACE-inhibitors, AT1-antagonists, Calcium-antagonists, Alpha-blockers, direct vasodilatators, NSAIDs	

The blood pressure lowering effect of renal denervation is inversely related to kidney function

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Abstract

Objectives

In renal denervation (RDN) a wide range in the blood pressure (BP)-lowering effect has been reported. Based on the current knowledge of pathophysiology, we hypothesised that the BP-lowering effect of RDN would be inversely related to kidney function. Secondly, we investigated whether direct and indirect variables of the renin angiotensin aldosterone system (RAAS) and the sympathetic nervous system (SNS) would be related as well.

Methods

Sixty-seven patients from a prospective cohort of patients treated with RDN with completed 6 months follow-up were included. Data collected during routine standardized work-up before RDN were used: 24-h urine excretion of creatinine, albumin, sodium and catecholamines, plasma creatinine, renin activity and aldosterone, ambulatory BP-monitoring and a captopril challenge test. When considered safe, anti-hypertensive drugs were stopped before these investigations.

Results

The BP-lowering was inversely related to eGFR in patients who stopped antihypertensive drugs prior to testing (ß:0.46; P=0.013). There was a positive relation between respectively SBP at baseline (ß:-0.55mmHg per mmHg; P<0.001). Parameters related to the renin angiotensin system (aldosterone, captopril test) and the sympathetic nervous system (dipping pattern and catecholamines in urine) positively related to the BP-lowering effect of RDN.

Conclusions

The present explorative study shows an inverse relation between the BP-lowering effect of RDN and eGFR. Secondly, we found relations between variables of the RAAS and SNS with the BP-lowering effect of RDN. The data complement current concepts on pathophysiology of sympathetic hyperactivity and hypertension and may give some insight in the wide range of the effect of RDN.

Introduction

The Symplicity HTN-1 and HTN-2 studies suggested that renal denervation (RDN) is a safe and effective treatment for resistant hypertension.[1-3] Office systolic blood pressure (SBP)/ diastolic blood pressure (DBP) reduced by 32/12mmHg, six months after RDN.[2] However, the wide standard deviation (SD) of the observed effect (23/11mmHg) and the percentage of about 16% non-responders (defined as a decrease of SBP <10 mmHg)[2], implies that some patients have little or no effect of RDN at all. Recently, Medtronic announced that the HTN-3 trials failed to meet its primary efficacy endpoint (change in office BP 6 months after RDN), while meeting the safety endpoint.[4] This indicates that RDN is not an effective treatment for all hypertensive patients. Therefore, it is of value to define factors that relate to the BP-lowering effect of RDN. Recently, only baseline SBP and the use of central sympatholytic agents were identified as significant independent factors determining the extent of decrease in SBP after RDN.[3, 5]

The purpose of RDN is to (partially) disrupt afferent and efferent renal nerves located in the adventitia of the renal arteries. Mechanistically, it seems likely that RDN will be especially effective when these nerves are particularly active i.e. involved in pathogenesis of hypertension. Earlier, we and several others have argued that this is especially the case in patients with kidney failure.[6-12] Based on this, we hypothesized in the present study that the BP-lowering effect of RDN would be inversely related to kidney function. Experimental evidence supports the idea that kidney ischemia rather than kidney failure per se, could increase afferent renal nerve activity.[13] Therefore, we addressed the question whether direct and indirect variables of the renin angiotensin aldosterone system (RAAS) and the sympathetic nervous system (SNS) would be related as well. A unique feature of the present study was that in part of our patient population we temporarily stopped antihypertensive medication to improve standardization of the study conditions of the pre-RDN assessments.

Methods

Study population

A highly standardized screening protocol was applied to patients referred to our centre for suspected resistant hypertension.[14] We consider this protocol standard patient care, because it is in line with the recommendations of the European Society of Hypertension and the European Society of Cardiology.[15, 16] Our standardized screening protocol is described in detail in a recent paper.[14] In brief, the presence of hypertension was confirmed using 24-h ambulatory BP monitoring (ABPM), secondary forms of hypertension were excluded and non-invasive imaging (by magnetic resonance angiography or computed tomography angiography) of the anatomy of the renal arteries was obtained.

Primarily patients with resistant hypertension (defined as a SBP ≥160 mmHg, despite use of ≥3 antihypertensive drugs) were considered eligible for RDN. In addition, patients fulfilling the same BP criteria, but without optimal pharmacological treatment due to intolerance for antihypertensive drugs were accepted. Major contraindications for RDN were: an estimated glomerular filtration rate (eGFR) <30 mL/min/1.73m^2, secondary causes of hypertension, and a history of renal artery stenting or severe co-morbidity.

Patients were followed as a prospective cohort. The present analysis was performed in patients from this cohort, with complete 6 months follow-up.

Temporal medication stop

As part of the standardized screening protocol, all patients were discussed in our multidisciplinary team. We considered whether it would be safe to stop antihypertensive medication for two weeks prior to the tests (supplemental data: table 1). This was done as part of our standardized clinical work-up for patients with complicated hypertension[14] to improve standardization of the test conditions. Temporal wash out of interfering drugs is advised by the European Society of Hypertension and the Endocrine Society in the diagnostic work-up for primary hyperaldosteronism.[17, 18] The decision to stop antihypertensive drugs was

based on clinical judgement with emphasis on (cardiovascular) medical history. During the medication free interval, patients were regularly contacted by telephone by trained nurses or a physician. Also, patients are informed to contact when they develop symptoms. If considered unsafe to stop antihypertensive medication, patients continued their medication or used 'escape-medication', consisting of doxazosin and/or diltiazem. These drugs do not influence eGFR, plasma aldosterone concentration (PAC), plasma renin activity (PRA) and urine catecholamine levels.[17, 19] After the testing period, patients restarted their medication.

Measurements

In all patients, we used data collected from the standardized work-up protocol for the present analysis. During a visit to the outpatient department, medical history was taken, detailed information on medication use was obtained and standard physical examination including anthropometrics was performed. BP was assessed in sitting position, at both arms, after 15 min of rest using a non-invasive automated device (Welch Allyn Inc. Skaneateles Falls, NY, USA). The mean of the last three values was calculated. Baseline BP (to quantify the BP-lowering effect of RDN) was taken in the same standardized way at the arm with the highest BP.

All patients visited the clinical research department. One week before the scheduled visit, plasma potassium concentration was controlled. In case of hypokalemia, supplementation was prescribed to prevent hypokalemia during measurements of PAC and PRA. Three days before the investigations, patients were asked to maintain a constant diet in order to avoid large fluctuations in sodium balance.

During the medication-free interval, an ABPM was done and 24-h urine was collected. ABPM was taken non-invasively using WatchBP O3 (Microlife Inc., Widnau, Switzerland), with readings taken every 30 min during day and every 60 min at night. Sodium (mmol/24-h), creatinine (mmol/24-h), albumin (mg/24-h), noradrenaline (nmol/24-h), vanillylmandelic acid (VMA, μmol/24-h), metanephrine (μmol/24-h) and normetanephrine (μmol/24-h) were analysed in 24-h urine using standard laboratory procedures (supplemental data).

At the clinical research department blood samples were obtained from all patients for routine lab including creatinine (μmol/L). In patients who temporarily stopped medication or used 'escape-medication', the protocol was extended with tests of the RAAS. Blood samples were drawn to measure PAC (pmol/L) and PRA (fmol/L/s) after 90 min of standing and after 90 min in supine position. Subsequently, a captopril challenge test (CCT)[20] was performed while the patient was in supine position. After baseline BP assessments using a non-invasive BP measuring device, 25 mg of captopril was taken orally, where after BP was measured at 30, 60, 90 and 120 min.

Follow-up measurements

Six months after RDN, patients visited the clinical research unit again. Detailed information on drug use was collected. For quantification of the effect of RDN, BP was assessed on the same arm as at baseline, in sitting position after 15 min of rest using a non-invasive automated device. The mean of the last three values was calculated.

Percutaneous renal denervation

RDN was performed using the Symplicity Catheter System, a 6Fr compatible, single-use RF probe. Before introduction of the RF–probe, renal angiograms were performed to confirm anatomic eligibility. Subsequently, the system was introduced in the renal arteries and the catheter electrode was positioned in contact with the vessel wall at desired locations and the catheter was connected to an automated RF-generator. Multiple (≥4) applications of RF energy in a spiral pattern along the renal arteries with 5 mm interspace were performed. After the procedure, the puncture site was closed with a closure device and

the groin was compressed for 4 to 6 h. Patients took 100mg of acetylsalicylic-acid 5 days before- and 4 weeks after the procedure.

Data analysis

The differences between means of BP at baseline and at 6 months were calculated and used for the present analysis. A negative value represents a decrease in SBP 6 months after RDN. Percentage dipping of SBP during night-time was calculated as [(mean daytime SBP –mean night-time SBP)/mean daytime SBP] * 100%.[21] Subjects were subdivided into 2 groups: dippers (percentage ≥10%), non-dippers (<10%).[22]

Estimated glomerular filtration rate (eGFR) is calculated on the basis of the CKD-epi formula.[23] The change in SBP after administration of captopril was calculated as average of the measurements taken at 60 and 90 min after intake. Albumin-creatinine ratio in urine was calculated.

Prescribed dosages of antihypertensive drugs were converted to defined daily doses (DDD) using conversion factors as provided by the World Health Organization (WHO) Drug Classification (http://www.whocc.no/atcddd/). Using DDD's and the total prescribed dosages, daily use (DU) of all antihypertensive drugs was calculated.

Statistical analysis

Because not all tests were done in all patients, the actual number of tests is mentioned in the tables for all variables. Since antihypertensive drugs interfere with some laboratory parameters and influence ABPM, results were analysed for the whole patient cohort and for the subgroup of patients who stopped antihypertensive drugs or used 'escape-medication'. All variables were reported as mean ± SD, median (min-max), or as proportion when appropriate. Wilcoxon signed rank test was used for paired sample analysis.

The current study was an explorative but hypothesis based analysis, investigating the relation between predefined variables and the BP-lowering effect of RDN. The relation between baseline characteristics (independent variables) and the change in SBP 6 months after RDN (dependent variable) was analysed using linear regression models. Multivariable linear regression models were used to adjust for eGFR (table 3 and 4), to examine whether a relation was caused by kidney function per se. When considered appropriate, adjustments were also done for baseline SBP, age and gender (table 2). Multivariable analysis was restricted by the limited sample size. Interpretation of results focused on comparison of regression coefficients (ß's) of the univariable- and multivariable analysis, to check for abolishment of the effect after adjustment for eGFR. For multivariable analysis the rule of thumb of 10 cases per variable was applied, to avoid an over fitted model. A two sided *P* value of <0.05 was considered to be statistically significant. All analyses were performed with the SPSS statistical package version 20 (IBM SPSS Data Collection, Chicago, IL).

Results

Baseline characteristics

Sixty-seven patients were included in this study. Baseline characteristics are shown in table 1. Forty-seven (70%) patients temporarily stopped antihypertensive treatment or used 'escape-medication' prior to the screening-tests.

Table 1: Baseline characteristics

	All patients n=67	Patients who temporarily stopped antihypertensive drugs n=47	Patients who did not temporarily stop antihypertensive drugs n=20
Age (yrs)	59(±10)	58 (±10)	63 (±10)
Sex (male/female)	33/34	25/22	8/12
Reason for treatment with RDN			
Resistant hypertension	55 (82%)	39 (83%)	16 (80%)
Intolerance	12 (18%)	8 (17%)	4 (20%)
Comorbidity			
Hypercholesterolemia	43 (64%)	32 (68%)	10 (46%)
Diabetes Mellitus Type II	12 (18%)	11 (23%)	1 (5%)
Cardiovascular diseases	20 (30%)	12 (26%)	8(40%)
Antihypertensive medication			
Number of antihypertensive drugs	4 (0-8)	4 (0-8)	4 (1-7)
Daily use of antihypertensive drugs	5.7 (±3.3)	5.2 (±3.2)40 (85%)	6.2 (±3.8)
ACEi/ARB/Renin inhibitor	58 (87%)	27 (57%)	18 (90%)
ß-Blocker	44 (66%)	32 (68%)	17 (85%)
Calcium-channel blocker	46 (69%)	7 (15%)	14 (70%)
α-Blocker	10 (15%)	34 (72%)	3 (15%)
Diuretic	50 (75%)	2 (4%)	16 (80%)
Central Acting	4 (6%)		2 (10%)
Office SBP (mm Hg) under medication	195 (±27)	193 (±29)	202 (±21)
Office DBP (mm Hg) under medication	106 (±14)	105 (±15)	108 (±13)
PP (mm Hg) under medication	89 (±23)	87 (±25)	94 (±18)
Mean day-time* SBP (mm Hg) ‡	169 (±17)	174 (±15)	157 (±15)
Mean day-time* DBP (mm Hg) ‡	100 (±12)	103 (±11)	94 (±14)
Mean day-time HR† (bpm) ‡	77 (±13)	78 (±14)	76 (±11)
Body-mass index (kg/m^2)	29.1 (±5.5)	29.4 (±5,8)	28,3 (±4.8)
eGFR‡ (mL/min/1.73m^2) ‡	74 (±18)	74 (±18)	75 (±17)
Log-Albumin-creatinine ratio (mg/mmol) ‡	2.6 (0.19-119.6)	2.6 (0.19-119.6)	3.1(0.5-30.7)
Sodium (mmol/24-h)‡	158.3(65.4)	147.2 (61.5)	200.7 (65.0)

Continuous variables are displayed as a mean (SD), or as median (min-max) when applicable. Categorical variables are displayed as a number (percentage). Yrs indicates: years, ACEi: angiotensin-converting enzyme inhibitor, ARB: angiotensin II receptor blocker, SBP: systolic blood pressure, DBP: diastolic blood pressure, PP: pulse pressure, HR: heart rate, bpm: beats per minute, eGFR: estimated glomerular filtration rate.
**Mean SBP, determined using ABPM.*
† Mean HR during daytime, determined using ABPM .
‡ Determined during the medication free interval when possible.

Renal denervation

Eligible anatomy was confirmed by angiogram, before delivery of RF-energy to the treatment site. On average, 12.0 (±2.1) ablations were applied per patient. Three patients had a complication: two patients had a minor bleeding at puncture site that was treated with compression and one patient was admitted 2 weeks after RDN because of hypotension, requiring fluid administration and cessation of antihypertensive drugs. No complications with long term consequences or adverse events related to the procedure occurred during 6 months of follow-up.

Effect on blood pressure and kidney function

Office SBP/DBP decreased significantly with -30(±27)/ -11(±13) mmHg (P<0.001 and P<0.001, respectively). The daily use of antihypertensive drugs decreased significantly from 5.7 (±3.3) at baseline to 4.7 (±3.4) 6 months after treatment (P=0.008). Over a period of 6 months, kidney function remained stable (eGFR: 73 (± 19) mL/min/1.73m^2 at 6 months (P=0.90)).

Table 2: Multivariable regression analysis of baseline characteristics and the change in SBP after RDN: adjustment for eGFR, baseline SBP, gender and age

	N	β	95%CI	p
All patients				
Gender	67	0.93	-12.55-14.40	0.89
Cardiovascular diseases	67	-18.95	-32.23- -5.67	0.006
Office SBP (mmHg)*	67	-0.55	-0.82- -0.29	<0.001
Office DBP (mmHg)	67	-0.67	-1.17- - 0.17	0.009
Presence of a non-dipping profile	61	-13.09	-26.18- -0.0	0.05
Only patients who temporarily stopped antihypertensive drugs				
ABPM: Mean night-time SBP (mmHg)‡	45	-0.52	-0.83- -0.20	0.002
Presence of a non-dipping profile‡	45	-17.29	-28.96- -5.61	0.005
eGFR† (mL/min/1.73m^2) ‡	47	0.46	0.10-0.781	0.013
Log-Albumin (mg/24-h) ‡	43	-12.85	-23.75- -1.95	0.02
Log-Albumin-creatinine ratio (mg/mmol) ‡	43	-13.38	-24.22- -2.54	0.02

ß: regression coefficient, 95%CI: 95% confidence interval, N: number of patients, SBP: systolic blood pressure, DBP: diastolic blood pressure, eGFR: estimated glomerular filtration rate.
**Only adjusted for gender age and eGFR. ‡Only adjusted for baseline SBP, gender and age*

Table 3: Univariable and multivariable analyses of baseline characteristics and the change in SBP after RDN in all patients

		Univariable model			Multivariable model*		
	N	β	95%CI	p	β	95%CI	p
General characteristics							
Age (yrs)	67	-0.29	-0.94-0.37	0.39			
Gender (female)	67	-12.1	-25.12-0.93	0.07			
Nr of antihypertensive drugs	67	0.18	-3.49-3.85	0.92			
Total DU antihypertensive drugs†	67	0.30	-1.73-2.32	0.77			
Medical history							
Hypercholesterolemia	67	0.81	-13.12-14.75	0.91			
Diabetes Mellitus Type II	67	8.17	-8.60-24.95	0.33			
Cardiovascular diseases	67	-20.25	-33.96- -6.54	0.004			
Physical examination							
Body mass index (kg/m^2)	67	0.28	-0.96-1.53	0.65			
Office SBP (mmHg)	67	-0.53	-0.74- -0.32	<0.001	-0.53	-0.75- -0.31	<0.001
Office DBP (mmHg)	67	-0.66	-1.10- -0.21	0.005	-0.67	-1.13- -0.22	0.004
PP (mmHg)	67	-0.50	-0.77- -0.23	<0.001			
ABPM							
Mean day-time SBP (mmHg)	62	0.07	-0.35-0.50	0.73			
Mean night-time SBP (mmHg)	61	-0.13	-0.51-0.25	0.51			
Mean day-time PP, (mmHg)	62	0.01	-0.50-0.52	0.97			
Mean HR during day-time (bpm)	62	-0.05	-0.58-0.49	0.87			
Presence of a non-dipping profile	61	-13.97	-28.09- 0.15	0.05	-15.01	-29.30- -0.72	0.04
Laboratory parameters: Blood							
eGFR‡ (mL/min/1.73m^2)	67	0.06	-0.33-0.44	0.77			
Laboratory parameters: Urine (24-h)							
Log-Albumin (mg/mmol)	56	-7.75	-19.92-4.42	0.21			
Log-Albumin-creatinine ratio (mg/mmol)	56	-9.27	-21.29-2.76	0.13			
Sodium (mmol/mmol)	58	-0.09	-0.20-0.02	0.10			
Noradrenaline (nmol/24-h)	46	-0.05	-0.10- 0.00	0.05	-0.05	-0.10 - -0.001	0.06
VMA (μmol/24-h)	44	-0.77	-1.71-0.17	0.11	-0.74	-1.71-0.124	0.14
Metanephrine (μmol/24-h)	56	-11.70	-25.96-2.56	0.11	-12.25	-27.12- 2.62	0.10
Normetanephrines (μmol/24-h)	56	-4.56	-10.70-1.57	0.14	-4.23	-10.56-2.10	0.19

ß indicates regression coefficient, 95%CI: 95% confidence interval, N: number of patients, yrs: years, DU; daily use, Nr: number, SBP: systolic blood pressure, DBP: diastolic blood pressure; PP: pulse pressure, ABPM: ambulatory blood pressure monitoring, HR: heart rate, bpm: beats per minute, eGFR: estimated glomerular filtration rate, VMA: vanillylmandelic acid.
** Adjustment for eGFR.*
† Calculated with the use of defined daily dosages.

General determinants of the BP-lowering effect:
The presence of cardiovascular diseases (CVD; composite of transient ischemic attacks, strokes and coronary artery disease), was related to a greater BP-lowering effect of RDN (ß: -18.95, P=0.006, table 2) after adjustment for baseline eGFR, baseline SBP, gender and age.
Baseline SBP and DBP were related to the BP-lowering effect of RDN in a multivariable model (adjustment for eGFR, table 3) and in a second multivariable model (adjustment for gender age and eGFR, table 2) including all patients.
The change in SBP was not related to change in daily use of antihypertensive drugs (P=0.96).

Hypothesis 1: The effect of RDN is related to kidney function.
In patients who stopped antihypertensive drugs prior to the tests, the decrease in SBP after RDN was inversely related to eGFR at baseline in multivariable models (adjustment for baseline SBP, gender and age, β:0.46 mmHg per mL/min/1.73m^2, P=0.013, table 2 and adjustment for CVD: β:0.34 mL/min/1.73m^2, P=0.056). Addition of an interaction-term to the model showed that the relation between baseline eGFR and change in SBP after RDN was different in patients who temporarily stopped antihypertensive drugs prior to the test (ß interaction term:1.41) compared to patients who did not stop antihypertensive drugs (P<0.001).
In patients in whom urine was collected during a medication-free interval, the BP-lowering effect of RDN was related both to 24-h urinary albumin excretion (ß:-12.85, P=0.02, table 4) as well as albumin-creatinine ratio (ß:-13.38, P=0.02, table 4).

Hypothesis 2: Parameters of the RAAS and SNS are related to the effect of RDN.
2.1: Direct and indirect variables of the RAAS are related to the effect of RDN.
A univariable model suggested a direct relation between PAC in standing position and the decrease in SBP (β: -0.02mmHg per pmol/L, P=0.12, table 3, supplemental fig 1B) and an inverse relation to eGFR (ß: -9.48, P<0.001). After adjustment for eGFR in a multivariable model, the suggested relation between PAC and the BP-lowering effect of RDN abolished. (β:-0.006 mmHg per pmol/L, P=0.63, table 4). PRA levels were not related to the BP-lowering effect of RDN. (Table 3) A univariable model suggested a relation between the BP-lowering effect of captopril and change in SBP after RDN (β:0.61 mmHg per mmHg, P=0.05, table 4, fig 1D). This relation partly disappeared after correction for eGFR (table 3).

2.2: Direct and indirect variables of the SNS are related to the effect of RDN.
In patients who temporarily stopped antihypertensive drugs before ABPM, the BP-lowering effect was related to mean night-time SBP in a multivariable model adjusting for eGFR (β:-0.50, P=0.005, table 4, Fig 1A) and in a second multivariable model adjusting for baseline office SBP gender and age (β:-0.52, P=0.002, table 2). Patients classified as non-dipper had a significant greater decrease in SBP after RDN (table 2,3,4). Among patients who temporarily stopped antihypertensive drugs, SBP decreased by 28 (±21) mmHg in non-dippers (n=29) and by 10 (±18) mmHg in dippers (n=16) (table 4: P=0.001 adjustment for eGFR, table: 2: P=0.005 adjustment for baseline SBP, gender and age).
Univariable models suggested a relation between the BP-lowering effect of RDN and 24h-urinary output of noradrenaline, VMA and metanephrine (respectively β:-0.05mmHg per nmol/24-h, P=0.05 and β:-0.77mmHg per µmol/24-h, P=0.11, and ß:-11.70 mmHg per µmol/24-h, P=0.11, table 3, supplemental fig 1C and 1D). After adjustment for eGFR, these relations were comparable (table 3). Since antihypertensive drugs may affect catecholamine excretion in urine, this relation was also tested in patients who temporarily stopped antihypertensive drugs. The correlation coefficients (ß's) were similar in these patients; however significance level decreased (Table 4).

Figure 1: Determinants of effect plotted against changes in SBP, 6 months after treatment in patients who temporarily stopped antihypertensive treatment

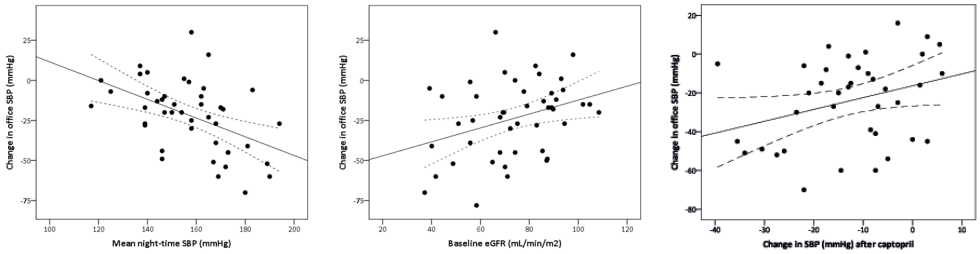

Figure 1A: Mean night-time SBP (mmHg) at baseline, vs. change in SBP.*
Figure 1B: Baseline eGFR (mL/min/1.73 m^2*), vs. change in SBP.*
Figure 1C: Change in SBP (mmHg) after captopril (CCT at baseline), vs. change in SBP.
All Y-axes show the change in SBP in mmHg.
SBP indicates systolic blood pressure, eGFR: estimated glomerular filtration rate, CCT: captopril challenge test.
** Calculated from ABPM.*

Table 4: Univariable and multivariable analyses of baseline characteristics and the change in SBP after RDN in patients who temporarily stopped antihypertensive treatment

		Univariable model			Multivariable model*		
	N	β	95%CI	p	β	95%CI	p
ABPM							
Mean day-time SBP (mmHg)	46	-0.31	-0.74-0.13	0.16			
Mean night-time SBP (mmHg)	45	-0.54	-0.87- -0.21	0.002	-0.50	-0.84- -0.16	0.005
Mean day-time PP, (mmHg)	46	-0.23	-0.68-0.23	0.32			
Mean HR during day-time (bpm)	43	0.05	-0.42-0.53	0.82			
Presence of a non-dipping profile	45	-18.57	-31.22- -5.92	0.005	-21.16	-32.75- -9.58	0.001
Laboratory parameters: Blood							
eGFR (mL/min/1.73m^2) †	47	0.43	0.07-0.80	0.02			
PAC – standing (pmol/L)	43	-0.02	0.04--0.01	0.12	-0.006	-0.03-0.02	0.63
PAC – supine (pmol/L)	41	-0.04	-0.11-0.03	0.28			
Log-PRA – standing (fmol/L/s)	43	-0.75	-18.85-17.35	0.93			
Log-PRA – supine (fmol/L/s)	41	-3.89	-23.60-15.81	0.69			
Laboratory parameters: Urine (24-h)							
Log-Albumin (mg/24-h)	43	-12.24	-23.03- -1.44	0.03			
Log-Albumin-creatinine ratio (mg/mmol)	43	-11.76	-21.75- -1.77	0.02			
Noradrenaline (nmol/24-h)	37	-0.05	-0.11- 0.01	0.13	-0.04	-0.09-0.02	0.24
VMA (μmol/24-h)	34	-0.57	-1.50-0.36	0.22			
Metanephrine (μmol/24-h)	46	-9.03	-23.63-65.58	0.22			
Normetanephrines (μmol/24-h)	46	-3.83	-10.59-2.92	0.26			
CCT effect							
Δ SBP (mmHg) after captopril	39	0.61	-0.005-1.21	0.05	0.57	-0.002-1.12	0.05

*ß indicates regression coefficient, 95%CI: 95% confidence interval, N: number of patients, SBP: systolic blood pressure, PP: pulse pressure, ABPM: ambulatory blood pressure monitoring, HR: heart rate, bpm: beats per minute, PRA: plasma renin activity, PAC: plasma aldosterone concentration, eGFR: estimated glomerular filtration rate, VMA: vanillylmandelic acid, CCT: captopril challenge test, Δ: change. *Adjustment for eGFR.*

Discussion

The main new finding of this study is that the BP-lowering effect of RDN inversely relates to eGFR. We found this relation in patients who temporarily stopped their antihypertensive medication. This relation seems consistent with present knowledge of pathophysiology. This suggests that patients with kidney failure are especially likely to show a reduction in BP after RDN. Secondly, we found relations between variables of the RAAS and SNS with the BP-lowering effect of RDN.

The current analysis confirms the BP-lowering effect of RDN in a clinical setting. The mean decrease in office BP after RDN was comparable to the Symplicity HTN-2 trial.[2] In Symplicity the SD of this effect was already wide. An even wider range of effect was observed in present study. We also confirmed the reported positive relation between baseline BP and the BP-lowering effect of RDN.[1]

The present study gives support to our first hypothesis. Patients with decreased kidney function are especially likely to show a BP-lowering effect. Importantly, eGFR is a variable easily available in daily clinical practice. Further, albuminuria, which is generally accepted as a sign of kidney injury, seems to be related to a greater BP-lowering effect of RDN.

The inverse relation between eGFR and the decrease in BP after RDN -even after adjustment for baseline SBP, gender and age- was only present in patients who temporarily stopped their medication. This may be explained by the effect of many antihypertensive agents on eGFR and may be the reason why this relation was not found in other studies.[1, 5] However the effect of patient selection on this relation, by selecting patients in whom it was considered safe to stop medication, cannot completely ruled out. Also the range of eGFR in the present study was slightly larger than in previous studies. Patients with an eGFR < 30 mL/min/1.73m^2 were excluded from treatment with RDN. However, based on present knowledge of pathophysiology which we outlined elsewhere[6-8], it seems very likely that the results are generalizable to patients with more severe kidney failure and give strong rationale to continue research in this line of thinking.

Secondly, our study suggests a relation between indirect and direct parameters of the RAAS (PAC and BP change after captopril) and SNS (urinary output of catecholamines and day-night BP pattern) and the BP-lowering effect of RDN. Several studies have shown that the SNS and the RAAS are especially active in the presence of ischemic kidneys. Although we did not directly measured kidney oxygenation in our study and therefore did not quantify tissue ischemia, our data support the hypothesis that variables suggestive for the presence of kidney ischemia might be related to the effect of RDN. In patients who stopped medication, a higher level of BP during night-time and a non-dipping pattern were related to a greater change in BP after RDN. The fact that we found this relation only in patients who temporarily stopped medication, probably explains why others were unable to find this. Grassi et al. showed that day-night BP difference is inversely related to sympathetic activity, expressed as muscle sympathetic nerve activity (MSNA).[22] Moreover MSNA is positively related to the nocturnal BP-level.[22]

There was a suggestion for a relation between PAC levels -an end product of the renin-angiotensin cascade- in standing position and the change in office SBP after RDN. PAC inversely related to eGFR, indicating that an activated RAAS was especially a feature of patients with kidney failure. Indeed, after adjustment for eGFR, the suggestion of a relation between PAC and the effect of RDN, disappeared. The absence of a significant relation between PAC in supine position and the effect of RDN can possibly be explained by the fact that in standing position, effective plasma volume is slightly decreased, activating the RAAS. The decrease in BP after a single tablet of captopril tended to be positively related to the effect of RDN. This gives further support to a relation between activation of the RAAS and the effect of RDN. ACE inhibitors especially work in presence of an activated RAAS. After adjustment for eGFR, the relation between the decrease in BP after captopril and the effect of RDN, partly disappeared, indicating that this relation can partly be explained by eGFR.

The exact origin of catecholamines in urine is uncertain. It is supposed to represent both filtration and local production.[24, 25] There was a suggestion of a positive relation between urinary excretion of catecholamine and the decrease in BP after RDN, which remained after adjustment for kidney function. This suggests that increased catecholamine excretion is (partly) independent of kidney failure per se. A possible explanation for this can be that sympathetic activity already increases in early stages of kidney impairment.[26-28]

The present study gives some suggestion to support the idea that activation of the RAAS and SNS are the mechanisms of the inverse relation between eGFR and the effect of RDN. Activation of the RAAS and SNS run in parallel.[13, 29] Kidney ischemia is the putative common mechanism stimulating both systems.[13] BP-elevation after intrarenal injection of phenol in a rat model, can be prevented by RDN[26], demonstrating a crucial role of intact renal nerves. Convincing evidence shows that these mechanisms are also operational in humans. MSNA increases with decreasing eGFR[29, 30]: MSNA is increased in patients with impaired kidney function and is normal in bilaterally nephrectomised patients.[31, 32] Also the suggestion of

a relationship with urinary sodium excretion could fit in that model. BP is especially sodium sensitive in presence of an activated RAAS and/or SNS.

The present data provide some insight in factors responsible for the wide range of the BP-lowering effect of RDN. Earlier, we argued that this could conceptually be explained by (at least) two mechanisms.[6] It could be due to a failure of the intervention itself, i.e. the renal nerves were not (sufficiently) interrupted by RDN. Secondly, it is also possible that there are individual differences in involvement of the kidneys and renal nerves in pathophysiology of hypertension. Indeed, the present study supports the latter. We feel that this should be further explored since it will help to identify patients most likely to benefit and hence improve cost effectiveness of RDN.

So far, there seems to be more or less consensus that RDN should be applied to patients with relatively well preserved kidney function.[15, 16] Earlier studies, both in relatively normal kidney function and with advanced kidney failure as well as present data did not show any detrimental effect of RDN on eGFR during short term follow up.[2, 3, 33] Experimental data in various models of kidney disease suggest that RDN may even retard disease progression.[34] However, special attention for safety of RDN should be paid: Templin et al. showed thrombus formation by optical coherence tomography (OCT) after RDN.[35]

To the best of our knowledge, this is the first study that identified these factors. Most of them only showed a relation with the BP effect in patients temporarily taken off medication. A limitation of current study is the relatively small number of patients. Since the present study was explorative of nature with predefined variables to be investigated, univariable analyses seem appropriate. Restricted by sample size, adjustments were done. Adjustment for eGFR was done to examine whether a relation was caused by kidney function per se. The current study should be interpreted as exploratory. The variables should be studied in larger trials with sufficient power for a robust multivariable analysis. Ultimately prediction rules have to be made to identify responders of RDN. The effect of RDN was determined using office BP which could be seen as a limitation. However, BP measurements were done in a standardized way using a non-invasive automated device. The present results need to be confirmed using ABPM.

In conclusion, to the best of our knowledge, the present explorative study is the first to show that the BP-lowering effect of RDN is inversely related to eGFR. Secondly, we found relations between variables of the RAAS and SNS with the BP-lowering effect of RDN. The data complement current concepts on pathophysiology of sympathetic hyperactivity and hypertension and may give some insight in the wide range of the effect of RDN.

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Renal denervation in multiple renal arteries

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Structured abstract

Background

In most previous studies investigating efficacy of renal denervation (RDN), patients with multiple renal arteries are generally excluded from treatment. This study was designed to determine the prevalence of multiple renal arteries in patients referred for RDN, to propose a classification for anatomical eligibility, and to investigate the relation between presence of multiple arteries and blood pressure (BP)-lowering effect.

Materials and methods

Patients referred for RDN who underwent non-invasive imaging of the renal arteries before treatment were included in present analysis. Eligible patients were treated. Renal function and BP were evaluated 6 months after treatment.

Results

Hundred-twenty-six patients referred for RDN were included in present analysis. Thirty-four per cent had multiple arteries. Sixty-nine patients underwent RDN. Office BP significantly reduced from 195(±26)/ 106(±14) mmHg to 165(±24)/ 95(±14) mmHg (P<0.001). BP-reduction in patients with multiple arteries which were all treated was comparable to patients with solitary arteries. However patients with multiple which were not all treated showed a trend towards a less pronounced effect of RDN (ß: 11.6, P=0.11). The proposed classification appeared useful by identifying eligible anatomy. Renal function at 6 months did not differ from baseline in all subgroups.

Conclusions

Based on our results and the high prevalence of multiple arteries; it seems reasonable not to exclude patients with multiple renal arteries from RDN. Current analysis suggests that BP reduction may be less pronounced in patients with multiple renal arteries of whom not all arteries were treated.

Introduction

A considerable subgroup of the patients diagnosed with hypertension has resistant hypertension[1], defined as a blood pressure (BP) above treatment goals despite concurrent use of at least three antihypertensive drugs, one ideally being a diuretic[2]. Increased activation of the sympathetic nervous system (SNS) has been identified as an important factor in the development of hypertension[3], especially in resistant hypertension. In this context, a catheter-based approach has been developed to disrupt renal sympathetic nerves, using radiofrequency (RF) energy[4]. The safety and BP-lowering effect of renal denervation (RDN) has been reported in a number of studies[5-8], showing that renal denervation is a promising therapy for resistant hypertension. In the available clinical studies, patients with multiple renal arteries were generally excluded. This is also recommended in the position paper of the European Society of Hypertension (ESH)[9]and expert consensus document from the European Society of Cardiology (ESC)[10]. Multiple arteries are however not exceptional, with prevalence estimates ranging from 15% to 28%[11-16]. A link between presence of multiple renal arteries and hypertension has long been suspected[17-19]. In our centre, it was decided not to exclude patients based on presence of multiple renal arteries when the overall anatomy was eligible for RDN. This is a pragmatic approach, prompted by the high prevalence of multiple renal arteries.

The aims of this study were to determine the prevalence of multiple renal arteries in patients referred for RDN. Secondly a classification for renal artery eligibility is proposed. Finally, the relation between presence of multiple renal arteries and the BP-lowering effect after RDN was investigated.

Materials and methods

Study population

Between August 2010 and March 2013, all patients referred to the University Medical Center Utrecht (ESH Excellence Center) for treatment with RDN were screened using a standardized protocol[20]. The aims of this protocol were: to confirm the diagnosis of resistant hypertension using 24-h ambulatory BP monitoring (ABPM), to exclude secondary causes of hypertension, and finally to determine whether anatomy of the renal arteries was eligible for RDN. To determine the prevalence of multiple arteries all patients referred to our centre were analysed. However, not all referred patients were treated with RDN due to secondary causes of hypertension or ineligible anatomy[20].

For the second question –to investigate the relation between presence of multiple arteries and BP-reduction– only patients treated with RDN were analysed.

Primarily, patients with resistant hypertension were considered eligible for RDN. This condition was defined as an office systolic BP (SBP) of ≥160 mmHg, despite use of ≥3 antihypertensive drugs. Secondly, patients fulfilling the same BP criteria -but without optimal pharmacological treatment due to recorded intolerance for antihypertensive drugs- were accepted. Major contraindications for RDN were: an estimated glomerular filtration rate (eGFR) <30 mL/min/1.73m^2, severe co-morbidity, and non-eligible renal artery anatomy. The latter was defined as renal arteries with a diameter < 4 mm and/or a length <20 mm (also see section 'classification of renal arteries'). Moreover patients with a history of renal artery stenting or significant renal artery stenosis were excluded from treatment.

Reporting of the study conforms to STROBE along with references to STROBE and the broader EQUATOR guidelines[21].

Measurements

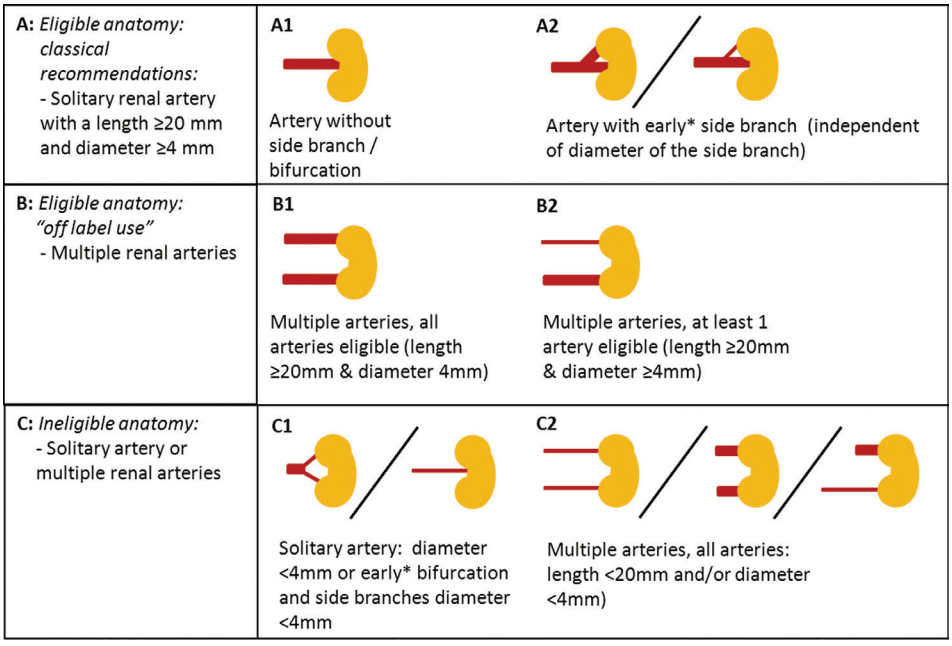
All measurements used for the current study were performed as standard care. Detailed information on drug use and medical history was collected and physical examination was performed. BP was assessed three times in sitting position after 15 minutes of rest using a non-invasive automated device. ABPM was taken using the Microlife WatchBP O3 device (Microlife Inc., Widnau, Switzerland).

Non-invasive imaging of the renal arteries was done to evaluate anatomical eligibility. Preferably magnetic

resonance angiography (MRA) was performed. When MRA was contraindicated, computed tomography angiography (CTA) was performed.
Six months after RDN, patients visited the out-patient department. BP was again taken in the same way as before treatment. Renal function was estimated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula [22].

Classification of renal arteries
A classification to identify patients eligible for RDN was made (figure 1). This classification consists of three classes including subclasses. Renal arteries are classified per kidney. The first class is 'A': 'eligible anatomy according to classical recommendations': A1 indicates a kidney with a solitary artery with a diameter ≥4 mm and a length ≥20 mm without an early bifurcation (<20 mm), A2; a kidney with a solitary artery with diameter ≥4 mm and early bifurcation, whereby at least one of the branches after the bifurcation has a diameter ≥4 mm. The second category consists of kidneys with multiple renal arteries: 'B': 'off label use'. B1 indicates that all arteries are eligible for RDN (diameter ≥4 mm and a length ≥20 mm), B2; indicates that not all arteries are eligible. The final category is 'C': 'Ineligible anatomy'; C1 indicates a solitary artery with either a diameter <4 mm or an early bifurcation (<20 mm) where after branches have diameters <4mm; C2 indicates multiple renal arteries, all with a diameter <4 mm and/or an early bifurcation (<20 mm). When a patient had at least one kidney classified in category C, he/she was excluded from RDN. A schematic overview of the classification is given in figure 1. Reproducibility of the classification was assessed by 2 authors (WV and EV), blinded to each other classification. In case of different scores, the radiologist was asked for final agreement.

Figure 1: Classification to identify anatomical eligibility for renal denervation



**early is defined as length of the renal artery < 20 mm*

Renal Denervation

Renal angiograms were performed to confirm anatomic eligibility. A bilateral treatment of the arteries was performed using series of 2-minute RF energy deliveries along each artery. These treatment points were made in a circumferential way with a minimum of 5 mm distance in between the treatment points. A control angiography was performed after the procedure. The majority was treated using the Symplicity Flex™ device (Medtronic), 2 patients were treated using the EnligHTN™ (St Jude) and 1 patient with Oneshot™ (Covidien).

Data analysis

Office BP was measured three times at baseline and during follow up. The mean BP level from these 3 recordings was used for analysis. The difference between the means of BP at baseline and at 6 months was calculated and used for the present analysis. A negative number represented BP-reduction 6 months after RDN. Prescribed dosages of antihypertensive drugs were converted to daily defined doses (DDD) using conversion factors as provided by the World Health Organization (WHO) Drug Classification (http://www.whocc.no/atcddd/). Using DDD's and total prescribed dosages; daily use (DU) of all antihypertensive drugs was calculated.

Statistical analysis

All variables were reported as mean (±SD), median (range), or proportion when appropriate. Prevalence estimates were expressed as percentages. The Wilcoxon signed rank test was used for paired sample analysis, Mann Whitney U test was used for non-paired sample analysis. Possible differences in percentages between non-paired groups were tested using the Chi-square test. The relation between change in SBP 6 months after RDN (dependent variable) and renal artery anatomy was analysed with a linear regression model. Possible confounders for the BP-lowering effect of RDN (baseline characteristics) were selected for a multivariable linear regression model. Dummy variables were made for renal artery anatomy: Patients with dual solitary arteries versus patients with multiple arteries in whom all arteries were treated (either: A1B1 or A2B1 or B1B1) and patients with multiple arteries in whom not all arteries were treated (either: A1B2, A2B2, B1B2, B2B2). A two-sided *P* value of <0.05 was considered to be statistically significant. All analyses were performed with the SPSS statistical package version 20 (IBM SPSS Data Collection, Chicago, IL).

Results

Baseline characteristics

126 patients underwent non-invasive imaging of the renal arteries as part of the work-up before RDN (4 patients (5%) underwent CTA; 122 (95%) MRA). Baseline characteristics are shown in table 1. Fifty-seven (45%) patients were excluded from RDN, e.g. because of a secondary cause of hypertension. Fourteen per cent was excluded because of ineligible renal artery anatomy (6 patients with a kidney classified in category 'C' and 2 patients with a significant renal artery stenosis).

In total 69 patients were treated with RDN: 80% because of resistant hypertension and 20% because of proven medication intolerance.

Table 1: Baseline characteristics

	All patients n= 126	Solitary arteries n= 83	Multiple arteries n= 43	P-value
Age (yrs)	59 ± 10	59 ± 10	60 ± 10	0.48
Sex (male/female)	73/53	49/34	24/19	0.73
Body-mass index (kg/m^2)	29.1 ± 5.3	28.4 ± 4.7	30.3 ± 6.1	0.024
eGFR† (mL/min/1.73m^2)	74 ± 18	76 ± 17	71 ± 17	0.14
Office SBP (mm Hg)	187 ± 28	188 ± 7	187 ± 29	0.85
Office DBP (mm Hg)	102 ± 15	102 ± 14	101 ± 16	0.82
PP (mm Hg)	85 ± 23	85 ± 23	85 ± 24	0.95
Mean day-time* SBP (mm Hg)	166 ± 18	163 ± 18	171 ± 18	0.02
Mean day-time* DBP (mm Hg)	99 ± 12	97 ± 2	102 ± 13	0.05
Mean day-time HR* (bpm)	77 ± 13	75 ± 11	80 ± 14	0.03
Comorbidity				
Hypercholesterolemia	75 (60%)	49 (59%)	26 (60%)	0.87
Diabetes Mellitus Type II	22 (17%)	14 (17%)	8 (19%)	0.38
TIA/stroke	15 (12%)	10 (12%)	5 (12%)	0.95
CAD	30 (24%)	20 (24%)	10 (23%)	0.92
Antihypertensive medication				
Nr of antihypertensive drugs	4 (0-8)	4 (0-8)	4 (0-8)	0.27
ACEi/ARB/Renin inhibitor	102 (81%)	67 (81%)	35 (81%)	0.93
ß-Blocker	85 (67%)	57 (69%)	28 (65%)	0.67
Calcium-channel blocker	86 (68%)	60 (72%)	26 (60%)	0.34
α-Blocker	22 (17%)	18 (22%)	4 (9%)	0.17
Diuretic	90 (71%)	62 (75%)	28 (65%)	0.33
Central Acting	8 (6%)	5 (6%)	3 (7%)	0.57

*Continuous variables are displayed as a mean (SD), except for number of drugs, this is displayed as median (range). Categorical variables are displayed as a number (percentage). Yrs indicates: years, TIA transient ischemic attack, CAD: coronary artery disease, ACEi: angiotensin-converting enzyme inhibitor, ARB: angiotensin II receptor blocker, SBP: systolic blood pressure, DBP: diastolic blood pressure, PP: pulse pressure, HR: heart rate, bpm: beats per minute, eGFR: estimated glomerular filtration rate. * Determined using ABPM.*

†Calculated on the basis of the Chronic Kidney Disease Epidemiology Collaboration formula.

Table 2: Prevalence of multiple arteries

	All patients n= 126	Treated patients n= 69	Excluded patients n= 57
Solitary arteries	83 (66%)	47 (68%)	36 (63%)
Three renal arteries:	30 (24%)	17 (25%)	13 (23%)
Multiple arteries at right side	10 (8%)	7 (10%)	3 (5%)
Multiple arteries at left sight	20 (16%)	10 (15%)	10 (18%)
Four renal arteries:	11 (9%)	5 (7%)	6 (11%)
Multiple arteries at both sides	9 (7%)	5 (7%)	4(7%)
Right side: 2 extra arteries	1 (1%)	NA	1 (2%)
Left side: 2 extra arteries	1 (1%)	NA	1 (2%)
Five renal arteries:	1 (1%)	NA	1 (2%)
Left side: 1 extra artery, right side: 2 extra arteries	1(1%)	NA	1 (2%)
Six renal arteries:	1 (1%)	NA	1 (2%)
Left side: 3 extra arteries, right side 1 extra artery	1 (1%)	NA	1 (2%)

All variables are displayed as a number (percentage)

Prevalence of multiple renal arteries

Thirty-four per cent of all patients who underwent non-invasive imaging had multiple renal arteries. This percentage was similar in both treated and excluded patients (table 2). The majority of patients with multiple arteries had an additional artery at the left side. Baseline ambulatory BP, heart rate and BMI were higher in patients with multiple arteries (table 1).

Classification

The classification (analysed per kidney) was applied to all 126 patients, 252 kidneys in total. Category 'A' was most prevalent (198 kidneys (79%); A1: 185 kidneys (73%), A2: 13 kidneys (5%)). Forty-eight kidneys (19%) were classified a 'B' (B1: 12 kidneys (5%), B2: 36 kidneys (14%)). Six kidneys (2%) were classified as 'C' (C1: 2 kidneys and C2: 4 kidneys).

Forty-six of the treated patients (67%) were classified twice in category 'A' and met classical recommendations. Five patients (7%) had multiple renal arteries at both sides and were classified twice in category 'B'. Seventeen patients (25%) had multiple arteries at one site, classified as 'AB'. Patients with one or both kidneys in category B would be excluded from treatment with RDN based on 'classical recommendations'.

Of patients excluded from treatment, 6 (11%) had a kidney classified in category 'C'. The majority of excluded patients was classified twice in category 'A' (36 patients; 63%), 11 patients (19%) had a combination of category 'A' and 'B' and 6 patients (11%) were classified twice in category 'B'.

Renal denervation

In all 69 treated patients, angiography confirmed eligibility of the renal arteries as assessed by MRA or CTA. On average 12.4 (±2.2) ablations were applied per patient. Twenty-three of the treated patients (33%) were treated with "off label indication" since they had multiple arteries in one or both kidneys. Only 4 of these patients were denervated in all arteries (classified as B1). The other kidneys with multiple arteries were classified as B2, therefore only the main renal artery was denervated. The majority (96%) was treated using the Symplicity flex device (Medtronic), 2 patients were treated using the EnligHTN (St Jude) and 1 patient with Oneshot (Covidien).

BP-lowering effect of renal denervation

Sixty-nine patients were treated with RDN. Baseline characteristics are shown in table 3. eGFR was significantly lower and mean day-time SBP was significantly higher in patients with multiple renal arteries. Office BP significantly decreased from 195(±26)/ 106(±14) mmHg to 165(±24)/ 95(±14) mmHg, 6 months after RDN (P<0.001, Figure 2). Heart rate decreased significantly from 73 (±14) bpm to 68 (±12) bpm (P=0.049).

Systolic BP decreased by 33 (±28) mmHg in the group of patients with solitary arteries (n=47; 68%). Systolic BP decreased by 29 (±33) mmHg in patients (n=4; 6%) with multiple arteries which were all treated with RDN, and 23 (±22) mmHg in patients (n=18; 25%) with multiple arteries in whom not all multiple arteries were treated (figure 1). Univariable analysis showed no significant difference in the effect of RDN on SBP in patients with multiple arteries (all treated, B1) and respectively patients with multiple arteries (not all treated, B2) compared to patients with solitary arteries (classified as A) (table 4).

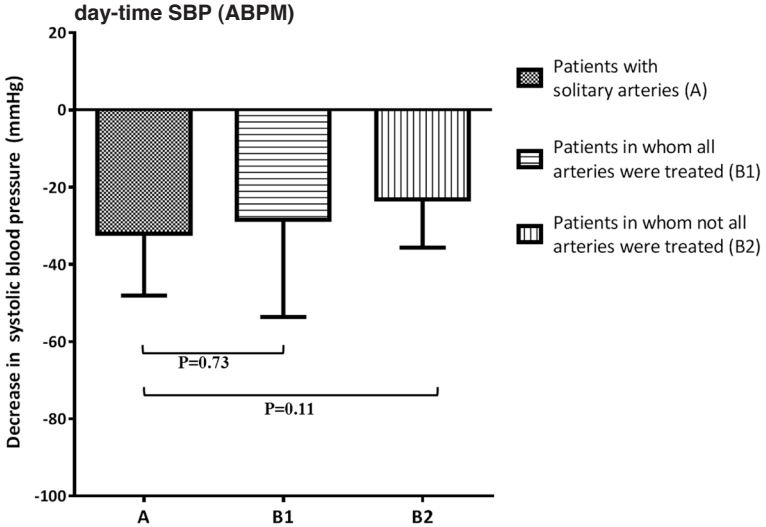
The use of antihypertensive drugs, expressed as DU changed non-significantly from 5.65 (1.00-17.50) units per day at baseline to 4.67 (0.25-14.00) units per day at 6 months follow-up (P=0.12). The change in antihypertensive drugs was greater in patients with multiple arteries: DU significantly decreased in patients with multiple arteries (all treated, B1) with 2.25 (2.00-12.17) units per day versus 0.00 (-7.96 – +5.5) units per day in patients with solitary arteries (P=0.006). Since baseline eGFR and day-time SBP were significantly different between the groups a multivariable analysis was performed. After adjustment for age, gender, baseline eGFR, baseline SBP, baseline day-time SBP (ABPM), and change in antihypertensive drugs (DU), the SBP-reduction after RDN in the groups with multiple renal arteries (all treated; B1, n=4) was comparable to the group with solitary arteries(n=47) (table 4 and figure 2). However, patients with multiple arteries (not all treated, B2), seem to have a less pronounced effect of RDN on SBP (ß:11.6 95%CI: -2.6 – 23.3, table 4) in a multivariable model.

Table 3: Characteristics treated patients with 6 months follow-up data

	Solitary arteries n= 47	Multiple arteries n= 22	P-value
Age (yrs)	59 ± 11	59 ± 9	0.85
Sex (male/female)	23/22	8/12	0.41
Body-mass index (kg/m^2)	28.4 ± 4.6	30.6 ± 6.9	0.20
eGFR† (mL/min/1.73m^2)	77 ± 17	67 ± 16	0.03
Office SBP (mm Hg)	195 ± 25	196 ± 31	0.91
Office DBP (mm Hg)	106 ± 13	105 ± 16	0.65
Office HR (bpm)	73 ± 15	72 ± 12	0.73
PP (mm Hg)	89 ± 22	91 ± 26	0.70
Mean day-time* SBP (mm Hg)	165 ± 17	176 ± 14	0.01
Mean day-time* DBP (mm Hg)	98 ± 13	104 ± 11	0.05
Comorbidity			
Hypercholesterolemia	30 (64%)	15 (68%)	0.72
Diabetes Mellitus Type II	9 (19%)	4 (18%)	0.77
TIA/Stroke	6 (13%)	1 (5%)	0.29
CAD	12 (26%)	3 (14%)	0.26
Antihypertensive medication			
Nr of antihypertensive drugs	4 (0-8)	4 (0-8)	0.57
ACEi/ARB/Renin inhibitor	41 (87%)	18 (82%)	0.55
ß-Blocker	31 (68%)	12 (59%)	0.47
Calcium-channel blocker	34 (72%)	14 (64%)	0.46
α-Blocker	7 (15%)	4 (18%)	0.73
Diuretic	35 (75%)	15 (68%)	0.59
Central Acting	5 (11%)	0 (0%)	0.11

Continuous variables are displayed as a mean (SD), except for number of drugs, this is displayed as median (range). Categorical variables are displayed as a number (percentage). Yrs indicates: years, TIA transient ischemic attack, CAD: coronary artery disease, ACEi: angiotensin-converting enzyme inhibitor, ARB: angiotensin II receptor blocker, SBP: systolic blood pressure, DBP: diastolic blood pressure, PP: pulse pressure, HR: heart rate, bpm: beats per minute, eGFR: estimated glomerular filtration rate.
** Determined using ABPM.*
†Calculated on the basis of the Chronic Kidney Disease Epidemiology Collaboration formula.

Figure 2: Change in systolic and diastolic BP, adjusted for age, gender, eGFR , baseline SBP and day-time SBP (ABPM)



Multivariable regression analysis of mean decrease in blood pressure.
SBP indicates systolic blood pressure. eGFR: estimated glomerular filtration rate.

Table 4: The relation of renal artery anatomy with the change in SBP 6 months after RDN

Univariable analysis Determinant	ß	p-value
Patients with multiple arteries which were all treated (n=4)	4.2	0.77
Patients with multiple arteries which were not all treated (n=18)	10.2	0.18
Multivariable analysis* **Determinant**	**ß**	**p-value**
Patients with multiple arteries which were all treated (n=4)	-5.0	0.73
Patients with multiple arteries which were not all treated (n=18)	11.6	0.11

ß indicates regression coefficient, i.e., mean change in SBP from baseline (in mmHg) compared to mean change in the reference group (patients with dual solitary arteries). A positive estimate means less blood pressure reduction as compared to reference group.
**Adjusted for: age, gender, baseline eGFR, baseline SBP, change in daily units and baseline day-time SBP (ABPM).*

Renal function and clinical outcome

Mean eGFR at 6 months did not differ from baseline in the total group (-0.4 mL/min/1.73m^2 P=0.81) nor in the subgroups of patients with solitary (-1.4mL/min/1.73m^2 P=0.58) or multiple arteries (+1.1 mL/min/1.73m^2, P=0.67). No complications with long term consequences or adverse events related to the procedure occurred during 6 months of follow-up.

Discussion

The first aim of present study was to analyse prevalence of multiple renal arteries in patients referred for RDN. Multiple renal arteries were present in 35% of referred patients, resulting in a proposed renal artery classification. No differences in prevalence were observed between excluded and treated patients. Finally, an explorative analysis showed that BP-reduction was different in subgroups of patients with solitary arteries compared to patients with multiple arteries categorized as B2.

The prevalence of 34% in the current analysis is higher than the previous articles that described a prevalence of multiple arteries ranging from 15% to 28%[11-16]. This difference may be explained by the preselected population of patients referred for RDN in the present study, since a relation between presence of multiple renal arteries and hypertension has been suggested[17, 18]. Glodny hypothesized that hypertension in patients with multiple arteries can be explained by the smaller diameter of the multiple artery what leads to localized hypoperfusion and consequent increased RAS-activity[19].

The classification made in our centre was designed as an easily applicable tool to optimize patient selection for RDN. Renal denervation has renewed the interest in renal artery anatomy. In the future, this classification can be helpful for standardization when eligibility for RDN is reported in different studies and daily clinical practice. However, vascular pathology, like stenosis or fibromuscular dysplasia, was not taken in to account. Moreover it is important to realize that eligible renal artery anatomy in the current classification (diameter ≥ 4 mm and length ≥20 mm) is inspired by the use of the Medtronic Symplicity catheter. The classification may need adjustment when other devices with different properties become available.

The current study confirmed the BP-lowering effect of RDN in a real life setting. The decrease in office BP of -30/-11 mmHg 6 months after RDN was comparable to the decrease in the treatment-arm of the Symplicity HTN-2 trial[7]. Recently, the HTN-3 trial showed that there was no reduction in systolic BP after 6 months between the treated group and the sham group.[23] The current significant BP reduction should therefore be interpreted with caution as we did not include a control group.

The decrease in BP was similar in patients with solitary arteries compared to patients with multiple arteries all treated (B1). However patients with multiple arteries not all treated (B2) showed a trend towards a less pronounced effect of RDN. Of course, this finding should be confirmed in a larger and randomized trial. Consequently, treatment of small multiple arteries may be of particular interest for development of future devices.

In the present analysis, RDN appeared safe in both groups. However, this study was not powered for detection of such small changes in for instance kidney function. Based on results of the current study (equal BP-lowering capacity and less antihypertensive drugs after 6 months), we feel that patients with multiple renal arteries of sufficient size (B1) should not be excluded from RDN. Thirty-three per cent of patients treated in our centre would not have been treated with RDN when 'classical recommendations' had been applied. In only 4 of the patients with available follow-up data, all multiple arteries could be treated; the multiple arteries of remaining 18 patients had a diameter or length too small for currently available devices.

Multiple non-invasive techniques are available for imaging of the renal arteries. In the current work-up MRa was chosen because of excellent vascular imaging without radiation exposure and use of a gadolinium-like contrast agent which can be applied safely in patients with kidney failure[24]. When MRA was contraindicated, CTA was performed. CTA is an accurate alternative, but exposes patients to both ionizing radiation and iodinated contrast agent[25]. Doppler Duplex ultrasonography, a relatively inexpensive technique, provides both functional and some anatomical information. However, it is time consuming and operator dependent, especially in obese patients[25].

Current study was hindered by some limitations, including the small sample size of patients, especially patients classified as B1. Therefore, the results should be interpreted as explorative. Furthermore it is a single-centre, non-randomized study using data of standard patient care.

In conclusion, to our knowledge, we are among the first to evaluate the effect of RDN in patients with multiple arteries. The current analysis suggests that RDN is effective in patients with multiple renal arteries, specifically in those patients with arteries which could all be treated (classified as B1). Based on these results and the high prevalence of multiple renal arteries among patients with complicated hypertension, it seems reasonable not to exclude patients with multiple arteries. A classification is offered as a uniform strategy to identify eligible anatomy for clinical and research purposes.

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Part 3. The effect of renal denervation in hypertensive patients

Percutaneous renal denervation for the treatment of resistant essential hypertension; the first Dutch experience

Neth Heart J. 2011 Aug;19(7-8):319-23.

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Abstract

Background
In a subpopulation of patients with essential hypertension, therapeutic targets are not met, despite the use of multiple types of medication. In this paper we describe our first experience with a novel percutaneous treatment modality using renal artery radiofrequency (RF) ablation.

Methods
Patients who were resistant to at least 3 types of anti-hypertensive medical therapy (office systolic blood pressure ≥ 160 mm Hg; n= 9) or who did not tolerate medication (n= 2) were selected. Between July and November 2010, a total of 11 patients received percutaneous RF treatment. Patients were followed up to 1 month after treatment. Urine and blood samples were taken to evaluate the effects on renal function and neurohumeral factors.

Results
No periprocedural complications or adverse events during follow-up were noted. A reduction of mean office blood pressure was seen from 203/109 ± 32/19 mm Hg at baseline to 178/97 ± 28/21 mm Hg at 1 month follow-up (mean difference 25±12 mm Hg, P< 0.01). Also, we noted a significant decrease in aldosteron level (391 ± 210 pmol/L versus to 250 ± 142 pmol/L; P= 0.03), while there was no decrease in plasma renin activity (190 ± 134 fmol/L/s versus to 195 ± 163 fmol/L/s; P= 0.43). No change in renal function was noted.

Conclusion
Catheter-based renal denervation seems an attractive novel minimally invasive treatment option in patients with resistant hypertension, with a low risk of serious adverse events.

Background

Using the World Health Organisation criteria of 2001 (systolic blood pressure ≥140mmHg and / or a diastolic blood pressure ≥90mmHg), 34% of adult men and 30% of women in the Netherlands suffer from hypertension.[1] This prevalence increases strongly with age in both men and women.

Treatment of hypertension remains suboptimal. Despite the availability of numerous safe and effective pharmacological therapies, the percentage of patients achieving adequate blood-pressure control to guideline target values remains inadequate. The Julius Centre for Health Sciences and Primary Care in Utrecht recently reported that among patients with hypertension, only 33.7% was aware of the condition. Of those patients aware, only 59.4% was treated. Of those patients treated, 41.9% had blood pressure at or below the advised level.[2] Frequent failure of the pharmacological strategy to attain adequate blood-pressure control can be attributed to both physicians' negligence as well as patient non-compliance to a lifelong pharmacological therapy for a mainly asymptomatic disease. Thus, the development of new approaches for the management of hypertension, especially those that could help overcome these issues, is a priority. These considerations are especially relevant to patients with drug resistant hypertension and/ or patients with severe intolerance to medication. The exact prevalence of therapy resistant hypertension is not exactly known, but cross-sectional studies suggest that it affects approximately 10–15% of patients being treated for hypertension by primary care physicians.[3]

Renal sympathetic efferent and afferent nerves, which lie within and immediately adjacent to the wall of the renal artery, are crucial for initiation and maintenance of systemic hypertension. Radical surgical methods for sympathetic denervation have been successful in lowering blood pressure in severely hypertensive patients. However, these methods were associated with high peri-operative morbidity and even mortality and also long-term complications.[4] Recently, a percutaneous, catheter-based approach using radiofrequency energy (RF) has been developed to disrupt renal sympathetic nerves. This resulted in no severe (long-term) vascular or renal injury. Importantly, catheter-based renal nerve ablation was associated with a significant reduction in both systolic and diastolic blood pressure on top of maximal medical therapy, which persisted through out 12 months follow-up in the first-in-man study[5] The Symplicity HTN-2 Trial was recently published, which was the first randomized controlled study using this technique of renal denervation, confirming the findings of the first-in-man study.[6]

Here, we report the results of the first Dutch experience regarding this novel treatment modality.

Methods

Patient group

Patients were eligible if they have an office systolic blood pressure of 160 mm Hg or more, despite being treated with at least three antihypertensive drugs, or confirmed intolerance to medication. Blood pressure measurements were performed in a seated position in at least two subsequent visits in both arms. Blood pressure check was performed before intervention and at 1 month follow-up.

Also, renal function and changes in neurohumeral factors were obtained during follow-up.

The renal artery anatomy was considered suitable in case of a vessel diameter of ≥ 4mm, no prior renal angioplasty/stenting and no significant stenosis or other abnormalities.

Exclusion criteria for this treatment modality were pregnancy, age below 18 years, patients with any known secondary cause of hypertension and a glomerular filtration rate estimated being <45 mL/min/1.73m². Also, patients with type 1 diabetes, hemodynamically significant valvular disease, implantable cardioverter defibrillators, or who are on treatment with clonidine, moxonidine, rilmenidine, or warfarin, were excluded from intervention.

Procedure

Patients were pretreated with diazepam 5 mg and midazolam 1 mg. Using local anesthetics, cannulation of the femoral artery was performed using the standard Seldinger technique. An 8Fr sheath was introduced

and unfractionated heparin was given using an i.v. bolus of 1.000 IE/kg bodyweight with a target ACT >250 sec. Using an 8Fr RDC or LIMA renal guiding catheter and a 5Fr soft tip straight delivery catheter, a steerable catheter with radiofrequency energy electrode tip was delivered into the renal artery. Before treatment, a starting dose of fentanyl of 50 μgram was given. A bilateral treatment of the renal arteries was performed with the use of series of 2-minute RF energy deliveries along each artery, aiming at 4-6 treatment points per artery (approximately 8 Watts of energy per treatment point). These treatment points are made with a minimum of 5mm distance in between and with a pullback from distal to proximal in a circumferential way. A control angiography was performed after the procedure. Also in a subset of three patients, intravascular ultrasound (IVUS) was performed.

Statistical analysis

Continuous variables are described with mean ± standard deviation. Dichotomous variables are reported as numbers (percentages). For comparison within different time points, a paired t test was used. A two-sided alpha level of 0.05 was used for superiority testing. All statistical analyses were done with PASW Statistics version 17.0 (IBM SPSS, Somers, NY, USA).

Results

The baseline characteristics of the patient group are listed in Table 1. The mean time of the procedure (i.e. from puncture of the femoral artery to closure) was 74 ± 9 minutes. Mean fluoroscopy time was 15 ± 2 minutes. The ACT time achieved was 298 ± 74 seconds. The mean use of contrast was 208 ± 35 ml. A mean dose of fentanyl of 164 ± 29 μgram was given (including the starting dose of 50 μgram). For midazolam the mean periprocedural dose was 3 ± 1.4 mg (including the starting dose of 1 mg). In total, an average of 5.1 ± 1 RF ablations was done in the left renal artery, and 5.6 ± 1 RF ablations in the right renal artery.

Table 1: Baseline characteristics

	All patients (n=11)
Age (yrs)	68 ± 12
Gender (female)	10 (91%)
Etnicity (Caucasian)	11 (100%)
Weight (kg)	82 ± 20
Body-mass index (kg/m^2)	30 ± 8
eGFR (ml/min/1.73m^2)	74 ±14
Co-morbidity	
Coronary artery disease	5 (45%)
CVA/TIA	2 (18%)
Diabetes	2 (18%)
Hypercholesterolemia	4 (36%)
Office SBP (mm Hg)	203 ± 32
Office DBP (mm Hg)	109 ± 19
Number of antihypertensive drugs	3.1 ± 1.5
Patients on medication	
Beta blockers	8 (73%)
ACE inhibitors/ ARB's	9 (82%)
Calcium channel antagonists	3 (27%)
Vasodilators	2 (18%)
Diuretics	5 (45%)
Renin blockers	2 (18%)
Alpha blockers	2 (18%)

ACE = angiotensin-converting enzyme, ARB = angiotensin receptor blocker, GFR = glomerular filtration rate, CVA = cerebro vasculair accident, TIA = transient ischaemic attack
Continuous variables are displayed as a mean (SD), categorical variables are displayed as a number (percentage). Yrs indicates: years, eGFR: estimated glomerular filtration rate, TIA transient ischemic attack, CVA = cerebro vasculair accident, SBP: systolic blood pressure, DBP: diastolic blood pressure, ACEi: angiotensin-converting enzyme inhibitor, ARB: angiotensin II receptor blocker.

No patients showed endovascular damage at final angiography. In a small subgroup IVUS was performed, which showed no dissections or other intra-vascular complications (n=3).

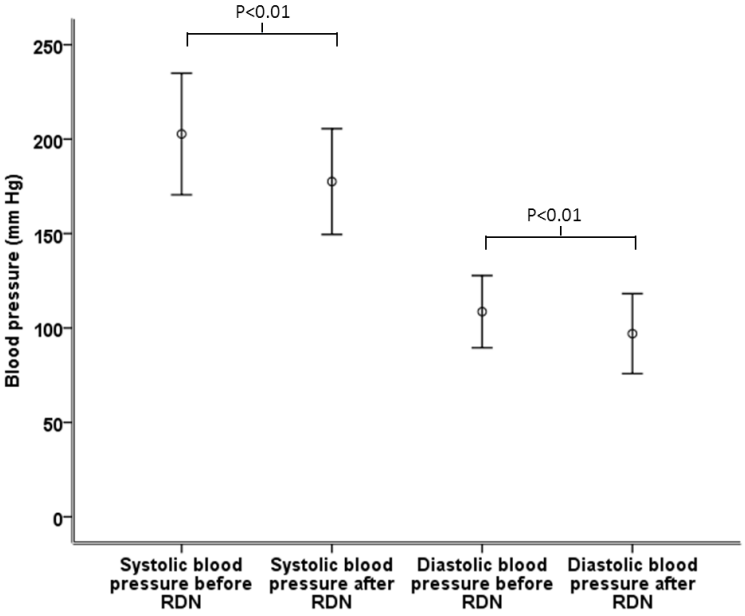
After the procedure, there was no change in serum creatinine (78 ± 17 μmol/L before compared to 78 ±

16 μmol/L; P=0.92). There was a statistically significant, but clinically not relevant, drop in haemoglobin of 9.0 ± 0.7 mmol/L to 8.6 ± 0.7 mmol/L; P< 0.01). In general, there were no peri-procedural (particularly access site) complications and / or complications during follow-up. No changes in medication were noted at 1-month follow-up.

As depicted in Figure 1, the systolic office blood pressure decreased from 203 ± 32 mm Hg at baseline to 178 ± 28 mm Hg at 1 month follow-up. This is a decrease of 25 ± 12 mm Hg (P< 0.01). The diastolic blood pressure changed from 109 ± 19 mm Hg at baseline to 97 ± 21 mm Hg at follow-up (decrease of 12 ± 11 mm Hg; P< 0.01).

The plasma renin activity did not change (190 ± 134 fmol/L/s versus to 195 ± 163 fmol/L/s; P= 0.43). Interestingly, there was a decrease in aldosteron level (391 ± 210 pmol/L versus to 250 ± 142 pmol/L; P= 0.03). In urine samples taken before and 1 month after the procedure, no significant decrease in microalbuminuria (39 ± 80 mg/L versus to 27 ± 55 mg/L; P= 0.22) and total amount of protein in the urine was noted (0.14 ± 0.10 g/L versus to 0.13 ± 0.07 g/L; P= 0.35).

Figure 1: Mean systolic and diastolic blood pressure before and after renal denervation.



Discussion

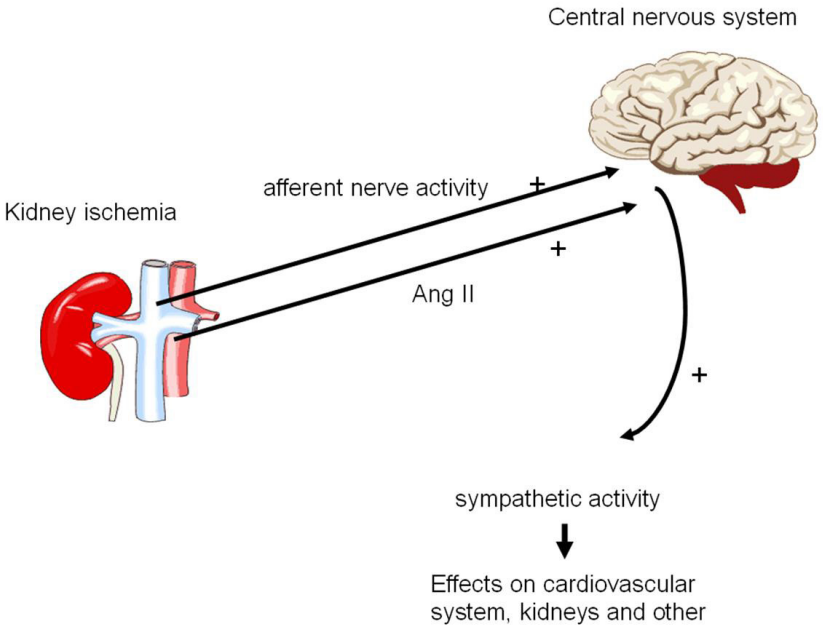
Our first experience with renal sympathetic denervation, using a percutaneous approach, confirms the results of the previous proof-of-principle and recent randomized study, showing the safety and efficacy of this new treatment modality in daily clinical practice for patients with therapy resistant hypertension.[5, 6] The decrease of blood pressure achieved in our patient population is comparable to the one achieved in the previous studies and most likely will be clinically relevant, although current guideline target values were not met in our patients with extreme hypertension (baseline blood pressure 200/106 mm Hg).[7] A recent meta-analysis from Law et al showed that irrespective of the type of medication used, the incidence of coronary heart disease events was reduced by 22% after a systolic blood pressure reduction of 10 mm Hg or a diastolic blood pressure reduction of 5 mm Hg. Even more, the incidence of stroke was reduced by 41%.[8] Assuming that the effects of renal denervation are as effective in reducing clinical events as a pharmacological approach for the treatment of hypertension, the observed blood pressure reduction of 25/12 mm Hg in our patients most likely will be highly beneficial.

The efficacy of this new treatment option should not only be present during short term, but particularly during long term follow-up. Several patients treated with this new technique are now approaching the 2-year follow-up, and the blood pressure reductions observed appear to be sustained over this period, suggesting the absence of nerve fiber recovery, nerve fiber regrowth, or development of counter-regulatory blood pressure–elevating mechanisms.[9]

Besides efficacy, safety remains an equally important issue in a therapy for (secondary) prevention of disease. No adverse events were noted in our first patients peri-procedural and/or at follow-up. In the first cohort study performed in a multi-center setting, no renal artery stenosis occurred as verified using follow-up renal magnetic resonance angiogram at 6 months.[5] Among all patients treated worldwide, a local dissection without sequelae was noted during the procedure in 2 patients, a few access site bleedings were reported, but no (long-term) side effects have been published up till now.[6] Particularly, no change in renal function has been noted.

There is accumulating preclinical and clinical evidence compelling for a primary role of renal sympathetic activation in the pathogenesis of hypertension as described in recent review articles.[10, 11] A crosstalk between the central nervous system and the kidneys is present (Figure 2). Blocking sympathetic nerves leading to the kidney ('efferent') will reverse fluid and salt retention. By blocking sympathetic nerves emanating from the kidney ('afferent'), renal denervation may also decrease the stimulation of other members of the sympathetic nervous system, such as the heart and blood vessels, leading to an additional anti-hypertensive effect.

Figure 2: Schematic representation of the involvement of sympathetic hyperactivity in the pathogenesis of hypertension



Increased plasma levels of angiotensin II and / or increased afferent renal nerve activity stimulates the central nervous system to increase central sympathetic outflow.

The earliest insight into the influence of intervention of the sympathetic nerve activity on renal function in hypertension is the one of Claude Bernard in 1859.[12] He observed that by cutting the greater splanchnic nerve, he caused an increased diuresis, whereas electrical renal sympathetic nerve stimulation produced a reduced diuresis. However, the surgical approach of (non-specific) renal denervation coincided with

severe side effects as observed in studies from the 1930s using surgical denervation of the sympathetic system of the thoracico-lumbar region and has therefore been abandoned.[13, 14] Using the catheter-based renal denervation in patients as described in this paper, we may have overcome these side effects of non-specific denervation of the lumbar region.[15]

Pathophysiological proof of concept of the denervation of the renal artery has also been shown in a small subset of patients.[16] Schlaich et al showed that the 'so called' norepinephrine spillover showed a decrease of 40-50%, which was accompanied by halving of renin activity and an increase in renal plasma flow. In our population, only a decrease in aldosterone was shown. Most likely, because of the small size of the population, no uniform effects of the intervention could be shown on the neurohumeral level in our population. Moreover, it was shown that microneurography at baseline and at follow-up showed a reduction in muscle sympathetic-nerve activity to normal levels.[16]

For future therapeutic application of sympathetic denervation of the renal arteries, further research is needed to identify groups of patients who might benefit from this intervention. In this light, searching for efficacy in patients with for instance chronic kidney disease, patients with heart failure, diabetes and obesity will be interesting. Also, studies in milder forms of essential hypertension should be the next goal of research using this percutaneous technique. Hereby, so-called 'hard endpoint' studies are warranted to proof the value of this new percutaneous technique in daily clinical practice.

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The effect of percutaneous renal denervation on muscle sympathetic nerve activity in hypertensive patients

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Abstract

Objective

The rationale of percutaneous renal denervation (RDN) is based on extensive studies suggesting that renal nerves contribute to hypertension and that they comprise a sensible treatment target. Sympathetic Nerve Activity (MSNA) is considered to be one of the few reliable methods to quantify central sympathetic activity. The aim of this current study is to determine the effect of RDN on MSNA in a standardized fashion.

Methods

MSNA was determined in 13 patients before and 6 months after RDN. Anti-hypertensive medication was stopped before MSNA. If cessation of medication was considered unsafe, a patient was instructed to use the exact same medication on both occasions.

Results

Ten sets of MSNA recordings were of good quality for analysis. Mean age was 57±3 years, mean eGFR was 85±18mL/min/1.73m^2. MSNA was determined twice during a medication free interval in 5 patients, 1 patient used the exact same medication twice, and 4 patients used different drugs. Mean BP, changed from 206±7/116±4 mm Hg, to 186±6/106±3 mm Hg, 6 months after RDN (P=0.06 /P= 0.04). Mean resting heart rate did not change (P=0.44). MSNA, did not change after RDN: respectively 37±4 bursts/min at baseline and 43±4 bursts/min (P=0.11) after RDN. In the 6 patients with standardized medication use during the MSNA sessions, results were comparable.

Conclusions

Treatment with RDN did not result in a change in MSNA. Changes in BP did not correlate with changes in MSNA.

Introduction

Globally, 34% of the adult population has hypertension and this prevalence is still rising.[1] Despite a broad availability of effective pharmaceutical agents, only about 30%of the treated patients reach treatment goals.[2] Increased activation of the sympathetic nervous system is identified as an important factor in the development and progression of hypertension.[3-6] In this context, a catheter-based approach has been developed to disrupt the renal sympathetic nerves, using radiofrequent energy. The first clinical studies, in a relatively small number of patients, showed that this technique appears safe and effective.[3-6] Office systolic blood pressure (SBP)/ diastolic blood pressure (DBP) reduced by 32/12mmHg six months after RDN.[6]

The central hypothesis of this procedure is that by interruption of the renal efferent and afferent nerves by percutaneous renal denervation (RDN), central sympathetic outflow decreases towards the kidneys and various other organs, resulting in a BP lowering effect. Muscle Sympathetic Nerve Activity (MSNA) is considered to be one of the few reliable methods to quantify central sympathetic activity. MSNA is the centrally originated postganglionic sympathetic nerve activity directed towards the resistance vasculature. There is convincing evidence that MSNA is modulated by renal afferent nerve activity.[7] So, it seems attractive to use MSNA as a surrogate for afferent nerve activity and to hypothesize that RDN lowers MSNA. We have vast experience with this technique.[4, 8-13]The within-subject reproducibility of the basal supine MSNA signal is very good, so this technique has extensively been used to quantify chronic effects of interventions.[4, 8-13]

A few studies investigating the effect of RDN on MSNA are recently published, and report mixed effects of RDN on MSNA.[14-16] A possible limitation of these studies is that patients used diverse antihypertensive drugs with various effects on MSNA during the recordings. The aim of current study was to determine the effect of RDN on MSNA while taking particularly care of standardization of medication use at the time of the two measurements.

Methods

Study population

Thirteen patients with resistant hypertension (defined as a SBP ≥160 mmHg, despite use of ≥3 antihypertensive drugs), or fulfilling the same BP criteria but without optimal pharmacological treatment due to intolerance for antihypertensive drugs, planned to be treated with RDN were included in this study. Before treatment with RDN, patients were screened using a standardized protocol; firstly to confirm the diagnosis of hypertension, secondly to exclude secondary forms of hypertension and finally to obtain renal artery imaging to assess renal artery anatomy before treatment with RDN.[17]

At baseline 24 hour ambulatory BP monitoring was taken noninvasively using the Microlife WatchBp 03 device (Microlife Inc., Widnau, Switzerland), to exclude patients with white coat hypertension.

Before RDN (within one week) and 6 months after RDN a set of measurement was performed: BP-measurements, heart rate and MSNA. Anti-hypertensive medication was stopped, when considered safe, before these measurements as described in table 1. This had been done by our group in previous studies.[8, 18] The decision to stop antihypertensive drugs was based on clinical judgement with emphasis on (cardiovascular) medical history. During the medication free interval, patients were regularly contacted by a physician. Also, patients are informed to contact when they develop symptoms. If cessation of medication was considered unsafe, a patient was instructed to use the exact same medication twice for both sessions.

The study protocol was carried out with the approval of the Ethics Committee of the University Medical Center Utrecht, and all patients gave written informed consent.

Table 1: Scheme of gradual discontinuation of medication

4 weeks before measurements	Stop: diuretica (including aldactone) and aliskiren	
	Gradually reduced: Bèta blockers and central working antihypertensive drugs are reduced in two weeks:	
	Day 1: 100%	Day 8: 0%
	Day 2: 50%	Day 9: 25%
	Day 3: 50%	Day 10: 0%
	Day 4: 50%	Day 11: 25%
	Day 5: 50%	Day 12: 0%
	Day 6: 0%	Day 13: 25%
	Day 7: 50%	Day 14: 0%
2 weeks before measurements	Stop: ACE-inhibitors, AT1-antagonists, Calcium-antagonists, Alpha-blockers, direct vasodilatators.	

Measurements

All subjects underwent an identical set of measurements in the morning, in supine position in a quiet room with an ambient temperature of 22 to 24°C. Patients were asked to empty their bladder to minimize possible sympathetic activity caused by bladder extension. MSNA was recorded with a unipolar tungsten microelectrode placed in a muscle nerve fascicle of the right peroneal nerve using the technique of Wallin et al.[19], as described by us previously.[8-11, 13, 18, 20, 21] After instrumentation, subjects rested for 20 minutes. The correct position of the electrode was evaluated by means of a Valsalva maneuver, while electrocardiogram (ECG), heart rate and MSNA were continuously recorded. During restart of breathing after the Valsalva maneuver, a short pause in neural activity can be seen, this was considered to be the background noise. This procedure was done at the beginning and at the end of the study session. BP was measured in supine position during the MSNA session after the needle has been positioned in a stable position, at the arm with an automatic non-invasive calibrated BP-device. So BP was measured while patients were pain free and in a relaxed and standardized position. Means of at least three measurements are presented.

The neural signal was filtered (bandwidth, 500–2000 Hz), rectified and integrated (time constant, 0.1 s). Nerve activity was monitored online (software: Poly 5, Inspectors Research Systems, Amsterdam, the Netherlands) and stored on disc together with ECG, both at a sample frequency of 200Hz, for offline analysis. Sympathetic bursts were identified by their characteristic morphology and relationship to R waves on the ECG. We have previously reported that intra-observer and inter-observer reproducibility are 4.5 ± 0.5%, respectively 6.2 ± 0.7%.[10] Heartbeat intervals were measured from the ECG. The stored integrated MSNA signal was analyzed by software specially developed by our group.[22-25]

Glomerular filtration rate (eGFR) was estimated (at the day of MSNA measurement) on the basis of the CKD-epi formula.[26] Some data were used from the clinical work-up for patients with hypertension: urine was collected during 24-h to determine the albumin-creatinine ratio. Blood samples were drawn to measure plasma aldosterone concentration (PAC; pmol/L) and and plasma renin activity (PRA; fmol/L/s) after 90 min of standing.

Percutaneous renal denervation

RDN was performed using the Symplicity Catheter System, a 6Fr compatible, single-use RF probe. Before introduction of the RF –probe, renal angiograms were performed via a transfemoral approach to confirm anatomic eligibility. Subsequently, the system was introduced in the renal artery and the catheter electrode was positioned in contact with the vessel wall at the desired location and the catheter was connected to an automated RF generator. Multiple applications of RF energy in a spiral pattern along the renal artery with 5 mm interspace were performed. After the procedure, the puncture site was closed with

a closure device and the groin was compressed for 4 to 6 h. Patients took 100mg of acetylsalicylic-acid 5 days before- and 4 weeks after the procedure.

Data analysis

Continuous baseline characteristics are given as mean ± SD. Categorical baseline data are given as number and percentage. Data which are compared (baseline vs follow-up) are shown as mean ± SEM. MSNA is expressed as the number of bursts of sympathetic activity per minute and as the number of bursts per 100 heart beats to correct for differences in heart rate. The change in SBP(an average of 3 measurements per session), heart rate and MSNA were calculated. A negative value represents a decrease in SBP 6months after RDN. The Wilcoxon signed rank test was used for paired sample analysis. Spearman correlation was used to test correlations. A two sided *P* value of <0.05 was considered to be statistically significant. All analyses were performed with the SPSS statistical package version 20 (IBM SPSS Data Collection, Chicago, IL).

Results

Thirteen patients were included in this study, one patient died during follow-up from a non-procedure related cause, 12 patients completed follow-up. Ten sets of MSNA recordings were of sufficient quality for analysis: two recordings of the same patient (pre and post recoding) were excluded because of a signal to noise ratio, making it impossible to appropriately recognize bursts. Baseline characteristics of these 10 patients are depicted in table 2. Secondary causes of hypertension were excluded before treatment with RDN. Four patients had micro-albuminuria and one macro-albuminuria. No adverse events related to the medication stop occurred.

Table 2: Baseline characteristics

	N = 10
Age, yrs	57 ± 3
Sex (male/female)	4/6
Comorbidity	
Hypercholesterolemia	6 (60)
Diabetes Mellitus Type II	3 (30)
Cardiovascular diseases	
TIA/stroke	1 (10)
CAD	1 (10)
Antihypertensive medication	
Number of antihypertensive drugs	4.3 ± 0.5
ACEi/ARB/Renin inhibitor	10 (100)
ß-Blocker	8 (80)
Calcium-channel blocker	6 (60)
α-Blocker	3 (30)
Diuretic	6 (60)
Aldosterone antagonist	1 (10)
Office BP (mm Hg)	203 ± 8 / 115 ± 4
Mean 24-h BP (mm Hg)	174 ± 6 / 99 ± 3
Mean daytime BP (mmHg)	180 ± 17 /103 ± 10
Mean nighttime BP (mmHg)	159 ± 16 / 90 ± 7
Presence of a dipping profile	3 (30)
HR (bpm)	70 ± 3
Body-mass index (kg/m^2)	30.2 ± 1.8
Laboratorial variables	
eGFR (mL/min/1.73m^2)	85 ± 18
Albumin-creatinine ratio (mg/mmol)	2.6 (0.71-78.0)
PAC (pmol/L)	380 (40-580)
PRA (fmol/L)	250 (75-580)

Continuous variables are displayed as amean (SD) or asmedian (range) when applicable. Categorical variables are displayed as a number (percentage). TIA indicates transient ischaemic attack, CAD: coronary artery disease, ACEi: angiotensin-converting enzyme inhibitor, ARB: angiotensin II receptor blocker, HR: heart rate, bpm: beats per minute.

MSNA was determined twice during identical conditions in six patients: a medication free interval in 5 patients and 1 patient used the exact same medication twice. Although aimed to do in identical conditions, in 4 patients medication use showed dissimilarities during the 2 study sessions. Table 3 shows the individual data on medication during the MSNA sessions. Unfortunately, it was not possible to stop antihypertensive drugs twice in all patients; patient 5 did have a transient ischemic attack during follow-up, therefore it was considered unsafe to stop all antihypertensive drugs again. Patient 4 did not want to stop all drugs a second time and patient 8 did not stop antihypertensive treatment at baseline due to previous hypertensive crises with neurologic complaints. Because of a great BP-reduction, antihypertensive medication was reduced during follow-up. Therefore temporary use of the same treatment comparable to baseline was therefore not feasible in this patient.

Eligible anatomy was confirmed before delivery of RF-energy to the treatment site. On average 11.6 (±1.3) denervation points were applied per patient. One patient was admitted 2 weeks after RDN because of hypotension, requiring fluid administration and cessation of antihypertensive drugs. No complications with long term consequences or adverse events related to the procedure occurred during 6 months of follow-up. Kidney function did not change after RDN (P=0.33).

Mean BP, recorded during the MSNA session, changed from 206(±7) / 116(±4) mm Hg at baseline, to 186(±6)/ 106(±3) mm Hg 6 months after RDN(P=0.06 for SBP and 0.041 for diastolic BP (DBP)). Mean resting heart rate during the MSNA session, did not change: 69(±3) bpm before- and 67(±2) bpm after RDN (P=0.44). In the total group, MSNA expressed as bursts per minute, did not change after RDN: 37(±4) bursts/min at baseline compared to 43(±4) bursts/min (P=0.11) after RDN. MSNA corrected for changes in heart rate, did even increase: 54(±6) bursts/100HB at baseline versus 65(±6) 6 months after RDN (P=0.01). (Table 4)

Figure 1 and table 3 show the individual changes in BP, heart rate and MSNA. In 3 subjects MSNA, expressed as number of bursts/min, decreased after RDN. After correction for the change in heart rate, this effect disappears. In the six patients with standardized medication use during the two MSNA sessions , SBP, HR and MSNA -expressed as bursts per minute-, did not change: respectively: SBP; 197(±6) mm Hg versus 193±6 mm Hg (P=0.46), HR 67(±4) bpm versus 66(±2) bpm (P=0.75) and MSNA; 37(±6) bursts/min versus 47(±4) bursts/min(P=0.12).MSNA corrected for HR tended to change in these patients after RDN: 57(±9) bursts/100HB at baseline and 72(±7) bursts/100HB (P=0.06).

Changes in SBP did not relate to changes in MSNA (MSNA expressed as bursts/min: P=0.48, MSNA expressed as bursts/100HB: 0.75) or heart rate (P=0.52). Finally, the change in SBP 6 months after RDN, does not depend on baseline MSNA (both expressed as bursts/min (P=0.21) as well as bursts/100HB(P=0.19)) both in the total group as well as in the standardized patients.

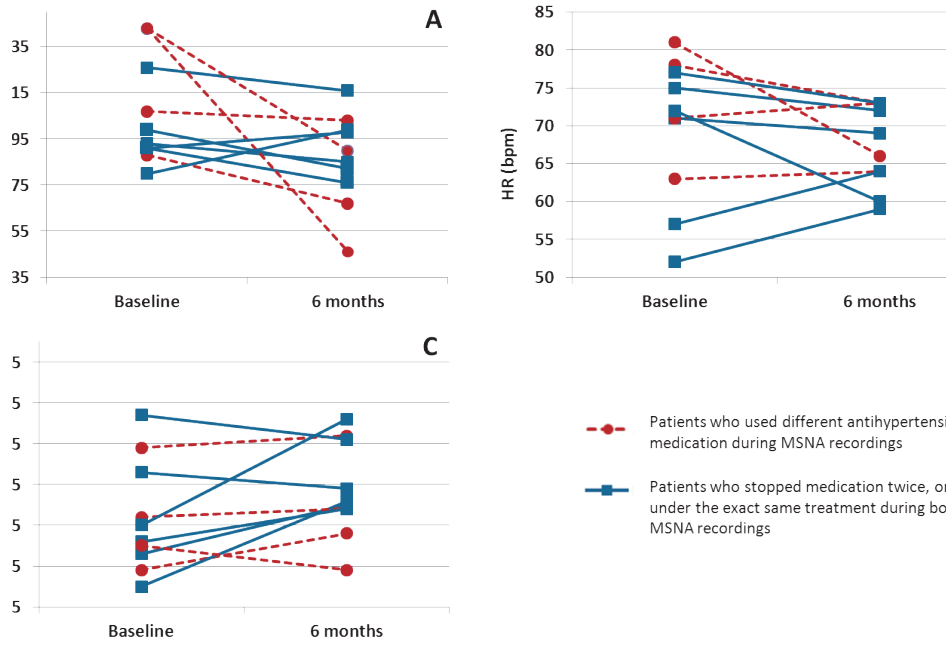
Table 3: Individual data at baseline and 6-months of follow-up

Nr	Sex	Age (yrs)	Baseline					
			Antihypertensive medication*	eGFR (mL/ min/1.73)	SBP/DBP (mmHg)	HR (bpm)	MSNA b/min	MSNA b/100hb
1	M	57	Verapamil	68	188/107	63	24	38
2	M	53	None	102	191/115	75	20	27
3	F	67	None	58	226/133	71	28	39
4	F	65	None	93	243/107	71	54	76
5	F	42	None	107	207/117	78	37	47
6	M	54	None	87	199/109	57	35	62
7	M	66	Labetolol	88	193/115	52	31	59
8	F	39	Captopril, clonidine, metoprolol, hydro-chloorthiazide	101	243/139	81	30	37
9	F	66	None	93	180/107	72	62	87
10	F	63	None	56	191/106	72	48	67

Nr	Sex	Age (yrs)	6-months of follow-up					
			Antihypertensive medication*	SBP/DBP (mmHg)	Δ SBP/DBP (mmHg)	HR (bpm)	MSNA b/min	MSNA b/100hb
1	M	57	None	167/102	-11/-5	64	33	52
2	M	53	None	176/109	-15/-6	72	41	57
3	F	67	None	216/121	-10/-12	69	40	58
4	F	65	Amlodipine	190/97	-53/-10	73	57	78
5	F	42	Metoprolol, doxazosine, losartan	203/102	-4/-15	73	39	54
6	M	54	None	182/103	-17/-6	64	61	95
7	M	66	Labetolol	185/101	-8/-14	59	39	68
8	F	39	Metoprolol	146/102	-97/-37	66	24	38
9	F	66	None	199/121	-19/-16	60	56	93
10	F	63	None	198/99	+7/+7	71	44	61

M indicates male, F: female, SBP: systolic blood pressure, DBP: diastolic blood pressure, HR: heart rate, bpm: beats per minute, MSNA: muscle sympathetic nerve activity, b/min: bursts per minute, b/100hb: bursts per 100 heartbeats. Δ: change in.
a Antihypertensive medication used during MSNA-session.

Figure 1: Change in blood pressure, heart rate and MSNA



A: Systolic blood pressure (SBP; mm Hg) at baseline and after 6 months of follow-up.
B: MSNA, displayed as bursts/minute.
C: Heart rate (HR; bpm), displayed as beats per minute.

Patients who used different antihypertensive medication during MSNA recordings
Patients who stopped medication twice, or under the exact same treatment during both MSNA recordings

Table 4: Parameters determined during MSNA session, at baseline and 6 months of follow-up

	Baseline	6-months of follow-up	P
SBP (mm Hg)	206 (7)	186 (6)	0.06
DBP (mm Hg)	116 (4)	106 (3)	0.04
HR (bpm)	69 (3)	67 (2)	0.44
MSNA			
bursts per minute	37 (4)	43 (4)	0.11
bursts per 100 heartbeats	54 (6)	65 (6)	0.01
eGFR* (mL/min/1.73m^2)	87 (6)	84 (5)	0.52

Data are presented as mean (SEM). SBP indicates systolic blood pressure, DBP: diastolic blood pressure, HR: heart rate, bpm: beats per minute, MSNA: muscle sympathetic nerve activity.

Discussion

Present study shows, that RDN does not result in a reduction in MSNA (number of bursts per minute) in patients with hypertension. The study confirms that RDN has a highly variable effect on BP.

A few papers on the effect of RDN on MSNA have been published.[14-16] Our results are in line with the results of Brinkmann et al.[15] The unique feature of the present study is that we aimed to measure MSNA in a condition as standardized as possible. In 6 patients, we were successful in that, and in 4 patients medication use differed between the 2 study sessions. This approach is in contrast to that of the studies of Brinkmann and Hering in which the patients used diverse antihypertensive drugs with various effects on MSNA during the recordings.[14, 15] Based on the list of medication presented in the baseline table of both

articles, it is clear that both sympatho-inhibitory- (for instance RAAS inhibitors, moxonidine and clonidine) and sympatho-excitory- (for instance diuretics and amlodipine) medication was prescribed. Admittedly, despite of these considerations, our overall conclusion is identical to that of the study of Brinkman et al.[15]
Another interesting finding is the rather variable effect of RDN on BP, which was assessed in a highly standardized fashion (supine position during the MSNA session after stable positioning of the needle). With respect to SBP, patient 1,2,3,6 and 8 are clear responders (decrease in SBP > 10 mmHg) and the BP lowering effect in patient 4 seems (far) too large to be attributable to amlodipine alone. Patient 7 shows a modest effect. We found no relation between the effect on BP and the effect on MSNA.
Various possible explanations for the combination of these findings need to be discussed. Firstly, variability in the contribution of afferent and efferent nerves in the pathogenesis of hypertension in this study population. It has been shown that MSNA increases with decreasing GFR.[27] Indeed, eGFR in this group was relatively normal and MSNA in the majority of patients not very high. So, it is very well possible that even when the procedure effectively disrupted the function of renal nerves, the effect on both BP and MSNA could be highly variable and sometimes absent. This can be illustrated
by the study by Schlaich et al showing that RDN effectively reduced BP in 9 dialysis patients.[16] Two of these patients underwent MSNA , before and 12 months after RDN. In both patients MSNA decreased.[16] Baseline MSNA ranged from 20 to 62 bursts/min, with a mean value of 37 (±4) bursts/min. The population investigated by Brinkmann et al. had a comparable baseline MSNA value (34 (±2) bursts/min)[15], the population described by Hering et al had a higher baseline value (50 (±2) bursts/min).[14] All of our patients had clear hypertension, confirmed by ABPM. It is known that there is no linear relation between activation of the sympathetic nervous system and BP in the hypertensive population. The two gold standard methods to quantify sympathetic activity, MSNA and noradrenaline spillover, show that there is a range in sympathetic activation in studies of patients with essential hypertension.[28, 29] Besides, there is an overlap in sympathetic activation in normotensive- and hypertensive patients.[29] Based on this, it would be possible that the response to treatment with RDN depends on the baseline MSNA level. The change in BP, however did not relate with the baseline MSNA level.
Another possibility could be variability of efficacy of the procedure itself. Subjects were treated by an average of 11.6 denervation points (range 9-14), according to the recommendations of the manufacturer. All procedures were done by an experienced interventional radiologist. An important limitation of RDN is the absence of a peri-procedural-marker to confirm and (ideally) quantify procedural success. So, it is likely that there is a variable efficacy of the procedure to partially or (near) completely disrupt the function of either or both afferent and efferent renal nerves. Further, the relative contribution of afferent versus efferent nerves in various disease conditions is unknown. MSNA is a surrogate of afferent nerve activity. It is possible that in some cases mainly the efferent nerves are affected by RDN. Interruption of the efferent nerves may result in various effects including decrease of activity of the RAAS, change in salt sensitivity, decrease in renal vascular resistance.[7, 30] These variables were not assessed in this study.

Another explanation for the absence of an effect on MSNA could be that MSNA is not quantified correctly. We do not see that as a likely option. Our department has vast experience with MSNA to quantify chronic effects of interventions in multiple studies.[4, 8-13] An often heard explanation on the highly variable effect of RDN is that it can be explained by improved drug adherence in some patients, rather than by the procedure.
Finally within the light of the results of the recent HTN-3 trial, a randomized controlled trial including a shamprocedure, which failed to meet its primary efficacy endpoint (change in office BP 6 months after RDN)[31], it is possible that the achieved BP-lowering effect in our study is the result of regression to the mean, a placebo effect or a Hawthorne effect. Confounding by improved drug adherence can be excluded in our study.
Next studies should include patients with more advanced kidney failure, preferably a control group should

be included. Indeed, the first case reports[32, 33] and a first small observational study[16] of successful use of RDN in dialysis patients with uncontrolled hypertension are reported. We stress that such studies should be done in conditions as standardized as possible. In order to quantify the net effect of RDN on MSNA, ideally studies should be done when patients are taken off all medications known or likely to affect MSNA. For practical reasons or when it is considered unsafe to stop antihypertensive treatment, one could be tested while on a fixed multi-agent combination pill (eg, Exforge: valsartan, amlodipine, and hydrochlorothiazide).

The small number of patients included in our study is a limitation. Moreover, the current study did not include a control group and the effect of RDN was not determined using ambulatory BP monitoring which could be seen as a limitation. However, BP measurements were done in a highly standardized way in supine position during the MSNA session, after the MSNA needle has been placed in a stable position. Important strengths of this study are the measurements of MSNA by an experienced neurophysiologist, the standardization of antihypertensive drugs and of the BP measurements.

Conclusions

Increased activation of the sympathetic nervous system is identified as an important factor in the development and progression of hypertension. Overall, we found no effect of RDN on MSNA, despite of an effect on BP in most of the patients. In the present group of patients with relatively normal kidney function, the BP lowering effect of RDN does not seem to be explained by a decrease in MSNA.

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Effects of Renal Denervation on End Organ Damage in Hypertensive Patients

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Abstract

Background
Renal denervation (RDN) is believed to reduce sympathetic nerve activity and is a potential treatment for resistant hypertension. The present study investigated the effects of RDN on end organ damage (EOD).

Design
The present study was a prospective cohort study (registered as NCT01427049).

Methods
Uncontrolled hypertensive patients underwent a work-up prior to and 1 year after RDN. Cardiac magnetic resonance imaging (CMR) was used to determine left ventricular (LV)-mass; pulse wave analysis and pulse wave velocity (PWV) were used for evaluation of central blood pressure and arterial stiffness and 24-hour urine was collected for assessment of urinary albumin excretion. 24-hr ambulatory BP measurement (ABPM) was used to evaluate the effect of RDN on BP.

Results
Fifty-four patients gave informed consent for study participation. Mean age was 58±10 years, 50% was male. One year after RDN, mean ABPM decreased by 7±18 /5±11 mm Hg (P=0.01/P<0.01). In the patients followed-up in a standardized fashion ABPM decreased by 5±18 /4±12 mm Hg (n=34; P=0.11/P=0.09). Mean body surface area indexed LV-mass decreased by 3.3±11.5 g/m^2 (corresponding to a 3±11% reduction; P=0.09). PWV increased by 2.9 (-2.2 to +6.1) m/s (P=0.04). Augmentation index corrected for 75 beats per minute did not change (median increase 3.0 (-7 to +17) mm Hg; P=0.89). Urinary albumin excretion did not change during follow-up (mean decrease 10±117 mg/24hr; P=0.61).

Conclusion
In the current study, we observed a modest effect from renal denervation. Moreover, RDN did not result in a statistical significant effect on end organ damage 12 months after treatment.

Introduction

The prevalence of hypertension worldwide was approximately 40% in 2008[1, 2] and is expected to increase further.[2] The rationale to treat hypertension is based on the detrimental long-term effects of this condition. Albuminuria, left ventricular hypertrophy (LVH) and arterial stiffness are manifestations of hypertensive end-organ-damage (EOD) and their presence reflects a worse prognosis.[3, 4] Patients with resistant hypertension (defined as a blood pressure (BP) above target despite concurrent use at least three different antihypertensive classes)[5] have an increased risk of EOD, compared to patients with well-controlled hypertension.[6]

The sympathetic nervous system (SNS) plays a crucial role in the pathophysiology of (resistant) hypertension and EOD.[7] The effects of the SNS on EOD are mediated not only by an increased BP, but also through BP-independent direct negative effects on the cardiovascular system and metabolic disarray.[7] Renal denervation (RDN) has been developed as a catheter-based approach to target renal sympathetic nerves. The Symplicity HTN-1 and HTN-2 studies demonstrated a persistent BP-reduction up to 3 year.[8, 9] However, more recently the HTN-3 trial showed a smaller reduction in BP and a relevant placebo effect in a sham controlled study.[10] To our knowledge, no studies have yet investigated the effect of RDN on hard clinical endpoints. In the absence of hard clinical endpoints use of intermediate endpoints such as change in EOD can provide valuable insights beyond mere BP- effects. Measures that reflect EOD in patients with hypertension such as LVH, increased arterial stiffness and albuminuria have shown to be related to increased risk.[3, 4] Conversely, beneficial changes in EOD are thought to portend reductions in morbidity and mortality.[3, 4]

Purpose of the current study was to investigate the effects of RDN on EOD. We hypothesized that RDN would have a positive effect on EOD, through either blood pressure lowering and / or decreased SNS activity.

Methods

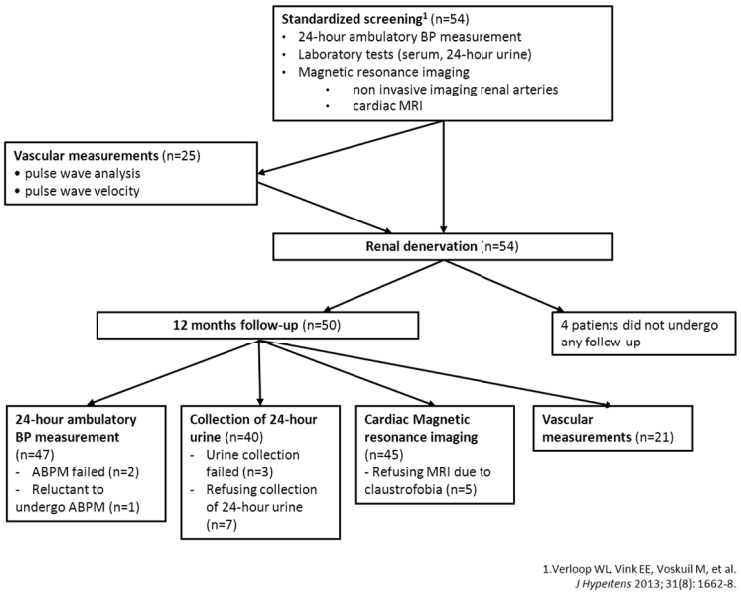
The current study was designed as a prospective cohort study in patients with resistant hypertension was initiated (NCT01427049). The local ethics committee of the University Medical Center (UMC) Utrecht approved the study in accordance with the Declaration of Helsinki. Patients were treated between August 2010 and February 2013 with follow-up planned after 1 year.

Study population

Both patients with resistant hypertension (n=46, defined as a SBP ≥160 mmHg, despite use of ≥3 antihypertensive drugs) as well as patients fulfilling the same BP criteria, but without optimal pharmacological treatment due to intolerance for antihypertensive drugs (n=8) were included in present study. Major contraindications for inclusion were: an estimated glomerular filtration rate (eGFR) <30 mL/min/1.73m^2, secondary causes of hypertension, and a history of renal artery stenting. All patients were screened using a standardized screening protocol.[11] The protocol had three aims: to confirm the presence of hypertension using ambulatory BP monitoring (ABPM); to exclude secondary forms of hypertension; and to confirm eligibility of the renal arteries for RDN by contrast-enhanced magnetic resonance angiography (MRA).

Inclusion of patients in the current study started in August 2010 and a consecutive cohort of patients were asked for study participation. Patients who gave informed consent were treated and followed-up by regular outpatient visits. The 1-year follow-up visit was combined with the study investigations as depicted in the flowchart (figure 1).

Figure 1: Flowchart of study design



ABPM: ambulatory blood pressure measurement; BP: blood pressure; MRI: magnetic resonance imaging

Temporary medication stop

At baseline and 12 months after RDN, we considered whether it would be safe to stop antihypertensive medication for two weeks prior to the tests.[11] This was done as part of our standardized clinical work-up for patients with complicated hypertension[11] to improve standardization of the test conditions since measurement of BP during a medication free interval enables to determine the net effect of RDN on BP and EOD.[12] The decision to stop antihypertensive drugs was based on clinical judgement with emphasis on (cardiovascular) medical history.[11] During the medication free interval, patients were regularly contacted by telephone by trained nurses. For patients in whom it was considered unsafe to stop the medication at baseline, it was attempted to reschedule them on exactly the same medication during follow-up.

Blood pressure and antihypertensive drugs

At baseline and at 1-year follow-up, ambulatory 24-hour BP measurement (ABPM) was measured using the Microlife WatchBP O3 device (Microlife Inc., Widnau, Switzerland), with readings taken every 30 min during day and night. During the screening at baseline and at the follow-up visits, detailed information on medication use and physical examination was obtained. Renal function was assessed at 12 months follow-up.

Measurement of left ventricular mass

Left ventricular (LV) mass was determined by cardiac magnetic resonance imaging (MRI). In all subjects standard steady state free precession (SSFP) cine MRI images were made before and after RDN in multiple orientations. In all acquisitions the slice thickness was 8 mm; the in-plane spatial resolution was 1.3x1.3 mm^2. To quantify LV mass short axis images were used. A trained operator blinded to the time point of acquisition and blinded to the BP values before and after RDN determined the diastolic phase with the largest LV cavity size. On these images, endocardial and epicardial contours were drawn on contiguous slices containing LV myocardium from apex to base, including the atrioventricular ring. Basal

slices were included when they contained LV myocardium in the end-diastolic phase over more than 180° of the circumference. LV muscle volume was calculated by summing the LV volumes from each slice using a validated software package (Mass, Software release 7, Medis, Leiden, The Netherlands). For LV-mass calculation, myocardial volume was multiplied by the specific density of myocardium (1.05 g/cm^3). Papillary muscles and left ventricular trabeculae were included in LV-mass [13]. All measurements were corrected for body surface area (BSA).

Pulse wave velocity and pulse wave analysis

Pulse wave analysis (PWA) and pulse wave velocity (PWV) were measured noninvasively using the SphygmoCor system (AtCor Medical) with applanation tonometry. As described previously, BP was measured both as peripheral BP and as a derived central aortic BP.[14] From derived central waveforms, data were obtained for central SBP, DBP, pulse pressure, and augmentation pressure.[14] Central augmentation index (AIx) was defined as the ratio of augmentation pressure to pulse pressure. AIx was also reported as normalized to a heart rate of 75 beats per minute (AIx@75bpm) to minimize the influence of heart rate.

Pulse wave velocity was performed to assess arterial stiffness. PWV was calculated as the carotid-femoral path length divided by the carotid-femoral transit time. PWA and PWV measurements were measured by an experienced technician blinded for BP response after RDN.

Albuminuria

To assess urinary albumin excretion patients were instructed to collect 24-hour urine. Albumin (mg/24-hr) was analysed using standard laboratory procedures. Three days before the investigations, patients were asked to maintain a constant diet to avoid large fluctuations in sodium balance. Albuminuria was classified as normal (<30 mg/24-hr), micro-albuminuria (30-300 mg/24-hr), or macro-albuminuria (>300 mg/24-hr).[15]

Renal denervation

Renal angiograms were performed to confirm anatomic eligibility. Intravenous narcotics and sedatives were used to manage pain during RDN. 51 of 54 patients were treated using the Symplicity Flex device (Medtronic, Minneapolis, USA). In these patients, using local anaesthetics, a 6Fr sheath was introduced via a femoral artery access site. Bilateral treatment of the arteries was performed using series of 2-minute radio frequency (RF) energy deliveries along each artery.[16] These treatment points were made in a circumferential way with a minimum of 5 mm distance in between the treatment points. In 2 patients the EnligHTN system (St Jude, St. Paul, USA) and in 1 patient the OneShot system (Covidien, Mansfield, USA) was used.

Data analysis

Pulse pressure was calculated as PP= SBP - DBP. Mean arterial BP was calculated as MAP= DBP + (1/3 * (SBP- DBP)).

Differences between findings at baseline and 12 months after RDN were calculated and used for the present analysis. A negative value represented a beneficial change, i.e., a decrease in BP, LV-mass, arterial stiffness (i.e., PWV and PWA) and urinary albumin excretion. Used dosages of antihypertensive drugs were converted to defined daily doses (DDD) using conversion factors as provided by the World Health Organization (WHO) Drug Classification (http://www.whocc.no/atcddd/). Using the DDD's and the total prescribed dosages, the daily use (DU) of all antihypertensive drugs was calculated.

Statistical analysis

All variables were reported as mean (± standard deviation (SD)), median (min-max), or proportion when appropriate. Since antihypertensive drugs interfere with albuminuria, vascular investigations and ABPM

results were analysed for the whole patient cohort and for the subgroup of patients who were investigated in a standardized fashion (i.e. patients that stopped antihypertensive drugs twice or used exact the same medication during follow-up and baseline). Either the paired student t-test or Wilcoxon signed rank test was used when appropriate for paired samples analysis. Differences in proportions between non-paired groups were tested using the Chi-square test.

The relation between change in BP (independent variable) and changes in EOD (dependent variables) 12 months after RDN were analysed using linear regression models. In the linear regression, only standardized patients were taken into account. Multivariable linear regression models were used to correct for age and gender, and baseline BP when appropriate. For multivariable analysis the rule of thumb of 10 cases per variable was applied, to avoid an over fitted model.[17]

A two sided *P* value of <0·05 was considered statistically significant. All analyses were performed with the SPSS statistical package version 20 (IBM SPSS Data Collection, Chicago, USA).

Results

Patient characteristics

Fifty-four patients were included in the current study, 50 of which completed follow-up. Mean follow-up duration was 12±1 months. Not all 50 patients who completed follow-up had all investigations performed. In the flowchart the numbers of participants in the different examinations are given (figure 1).

Baseline characteristics are listed in table 1, mean age was 58±10 years, halve of the included patients was male. Not all patients used a diuretic at baseline, this was due to intolerance. Thirty-four patients underwent follow-up examinations under the same medication regimen as the baseline visit (standardized fashion). Of these 34 patients, 25 patients temporarily stopped all antihypertensive drugs both at baseline and follow-up and 9 patients used the exact same antihypertensive drugs. Sixteen patients had a different drug regimen at follow-up compared to baseline. Reasons for a different drug regimen between baseline and follow-up were: 1) occurrence of an adverse event in the first 12 months after RDN (e.g. stroke, CAD, n=2) making it unsafe in the opinion of the multidisciplinary treatment team to stop medication again; 2) two patients refused to temporarily stop medication for the follow-up visit; 3) patients who needed rescue medication during baseline screening and not during follow-up (due to a decreased BP, n=6); and 4) six patients needed rescue medication during follow-up while this was not needed during the baseline visit or had a higher BP during follow-up so it was considered unsafe to stop medication again.

Blood pressure, renal function and antihypertensive drugs after renal denervation

Patients received an average of 11±4 ablations. One year after RDN, a reduction in ambulatory 24-hr systolic/diastolic BP was observed from 162±20/98±13to 155±21/93±15 mm Hg with a mean difference of -7±18 /-5±11 mm Hg (P=0.01/P<0.01) (table 2). In the 34 patients followed-up in a standardized fashion mean 24-hr systolic/diastolic BP decreased from 165±20/99±13 to 160±20/96±15 mm Hg (mean difference: -5±18 /-4±12 mm Hg; P=0.11/P=0.09).

During follow-up renal function remained stable (eGFR changed from 76±19 ml/min/1.73 m^2 at baseline to 78±20 ml/min/1.73 m^2 at follow-up; P=0.30). Mean BMI did not change during follow-up (baseline 29.2±5.5 kg/m^2 versus follow-up 29.0±5.4 kg/m^2; P=0.49). The use of antihypertensive drugs, expressed as DU decreased from 6±3 units per day at baseline to 5±3 units per day at follow-up (P=0.07). In univariable and multivariable analyses we found no relation between changes in eGFR, BMI or DU and changes in BP or EOD.

Table 1: Baseline characteristics

	All patients n= 54
Age (yrs)	58 ± 10
Sex (male/female)	27/27
Body-mass index (kg/m^2)	29.2 ± 5.2
eGFR† (mL/min/1.73m^2)	75 ± 19
Office SBP (mm Hg)	197 ± 28
Office DBP (mm Hg)	108 ± 14
Mean 24-hour SBP* (mm Hg)	162 ± 20
Mean 24-hour DBP* (mm Hg)	98 ± 13
Mean 24-hourPP* (mm Hg)	65 ± 16
Mean 24-hour HR* (bpm)	75 ± 12
Mean day-time* SBP (mm Hg)	167 ± 21
Mean day-time* DBP (mm Hg)	101 ± 14
Mean day-time HR* (bpm)	77 ± 13
Comorbidity	
Hypercholesterolemia	31 (59%)
Diabetes Mellitus Type II	8 (15%)
TIA/stroke	3 (6%)
CAD	9 (17%)
Antihypertensive medication	
daily use of antihypertensive drugs	6.1 ± 3.2
Nr of antihypertensive drugs	4 (0-8)
ACEi/ARB/Renin inhibitor	46 (85%)
ß-Blocker	37 (69%)
Calcium-channel blocker	37 (69%)
α-Blocker	10 (19%)
Diuretic	40 (74%)
Central Acting	4 (7%)

Continuous variables are displayed as a mean (SD), except for number of drugs, this is displayed as median (range). Categorical variables are displayed as a number (percentage). Yrs indicates: years, TIA transient ischemic attack, CAD: coronary artery disease, ACEi: angiotensin-converting enzyme inhibitor, ARB: angiotensin II receptor blocker, SBP: systolic blood pressure, DBP: diastolic blood pressure, PP: pulse pressure, HR: heart rate, bpm: beats per minute, eGFR: estimated glomerular filtration rate.
†Calculated on the basis of the Chronic Kidney Disease Epidemiology Collaboration formula.
** Determined using ABPM.*

Left ventricular mass after RDN

Forty-six patients underwent cardiac-MRI before and after RDN. LV-mass (including trabeculae) corrected for BSA decreased by 3±11% after RDN (baseline 88±20 g/m^2 versus follow-up 85±21 g/m^2; P= 0.09; figure 2). Change in LV-mass ranged from -51.3 g/m^2 to + 8.9 g/m^2 (per cent change: -37% to +12%). In univariable linear regression analysis, change in LV-mass was not related to reduction in mean 24-hr BP (ß= 0.53 g/m^2/mm Hg; 95%CI:-0.15 to 0.26; P=0.60, figure 3). Correction for age and gender in a multivariable model did not influence this relation. Baseline LV-mass was not related to a change in LV-mass (ß = -0.15 g/m^2/ g/m^2; 95%CI:-0.33 to 0.03; P=0.09). Peripheral resistance did not change during follow-up (baseline 19.7±1.6 mm Hg/mL versus follow-up 18.2±1.0 mm Hg/mL; P=0.30).

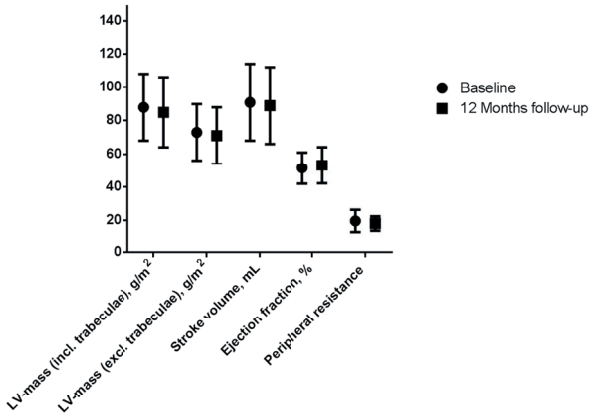
Table 2: Results on blood pressure, arterial stiffness, and renal function

	Standardized patients		
	Baseline	12 months	P value
Ambulatory blood pressure			
Mean 24-hour* SBP (mm Hg)	165 ± 20	160 ± 20	0.114
Mean 24-hour* DBP (mm Hg)	99 ± 13	96 ± 15	0.090
Mean 24-hour HR* (bpm)	72 ± 10	72 ± 10	0.659
Central hemodynamics and arterial stiffness			
Peripheral SBP (mmHg)	165(118-233)	178 (140-224)	0.500
Peripheral DBP (mmHg)	94 (75-152)	100 (81-122)	0.686
Peripheral PP (mmHg)	69 (39-99)	72 (53-114)	0.854
Central SBP (mm Hg)	143 (111-225)	165 (135-205)	0.410
Central DBP (mm Hg)	96 (76-153)	101 (82-124)	0.505
Central PP (mm Hg)	52 (31-81)	56 37-93)	0.080
Augmentation, mm Hg	16 (6-26)	20 (6-34)	0.420
Augmentation index, % (AP/PP)	30 (17-45)	32 (16-43)	0.539
Augmentation index @ 75 bpm (%)	26 (9-38)	29 (17-41)	0.441
Ejection duration (ms)	296 (263-363)	297 (260-351)	0.463
PWV (m/s)	10 (8-17)	13.85 (10-20)	0.010
Renal function			
Urinary Albumin (mg/24hr)	29 (3-663)	29 (4-1073)	0.432
eGFR* (mL/min/1.73m^2)	76 ± 19	78 ± 21	0.253

	All patients		
	Baseline	12 months	P value
Ambulatory blood pressure			
Mean 24-hour* SBP (mm Hg)	162 ± 20	155 ± 21	0.007
Mean 24-hour* DBP (mm Hg)	98 ± 13	93 ± 15	0.003
Mean 24-hour HR* (bpm)	74 ± 13	72 ± 10	0.094
Central hemodynamics and arterial stiffness			
Peripheral SBP (mmHg)	183 (118-233)	173(133-224)	0.218
Peripheral DBP (mmHg)	106 (75-152)	100 (75-122)	0.251
Peripheral PP (mmHg)	69 (39-115)	70 (47-114)	0.931
Central SBP (mm Hg)	171 (111-225)	159(125-205)	0.296
Central DBP (mm Hg)	107 (76-153)	101 (76-124)	0.296
Central PP (mm Hg)	57 (31-101)	55 (37-93)	0.837
Augmentation, mm Hg	17 (6-37)	19 (6-34)	0.852
Augmentation index, % (AP/PP)	30 (17-45)	31 (14-43)	0.710
Augmentation index @ 75 bpm (%)	27 (9-38)	27 (15-41)	0.767
Ejection duration (ms)	296 (263-363)	299 (260-351)	0.917
PWV (m/s)	10.30 (8-25)	12.55 (7-20)	0.053
Renal function			
Urinary Albumin (mg/24hr)	29 (1-663)	30 (4-1073)	0.871
eGFR* (mL/min/1.73m^2)	76 ± 19	78 ± 20	0.304

**Based on the Chronic Kidney Disease Epidemiology Collaboration formula.*
Continuous variables are displayed as a mean (SD) or as median (range). Categorical variables are displayed as a number (percentage). SBP: systolic blood pressure; DBP: diastolic blood pressure; bpm: beats per minute.

Figure 2: Results on cardiac parameters after RDN



Depicted is the change in LV-mass including trabeculae, the change in LV-mass excluding trabeculae, the change in stroke volume, the change in ejection fraction, and the change in peripheral resistance.

Pulse wave velocity and pulse wave analysis after RDN

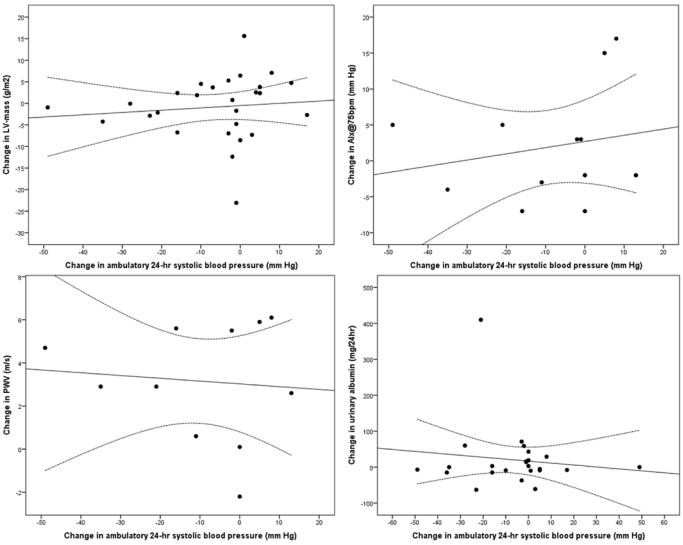
Twenty-one patients underwent PWV and PWA before and after RDN. Thirteen patients (62%) were measured in a standardized fashion. Table 2 shows the results of the vascular measurements. In both the total cohort of patients and in the standardized patients, no changes in central haemodynamics were observed. A change in Aix@75bpm was not related to a change in BP (ß= 0.09 mm Hg/mm Hg; 95%CI:-0.21 to 0.38; P=0.53).

PWV increased in both the total group of patients as well as in the standardized group of patients (standardized increased from 10 (8-17) m/s to 13.85 (10-20) m/s (P=0.01)). The change in BP was not related to the change in PWV (ß= -0.13 m/s/mm Hg; 95%CI:-0.12 to 0.10; P=0.79).

Albuminuria after RDN

Albumin measured in 24-hr urine was assessed in 40 patients before and after RDN. At baseline, 17 patients (43%) had micro-albuminuria, 3 patients (8%) had macro-albuminuria and 20 patients (50%) had a normal urinary albumin excretion. At follow-up, 20 patients (50%) had micro-albuminuria, 1 patient (3%) had macro-albuminuria and 19 patients (48%) had a normal urinary albumin excretion. Overall, a wide range in effect was observed from a reduction of 237 mg/24-hr to an increase of 410 mg/24hr. The mean difference was a reduction of 9.6±18.8 mg/24hr (P=0.61). Change in albumin was not related to the BP-change in the univariable regression analysis (β= -0.54 mg/24-hr/mm Hg; 95%CI: -2.46 to 1.38; P=0.57).

Figure 3: Change in blood pressure plotted against change in end organ damage



Only standardized patients are taken into account for these analyses.

Discussion

Although RDN with radiofrequency ablation is safe, the reduced sympathetic activity after renal denervation did not result in an overall measurable effect on EOD, although we found a wide range in interindividual responses. In comparison to prior studies we found a less prominent reduction in blood pressure in patients followed-up under identical conditions compared to baseline. The present observations are in line with the HTN-3 trial.[10]

If we observed an improvement in EOD, this occurred irrespective of the effect on BP.

An important consideration is that the exact rate of deterioration of EOD is not known, although EOD is generally assumed to worsen in a period of 12 months.[18] While no significant positive effects were observed after RDN, it may be that RDN halted progression of EOD.

In contrast to prior studies investigating the effect of RDN we aimed to standardize the antihypertensive drug-conditions under which measurements were performed, thereby excluding the potential disturbance by antihypertensive medication. It cannot be ruled out that the more favourable effect reported in other studies may be due to confounding by changes in pharmacological treatment during follow-up.[8, 19, 20] Moreover, in the present study the BP-lowering effect was determined using 24-hr ABPM, which is more reliable than office BP.[21] Although unlikely in our opinion, we cannot rule out that the observed lack of effect on end-organ damage in this study is the result of a lack of synergism of RDN with other BP medication.

Left ventricular mass

Left ventricular hypertrophy is an important marker of hypertensive EOD.[22] In the current study RDN was associated with a small, non-significant reduction in LV-mass, although there were large variations between individual patients. Our findings are in contrast to the studies of Brandt and Schirmer who found a regression of LV-mass in 46 and 66 patients respectively, using echocardiography.[23, 24] In the current study we chose CMR for LV mass assessment since this modality is known to have a higher accuracy and reproducibility than echocardiography in evaluation of LV-mass.[25]

Surprisingly, 9 patients showed a reduction in LV-mass although no effect in BP was observed after RDN. We postulate that this may be the result of a BP-independent effect of RDN.

Arterial stiffness

No reductions in PWA and PWV were observed in the current study and we were not able to confirm the effects observed by other larger studies.[26, 27] In the control population of Brandt et al. a more pronounced increase in both PWA and PWV during follow-up was observed compared to our population. This supports the hypothesis that in the current study RDN did indeed decrease the deterioration of arterial stiffness although no significant improvements were observed in the small population. Potentially, it might be that the haemodynamics of the studied population were already impaired to such an extent at baseline, that no improvement can be expected anymore: baseline parameters were compared with reference-values that could be expected based on age and BP. At baseline, AIx@75bpm was 15.5±6.2 mm Hg higher compared to the normal range for age and gender. Baseline PWV values were 0.17±2.97 m/s higher than expected. Similar results were observed in the CAFE-study that studied 2199 hypertensive patients. In this study it was hypothesized that differences in baseline central aortic pressures may be the mechanism to explain the different clinical outcomes between different treatment arms.[28] Although the above seems logical, it should be mentioned that the mean baseline PWV is still seemingly low for a population of resistant hypertension. Another explanation for the present observations might therefore be that not all patients included were hindered by an elevated sympathetic activity and that they could therefore not benefit from RDN.

Albuminuria

No improvement in (micro)albuminuric status was observed after RDN. Since albuminuria can be influenced by the use of antihypertensive drugs[29], analyses were performed in the subgroup of standardized patients. In this subgroup the results were comparable to the complete group. In different antihypertensive drug studies, an improvement in (micro)albuminuria was observed.[30, 31] It is known that the albuminuric state deteriorates during follow-up.[32] It may be that in the current study RDN in fact halted a further deterioration of albuminuric state.

Strengths and limitations

A limitation of the current study is the lack of a control group. Secondly, not all patients completed all study investigations. Another limitation is the relative small sample size. However, Grothues et al investigated the sample sizes needed to observe significant differences in LV-mass with MRI. They showed that a number of 15 patients is needed to observe a 10 gram change in LV-mass.[33]However, the number of subjects undergoing measures of arterial stiffness was very small. Therefore we cannot draw firm conclusions on these data.

One can wonder whether a follow-up duration of one year is enough time to see a change in all of the EOD of each of the measures studied. It is possible that much longer follow-up is needed to ascertain an effect on EOD. Although, other studies reporting about changes in EOD had even shorter follow-up durations[23, 26] we intend to follow these patients up to five years after treatment to study the effect on EOD after a longer follow-up period.

Another point of discussion is that we included patients with an eGFR <45 ml/min/1.73 m^2. The condition of an impaired renal function itself may already sustain EOD. However, we only included three patients with an eGFR <45 ml/min/1.73 m^2. All other patients had an eGFR >45 ml/min/1.73 m^2. In conclusion it would have been interesting to study urinary catecholamines and changes in vagal tone following RDN in the evaluation of EOD. However, we do not have these data.

Strength of our study was the comprehensive assessment of EOD and the attempt to measure EOD under identical conditions at baseline and 1-year follow-up, including a temporary stop in blood pressure medication. Since both baseline and follow-up assessment was obtained in a medication-free period, we were able to study the net BP-lowering effect of RDN. We believe this study design excludes the potential disturbance on ABPM, vascular measurements and urinary albumin by antihypertensive medication. Since it was not always possible to stop medication twice we ensured patients used exactly the same drugs at both time points. In 30% of patients it was unfortunately not possible to obtain standardized measurements.

A second strength was that different parameters of EOD were studied use state-of-the-art thorough methods.

How can current observations be explained?

Current results were surprising since, in contrast to prior studies, we did not observe significant improvements in LV-mass and arterial stiffness after RDN. In the light of the Symplicity HTN-3 trial, our observations are all the more relevant. Based on the Symplicity HTN-3 trial the discussion about the effects of RDN has emerged.[34]

At present there are no periprocedural markers for a successful denervation and it may be that renal sympathetic denervation was not complete in all patients in the current study. However, we performed an average of 11±4 ablations, a number that is larger than in previous studies.[8, 26] Authors of the HTN-3 trial discussed that they were not sure if renal nerves were adequately targeted in their trial.[10] Although Miller et al. indicated that electroanatomical mapping may provide such a tool[35], future research should focus on periprocedural markers of successful denervation.

In conclusion, the current study found no clear improvements in various measures of end organ damage 1 year after RDN in this population of hypertensive patients.

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Part 4. Renal denervation beyond resistant hypertension

Denervation of the renal arteries in metabolic syndrome: the DREAMS study

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Abstract

Chronic elevation of sympathetic nervous system is a key factor in metabolic syndrome (MetS). Since renal denervation (RDN) is believed to modulate sympathetic activity, we performed the DREAMS study to investigate the effects of RDN on insulin sensitivity and blood pressure (BP) in patients with MetS. Twenty-nine patients fulfilling the criteria for MetS and who used a maximum of 1 antihypertensive and/or 1 antidiabetic drug gave informed consent and were treated by RDN. Glucose tolerance tests (GTT) and 24-hour ambulatory BP measurements were performed at baseline, at 6 and 12 months follow-up. Moreover, we performed self-monitored BP measurements (SBPM) at home every month. To assess sympathetic activity we performed muscle sympathetic nerve activity and heart rate variability measurements at baseline and follow-up. The majority of the included patients was male (57%), mean BMI was 31±5kg/m^2. Median insulin sensitivity as assessed by the Simple Index assessing Insulin Sensitivity Oral GTT (SIisOGTT) did not change at 6 and 12 months follow-up (P=0.60, respectively P=0.77). Mean 24-hr BP decreased by 6±12/5±7 mmHg 12 months after RDN (P=0.04/0.01). However, SBPM data showed no reduction over time. Measurements of sympathetic activity showed no reduction in systemic sympathetic activity. In conclusion, renal denervation did not lead to a significant improvement of insulin sensitivity up to 12 months after treatment. Although a significant reduction in ambulatory BP was observed in this nearly drug-naïve population the SBPM data suggest that this may be explained by regression to the mean. Moreover, no effect in systemic sympathetic activity was observed.

Introduction

Metabolic syndrome (MetS) is a cluster of metabolic features that is associated with a twofold-increased risk of cardiovascular disease.[1] According to the statement of the American Heart Association, MetS is defined as the presence of three or more of the following five features: abdominal obesity, hyperglycemia, hypertension, hypertriglyceridemia, and low HDL cholesterol levels.[1] Metabolic syndrome is a worldwide problem with a high prevalence, being 22.9% of US adult population in 2010.[2] Hypertension as part of MetS is often resistant to usual antihypertensive drugs and patients have an increased risk to develop diabetes mellitus type 2.[3]

Chronic elevation of activity of the sympathetic nervous system (SNS) is common in MetS and has been identified by preclinical and clinical studies as being a key factor in MetS.[4, 5] The renal sympathetic nerves are a major contributor to the pathophysiology of elevated sympathetic nerve activity (SNA).[6] Percutaneous renal denervation (RDN) has been developed as a new therapy to lower SNA.[7] First and foremost, RDN was designed to lower blood pressure (BP) in patients with resistant hypertension. Although the first studies were promising[8, 9], the sham-controlled, double-blind HTN-3 trial showed no significant differences in BP-response between the treated group and the sham-group.[10] In a retrospective analysis, the effects of RDN on insulin sensitivity (IS) have been assessed in hypertensive patients.[11] Measures of IS significantly improved after RDN. However, the population was a heterogeneous hypertensive population including patients with a normal glucose level.[11]

In the current prospective study we aimed to investigate the effects of RDN on insulin sensitivity and blood pressure in a nearly drug-naïve population with MetS. We hypothesized that RDN would have a positive effect on both IS and BP. To evaluate the effect of RDN on sympathetic activity we performed muscle sympathetic nerve activity (MSNA) and heart rate variability (HRV) as secondary endpoints.

Methods

The current study was designed as a prospective cohort (pilot) study (NCT01465724). The local ethics review committee of the University Medical Center Utrecht approved the study in accordance with the Declaration of Helsinki and Title 45, U.S. Code of Federal Regulations, Part 46, Protection of Human Subjects. All participants provided written informed consent. Patients were treated between March 2012 and August 2013 with follow-up planned after 6 and 12 months.

Study population

Eligible patients were over 18 years, had the combination of a high fasting glucose, hypertension and one other metabolic feature to fulfill the criteria for MetS.[1] Hypertension was defined as a 24-hr ambulatory systolic BP (SBP) >130 mm Hg. High fasting glucose was defined as ≥5.6 mmol/L (≥100 mg/dL). Patients used a maximum of 1 antidiabetic and/or 1 antihypertensive drug at baseline. Before treatment, patients were screened using a standardized protocol as previously described.[12]

During the screening at baseline and at the follow-up visits, detailed information on medication use was obtained and physical examination was performed. Questionnaires were given to participants to inform about physical activity at that moment. To observe the net effect of RDN, we temporarily stopped antihypertensive or antidiabetic drugs (when used) during the baseline and follow-up visits according to protocol[12] when deemed safe. This resulted in drug-naïve patients during the baseline and follow-up visits.

Glucose Tolerance Test

At baseline and during both follow-up visits, a standard 75 gram glucose tolerance test (GTT) was performed with plasma samples obtained at 0, 30, 60, 90, and 120 minutes after the glucose load.[13] The primary endpoint was change in IS assessed with the formula of the Simple Index assessing Insulin Sensitivity Oral Glucose Tolerance Test (SIisOGTT). SIisOGTT was calculated using the formula: SIisOGTT = 1/ (log [∑ glucose t 0-30-90-120] [mmol/L] + log [∑ insulin t 0-30-90-120] [IU/µml]).[14] Additionally, HOMA-IR was calculated as: HOMA-IR= (glucose t_0 [mmol/L] x insulin t_0 [mIU/L]) / 22.5).[15]

Blood pressure monitoring

At baseline and 6 and 12 months after RDN, ambulatory 24-hr BP measurements (ABPM) and office BP measurements were taken (appendix).

Moreover, we measured self-monitored BP measurements (SBPM) at home (appendix) according to the ESH guidelines.[16] SBPM was performed using an automated WatchBP Home device (Microlife Inc., Widnau, Switzerland).

Sympathetic nerve activity

To obtain information about SNA, we performed MSNA and HRV measurements at baseline and during follow-up. The MSNA measurements were offered as a subset for which patients had to give informed consent separately. MSNA measurements were performed at baseline and at 6 months follow-up similar to the methods recently described in detail[17, 18] (appendix). MSNA is expressed as the number of bursts of sympathetic activity per minute and as the number of bursts per 100 heart beats to correct for differences in heart rate.

HRV testing was performed using an applanation tonometer interface with HRV software (Sphygmocor, Atcor Medical Systems Inc., Sydney, Australia). The outcomes of HRV measurement were frequency domain parameters: high frequency (HF) spectral power component of HRV (measured in absolute units, ms^2), low frequency (LF), total power (TP), and the LF:HF-ratio. High frequency generally represents parasympathetic activity. LF is influenced by both sympathetic and parasympathetic activity. The ratio of LF:HF represents the balance of parasympathetic and sympathetic activity.

Safety

At baseline and during follow-up visits, special attention was paid to occurrence of (serious) adverse events and patients were instructed to report any adverse event spontaneously. Renal function was assessed at baseline and during follow-up. The estimated glomerular filtration rate (eGFR) was calculated using the CKD-EPI formula.[19]

Renal denervation

Patients were treated using the Symplicity Flex device (Medtronic, Minneapolis, USA) as previously described[20] (appendix). A control angiography was performed after the procedure.

Statistical analysis

A sample size calculation was performed for the primary endpoint (SIisOGTT) on forehand. Based on the available literature[21], we expected a mean difference after treatment of 0.4±0.7. The desired power was set at 0.80, α (type I error) was set at 0.05. This yielded a sample size of 27 patients. To make sure that the study was not underpowered, we aimed to include 30 patients.

All variables were reported as mean ±standard deviation (SD), median (interquartile range), or proportion when appropriate. The changes in IS and BP were calculated 6 and 12 months after RDN. A positive value in SIisOGTT represents an improvement in IS. A negative value in BP, glucose, insulin, HOMA-IR, MSNA, or HRV represents an improvement. The student t-test or Wilcoxon signed rank test was used for paired sample analysis when appropriate.

The relation between change in SIisOGTT (independent variable) and changes in BP, renal function, or anthropometrics (dependent variables) 12 months after RDN were analyzed using linear regression models. Multivariate linear regression models were used to correct for age and gender.

For analysis of the SBPM we used linear mixed models (LMM) to evaluate the effect on BP over time. The effect over time is presented as mean ± standard error per month. We performed LMM with a random intercept and random slope or a random intercept alone (depending on the lowest Akaike's information criterion value) to model changes of systolic BP (SBP), diastolic BP (DBP), mean arterial pressure (MAP),

and heart rate (HR) over time. Subsequently we adjusted the models for baseline factors (gender, age) or change in DU, change in BMI, or change in eGFR. A two-sided *P* value of <0.05 was considered to be statistically significant. All analyses were performed with the SPSS statistical package version 20 (IBM SPSS Data Collection, Chicago, IL).

Results

Patient characteristics

Twenty-nine patients were included in the current study and fulfilled the inclusion-criteria. The baseline characteristics are listed in table 1. Most patients were male with a mean age of 60±9 years. Thirty-four percent (n=10) of patients did not use any antihypertensive drugs at baseline, 74% of patients (n=25) did not use any antidiabetic drugs at baseline. All included patients were Caucasian. Multiple renal arteries were observed in nine (31%) patients. In four patients the multiple arteries were of sufficient size for RDN and therefore all arteries were treated, in five patients only the main arteries at both sides were treated. During RDN, a mean number of 14.3 ±3.1 ablations were applied per patient.

During follow-up, BMI and waist circumference did not change as shown in table 2. Use of antihypertensive drugs also remained stable during follow-up (table 2). Physical activity did not change during follow-up (P=0.43).

Table 1: Baseline characteristics

Characteristics	All patients n=29	HRV sub-population n=26	MSNA sub-population n=10
Age (yrs)	60 ± 9	59 ± 9	54 ± 8
Sex (male/female)	17/12	16/10	6/4
Body-mass index (kg/m^2)	31.5 ± 5.0	31.8 ± 5.0	32.7 ± 4.8
eGFR* (mL/min/1.73m^2)	85 ± 15	84 ± 14	93 ± 11
Fasting glucose (mmol/L)	7.2 ± 1.7	7.0 ± 1.1	7.6 ± 2.4
Fasting insulin (mIU/L)	20.9 ± 10.6	22.2 ± 10.4	18.2 ± 10.6
Fasting C-peptide (pmol/L)	1316 ± 403	1358 ± 401	1281 ± 475
Office SBP (mm Hg)	162 ± 19	162 ± 20	154 ± 17
Office DBP (mm Hg)	98 ± 10	96 ± 10	93 ± 10
Mean 24-hr SBP (mm Hg)	145 ± 12	145 ± 12	144 ± 12
Mean 24-hr DBP (mm Hg)	89 ± 10	89 ± 10	91 ± 11
Mean 24-hr PP (mm Hg)	56 ± 8	56 ± 8	54 ± 6
Mean 24-hr HR (bpm)	75 ± 9	76 ± 9	73 ± 9
SlisOGTT	0.243 ± 0.022	0.242 ± 0.021	0.250 ± 0.023
HOMA-IR	6.6 ± 3.5	6.9 ± 3.5	6.1 ± 3.8
Comorbidity			
Diabetes Mellitus Type II	5 (17%)	4 (15%)	2 (20%)
CAD	3 (10%)	3 (11%)	1 (10%)
Use of antihypertensive- and antidiabetic drugs			
Patients using no antihypertensives	10 (34%)	9 (35%)	5 (50%)
Patients using no antidiabetics	25 (74%)	22 (85%)	9 (90%)

*Continuous variables are displayed as a mean (SD), categorical variables are displayed as a number (percentage). Yrs indicates: years, eGFR: estimated glomerular filtration rate, HR: heart rate, bpm: beats per minute, TIA transient ischemic attack, CAD: coronary artery disease, SBP: systolic blood pressure, DBP: diastolic blood pressure, PP: pulse pressure, *Calculated on the basis of the Chronic Kidney Disease Epidemiology Collaboration formula.*

Table 2: Change in anthropometrics and laboratory measurements

Anthropometrics and laboratory measurements	Baseline	6 months follow-up	P-value*	12 months follow-up	P-value†
Weight (kg)	96 ± 15	95 ± 15	0.08	95 ± 15	0.22
Body-mass index (kg/m^2)	31.5 ± 5.0	31.0 ± 4.9	0.09	31.0 ± 4.8	0.21
Abdominal waist (cm)					
Men	112 ± 13	111 ± 13	0.11	111 ± 12	0.68
Women	110 ± 11	111 ± 11	0.19	110 ± 11	0.83
eGFR‡ (mL/min/1.73m^2)	85 ± 15	88 ± 14	0.02	88 ± 14	0.06
Fasting glucose (mmol/L)	7.2 ± 1.7	7.4 ± 2.6	0.34	7.0 ± 1.3	0.34
Fasting insulin (mIU/L)	20.9 ± 10.6	20.1 ± 9.8	0.53	19.6 ± 11.1	0.53
Fasting C-peptide (pmol/L)	1319 ± 410	-	-	1306 ± 468	0.82
Daily use of antihypertensive drugs	1.2 ± 0.4	1.3 ± 0.5	0.41	1.3 ± 0.5	0.83

**Six months versus baseline; † Twelve months versus baseline*
‡Calculated on the basis of the Chronic Kidney Disease Epidemiology Collaboration formula.

Insulin sensitivity

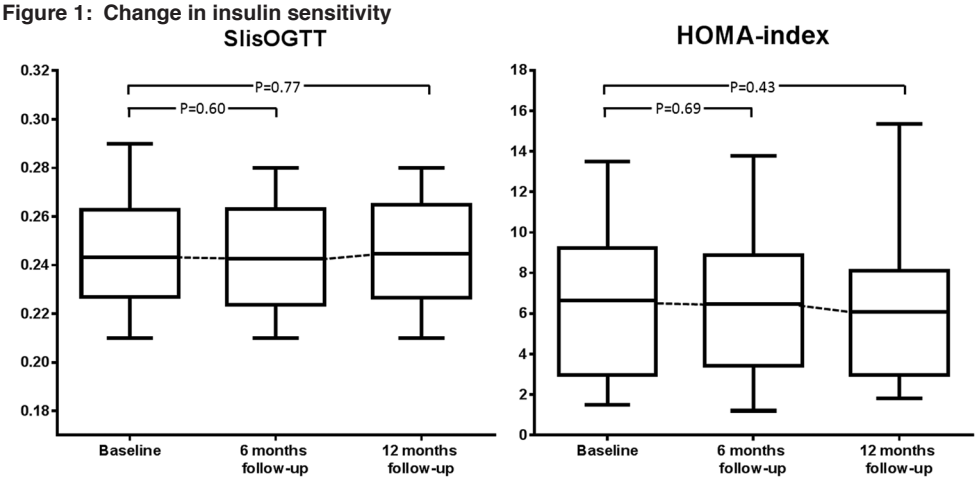
Table 2 represents the insulin, glucose, and C-peptide levels at baseline and follow-up. No significant changes were observed in these laboratory parameters.

The effect on IS is shown in figure 1. Six months after RDN median IS, as assessed by SIisOGTT, did not change (median change 0.00; IQR 0.0141; P=0.60). Twelve months after RDN median IS also did not alter significantly (median change -0.001; IQR 0.0194; P=0.77). Also, in the patients with a reduction of 5 mm Hg or more in 24-hour SBP (n=12) SIisOGTT also did not change (median change -0.0030; IQR 0.0248; P=0.88).

Age, gender, and baseline BP were not related to a change in SIisOGTT in a univariate linear regression (table 3), neither were changes in BP and systematic sympathetic activity. A more impaired SIisOGTT and more impaired HOMA-IR at baseline were related to deterioration in SIisOGTT in a univariate and multivariate linear regression (table 3).

HOMA-IR did not change at six and twelve months follow-up. Twelve months after RDN, HOMA-IR decreased numerically by -0.55±3.7 (P=0.43; figure 1) although this did not reach any statistical significance.

Figure 1: Change in insulin sensitivity



SIisOGTT indicates Simple Index assessing Insulin Sensitivity Oral Glucose Tolerance Test; HOMA-IR the Homeostasis Model of Assessment-Insulin Resistance.

Table 3: The relations between change in insulin sensitivity or ambulatory BP and baseline characteristics or changes in study parameters

	Univariate model			Multivariate model*		
	β	95%CI	p	β	95%CI	p
Change in SlisOGTT						
Baseline SlisOGTT	-0.249	-0.490– -0.009	0.04	-0.250	-0.491 – -0.008	0.04
Baseline 24-hour SBP	0.000	-0.001 – 0.000	0.54			
Baseline HOMA-IR	0.002	0.000 – 0.003	0.03	0.002	0.000 – 0.003	0.02
Age at baseline	0.000	0.000 – 0.001	0.26			
Gender	-0.006	-0.017 – 0.005	0.29			
Change in 24-hour SBP	2,22E-02	0.000 – 0.000	0.92			
Change in BMI	-0.002	-0.005 – 0.000	0.07	-0.002	-0.004 – 0.001	0.12
Change in MSNA (burst/100HB)	8,65E-02	0.000 – 0.001	0.71			
Change in LF-HF ratio	-0.001	-0.004 – 0.001	0.25			
Number of ablations	-0.001	-0.003 – 0.001	0.28			
Change in ambulatory BP						
Baseline 24-hour SBP	-0.593	-0.931 – -0.254	<0.01	-0.493	-0.820 – -0.166	<0.01
Baseline HOMA-IR	-0.292	-1.774 – 1.190	0.69			
Change in SlisOGTT	17.098	-329.64 – 363.84	0.92			
Change in BMI	-0.127	-2.402 – 2.149	0.91			
Change in MSNA (burst/100HB)	-0.174	-0.930 – 0.582	0.61			
Change in LF-HF ratio	-1.203	-3.088 – 0.682	0.20			
Number of ablations	-0.430	-2.015 – 1.156	0.58			

ß indicates regression coefficient, 95%CI: 95% confidence interval. SBP indicates systolic blood pressure, BMI: body mass index, MSNA: muscle sympathetic nerve activity, LF-HF: low frequency- high frequency
** Adjustment for age and gender.*

Blood pressure

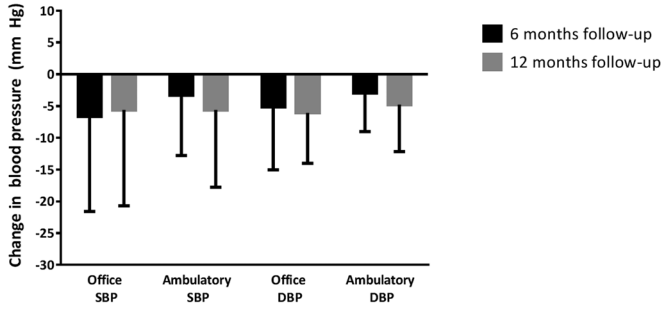
Six months after RDN office BP reduced by 7±15/5±10 mm Hg (P=0.02/0.01). This reduction in office BP persisted up to 12 months after treatment with a mean reduction of -7±14/-7±7 mm Hg compared to baseline office BP (P=0.01/ <0.01, figure 2).

Six months after RDN 24-hr BP reduced from 144±12/88±9 to 141±13/85±9 mm Hg with a mean difference of -3±9/-3±6 mm Hg (P=0.07/0.01). Twelve months after RDN ambulatory 24-hr BP was reduced – by 6±12/5±7 mm Hg compared to baseline (P=0.02/<0.01).

A higher ABPM at baseline was related to a more pronounced decrease in ambulatory BP in a univariate and multivariate linear regression analysis (table 3).

The linear mixed model showed that per month SBP, DBP, MAP, and HR all did not significantly change over time using SBPM. Mean SBP remained stable with a delta of -0.05 ±0.38 mmHg per month (P=0.89). When corrected for changes in daily units of antihypertensive medication mean SBP remained stable with a delta of +0.12 ±0.22 mm Hg per month (P=0.60). Corrected for BMI mean SBP remained stable with a delta of -1.4 ±1.30 mm Hg per month (P=0.27). Corrected for eGFR mean SBP remained stable with a delta of +0.54 ±1.11 mm Hg per month (P=0.63)

Figure 2: Change in blood pressure



SBP indicates systolic blood pressure; DBP: diastolic blood pressure.

Sympathetic activity

Ten patients gave informed consent to participate in the MSNA-substudy and had a complete set of MSNA-measurements. We had 26 complete sets of HRV measurements (no complete sets in three patients). MSNA expressed as bursts per minute did not change after RDN: 48(41) bursts/min at baseline compared to 48(31) bursts/min (p=0.86) at 6 months follow-up. MSNA corrected for changes in heart rate did also not change: 74(48) bursts/100HB at baseline versus 75(23) bursts/100 HB at 6 months follow-up (p=0.80).

No significant differences in HRV-measures were observed 12 months after treatment. Total power showed a median numerical increase of 4 (408)% (P=0.16). Median LF-power showed a numerical increase of 68 (727)% increase (P=0.08), median HF-power showed a numerical reduction of 22 (1241)% reduction (P=0.29). The consequent non-significant increase in LF:HF ratio was 59 (2388)% (P=0.15). The extensive results of MSNA and HRV are displayed in the appendix.

Safety

Renal function showed a trend towards improvement during 12 months follow-up (table 2). There were three adverse events in the study. One patient had a minor bleeding at the puncture site the day after treatment that was treated with compression. One patient had an ischemic stroke due to an stenosis of the arteria carotis two months after RDN and one patient had a transient ischemic attack ten months after RDN.

Discussion

To our knowledge, the present study is among the first prospectively investigating the effects of RDN on metabolic parameters and sympathetic activity in patients with MetS. We showed that RDN did not lead to an improvement of IS up to 12 months after treatment although we observed a significant reduction in ambulatory BP in this nearly drug-naïve population. Remarkably, we observed that RDN did not alter sympathetic activity as assessed by MSNA and HRV. In contradiction with changes in 24-hr BP it was found that repeated BP measurements at home showed no significant reduction over time.

Decreased IS is an important risk factor for the occurrence of cardiovascular disease.[22] Since direct measurement of IS is invasive[23], surrogate indexes have been developed using insulin and glucose levels at various OGTT sampling times.[23] Based on the retrieved OGTT-data we estimated IS by means of the SIisOGTT formula. SIisOGTT is strongly associated with directly measured IS by euglycemic-hyperinsulinemic clamping.[14, 23, 24]

Since the SIisOGTT showed that IS did not change after RDN, we could not confirm the impressive improvement in IS as reported previously.[11] As we did not include a control group, we cannot compare the

results of the treated patients against the natural course. Potentially, a further deterioration in metabolic state can be prevented using RDN, although this is highly speculative. The difference between the present study and the previously published study may be explained by patient selection. Mahfoud et al. included patients with resistant hypertension while we included nearly drug-naïve patients. It may therefore be that the sympathetic activation was higher in their population. Yet, HOMA-IR and other baseline characteristics in the present study were comparable to the previous study or even more impaired. Secondly, patients had a high baseline SNA with a mean of 48 (35) bursts per minute. In other studies investigating the change of sympathetic activity baseline values ranging from 34±2 to 50±2 burst/minute were observed.[25, 26]

Preferably, we would have stratified the present population to more or less severe metabolic classes to evaluate whether more diseased metabolic patients would respond differently. However, the current study population is too small for such a sensitivity analysis.

Although both office and ambulatory BP decreased significantly after RDN, the reduction was less impressive compared to earlier studies.[9, 27] The present results are more in line with the recent HTN-3 trial.[10] Similar to previously reported findings we observed a relation between a higher ambulatory SBP at baseline and a more pronounced reduction in SBP at 12 months follow-up.[28] However, SBPM showed no decrease over time after RDN. In contrast to previous studies, we used a linear regression model (LMM) to investigate the reduction in SBPM over time. We believe this LMM is more accurate since it takes intracorrelation into account and therefore minimizes the effect of regression of the mean that can be observed when analyzes are based on two measurements.[29]

Another interesting observation of the present study was that positive effects on IS or BP after RDN occurred independent of each other. Based on the influence of the SNS on MetS[4, 5], we expected that if RDN was successful, it would lead to an improvement in both IS and BP.

Based on the HTN-3 trial, the role of RDN has been criticized and it has been discussed whether any effect of RDN can be attributed to an improved drug adherence in the patients, rather than by the procedure itself.[30] In the present study we performed IS, BP, MSNA, and HRV measurements during baseline and follow-up in a medication free interval. This made us able to measure the net effect of RDN. Therefore we believe that improved drug adherence did not play a role in the significant reduction in both office and ambulatory BP. One may discuss that the observed reduction in OBPM and ABPM can simply be explained by the "regression to the mean" phenomenon. Asmar et al. showed that regression to the mean can lead to a mean 24-hr SBP reduction of 2.9 mm Hg, which is comparable to the decrease in ABPM in the present study.[31] The SBPM data of the present study may underscribe this hypothesis. SBPM is a very reliable means of BP monitoring since it offers the possibility to measure BP over many time points in a home setting.[32] Moreover, we used the LMM to evaluate the effect on SBPM. This LMM excludes the effect of regression to the mean.

Another point of discussion after the HTN-3 trial is whether RDN is able to lower systemic SNA. The present study cannot confirm that a reduced systemic SNA after RDN led to a decreased BP since we observed no relation between reduction in MSNA and reduction in BP. It may be that we did not correctly quantify MSNA. We do not consider that a plausible option since we have a vast experience with this technique and results have proven to be reproducible.[33-35] Moreover, both MSNA and HRV measures showed similar results.

Another explanation may be that denervation of renal nerves does not lower systemic SNA. However, this seems improbable since preclinical studies have proven that surgical denervation is able to lower sympathetic activity.[36] Therefore, it may be that the currently used catheter does not adequately lower SNS activity. In this light, a concern in the field of RDN is that we lack periprocedural markers. Thus, at present we are not able to evaluate whether we adequately target the renal sympathetic nerves. A

preliminary report by Chinushi et al. indicated that electroanatomical mapping may provide such a tool.[37] Future research should be focused on the identification of such periprocedural markers.

Limitations of the study

Although a sample size calculation was performed beforehand, the present study may have been underpowered to detect any significant differences in this low risk population. The mean difference we expected was much higher than the observed difference. It is important to realize that the small study group makes it difficult to draw firm conclusions. The present study was however set-up as feasibility study and should therefore be considered as hypothesis-generating. Our study lacked a control group and larger, (sham) controlled trial in patients with more severely impaired IS should be well powered to detect any differences.

Perspectives

The present study shows that RDN does not lead to an improvement of IS up to 12 months after treatment. Although we observed a significant reduction in ABPM, it is likely that this can be explained by regression to the mean. The measures of sympathetic activity suggested that SNA did not change after RDN, nor that a change in SNA was related to a change in BP or IS. In the light of the Symplicity HTN-3 trial the current results are all the more important. Exploring the role of sympathetic hyperactivity and RDN in IS should be performed more extensively in future, preferably randomized controlled trials. Hereby, important issues will be the choice of patient population, denervation technique / catheter type and hopefully a robust future per-procedural read-out technique to be able to directly evaluate the efficacy of the performed intervention.

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Appendix

Supplemental methods

Office blood pressure and ambulatory 24-hour blood pressure

ABPM was taken using the Mobil-O-Graph device (IEM Healthcare, Stolberg, Germany), with readings taken every 15 min during daytime and every 30 min at nighttime. Using mean SBP and DBP, pulse pressure (PP) was calculated as PP=SBP-DBP. Office BP was measured three times in sitting position after 15 minutes of rest using a non-invasive automated device (OMRON Healthcare Co. Ltd, Kyoto, Japan).

Self-monitored blood pressure

At last, we measured self-monitored BP measurements (SBPM) at home (online supplement). After RDN, patients were given a device to measure their BP at home. SBPM was performed using an automated WatchBP Home device (Microlife Inc., Widnau, Switzerland) that automatically saves measurements to a secure internet site (BP@home, MobiHealth B.V., the Netherlands). Every month patients were asked to measure BP during seven consecutive days. According to the ESH guidelines, patients were instructed to measure BP twice in the morning and twice in the evening. For analysis the average of all values and the averages of evening and morning measurements were used. The measurements of the first day were discarded, to avoid the stress component that may be involved.

Muscle sympathetic nerve activity

Muscle sympathetic nerve activity (MSNA) measurements were performed at baseline and at 6 months follow-up. A unipolar tungsten microelectrode was placed in a muscle nerve fascicle of the right peroneal nerve using the technique of Wallin et al. to record MSNA. After instrumentation, subjects rested for 20 minutes. The correct position of the electrode was evaluated by means of a Valsalva maneuver at the start and end, while heart rate and MSNA were continuously recorded. The neural signal was filtered (bandwidth, 500–2000 Hz), rectified and integrated (time constant, 0.1 s). Nerve activity was monitored online (software: Poly 5, Inspectors Research Systems, Amsterdam, the Netherlands) and stored on disc for offline analysis. Sympathetic bursts were identified by their characteristic morphology and relationship to R waves on the electrocardiogram (ECG). Heartbeat intervals were measured from the ECG, and stored together with the MSNA at a sample frequency of 200 Hz.

Renal Denervation

Patients were treated using the Symplicity Flex device (Medtronic, Minneapolis, USA). Patients received sedation, using a combination of midazolam (starting dose 1-2 mg and up-titrating with 1 mg steps if necessary) and fentanyl (starting dose 50 microgram and up-titrating with 50 microgram steps if deemed necessary). Using also local anesthetics, a 6Fr sheath was introduced via a femoral artery access site. Renal angiograms were performed to confirm anatomic eligibility. Bilateral treatment of the arteries was performed using series of 2-minute radio frequency (RF) energy deliveries along each artery. These treatment points were made in a circumferential way with a minimum of 5 mm distance in between the treatment points.

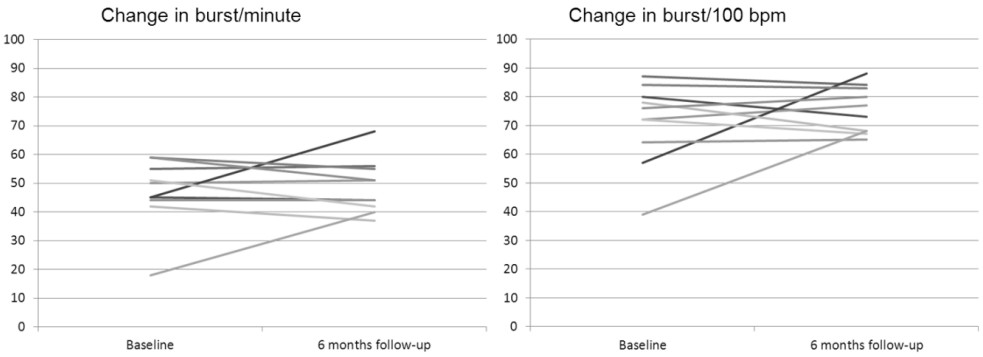
Supplemental results

Change in muscle sympathetic nerve activity (n=10) and heart rate variability (n=26)

	MSNA		
	Baseline	6 months follow-up	P-value
Heart rate during MSNA	67 ± 9	65 ± 6	0.37
Burst/min	47.5 (35)	47.5 (31)	0.86
Burst/HR	74 (45)	75 (23)	0.80
	HRV		
	Baseline	**12 months follow-up**	**P-value**
R-R interval (msec)	847.5 (506)	889.0 (645.0)	0.16
SDNN (msec)	34.05 (149.0)	32.5 (109.8)	0.72
Total power (ms2)	593.5 (55774)	503.5 (6213)	0.38
High frequency (ms2)	153.0 (8723)	119.0 (2705)	0.29
High frequency (nu, %)	58.4 (73.6)	47.7 (68.8)	0.04
Low frequency (ms2)	106.0 (3476)	135.0 (1602)	0.08
Low frequency (nu, %)	41.6 (73.6)	49.7 (68.8)	0.06
Ratio LF:HF	0.72 (3.54)	1.1 (10.3)	0.15

Categorical variables are depicted as mean ±SD or median (range). Except for heart rate the Wilcoxon signed rank test was used for paired sample analysis. The paired T-test was used for heart rate during MSNA.

Individual changes in MSNA



The individual changes in MSNA activity expressed in both burst/minute as burst/100 heart beats.

The impact of renal denervation on central blood pressure and arterial stiffness in a metabolic syndrome population

An observational substudy from the DREAMS study

Submitted

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Abstract

Background

Central blood pressure (BP) and arterial stiffness are seen as independent predictors of cardiovascular disease. Patients with metabolic syndrome (MetS) are known to have an increased arterial stiffness. Previous studies indicated that renal denervation (RDN) may improve central BP and decrease arterial stiffness in patients with resistant hypertension. The aim of the current study was to investigate whether RDN reduces central BP and arterial stiffness in patients with MetS.

Methods

In the current study, 26 patients with MetS were included and underwent RDN with measurements of central hemodynamics and arterial stiffness by means of pulse wave analysis (PWA) and pulse wave velocity (PWV), before and 12 months after the procedure. Patients were treated between March 2012 and August 2013 with subsequent follow-up. In all patients, 24-hr ambulatory BP measurements were used to evaluate the effect of RDN on BP.

Results

Mean 24-hr BP (SBP/DBP) decreased by 5±12/4 ±7 mm Hg 12 months after RDN (P=0.06/P<0.01). Peripheral BP decreased significantly by 9±14/4±9 mm Hg (P<0.01/P=0.02). Central BP decreased significantly during follow-up by 9±14/5±9 mm Hg (P<0.01/P=0.02). Although there was a trend, PWV did not change statistically during follow-up (from 12.0 (3.9) m/s at baseline to 10.2 (4.6) m/s at follow-up; P=0.13). Also, augmentation index remained unchanged during follow-up (from 27.5 (13.5) at baseline to 25.0 (13.5) mm Hg at follow-up; P=0.16).

Conclusions

The present study showed that RDN can positively influence central BP in patients with MetS. However, this was not accompanied with significant changes in arterial stiffness.

Introduction

Patients with the metabolic syndrome (MetS) have an increased risk for cardiovascular disease and type 2 diabetes, due to the combination of risk factors.[1] According to the statement of the American Heart Association, MetS is defined as the presence of three or more of the following five features: abdominal obesity, hyperglycaemia, hypertension, hypertriglyceridemia, and low HDL cholesterol levels .[2]

Patients with MetS show an increased arterial stiffness, mainly as a result of hypertension, central obesity, and hyperglycaemia.[3] Furthermore, arterial stiffness and central blood pressure (BP) have recently been identified as independent predictors of cardiovascular disease.[4] The increased arterial stiffness partially explains the increased cardiovascular risk in metabolic patients.[3] Reduction of BP is accompanied by a reduction of the risk for cardiovascular disease. The main treatment options to reduce BP are lifestyle changes and antihypertensive medication.[5] However, hypertension in patients with MetS is often resistant to antihypertensive drugs.[6] Because of the assumed constant elevation in activity of the sympathetic nervous system in patients with MetS,[7, 8] renal denervation (RDN) has been considered as a treatment option for hypertension and MetS (Verloop et al., submitted for publication). RDN has been designed to lower sympathetic activity and consequently BP in patients with resistant hypertension.[9]

A few studies showed that RDN may reduce arterial stiffness in patients with resistant hypertension.[10-12] The DREAMS-study prospectively investigated the effects of RDN on insulin sensitivity and blood pressure in patients with MetS (Verloop et al., submitted for publication). The current study is a substudy, investigating the effects of RDN on central BP and arterial stiffness in this patient cohort. We hypothesized that RDN will reduce central BP and arterial stiffness in patients with MetS.

Methods

Study population

All patients participating in the DREAMS study were asked to participate in this substudy. The inclusion and exclusion criteria for this study were similar to the main study (Verloop et al., submitted for publication). Patients were treated between March 2012 and August 2014 with subsequent follow-up of 12 months. The study protocol was carried out with the approval of the Ethics Committee of the University Medical Centre Utrecht, and all patients gave written informed consent in accordance with the Declaration of Helsinki.

Measurement of central blood pressure and arterial stiffness

Central BP and arterial stiffness were assessed at the day of the RDN procedure (baseline) and 12 months afterwards. Parameters of arterial stiffness were pulse wave velocity (PWV), augmentation pressure (AP), augmentation index (AIx), and central pulse pressure (PP). Pulse pressure was calculated as PP= SBP – DBP.

Peripheral BP was measured three times by an automatic oscillometric monitor (OMRON Healthcare Co. Ltd, Kyoto, Japan) on the right brachial artery. The highest BP measured was used for further analysis. Peripheral radial artery waveforms were registered by an applanation tonometer and analyzed using software of the SphygmoCor system (AtCor Medical, version 8.2). A validated generalized transfer function was used to generate the corresponding central aortic pressure waveform.[13] This transfer function allows physicians to perform non-invasive measurements of central pulse waves and is shown to strongly correlate with invasive central measurements.[14]

From the derived central waveforms, data were obtained to determine central BP and PP, augmentation pressure (the difference between the first and second systolic peak) and augmentation index (AP/PP ratio). By additionally registering carotid and femoral artery waveforms, PWV was calculated by dividing the pulse wave travel distance between carotid and femoral artery by the pulse wave transit time. Only PWV measurements with a standard deviation <15% of PWV at baseline and follow-up were included in the analysis. All measurements were performed by two experienced clinical assistants that were blinded for the BP response after RDN.

Blood pressure monitoring and renal function

At baseline and 6 and 12 months after RDN, ambulatory 24-hour BP measurements (ABPM) were taken using the Mobil-O-Graph device (IEM Healthcare, Stolberg, Germany), with readings taken every 15 min during day and every 30 min at night. Renal function was assessed at baseline and during follow-up. The estimated glomerular filtration rate (eGFR) was calculated on the basis of the CKD-EPI formula.[15]

Temporal medication stop

Eligible patients used a maximum of 1 antidiabetic and/or 1 antihypertensive drug. Since antihypertensive drugs influence the parameters of arterial stiffness and BP, we aimed to temporarily stop the antihypertensive drug when used during the baseline and follow-up visits as described previously.[16] The drug was stopped 2 weeks prior to the visit and restarted directly afterwards. Measurement of BP, PWA and PWV during a medication free interval enables to determine the net effect of RDN.

Renal denervation

Patients were treated using the Symplicity Flex device (Medtronic, Minneapolis, USA). A bilateral treatment of the arteries was performed using series of 2-minute radio frequency energy deliveries along each artery.[17, 18] The treatment points were made in a circumferential way with a minimum of 5 mm distance in between.

Data analysis

The differences between findings at baseline and 12 months after RDN were calculated and used for the present analysis. A negative value represents a beneficial change, i.e., a decrease in BP, PWV, and/or PWA measures.

Since PWV is dependent on age, we calculated the expected PWV values based on age and BP. We calculated the expected AIx values based on age, heart rate, gender, and height. For the expected values of PWV and AIx, we used the formulas as displayed in table 1.[19, 20] Subsequently, the observed PWV and AIx values were compared to the expected values as a reference.

Table 1: Formula to calculated expected PWV-values and expected AIx-values

Pulse wave velocity	
<30 yr.	PWV = 0.0472 x MAP + 2.20
30-39 yr.	PWV = 0.0423 x MAP + 2.20
40-49 jr.	PWV = 0.0646 x MAP + 1.41
50-59 yr.	PWV = 0.0731 x MAP + 1.35.
60-69 yr.	PWV = 0.0715 x MAP + 3.16
≥70 yr.	PWV = 0.0676 x MAP + 5.46
Augmentation index	
Men	AIx= 79.20 + 0.63 x age - 0.002 x age2 - 0.28 x HR - 0.39 x height
Women	AIx= 56.28 + 0.90 x age - 0.005 x age2 - 0.34 x HR - 0.24 x height

Statistical analyses

All variables were reported as mean ± standard deviation, median (interquartile range), or proportion when appropriate. Either the paired t-test or Wilcoxon signed rank test was used when appropriate for paired samples analysis. The unpaired t-test or Mann Whitney U test was used for non-paired sample analysis. Differences in proportions between non-paired groups were tested using the Chi-square test.

The relations between baseline characteristics or change in BP (independent variable) and the change in arterial stiffness (dependent variable) were analyzed with linear regression models. We only used those variables in the models, which we expected to influence the change in PWV or AIx. These were age, gender, baseline central SBP and change in central SBP (FU SBP – baseline SBP). Multivariate

linear regression models were used to adjust for age and gender. To avoid an overfitted model, the rule of thumb of 10 cases per variable was applied in the multivariate analysis.[21] A two sided *P* value of <0.05 was considered statistically significant. All analyses were performed with the SPSS statistical package version 20 (IBM SPSS Data Collection, Chicago, USA).

Results

Patient characteristics

Twenty-six patients underwent RDN in combination with measurements of central BP and arterial stiffness before and 12 months after the procedure. Mean follow-up duration was 362±7 days. The baseline characteristics are displayed in Table 2. The majority of the patients was male (n=16, 62%), patients had a mean age of 60±9 years with a mean 24-hr BP (SBP/DBP) of 145±12/89±10 mm Hg. We were able to perform all visits in a medication free interval in 23 patients. In three patients it was deemed unsafe to stop the antihypertensive drug at follow-up due to an increased BP.

Table 2: Baseline characteristics

	All patients (n= 26)
Age (yrs)	60 ± 9
Sexe (male/female)	16/10
Body-mass index (kg/m^2)	31.8 ± 4.9
eGFR (ml/min/1.73m^2)	84 ± 14
Smoking	
Current smoker	1 (4%)
Smoked in the past	17 (65%)
Comorbidity	
Diabetes Mellitus	4 (15%)
Hypercholesterolemia	16 (62%)
Cardiac vascular disease	3 (12%)
Cerebral vascular disease	1 (4%)
Office SBP (mm Hg)	162 ± 20
Office DBP (mm Hg)	96 ± 10
Mean 24-hr SBP (mm Hg)	145 ± 12
Mean 24-hr DBP (mm Hg)	89 ± 10
Mean 24-hr PP (mm Hg)	56 ± 8
Mean 24-hr HR (bpm)	75 ± 9
Mean daytime SBP (mm Hg)	151 ± 13
Mean daytime DBP (mm Hg)	93 ± 11
Antihypertensive medication	
Nr of antihypertensive drugs	1 (1.00-1.00)
ACEi/ARB/Renin inhibitor	3 (12%)
ß-Blocker	2 (8%)
Calcium-channel blocker	11 (42%)
α-Blocker	0 (0%)
Diuretic	1 (4%)
Central Acting	0 (0%)

Continuous variables are displayed as a mean (SD), except for number of drugs, this is displayed as median (interquartile range).Categorical variables are displayed as a number (percentage). Yrs indicates: years, TIA transient ischemic attack, CAD: coronary artery disease, ACEi: angiotensin-converting enzyme inhibitor, ARB: angiotensin II receptor blockTer, SBP: systolic blood pressure, DBP: diastolic blood pressure, PP: pulse pressure, HR: heart rate, bpm: beats per minute, eGFR: estimated glomerular filtration rate.
†Calculated on the basis of the Chronic Kidney Disease Epidemiology Collaboration formula.

Blood pressure, anthropometrics, and renal function after renal denervation

The changes in blood pressure, anthropometrics, and renal function are displayed in table 3. Mean 24-hr BP decreased significantly by 5±12/4 ±7 mm Hg 12 months after RDN (P=0.06/P<0.01; Table 3). During follow-up, mean BMI and mean abdominal waist did not change (table 3). Renal function improved 12 months after treatment (table 3). Medication use did not change during follow-up (from 1 (1.0-1.0) DU at baseline to 1 (1.0-1.33) DU at 12 months follow-up; P=0.50).

Table 3: Changes after renal denervation

	Baseline	12 months follow-up	p-value
Body-mass index (kg/m^2)	31.8 ± 5.0	31.2 ± 4.8	0.19
Abdominal waist (cm)			
Men	113 ± 13	112 ± 12	0.80
Women	110 ± 12	111 ± 12	0.82
eGFR (ml/min/1.73m^2)†	84 ± 14	88 ± 14	<0.01
Mean 24-hr SBP (mm Hg)	145 ± 12	140 ± 12	0.04
Mean 24-hr DBP (mm Hg)	89 ± 10	84 ± 8	<0.01
Mean 24-hr HR (bpm)	76 ± 9	74 ± 8	0.24
Augmentation pressure (mm Hg)	14.0 (9.0-19.3)	15.0 (7.8-19.0)	0.04
Augmentation index (%) (AP/PP)	27.5 (20.8-34.3)	25.0 (17.8-31.3)	0.16
Pulse wave velocity (m/s)	11.95 (10.0-13.9)	10.20 (8.5-13.1)	0.13

Continuous variables are displayed as a mean ± SD or median (IQR). SBP: systolic blood pressure, DBP: diastolic blood pressure, PP: pulse pressure, HR: heart rate, bpm: beats per minute, eGFR: estimated glomerular filtration rate. †Calculated on the basis of the Chronic Kidney Disease Epidemiology Collaboration formula.

Central blood pressure and arterial stiffness

Peripheral BP decreased significantly by 9±14/4±9 mm Hg (P<0.01/P=0.02, Figure 1). Central SBP and DBP also decreased significantly during follow-up by 9±14/5±9 mm Hg (P<0.01/P=0.02, figure 1). Central PP decreased significantly by 5±9 mm Hg (P=0.02). A higher baseline SBP was related to a more pronounced reduction in central SBP in the linear regression model (table 4).

The changes in arterial stiffness are displayed in table 3.

Valid assessment of PWV was possible in 12 (46%) patients. In 4 patients it was not possible to obtain a valid PWV due to obesity, in 10 patients the SD of PWV was more than 15% and therefore the measurements of these patients were invalid. In the 12 patients analyzed, there was a trend towards a drop in median PWV with a median difference of -0.75 (-3.3 – +0.4) m/s (P=0.13; table 3). Excluding the three patients who did not stop antihypertensive drugs at follow-up did not alter the data as these patients were already excluded based on invalid PWV measurements. The effect of RDN on PWV was significantly different between sexes (men -3.3 (-4.5 – -0.9) m/s versus women +0.5 (-0.6 – +2.7) m/s; P=0.01). As shown in table 4, female gender was related to an increase in PWV in the linear regression model. A change in PWV was not related to baseline central SBP, age, or change in central SBP.

Median augmentation pressure decreased during follow-up with a median reduction of 1.0 (-6 – +2) mm Hg (P=0.04, table 3). Median augmentation index did not change during follow-up with a median difference of -0.5 (-6 – +2) (P=0.16, table 3). No differences between sexes were observed in the effect of RDN on AIx (P=0.70). Excluding the patients that did not stop antihypertensive drugs at follow-up did not alter the results. A change in AIx was related to a change in central BP in both the univariate and multivariate linear regression model (table 4).

The observed PWV and AIx values were compared to expected values (table 5).[19] Compared to baseline, at follow-up there were less patients with an observed value of arterial stiffness that was higher than the expected value.

Figure 1: The change in peripheral and central blood pressure

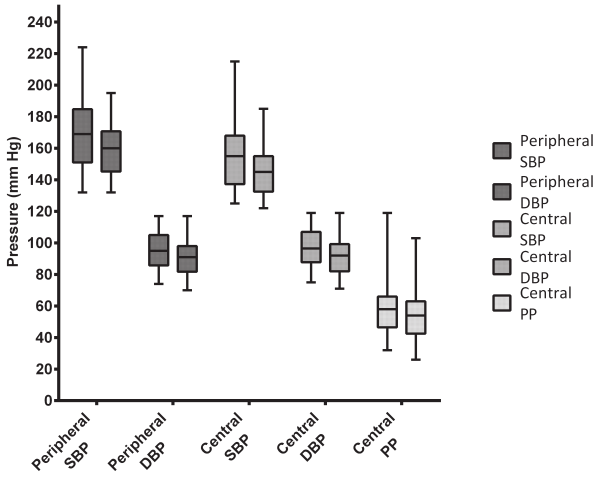

Table 4: The relations between change in central blood pressure or arterial stiffness and baseline characteristics or changes in central systolic blood pressure

	Univariate model			Multivariate model†		
	β	95%CI	p	β	95%CI	p
Change in pulse wave velocity						
Baseline central SBP	-0.050	-0.127 – 0.026	0.18			
Age	-0.023	-0.259 – 0.212	0.83			
Gender (female)	4.103	1.038 – 7.167	0.01	4.862	1.450 – 8.274	0.01
Change in central SBP	0.089	-0.021 – 0.198	0.10	0.026	-0.090 – 0.143	0.62
Change in central SBP						
Baseline central SBP	-0.352	-0.555 – -0.148	<0.01	-0.335	-0.550 – -0.121	<0.01
Change in augmentation index						
Age	0.135	-0.179 – 0.449	0.38			
Gender	1.512	-4.283 – 7.308	0.60			
Change in central SBP	0.218	0.035 – 0.402	0.02	0.221	0.020 – 0.421	0.03

Continuous variables are displayed as a mean ± SD or median (IQR). SBP: systolic blood pressure, DBP: diastolic blood pressure, PP: pulse pressure, HR: heart rate, bpm: beats per minute, eGFR: estimated glomerular filtration rate. †Calculated on the basis of the Chronic Kidney Disease Epidemiology Collaboration formula.

Table 5: Observed and expected values of pulse wave velocity and pulse wave analysis

		Observed	Expected
Pulse wave velocity	Baseline	12.0 (10.0-14.0)	10.3 (9.7-12.1)
	Follow-up	10.2 (8.5-13.1)	10.0 (8.9-11.1)
Augmentation index	Baseline	23.2 (19.5-25.4)	27.5 (20.8-34.3)
	Follow-up	24.2 (20.7-27.7)	25.0 (17.8-31.3)

Continuous variable are depicted as median (IQR)T.

Discussion

The present small, but prospective study in a homogenous patient cohort with MetS shows that RDN not only reduced ambulatory BP, but also central BP in patients with MetS. Although there was a trend, arterial stiffness did not change statistically during follow-up. It may well be that the study was underpowered to show a statistical significant difference in arterial stiffness after RDN, since it was a secondary outcome measure of the main study (Verloop et al., accepted for publication in Hypertension). Primary outcome of the main study (insulin sensitivity parameters) has been reported previously (Verloop et al., accepted for publication in Hypertension).

Since the parameters of arterial stiffness are considered to be independent predictors of cardiovascular disease,[4] it is important to learn whether these parameters are influenced by RDN in patients with MetS. To our knowledge, we are the first that performed measurements of central BP and arterial stiffness in a metabolic population treated by RDN. Previous studies investigated the effect of RDN on central BP and arterial stiffness in patients with resistant hypertension.[10-12] However, outcomes of these studies were not unambiguous.

Due to the fact that we did not include a control group, we cannot compare the observed results in the present study to the natural course regarding central BP and arterial stiffness. However, we calculated the expected values based on large cohort studies. These expected values represent the expected change in a natural course. These expected values were compared to the observed values. From the literature it is known that central BP and arterial stiffness deteriorate over time. Zieman et al. clearly set out that arterial stiffness develops from a complex interaction between stable and dynamic changes involving structural and cellular elements.[22] Main reasons for increase of arterial stiffness are aging, hypertension and diabetes mellitus.[22] In patients with MetS arterial stiffness has been observed across all age groups. A core feature appears to be insulin resistance due to the positive correlation between arterial stiffness and insulin resistance.[22]

Based on the metabolic status and insulin resistance of the patients included in the DREAMS study, one may expect that arterial stiffness will deteriorate over time in the present population. However, we do not know whether a period of one year is long enough to expect any effect of RDN regarding arterial stiffness. Potentially we should have performed the measurements of PWV and PWA after a longer period of follow-up. Ong et al. performed a meta-analysis on 15 randomized, controlled, double-blind trials including seven studies with short-term data (treatment duration 3 hours to 8 days) and nine studies with long-term data (treatment duration 1-6 months).[23] In both the short-term and the long-term trials a significant reduction in PWV was observed in the treatment arms compared to an increase in PWV in the placebo arms.[23] The long-term trials reported a more pronounced reduction in PWV compared to the short-term trials.[23] Ait-Oufella et al. followed 97 patients over a period of 5.3±1.3 years to investigate whether a reduction in arterial stiffness can occur after several years of antihypertensive treatment.[24] During the 5.3 years follow-up, PWV decreased by 3.17 m/s. The rate of change was -0.7±0.07 m/s per year.[24] This reduction per year is in line with the observations from the present study. Based on the studies of Ong et al. and Ait-Oufella et al., we believe that one year follow-up is sufficient to evaluate changes in arterial stiffness. However, future studies should consider following patients of a longer period of time.

Furthermore it should be mentioned that it is not clear whether the reduction in arterial stiffness after any antihypertensive treatment is caused by the BP lowering effect. It may also be that this effect is BP-independent and mediated by a reduction in the peripheral arteriolar resistance. Recently Laurent et al. demonstrated that higher dosages of olmesartan were able to significantly remodel and destiffen the arterial wall during long-term treatment and that this effect was partly independently of BP, compared to a lower dosage of olmesartan.[25] Based on the observations from the latter study, it may be that the observed effect on arterial stiffness in the present study was BP-independent since the observed effect on ABPM was only moderate.

The sympathetic nervous system (SNS) is increasingly considered as a regulator of not only short-term peripheral vasomotor tone and BP regulation, but also of long-term BP and systemic arterial stiffness.[26] Several complex mechanisms including vascular wall composition and smooth muscle cell tone can be mediated by the SNS. Overactivity of the SNS may therefore have a detrimental effect on arterial stiffness. Casey et al. demonstrated a positive relationship between sympathetic activity and augmentation index in men.[27] However, this relation was inverse in women.[27] In line with this positive relationship in men, we observed a significant difference between sexes favouring a positive effect on PWV in men (P=0.01). However, we could not confirm this gender difference in AIx (P=0.70). This observation is in line with another study from Caset et al. that also found no difference in AIx between sexes after β-adrenergic blockade.[28] Casey et al. advocated that the offset of arterial stiffness in women is influenced by other factors than the SNS. The observed gender difference in PWV after RDN in the present study strengthens this hypothesis. However, the absence of a gender difference in change of AIx argues against it. Both studies illustrate that the available literature regarding the effect of sympathetic inhibition and arterial stiffness is scarce. Secondly, the present study was underpowered to perform a reliable analysis for gender differences. Moreover, measures of sympathetic activation should be included to be able to state something about a possible positive or negative relation. In the main paper of the DREAMS we studied systemic sympathetic activity before and after RDN in a subpopulation of 10 patients. In this subpopulation undergoing MSNA changes in PWV or AIx were not related to a change in muscle sympathetic nerve activity (MSNA) (Verloop et al., accepted for publication in Hypertension). The subpopulation that underwent MSNA was too small to investigate whether gender was a determinant for a relation between change in sympathetic activation and changes in arterial stiffness. Future studies investigating the effect of sympathetic inhibition on arterial stiffness should consider including measures of sympathetic activity to investigate whether a reduction in sympathetic activity is related to a reduction in arterial stiffness in men and/or women.

Among the possible explanations for the lack of effects of RDN on arterial stiffness, it is likely that the present study was simply underpowered to detect any significant changes. Another explanation may be that we did not select patients likely to benefit from RDN. As mentioned, we evaluated the average arterial status by looking at the reference ranges. Only 73% of the patients had a higher AIx at baseline than expected. This reflects the fact that our population did not have severe arterial stiffness, compared to a patient cohort described before.[10] However, the MSNA values in this population were high implicating that the population had a high sympathetic activity at baseline (Verloop et al., accepted for publication in Hypertension). Therefore we believe that we did select patients likely to benefit. Potentially the gender difference was a determinant for the observations from the present study. In the present study 62% of the patients were male. In the study from Brandt et al, 70% of patients in the RDN group and 80% of the control patients were male. Given the relation between SNS and arterial stiffness in men,[27] it may be that the higher percentage of male patients in the Brandt study explained the more pronounced effect in arterial stiffness.

The Sphygmocor system provides, next to augmentation pressure and AIx, also the AIx normalized for a heart rate of 75 beats per minute (bpm). This normalized AIx is called AIx@75bpm. It has been matter of debate whether the AIx or AIx@75bpm should be considered more appropriate. Recently Stoner et al. performed a meta-analysis in 12 studies and they concluded that AIx is more appropriate.[29] Authors stated that the use of AIx@75bpm may be physiologically and statistically inappropriate based on the important physiological chronic interaction between heart rate and arterial stiffness.[29] Based on this review we considered the AIx to be more reliable and therefore did not analyse the AIx@75bpm.

Limitations and strengths

The major limitations of this study are the small sample size (26 patients) and the lack of a control group. Also, we cannot speculate about clinical outcome for this population after RDN since we only have follow-up up to 12 months. Therefore it would be interesting to design a trial with a larger cohort and longer follow-up, or to follow-up these patients for two more years.

A strength of the current study is that the measurements were performed under standardized circumstances i.e., a medication free interval. It might be that medication did influence other studies, where medication-use is not clearly described and no temporary stop was planned. A second strength is that we combined the ABPM and central BP measurements to evaluate the effect of RDN on BP. The measurement of central BP in hypertensive patients raises increasing interest because of both its predictive value for cardiovascular events and the differential effect of antihypertensive drugs, compared with brachial BP.[5]

In the light of the Symplicity HTN-3 trial we believe that this study is all the more relevant. The early clinical studies investigating RDN reported impressive reductions in blood pressure and measures of end organ damage. However, more recently the HTN-3 trial among others showed disappointing results. We believe that more research in the field of RDN is needed before this technique should be implemented widely in clinical daily care.

In conclusion, this study showed that RDN positively affects central BP in even a small patient population. However, no statistical drop in arterial stiffness could be shown. Potentially, we halted further deterioration. However, this is of course highly speculative. To investigate this topic further, a larger, controlled trial is needed with longer follow-up to observe whether improved arterial stiffness has a positive influence on cardiovascular outcome. Moreover, future studies should emphasize on the relation between the sympathetic nervous system, augmentation index and possible gender differences.

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A systematic Review concerning the Relation between the Sympathetic Nervous System and Heart Failure with Preserved Left Ventricular Ejection Fraction

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Abstract

Background

Heart failure with preserved left ventricular ejection fraction (HFPEF) affects about half of all patients diagnosed with heart failure. The pathophysiological aspect of this complex disease state has been extensively explored, yet it is still not fully understood. Since the sympathetic nervous system is related to the development of systolic HF, we hypothesized that an increased sympathetic nerve activation (SNA) is also related to the development of HFPEF. This review summarizes the available literature regarding the relation between HFPEF and SNA.

Methods and results

Electronic databases and reference lists through April 2014 were searched resulting in 7722 unique articles. Three authors independently evaluated citation titles and abstracts, resulting in 77 articles reporting about the role of the sympathetic nervous system and HFPEF. Of these 77 articles, 15 were included for critical appraisal: 6 animal and 9 human studies. Based on the critical appraisal, we selected 9 articles (3 animal, 6 human) for further analysis. In all the animal studies, isoproterenol was administered to mimic an increased sympathetic activity. In human studies, different modalities for assessment of sympathetic activity were used. The studies selected for further evaluation reported a clear relation between HFPEF and SNA.

Conclusion

Current literature confirms a relation between increased SNA and HFPEF. However, current literature is not able to distinguish whether enhanced SNA results in HFPEF, or HFPEF results in enhanced SNA. The most likely setting is a vicious circle in which HFPEF and SNA sustain each other.

Introduction

Heart failure with preserved left ventricular ejection fraction (HFPEF) affects about half of all patients with a clinical presentation of heart failure (HF).[1, 2] There is no consensus concerning the definition of HFPEF. The European guidelines define HFPEF as a clinical syndrome in which classical HF symptoms are present, accompanied by a normal or only mildly reduced left ventricular (LV) systolic function.[3] Using this definition, HFPEF becomes a mixed collection of different underlying causes of HF. The American Heart Association (AHA) guidelines and the consensus statement of the European Society of Cardiology (ESC) define HFPEF as a clinical HF state, which is accompanied by objective evidence of diastolic dysfunction (DD).[4, 5]

Irrespective of the definition, we still have much to learn about HFPEF. This is all the more important since no successful treatment is available yet.[3, 4] A number of studies have been conducted investigating different pharmacological treatment strategies for HFPEF. Unfortunately, these studies failed to provide unambiguous results.[6-10]

Even though HFPEF has been the focus of various mechanistic studies, the exact pathophysiology is still unknown.[11] It is generally accepted that HFPEF is characterized by prolonged isovolumic LV relaxation, slow LV filling, and an increased diastolic LV stiffness.[12] The consequent impairment of diastolic filling leads to an inappropriate pressure increase after volume load.[13] Eventually, this may lead to heart failure.[14, 15] The sympathetic nervous system (SNS) may play an important role in the genesis of HFPEF when accompanied by DD.[16] The underlying structural changes in the myocardium seen in HFPEF include the same spectrum of changes associated with catecholamine-induced cardiomyopathies.[17, 18]

However, while the role of the increased sympathetic nerve activity (SNA) in the development and progression of HF with reduced ejection fraction (HFREF) is well established[19, 20], to our knowledge, no systematic review has yet evaluated the relationship between SNA and HFPEF. Therefore, the objective was to systematically evaluate the role of SNA in HFPEF. In this respect, only HFPEF in combination with DD is taken into account.

The activity of the SNS can be measured in different ways. Examples are measurement of plasma or urinary norepinephrine (NE) level, assessment of local NE spillover, muscle sympathetic nerve activity (MSNA), iodine 123-metaiodobenzylguanidine (MIBG), or heart rate variability (HRV).

Methods

Search strategy

This systematic review was conducted and reported in accordance with the "preferred reporting items for systematic reviews and meta-analyses" (PRISMA) statement.[21] We conducted a systematic review to determine if there is a relationship between the sympathetic nervous system and heart failure with preserved LVEF. All available literature in the PubMed, Embase and Cochrane databases was searched using a pre-defined search strategy (appendix S1). A librarian checked the syntax before the search was conducted. The titles and abstracts of the retrieved articles were reviewed by three authors (WLV, MMAB, BTS). Full-text papers were retrieved from abstracts selected for further review. The references of these papers were also reviewed to identify relevant articles that may have been missed by the search strategy, e.g. studies that were not found due to negative results. If necessary, individual researchers were contacted by e-mail to obtain the full text, or to enquire about unpublished or unreported results.

All full-text articles were reviewed by 3 authors (WLV, MMAB, BTS) using pre-defined inclusion/exclusion criteria (Table 1). Articles were only included when the inclusion criteria of HFPEF were clearly defined. Only articles that included DD in the definition of HFPEF were included, studies about HFPEF based on valvular dysfunction or other disease entities were excluded. Citations from journals in languages other than English were not included. No pre-specified limitations were placed regarding species (human or animal) or NYHA functional class. Individual case reports, editorials, expert opinions, and review articles were excluded, as were studies regarding the diagnosis or treatment of HFPEF. Studies investigating the

prognosis of patients with HFPEF were also excluded. To minimize the risk of multiple publication bias, we only included publications with a pathophysiological objective when they contained original data. We pre-specified the data to be extracted from the included studies before reading the articles. Two authors (WLV, MMAB) independently extracted these data and listed them in a table.

Table 1: Pre-set inclusion and exclusion criteria

Inclusion criteria	Exclusion criteria
Investigating the relationship between HFPEF and the sympathetic nervous system	Investigating systolic heart failure
	Only investigating LVH without giving information about diastolic dysfunction
Investigating the relationship between diastolic dysfunction and the sympathetic nervous system	No original data (i.e. review, expert opinion)
	Study does not investigate relation SNA and HF
	Only abstract
	Full text in language other than English
	Therapeutic study
	HFPEF based on valve dysfunction, myocardial ischemia or hypertrophic cardiomyopathy
	Prognostic study

Critical appraisal and analysis

Critical appraisal was independently performed using pre-set criteria. These criteria are outlined in table 2 and table 3. In advance we decided only to include an article in the final analysis if it scored at least half of the maximum available points. Since we expected that there would be a large diversity in outcome measures, no pre-defined principal summary measures were composed. Studies were graded for the modality used to measure sympathetic activity, as reliability of these modalities differ.[22, 23] Local NE spillover, MSNA, and MIBG are considered as the best and most direct measures of sympathetic activity.[24, 25] Serum levels of NE and HRV are an indirect measure of SNA and are considered as less qualitative measures of sympathetic activity.[23-25]

Results

The search was conducted on October 31st, 2013 and identified 7722 unique articles. The search was updated on April 10th, 2014. A flowchart of the search is depicted in figure 1. After screening the titles and abstracts 77 articles remained that met the criteria for full text review. Fifteen full-text articles (6 animal studies; 9 human) were considered relevant to the study and were included in the critical appraisal.[26-40] A summary of these 15 studies is outlined in appendix S2.

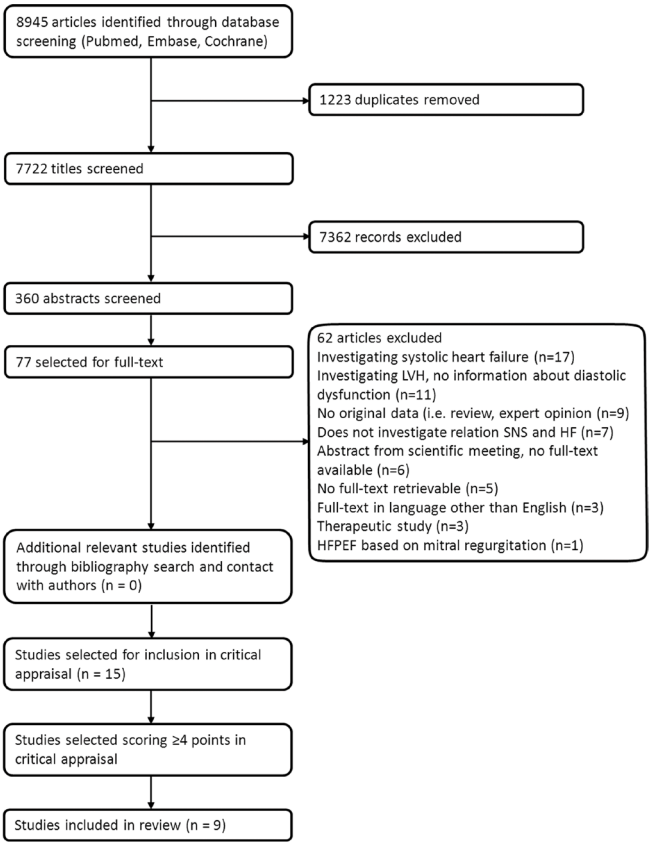
Animal studies

The animal studies that met the criteria for critical appraisal investigated whether a change in activity of the sympathetic nervous system might induce diastolic dysfunction. All animal studies used a model of isoproterenol (ISO) to mimic an increased SNA. Isoproterenol is a non-selective β-adrenergic agonist structurally similar to epinephrine. The assumed increase in SNA was not measured in any of the animal studies.

Three animal studies scored high (4 points or more) in the critical appraisal and were therefore further evaluated.[26, 27, 31] Rejected studies scored less than half of the available points mainly because the study-aim was insufficiently focused on DD or HFPEF.

In brief, the three selected animal studies demonstrated that administration of a β-adrenergic agonist established diastolic dysfunction in an experimental setting: the first study is the study from Grimm et al. Authors observed that dosages up to 150 mg/kg ISO led to diastolic dysfunction in mice as evaluated by echocardiography.[27] Dosages higher than 150 mg/kg led to systolic heart failure or death.[27] In the second study: the study of Brooks et al. an abnormal LV diastolic pressure-volume (PV) relationship and an increased myocardial stiffness in mice treated with 10 mg/kg ISO for 5 days was observed

Figure 1: Flowchart of the search



without impairment of systolic function.[26] The third study: the study of Yoshikawa et al. treated rats with 2.4 mg/kg/day ISO for 7 days and observed a significant increase in fibrosis, accompanied by an increase in LV hypertrophy by histology and a decrease in diastolic function by echocardiography.[31]

A major drawback of these three studies is that the percentage of animals in which ISO administration did or did not lead to DD or HFPEF ('responder rate') was not clearly reported. Impairment of DD was already observed after a dosage of 2.4 mg/kg/day ISO for 7 days. A single dosage of 150 mg/kg ISO also induced DD. Higher dosages led to systolic heart failure.

Human studies

No studies could be found that prospectively studied a human cohort whether an increased SNA leads to HFPEF during follow-up. In most human studies evaluated by the critical appraisal, patients were included that already had DD based on echocardiographic data. Therefore, the natural progression to HFPEF could not be studied.

All studies were hindered by a small sample size of patients (max. 34 patients). Another limitation is that not all studies reported the diastolic parameters: Sugiura and Arora et al. included HF-patients with a good ventricular function (LVEF>45%) irrespective of diastolic parameters.[32, 38] Echocardiographic examination was often limited to E/A ratio and deceleration time, despite that E/e' is nowadays considered a more reliable parameter.[5] An explanation may be the evolving guidelines on assessment of diastolic function that did not involve E/e' at the time the first studies were conducted. For the assessment of SNA, different entities were used in the human studies (i.e. heart rate variability (HRV), MSNA, MIBG, or norepinephrine levels). Six out of nine human studies scored 4 points or more in the critical appraisal and were therefore further evaluated.

In brief; the 6 human studies selected for further evaluation confirmed a relationship between SNA and HFPEF. Arora et al. observed that patients with HFPEF exhibit a reduction in HRV compared to control subjects.[32] Patients with HFREF had more decreased HRV values compared to patients with HFPEF. In the study of Grassi et al. abnormal baroreflex modulation and increased MSNA-levels were observed in hypertensive patients with DD compared to hypertensives without DD and normal controls.[33] Nixdorff et al. observed an increase of peak early (E-wave) and late (A-wave) diastolic filling velocities and a shortening

of deceleration time after administration of even the lowest dose of ISO (0.1 ug/min).[35] Piccirillo et al. performed HRV and observed that hypertensives with diastolic dysfunction have a higher sympathetic and lower vagal modulation of the sinus node compared to hypertensives without DD and normotensive controls.[36] deSouza et al. observed that patients with HFPEF had higher MSNA values compared to hypertensive patients with normal diastolic function although HRV values were similar among the groups.[37] In the study of Sugiura et al. it was concluded that cardiac SNA as assessed by MIBG increases proportionally with severity of HFPEF.[38]

Table 2: Critical appraisal of animal studies

First author, year	Study aim	Clearly defined hypothesis	Model to induce HFPEF	Assessment of diastolic dysfunction	Assessment of sympathetic activity	Clear report of findings	Value of study	Score
Grimm, 1998	+	+/-	+	+	+/-	+/-	+	4
Krishnamurthy, 2007	-	+/-	+	+	+/-	+/-	+/-	1
LaCroix, 2008	-	+	+	+	+/-	+	-	2
Brooks, 2009	+	+/-	+	+	+/-	+	+/-	4
Ma, 2011	-	+/-	+	+	+/-	+/-	+/-	1
Yoshikawa, 2012	+	+	+	+	+/-	+/-	+/-	4

Study aim:*+: study is focused on interpreting the relation between sympathetic activity (SNA) and diastolic dysfunction (DD); -: study is not focused on interpreting the relation between SNA and DD.* **Clearly defined hypothesis:** *+: hypothesis clearly defined; +/-: aim of study clearly defined, no hypothesis formulated; -: no clear aim nor hypothesis.* **Model to induce HFPEF:** *+: ISO infusion; -: transaortic constriction.* **Assessment of diastolic dysfunction:** *+: invasive measurement of LV diastolic filling pressures or echocardiographic evaluation of DD according to latest ESC guidelines; +/-: echocardiographic evaluation without use of E/E'; -: confirmation of normal LVEF only.* **Assessment of sympathetic activity:** *+: yes; +/-: no.* **Clear report of findings:** *+: results clearly described AND critical about own research; +/-: results clearly described OR critical about own research; -: results not clearly described AND not critical about own research.* **Value of study:** *To what extent is the study relevant to answering the current question.*
The score displayed in the right column is the sum of scores: "+" accounts for 1 point; "+/-" for 0 points; "-" for -1 point.

Table 3: Critical appraisal of human studies

First author, year	Study design	Number of patients	Study aim	Clearly defined study aim	Patient selection	Assessment of diastolic dysfunction	Assessment of sympathetic activity	Clear report of findings	Value of study	Score
Nixdorff, 1997	Cohort	10	++	+	N/A	+/-	+/-	+	+/-	4
Hirono, 2001	Cohort	26	-	-	+	+/-	+/-	+	-	-1
Vinch, 2003	Cross-sectional	14	-	+	+	+/-	-	+/-	-	-1
Arora, 2004	Cross-sectional	19	++	+	+	-	+/-	+	+	5
Piccirillo, 2006	Cross-sectional	30	++	+	+	+/-	+/-	+	+	6
Sugiura, 2006	Cohort	34	+	+	+	+/-	+	+	+	6
Tsuchida, 2007	Cross-sectional	8	-	+	+	-	+	+	-	1
Grassi, 2009	Cross-sectional	17	+	+	+	+/-	+	+	+	6
deSouza, 2013	Cross-sectional	15	+	+	+	+	+	+	+	7

Number of patients =: *Number of patients with diastolic dysfunction/HFPEF.* **Study aim:** *++: study is focused on interpreting the relation between SNA and DD* **AND** *patient selection was clearly explained (diastolic dysfunction defined and not just distinction between LVEF </> 45%)* **AND** *data collection was clear. +: study is focused on interpreting the relation between SNA and DD* **AND** *patient selection was clearly explained* **OR** *data collection was clear; -: study is focused on interpreting the relation between SNA and DD* **OR** *patient selection was clearly explained* **OR** *data collection was clear; --: study is not focused on interpreting the relation between SNA and DD* **AND/OR** *patient selection was not clearly explained* **AND/OR** *data collection was not clear.* **Patient selection:** *+: Sole HFPEF or clear distinction between HFPEF and HFREF; -: no clear distinction between HFPEF and HFREF.* **Assessment of diastolic dysfunction:** *+: invasive measurement of LV diastolic filling pressures OR echocardiographic evaluation of DD according to latest ESC guidelines; +/-: echocardiographic evaluation without use of E/E'; -: confirmation of normal LVEF only.* **Evaluation of sympathetic activity***: ++: NE-spillover locally measured; +: MSNA OR MIBG; +/-: HRV or adrenergic stimulation; -: plasma NE concentration.* **Clear report of findings:** *+: results clearly described AND critical about own research; +/-: results clearly described OR critical about own research; -: results not clearly described AND not critical about own research.* **Value of study:** *To what extent is the study relevant to answering the current question.*
The score displayed in the right column is the sum of scores: "+" accounts for 1 point; "+/-" for 0 points; "-" for -1 point.

Discussion

To our knowledge, this is the first systematic review investigating the relationship between sympathetic nerve activity and HFPEF in combination with DD. Based on the animal studies we concluded that administration of ISO and therefore increased SNA is related to HFPEF in an experimental setting. Based on the human studies, we concluded that an increased SNA - irrespective of the method of assessment - is indeed related to diastolic dysfunction and/or true HFPEF. However, the available literature about this topic is very scarce, let alone that results could be pooled. Moreover, administration of ISO in animals is not exactly the same as the increase in SNA in humans. However, no animal model to induce HFPEF has been accepted so far and we consider ISO administration the best available at present.

As mentioned above, different definitions are used for HFPEF.[3, 5] In the current review, the recommendations from the AHA guidelines and the ESC consensus document were followed, thereby excluding HFPEF based on hypertrophic cardiomyopathy among others.[4, 5] We chose to follow this line in order to obtain a set of studies with a more or less homogeneous patient population.

In the human studies, different ways of assessing sympathetic activity were used. The most straightforward method to measure SNS is measurement of plasma or urinary (NE) level. Plasma NE concentrations however are a resultant of removal rates and not selectively release rates.[41] Also, the precise origin of urinary NE levels is a matter of debate. Assessment of local NE spillover by a radiotracer technology displays the rate at which NE is released from the sympathetic nerves into the circulation. This is quantified by intravenous infusion of titrated NE combined with regional sampling. MSNA is a real-time measure of sympathetic nerve activity. Multiunit recordings of efferent postganglionic MSNA are obtained with a tungsten microelectrode into a muscle fascicle of the peroneal nerve.[42] MIBG imaging uses a norepinephrine analogue labeled with a radioactive isotope to image adrenergic receptors in many organs, including the heart.[43] MIBG imaging has been shown a very reliable marker of sympathetic activity in both HFPEF and HFREF disease states.[44] Moreover, Nakata et al demonstrated the long-term prognostic value of altered cardiac sympathetic function as assessed by MIBG imaging in HF patients.[45] HRV displays the variability of the resting heart rate and is a measure of the balance between the sympathetic- and parasympathetic nervous system.[46] HRV is linked through the baroreceptor reflex and a more indirect way to measure SNA.[23] Not all ways of SNA measurement are even reliable. This supposed difference in reliability was taken into account in the critical appraisal. Although NE-spillover and MSNA are among the most reliable ways to measure SNA, they are semi-invasive and time-consuming. Moreover, MSNA is hard to obtain in small animals like mice.[25] MIBG washout rate has a strong correlation with MSNA and therefore allows non-invasive assessment of general sympathetic nerve activity.[24] In the studies selected for critical appraisal, local NE spillover was not used as a method to quantify sympathetic activity.

The study of Grassi et al. showed conflicting results; no difference in plasma NE-concentration was observed whereas MSNA values were altered in the patients with DD.[33] In our analysis, the results of MSNA were taken into account whereas the NE-results were not due to the limited sensitivity of these markers of sympathetic tone. In the studies of Arora et al. and Piccirillo et al., HRV was assessed to obtain information about the parasympathetic nervous system.[32, 36] In the critical appraisal these studies scored less due to the use of HRV. Since HRV is an indirect measurement of SNA, it is not known whether the results of Arora et al. may lead to an over- or underestimation of the relation between HFPEF and SNA.[23]

Can the results be explained by more diseased states?

From a critical point of view, some experts argued that the increased SNA in patients with DD can be attributed to the higher BP since that is often present in patients with DD.[47] To respond to this criticism, Grassi et al. and Piccirillo et al. only included patients with similar blood pressure (BP) levels.[33, 36] Based on these 2 studies, we argue that increased sympathetic activation seen in DD is not attributable to a more diseased hypertensive state.[33, 36] As left ventricular hypertrophy (LVH) is also related to an increased

SNA and often present in DD, the presence of LVH may have caused us to overestimate the increased SNA.[48] Yet Grassi et al. found similar LV masses among both hypertensive groups (with and without DD).[33] Since β-adrenergic stimulation often causes both HFPEF and HFREF, SNS-induced HFPEF could be a precursor state of HFREF or both diseases could be entities resulting from increased SNA.[49] The study of Grimm et al. contributes to this discussion by showing systolic HF after administration of higher dosages of ISO.[27] Seeland et al. described comparable results in a mouse model undergoing adrenergic stimulation: five months after stimulation LVH was observed in all mice.[50] However, 12 months after stimulation ventricular dilatation and accompanying systolic dysfunction was observed in all mice.[50] More recent studies, however, have made clear that the two diseases are indeed two separate entities.[49, 51]

Diastolic dysfunction and SNA; the chicken or the egg?

As Rosendorff previously discussed, it is not yet clear whether DD potentiates the sympathetic activation or whether the increased SNA causes DD.[47] Based on their results, Grassi et al. concluded that DD enhances the already elevated MSNA.[33] However, the authors admitted that their data did not allow them to determine whether the greater SNA observed in patients with DD is the cause or the consequence of the cardiac alteration.[33] Other studies included in the current review concluded that it should be the other way around.[26, 35] Moreover, sympathico-inhibition has shown to delay the progression of DD.[52-55] Finally, Leite-Moreira et al. showed that β-adrenergic stimulation influences cyclic AMP, resulting in a changed diastolic relaxation.[56] Rosendorff set out 2 plausible ways that increased SNA causes DD: an indirect and a direct way. In the indirect way SNA induces hypertension, which imposes a mechanical load on the LV and consequent stiffening of the ventricles.[47] In the direct way, SNA has a direct effect on both hypertension and diastolic dysfunction.[47] Increased SNA activity plays a role in cardiac remodeling.[57] This is illustrated by the fact that sympathetic stimulation can induce pro-inflammatory cytokine expression[58] and can induce alterations in the sarcoplasmic reticulum, plasma membrane, and cytoskeletal proteins.[12, 56] However, one crucial question cannot be answered with the above theories: what is the trigger for an increased SNA? A mechanism is needed that is responsible for SNS stimulation. In HF*R*EF an ischemic model can be the precursor of increased SNA.[59] However, in HFPEF ischemia is often not present as a precursor of SNA.[4]

With the current available evidence, we cannot simply state that one causes the other. Paulus et al. has clearly set out that different comorbidities contribute to a systemic inflammatory state, which induces oxidative stress in the coronary microvascular endothelium. [60] The presence of reactive oxygen species (ROS) is important in the paradigm proposed by Paulus et al.[60] ROS are strongly believed to be related to increased SNA.[61, 62] We believe that a vicious circle is present in which HFPEF and SNA sustain each other. It is highly likely that other factors like the metabolic syndrome or renal ischemia are triggers for this circle.[63]

The effect of sympathico-inhibition on HFPEF and DD

Until now, no treatment has yet convincingly shown to improve clinical status, morbidity and mortality in HFPEF.[6, 64] It is of relevance whether therapies targeting the sympathetic nervous system are successful in HFPEF. Based on their sympatholytic effect, beta-blockers may be useful in HFPEF. The SENIORS trial suggested that nebivolol may be beneficial in elderly patients with HFPEF.[65] As a derivative of SNA, angiotensin II may be a target for treatment. While RAS inhibition has been shown to reduce SNA,[66] the CHARM study found no clear benefit in patients with HFPEF treated by RAS inhibition.[67] Varying results were reported in other studies investigating the effect of RAS-inhibition in patients with HFPEF: in a meta-analysis, RAS-inhibition was not associated with consistent reduction in HF hospitalization or mortality in HFPEF-patients.[68]

Echocardiography is a reliable tool to objectively assess diastolic function. The effects on echocardiography should be taken into account when conducting a therapeutic study in a HFPEF

population. Echocardiographic parameters have been used in some studies investigating aldosterone antagonists, beta-blockade, exercise training, and RAS-inhibition. Although some studies reported an improvement in clinical state, no clear effects on echocardiographic parameters were observed.[10, 69, 70] Based on our results, it is plausible that modulation of SNA can improve the clinical status of patients with HFPEF. Unfortunately, no studies investigating a treatment for HFPEF have evaluated SNA after treatment. One study of interest is that of Brandt et al. who recently showed an improvement in diastolic function after renal denervation.[71] The authors established a decrease in sympathetic activity by renal denervation and consequently observed improvement in echocardiographic parameters.[71] Therefore, renal denervation may be an attractive option for the treatment of HFPEF by disrupting the vicious circle between HFPEF and hyperactive SNA.

Limitations of the current review

First of all, the available evidence is limited and heterogeneous in design. This heterogeneity may have influenced our results. We tried to uniform the different studies by using the critical appraisal.

Although our search strategy was extensive and also focused on studies that showed HFPEF and SNA to be unrelated, it is possible that our search resulted in a relative over-representation of positive studies. By checking references of selected articles we tried to obtain studies that reported negative findings about the suggested relation. This search did not result in any relevant full text articles.

In the human studies, we could not report about medical history, medication use and other determinants for heart failure because these determinants were not clearly reported. It should, however, be taken into account that most patients studied were treated with drugs that affect sympathetic activity. Since most antihypertensive drugs (indirectly) lower SNA[72], the currently observed relation between SNA and HFPEF may be an underestimation. Potentially, an even stronger association between SNA and HFPEF does exist.

Not all human studies included patients with HFPEF; half of the human papers studied patients with DD instead of HFPEF. However, DD is an important, if not the main, precursor of HFPEF. Moreover, Sugiura et al. showed a higher cardiac sympathetic activity in HFPEF patients with a higher NYHA class.[38] Though, at this point we should keep in mind that the clinical symptoms in HFPEF are not solely explained by DD, but can also be explained by reduced chronotropic, vasodilator, and cardiac output reserve during exercise.[73]

A possible confounding factor in this review is that HFPEF-patients are more often older. Age itself is related to sympathetic activity and may be a confounder in the available literature.[4] Selected studies did not report whether they corrected for the baseline characteristics. To complicate matters, HFPEF has only recently been recognized as an important clinical problem and preserved ejection fraction was previously often considered as a diagnosis of exclusion.[16] Therefore the available literature is less extensive compared to HFREF.

Conclusion

Based on our results, we conclude that current literature confirms a relation between increased SNA and HFPEF. However, current literature is not able to distinguish whether enhanced SNA results in HFPEF, or HFPEF results in enhanced SNA. The most likely setting is a vicious circle in which HFPEF and SNA sustain each other. Disruption of this vicious circle may be an attractive treatment option.

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General discussion

The purposes of this thesis were to understand the working mechanism behind renal denervation (RDN), to determine which patients benefit most from treatment, and to study the effects of RDN. The thesis is subdivided into four parts. **Part I** is focused on the pathophysiologic studies of the working mechanism behind RDN and possible markers of successful therapy. **Part II** is focused on the selection of eligible patients for RDN. **Part III** is focused on the effects of RDN in hypertensive patients. **Part IV** is focused on the effects of RDN beyond resistant hypertension.

Renal denervation, how does it work and how do we know if it has been successful?

The sympathetic tone is chronically elevated in resistant hypertension.[1] The kidneys and especially the renal sympathetic nerves contribute to a state of elevated sympathetic activity.[1] The SNS comprises both efferent and afferent nerves. Based on the involvement of the sympathetic nervous system (SNS) in resistant hypertension, RDN has been developed as a new treatment option for patients with resistant hypertension. It is likely that both afferent and efferent nerves are affected by RDN. At present, it is not exactly known what the effect is of disruption of the renal nerves in the kidneys itself and in the feedback going through the afferent nerves to the brain. We hypothesized that RDN will lead to a decreased microvascular resistance in the kidneys itself and a consequent increase in renal blood flow. In *Chapter 2* we tested this hypothesis in a porcine model. We observed that microvascular resistance decreased and renal blood flow increased directly after RDN. At follow-up the renal resistance reserve (RRR) decreased even further in the treated arteries. However, all other initial effects on microvascular resistance and renal blood flow had disappeared after follow-up of three to twelve weeks. Next to the effects on renal haemodynamics we studied the histological changes after RDN. We observed that RDN induced a variety in vascular damage to and around the artery wall and a limited penetration up to 2 mm deep. Moreover, a large part of the renal nerves were remote to the vascular lumen and therefore not reached by the RF-energy. We evaluated whether vascular changes were related to hemodynamic changes and observed a relation between a reduction in RRR and more severe adventitial damage. This may imply that RDN is only effective when the RF-energy is delivered deep enough.

Based on the observations in pigs, we started a prospective study, as described in *chapter 3*, to evaluate whether similar hemodynamic changes could also be observed in hypertensive patients directly after RDN. We hypothesized that the positive effects observed in the porcine model directly after RDN would be more prominent in hypertensive patients since they are subject to a long lasting hypertension and hindered by an elevated sympathetic activity. Previous studies investigated a model of sympathetic stimulation to observe the effect of RDN directly after the intervention.[2, 3] Chinushi et al. showed that before RDN stimulation of the renal nerves within the renal artery led to an increase in blood pressure (BP).[3] After RDN, stimulation no longer established a higher BP. Although these results were very promising, no translation to the clinical practice has been made so far. Our hypothesis in *chapter 3* was that RDN would positively influence microvascular resistance and renal blood flow. Secondly, we aimed to investigate whether a changed microvascular resistance would be a marker of a successful treatment. Directly after RDN, we observed a reduction in the arteriolar resistance. However, the velocity of the blood flow remained the same at a reduced pressure in the aorta. These results may imply that indeed an increase in blood flow has occurred since the blood flow remained stable despite a pronounced reduction in aortic pressure. Normally, the blood flow follows the pressure of the aorta; a consequent decrease of the flow would therefore be expected. Now that the velocity remained stable seems to indicate a relative increase. Moreover, we observed that acute changes in HMR and AR following RDN were significantly associated with the change in systolic BP (SBP) during follow-up, independent of the magnitude of baseline SBP. These findings suggest that invasive assessment of the immediate physiological response to renal denervation can provide markers of a successful renal denervation procedure, or an adequate patient response to RDN. Future studies should further investigate this topic as to explore whether a change of hyperaemic resistance actually is a per procedural marker for successful denervation.

In *chapter 4* we reviewed the available literature for the role of reactive oxygen species in sympathetic hyperactive states. Based on the literature we concluded that local kidney ischemia may be causative in the production of ROS. The production of ROS may consequently play a central role in the cascade resulting in a sympathetic hyperactivity. A future step would be to define the exact response of ROS after RDN in the treatment of the different SNS related disease states. If it is possible to measure the change in ROS after RDN, it may be a simple, non-invasive way to investigate whether RDN decreases systemic sympathetic activity.

Which patients are eligible for RDN?

The potential amount of success of any intervention depends on the ability to select patients most likely to benefit from this intervention. Especially, this holds true for an intervention like RDN since it is irreversible and invasive. A standardized screening before treatment with RDN can exclude patients not likely to respond to RDN. In *Chapter 5* we investigated how many patients referred for RDN were indeed eligible for treatment. We showed that a relevant number of patients (two-third) were excluded from treatment. About 25% of the excluded patients appeared to have pseudoresistant hypertension due to a white coat effect (WCE). This WCE can simply be excluded using an ambulatory 24-hour BP measurement (ABPM). An ABPM offers a large number of BP measurements during both day- and night-time. This results in a more precise assessment of BP than can be obtained from single measurements.[4] Another important finding is the high prevalence of secondary causes of hypertension in this population. It is remarkable that all referred patients had an extensive history of hypertension and the majority had already been screened in some way for secondary causes before referral. Based on the results of *chapter 5* we proposed that all hospitals performing RDN should implement a standardized screening with three aims: the confirmation of the diagnosis of (resistant) hypertension using ABPM, the exclusion of secondary causes of hypertension (preferably during a medication-free interval), and non-invasive imaging of the renal arteries.

Based on the pathophysiology, disease states characterized by an overactive SNS are likely to respond to treatment with RDN. A disease state characterized by sympathetic hyperactivity is chronic kidney disease (CKD).[5, 6] *Chapter 6* describes the results of a study showing that the BP-lowering effect of RDN is related to eGFR, a parameter generally available in daily clinical practice. Secondly, we observed that determinants of the renin angiotensin aldosterone system (RAAS) were related to a BP-reduction after RDN. Up to now patients with an eGFR <30 mL/min/1.73m^2 are generally excluded from treatment with RDN. It is therefore not yet possible to extrapolate our findings to this population. However, based on the present concepts on the pathophysiology of resistant hypertension[5, 6]it seems likely that results are generalizable to patients with more severe kidney disease.

In the present available position papers and consensus documents patients with multiple renal arteries are generally excluded from treatment.[7-9] In *chapter 7* we determined the prevalence of multiple renal arteries in patients referred for RDN and we investigated whether patients with multiple renal arteries respond different to treatment by RDN. We observed that 34% of patients referred for RDN had multiple renal arteries. Moreover, our analysis suggests that RDN is effective in patients with multiple renal arteries, specifically in those patients with arteries, which could all be treated. Based on these results and the high prevalence of multiple renal arteries among patients with complicated hypertension, it seems reasonable not to exclude patients with multiple arteries. To identify the eligible anatomy, we proposed a classification as a uniform strategy for clinical and research purposes. However, this study was a pilot study with a very small number of patients with multiple arteries that all could be treated. Therefore, these results should be interpreted with caution and confirmation in a larger population is needed.

What is the effect of renal denervation in hypertensive patients?

The first studies reporting about RDN showed very promising results with an approximately 30 mmHg reduction in systolic blood pressure up to three years after RDN.[10, 11] The Symplicity HTN-3 trial however

showed a less pronounced BP reduction and a relevant placebo-effect in the first randomized, sham-controlled, double blind trial.[12] Like other centres performing RDN we analysed the effects after RDN in our population. Unlike other centres we aimed to standardize the antihypertensive drug conditions under which measurements were performed, thereby excluding the potential disturbance by antihypertensive medication.

In *chapter 8* we described the BP reduction in the first 11 patients treated by RDN in the UMC Utrecht. We observed that RDN led to a mean reduction in office SBP of 25±12 mm Hg.

Chapter 9 is focused on the effect of RDN on muscle sympathetic nerve activity (MSNA). MSNA is a real-time measure of sympathetic nerve activity. Multiunit recordings of efferent postganglionic MSNA are obtained with a tungsten microelectrode into a muscle fascicle of the peroneal nerve.[13] MSNA is considered to be one of the few reliable methods to quantify central sympathetic activity. In this observational study we observed no effect on MSNA, despite a BP reduction in most patients.

In *chapter 10* we investigated whether RDN could have a beneficial effect on end organ damage (EOD) as an intermediate hard endpoint. In this observational study we observed a moderate reduction in ambulatory BP. However, we did not observe any significant effect on EOD 12 months after treatment. Remarkably, nine patients showed a regression in LV-mass although no reduction in BP was observed. This can be the result of a BP-independent effect of RDN, suggesting RDN was successful although no BP reduction was observed. However, it may also be that the medication adherence was improved in these patients leading to regression of the LV-mass.

Chapter 9 and 10 emphasize the fact that a wide variety in effects is observed after RDN. Moreover, we identified a higher number of non-responders and described a less pronounced reduction in BP than previous studies.[11, 14, 15] We believe that these studies have two major strengths to make them more reliable than the previous studies. Firstly, we standardized the antihypertensive drug-conditions to exclude disturbances by antihypertensive drugs. It cannot be ruled out that the more favourable effect reported in other studies may be due to confounding by changes in pharmacological treatment during follow-up.[11, 14, 15] Secondly, we used ABPM and central BP to evaluate the effect in BP. These are more reliable means to measure BP than office BP only. The fact that the proper conducted HTN-3 study also failed to reach the primary endpoint supports our observations that RDN is not always successful and should be implemented with caution.

Is there a role for renal denervation beyond resistant hypertension?

As stated before, disease states characterized by an overactive SNS are likely to respond to treatment with RDN. Besides hypertension, examples of these disease states are metabolic syndrome (MetS), heart failure (HF), or sleep apnoea.[16, 17] Based on the concept of an elevated sympathetic activity in patients with metabolic syndrome we designed the prospective DREAMS study. In *chapter 11* we evaluated the effect of RDN on insulin sensitivity and blood pressure. Secondly we investigated what the effects of RDN on sympathetic activity were by performing MSNA and heart rate variability (HRV) measurements. We showed that RDN did not lead to an improvement of insulin sensitivity up to 12 months after treatment. Yet, we observed a moderate reduction in ambulatory blood pressure in this nearly drug-naïve population. Remarkably, we observed that RDN did not alter systemic sympathetic activity as assessed by MSNA and HRV. In addition to the ambulatory BP, we also let the patients carry out self-monitored BP measurements (SBPM) at home. Surprisingly, we observed that the home-based SBP did not change during the 12 months after RDN. This may indicate that the decrease in ambulatory blood pressure is explained by the "regression to the mean" phenomenon and that no actual decrease in blood pressure occurred.

Chapter 12 is focused on the effects of RDN on central blood pressure and arterial stiffness. In this substudy of the DREAMS study we observed that the central BP decreased significantly in patients with MetS. However, the parameters of arterial stiffness (augmentation index and pulse wave velocity) did not significantly decrease after RDN. A potential explanation for our observations is that RDN halted

further deterioration of arterial stiffness since the expectation is that this would increase over a period of 12 months in a population as the DREAMS patients. However, this is only speculative since we did not include a control group in this study. Finally, it may also be that RDN simply is not effective as a modality to reduce arterial stiffness.

It is well known that a sympathetic hyperactivity is involved in the pathophysiology of HF with a reduced left ventricular ejection fraction. In *chapter 13* we performed a systematic review to investigate whether this also holds true for HF with a preserved ejection fraction (HFPEF). Based on our analysis we indeed concluded that a relation between sympathetic hyperactivity and HFPEF does exist. However, the current literature is not able to distinguish whether enhanced sympathetic activity results in HFPEF, or HFPEF results in an enhanced sympathetic activity. We proposed a vicious circle in which HFPEF and SNS sustain each other. Disruption of this vicious circle may be an attractive treatment option. Especially, since no other pharmacological therapies have yet proven to be effective in this disease state.

Interpretations and conclusions

Based on the observation from this thesis a few conclusions can be drawn.

Firstly, the results from the preclinical study show the added value of a translational approach in the light of RDN. This translational approach led to the conclusion that hemodynamic measurements may be used as a per-procedural marker of successful RDN. Furthermore it gave more insight in the intrarenal effects directly after RDN.

Secondly, this thesis showed that a multidisciplinary team improves the selection of eligible patients for RDN. All hospitals performing RDN should implement a multidisciplinary approach for a standardized screening of patients referred for RDN. Since a relevant number of referred patients is not eligible, they should be screened for secondary causes of hypertension and non-eligible renal anatomy. Moreover, the second part of this thesis provides some tools for a proper selection of patients eligible for treatment. We demonstrated that patients with multiple renal arteries may be eligible for treatment, moreover we showed that eGFR may be used as a predictor of effective treatment.

Based on the third part of this thesis we concluded that the effects of RDN have a wide variety and that a relevant number of patients is a non-responder after RDN.

Based on the fourth part of this thesis we concluded that RDN does not have a significant effect on insulin resistance in a metabolic population. Moreover, the BP-lowering effect in the studied metabolic population may be explained by regression to the mean.

In conclusion, this thesis shows that we observed a less pronounced BP effect of RDN compared to previously published studies. With the presentation of the negative results of the Symplicity HTN-3 trial the discussion about RDN has emerged.[18] We feel that our results are in line with the results from the HTN-3 trial. The reduction of BP in our non-controlled studies, as described in this thesis, is also very moderate. Moreover, we could not show improvements in EOD, as well as a reduction in systemic sympathetic activity. Different explanations can be given for the observation of the present thesis.

Renal denervation is not effective. Based on the extensive historical studies of surgical renal denervation and preclinical studies, this option seems unlikely. Already in the ’20s and ‘30s of last century, complex, non-selective, procedures such as surgical nephrectomy and even radical surgical sympathectomy were used as an effective treatment of severe hypertension before antihypertensive drugs became generally available.[19, 20]Also, preclinical studies have shown that renal denervation is a successful treatment for hypertension and other disease states associated with an increased sympathetic activity.[21] These preclinical studies also showed that RDN led to a reduction of norepinephrine spillover.[22] Spillover of norepinephrine from various organs has been used as an index of sympathetic nerve activity to those organs.[23] Based on the extensive evidence in this field, it is very likely that denervation of the renal sympathetic nerves is a potential strategy to lower the sympathetic activity and consequent reduce blood pressure.

We did not select the patients with an elevated sympathetic activity. This does not seem to be a very likely explanation, since we included patients with rather high MSNA values in both studies evaluating MSNA. We observed a mean baseline MSNA of 54±6 burst/100 heartbeats in the hypertensive population and a mean baseline MSNA of 71±14 burst/100 heartbeats in the metabolic population. In other studies investigating the change of sympathetic activity baseline values ranging from 47±19 to 79±3 burst/100 heartbeats were observed.[24, 25] Both these studies observed a reduction in MSNA after different interventions.[24, 25]

We did not correctly measure sympathetic activity. We do not consider this as a plausible option since we have a vast experience with this technique and we have previously reported that intra-observer and inter-observer reproducibility is low: 4.5 ±0.5%, respectively 6.2 ±0.7%.[24, 26, 27] Moreover, in the DREAMS study both the results of MSNA and heart rate variability led to the same conclusions.

Renal denervation was not applied correctly. Percutaneous RDN is a relatively simple intervention, especially for trained interventionalists. However, the present available generators lack a periprocedural variable to monitor the efficacy of the procedure itself. A per-procedural marker can help interventionalists to deliver the optimal treatment. Yet, this lack of per-procedural variables is no explanation for the difference in results between our observations and the results from colleagues who used the same system. Theoretically, it may be so that the procedure was not performed according to the recommended technique. However, this is unlikely since all patients in the UMC Utrecht are treated by two well trained and highly experienced interventionalists.

The current available catheters are not able to produce a sufficient denervation of the renal nerves. Before RDN was performed in a clinical setting, only a limited number of animal studies have been performed. Furthermore, these studies were industry-driven and not all presented for a peer review journal. In *chapter 2* we showed that RDN led to a limited penetration of the renal artery and that not all renal nerves were targeted by RDN in an experimental set-up. This observation was supported by the clinical observation of Vink et al. who demonstrated an incomplete denervation in a case study.[28]

Recommendations and future directions

To gain more insight into renal denervation and into the potential of this novel therapeutic modality, more research should be performed. This research should focus on two aspects: patient selection and technical improvement of the therapy.

Patient selection

Future studies should identify the patient groups most likely to benefit from RDN. At present, patients are selected for RDN based on BP levels and medical history. However, we among others observed a wide variety in effect and a relevant number of non-responders after RDN. For a more successful therapy it may be of added value to quantify the sympathetic activity in a patient before RDN is considered. An option may be to measure MSNA in all patients before they are scheduled for RDN. However, this is unrealistic in the daily clinical practice due to the extensiveness and invasiveness of the measurement. One should be able to identify patients with an elevated sympathetic activity easier. Moreover, we observed only a small reduction in BP despite high MSNA values at baseline. Just as other studies investigating the effects of RDN, we tried to identify these patients based on their medical history. With the extensive screening we expected to include the correct patients and indeed “on paper” they seemed to be the correct candidates. However, the present thesis shows that this strict selection of patients did not lead to a more predictable and pronounced effect. Perhaps we should use a different strategy to identify patients with an elevated sympathetic activity in the future. For example: the classification to diagnose a patient with metabolic syndrome requires that at least three out of five criteria should be present.[29] A similar classification (using different criteria) for an increased SNS might be an option to identify patients with “increased sympathetic activity”. Below I propose a classification with major criteria and their variables.

1. An objectification of sympathetic activity.
   Different ways to measure sympathetic activity do exist. The most reliable ways to measure SNS activity at present are local norepinephrine (NE) spillover, MSNA, and Iodine 123-metaiodobenzylguanidine (MIBG) imaging. Based on the results of chapter 4, it can be considered to measure production of reactive oxygen species by means of markers of oxidative stress present in blood, urine and leukocytes. Markers of oxidative stress associated with hypertension are thiobarbituric acid- reactive substances, asymmetric dimethylarginine, or oxidated LDL among others.
2. Presence of clinical signs associated with an elevated sympathetic activity.
   Examples are an elevated BMI or increase waist circumference.
3. Abnormalities at the laboratorial investigation suggestive for a disease associated with sympathetic hyperactivity.
   For example, an elevated BNP suggestive for heart failure, decreased eGFR suggestive for renal failure, increased insulin suggestive for insulin resistance, or a misbalance in variables of the renin-angiotensin-system.

As a suggestion, we might consider a patient as having sufficient sympathetic hyperactivity when of all three major criteria one variable is present.

Technical improvement of the therapy

As discussed, the effect of surgical renal denervation has been proven in preclinical studies, just as the complex surgical sympathectomy in clinical studies. Percutaneous denervation is a more elegant way to target the renal sympathetic nerves, but does not seem to be effective in all patients. Therefore, the present available catheters should be improved at some points.

The devices for RDN should be able to treat arteries with a wider range of sizes, particularly vessels smaller than 4 mm of diameter. This adjustment has two aims. Firstly, it will provide an option for patients with multiple arteries that have diameters of less than 4mm. In *chapter 7* we showed that a substantial percentage of patients referred for RDN had multiple renal arteries. Moreover, in an explorative study in the same chapter we showed that patients classified as B1 (all multiple arteries can be treated) seemed to benefit more from RDN than patients classified as B2 (not all multiple arteries can be treated). Secondly, Sakakura showed that most renal nerves are located more distally along the renal arteries.[30] Since the diameter of arteries tends to decrease distally, the diameter in the distal part is often too small to be treated at present.

The present available generators are simple and cannot provide objective information whether the delivered therapy was successful. In *chapter 2* and *chapter 3* we investigated whether a change in renal haemodynamics might be used as a per-procedural marker. We showed that a decreased hyperaemic microvascular resistance was related to a BP-reduction. However, *chapter* 3 was an explorative pilot study and these results should be further investigated. Another option for a per-procedural marker may be stimulation of the renal nerves to identify the location of the renal nerves and consequently target them. However, the available evidence at present is not enough to confirm this role.

Preclinical studies may be of added value in the design of more adequate percutaneous denervation and in the evaluation of different per-procedural markers. Consequently, we will be able to investigate these potential improvements again in clinical practice (‘from bench to bedside’). We should be cautious that these studies are not all sponsor-driven, but that they are also objectified by investigator driven studies.

In conclusion, renal denervation still seems a very promising therapy. However, we should not be blinded by the first, very encouraging, studies. It is likely that a BP reduction can be established, however not to such an extent as suggested in the first clinical studies. Even more, it may be so that RDN can play a beneficial role in other disease states of sympathetic hyperactivity. Until proven, we should only use RDN in the field of (investigator-driven) studies to learn more about particularly proper patient selection and more adequate techniques to disrupt the renal sympathetic nerves.

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Nederlandse samenvatting

Het sympathische zenuwstelsel

In het lichaam vinden veel acties plaats, uitgevoerd vanuit het onbewuste. Deze handelingen vinden min of meer automatisch plaats en zijn onafhankelijk van de wil. Ze worden gecontroleerd door een speciaal systeem van zenuwcellen en zenuwvezels: het autonome zenuwstelsel.[1] Het autonome zenuwstelsel is onderverdeeld in het sympathische zenuwstelsel (SNS) en het parasympathische zenuwstelsel (PSNS).[2] Een specifieke functie van het sympathische zenuwstelsel is het mobiliseren van het menselijk lichaam in acute, gevaarlijke en/of stressvolle situaties door het induceren van de vecht-of-vluchtreactie.[3] Echter, het SNS is voortdurend actief op een basisniveau om de zelfregulatie van organismen met behulp van een vastgestelde norm en negatieve terugkoppeling (zgn. homeostase) te handhaven.[3] Tijdens de vecht-of-vluchtreactie bewerkstelligt het SNS een aaneenschakeling van processen in het lichaam, waaronder de productie van cortisol door de bijnieren, de onderdrukking van het immuunsysteem, de vernauwing van de bloedvaten, de verhoging van de hartslag en productie van renine door de nieren.[4] Deze opeenvolging van activiteiten verloopt via sympathisch afferente en sympathisch efferente zenuwvezels die vanuit de hersenen naar de verschillende organen lopen (efferent) en weer terug naar de hersenen (afferent) .[4]

Naast de vecht-of-vluchtreactie is de sympathische activiteit chronisch verhoogd in een aantal cardiovasculaire ziekten zoals hypertensie, hartfalen, chronisch nierfalen, insulineresistentie en obesitas.[5] De nieren en vooral de renale sympathische zenuwen dragen bij aan deze verhoogde sympathische activiteit.[5]

Resistente hypertensie

Hypertensie (hoge bloeddruk) heeft wereldwijd een prevalentie van ongeveer 40%[6, 7] en is een belangrijke risicofactor voor hart- en vaatziekten. Hypertensie wordt geclassificeerd als primaire of secundaire hypertensie.[8] Primaire hypertensie geeft aan dat er geen specifieke oorzaak kan worden gevonden om te verklaren waarom een patiënt een verhoogde bloeddruk heeft.[8] Secundaire hypertensie geeft aan dat de verhoogde bloeddruk het resultaat is van een geïdentificeerde onderliggende aandoening.

Ondanks behandeling met een breed scala aan beschikbare antihypertensiva heeft een aantal patiënten nog steeds een ongecontroleerde bloeddruk.[7, 9] Deze patiënten kunnen worden gediagnostiseerd met resistente hypertensie. Resistente hypertensie wordt gedefinieerd als een ongecontroleerde bloeddruk ondanks het gebruik van drie antihypertensiva van verschillende klassen, waarvan 1 diureticum.[10] Ook patiënten bij wie de bloeddruk gecontroleerd is met vier of meer antihypertensiva, worden beschouwd als patiënten met resistente hypertensie.[10]

Zoals hierboven vermeld is het SNS, met name de renale sympathische zenuwen, betrokken bij de pathofysiologie van (resistente) hypertensie.[11] Op basis van de betrokkenheid van het SNS in resistente hypertensie is renale denervatie (RDN) ontwikkeld als een nieuwe behandelingsoptie voor patiënten met resistente hypertensie.

Percutane denervatie van de nierslagaders

Direct ingrijpen in het sympathische zenuwstelsel is een potentiële behandeling van resistente hypertensie, die een lange historie kent. Al in de jaren dertig van de twintigste eeuw is aangetoond dat chirurgische sympathectomie (het doornemen van de sympathische zenuwvezels) een aanhoudende bloeddrukverlaging tot gevolg had.[12] Naarmate er meer en betere medicijnen beschikbaar kwamen is deze zeer invasieve behandeling in onbruik geraakt in verband met het risico op complicaties.[13] Recent is er een selectieve, minder invasieve techniek beschikbaar gekomen om de sympathische nierzenuwen te onderbreken: percutane renale denervatie. Letterlijk vertaald betekent dit het onderbreken van de nierzenuwen. Deze percutane renale denervatie is een kathetergestuurde benadering waarbij er radiofrequente energie in de nierarteriën wordt afgegeven.[14] De nierzenuwen, gelegen rond de nierarteriën, worden op deze manier onderbroken.

De eerste studies waarin onderzoek werd gedaan naar RDN waren zeer veelbelovend.[14-16] Meer recent

verschenen er echter ook studies die een minder uitgesproken bloeddrukdaling laten zien.[17] Los van het effect op de bloeddruk bestaan er nog vele vragen over RDN. Allereerst is het van belang om inzicht te krijgen in het exacte werkingsmechanisme achter renale denervatie. In deel één wordt hierop ingegaan. Ondanks het feit dat de behandeling veelbelovend leek, is de denervatie niet altijd effectief. Om de behandeling alleen toe te passen bij patiënten met een hoge kans van slagen, is het van belang om patiënten te selecteren die het meest gebaat zijn bij de behandeling. Deel twee van dit proefschrift gaat daarom over de selectie van patiënten. In het derde deel van dit proefschrift worden de effecten van RDN beschreven in een populatie met resistente hypertensie.
Naast verlaging van de bloeddruk hebben sommige kleine studies gemeld dat renale denervatie ook een positieve rol kan spelen in de behandeling van hartfalen, insulineresistentie en metabole veranderingen. In deel vier wordt dan ook nader ingegaan op de effecten van denervatie op aandoeningen anders dan resistente hypertensie.

Deel I Pathofysiologie

Momenteel is niet exact bekend, wat het effect is van verstoring van de renale sympathische zenuwen in de nieren zelf en in de feedback door de afferente zenuwen naar de hersenen. Onze hypothese was dat renale denervatie leidt tot een verminderde microvasculaire weerstand in de nieren zelf en een daaruit voortvloeiende toename van de doorbloeding van de nier. In **hoofdstuk 2** testten we deze hypothese in een varkensmodel. Direct na RDN nam de microvasculaire weerstand af en de renale bloeddoorstroming toe. Echter, deze effecten bleken niet meer waarneembaar na een follow-up duur van drie tot twaalf weken. Naast de effecten op de renale hemodynamiek bestudeerden we de histologische veranderingen na RDN. Hiervoor hebben we de nierslagaders van de varkens uit de dieren gehaald nadat zij getermineerd waren. We observeerden dat de afgegeven energie ongeveer 2 mm diep reikt in de wand van de arterie. Dit is minder diep dan wij van tevoren verwachtten op basis van eerdere studies. Daarnaast observeerden we dat het merendeel van de zenuwen niet geraakt werd door de renale denervatie. Opvallend was dat wij een relatie zagen tussen diepte van de laesie en mate van zenuwschade. Dit kan impliceren dat een diepere beschadiging nodig is voor het bewerkstellen van zenuwschade.
Op basis van de observaties bij varkens, zijn we een prospectief klinisch onderzoek gestart dat beschreven is in **hoofdstuk 3.** In deze studie onderzochten we of de hemodynamische veranderingen, zoals geobserveerd bij gezonde varkens, ook bij hypertensieve patiënten kon worden waargenomen direct na RDN. Onze hypothese was dat de positieve effecten waargenomen in het varkensmodel direct na RDN meer prominent zouden zijn bij hypertensieve patiënten, omdat zij onderworpen zijn aan een langdurige hoge bloeddruk. Ten tweede hebben we geprobeerd om te onderzoeken of een veranderde hemodynamiek een marker van een succesvolle denervatie zou kunnen zijn. Direct na RDN observeerden we een significante afname van de weerstandsreserve in de nier. De snelheid van de bloeddoorstroming bleef echter gelijk bij een afgenomen druk in de aorta. Deze resultaten kunnen betekenen dat RDN zorgt voor een hyperemische staat, die niet verder vergroot kan worden door een hyperemische stimulus zoals papaverine. Bovendien kan er wel degelijk een toename van de bloeddoorstroming zijn opgetreden, aangezien die stabiel bleef bij een zeer forse daling van de aortadruk. Normaal gesproken volgt de bloeddoorstroming ook de druk van de aorta; bij een daling van de aortadruk zou dus ook een daling van de doorstroming worden verwacht. Dat deze nu stabiel bleef, lijkt te wijzen op een relatieve stijging. Naast deze veranderingen observeerden we ook een relatie tussen een verandering in hyperemische microvasculaire weerstand en een verandering in de bloeddruk. Toekomstige studies zouden deze mogelijke relatie verder moeten onderzoeken om te kunnen concluderen of een verandering van de hyperemische weerstand daadwerkelijk een periprocedurele marker is voor succesvolle denervatie.
In **hoofdstuk 4** hebben we de beschikbare literatuur geëvalueerd om te onderzoeken wat de rol van reactieve zuurstofradicalen (ROS) is in aandoeningen geassocieerd met een sympathische hyperactiviteit. Op basis van de literatuur concludeerden wij dat lokale nierischemie een rol kan spelen in de productie

van ROS. De productie van ROS kan zodoende een centrale rol spelen in de cascade van sympathische hyperactiviteit. Een toekomstige stap zou zijn om de exacte respons van ROS te bepalen na behandeling van de verschillende SNS gerelateerde ziekten middels RDN.

Deel II De selectie van patiënten voor behandeling met renale denervatie

Het potentiële succes van een interventie hangt af van het vermogen om de patiënten te selecteren die de meeste baat hebben bij deze interventie. Een gestandaardiseerde screening vóór de behandeling met RDN kan mogelijk bijdragen aan het selecteren van de juiste patiënten. In **hoofdstuk 5** onderzochten we hoeveel patiënten verwezen voor RDN inderdaad in aanmerking kwamen voor behandeling. Een relevant aantal patiënten (twee derde) werd uitgesloten van behandeling met RDN. De belangrijkste redenen om een patiënt af te wijzen voor RDN waren bloeddrukgerelateerd: 23 patiënten (19%) hadden een spreekkamer-bloeddruk lager dan 160 mmHg systolisch (bovendruk); 26 patiënten (22%) hadden een zogenaamd wittejasseneffect (verhoogde spreekkamer-bloeddruk, maar normale ambulante bloeddruk (ABPM)). Veertien patiënten (12%) hadden een tot dusver onontdekte secundaire oorzaak van hypertensie, meestal in de vorm van een primair hyperaldosteronisme (11 patiënten, 9%). Opvallend is dat alle genoemde patiënten een uitgebreide geschiedenis van hypertensie hadden en een deel zelfs al (vluchtig) gescreend was op secundaire oorzaken, voordat zij verwezen werden. Op basis van de resultaten van *hoofdstuk 5* hebben we gesuggereerd dat alle ziekenhuizen een gestandaardiseerde screening moeten uitvoeren met drie doelstellingen: het bevestigen van de diagnose van hypertensie (m.b.v. ABPM); de uitsluiting van secundaire oorzaken van hypertensie en niet-invasieve beeldvorming van de nierslagaders.

Op basis van de remmende werking op het SNS door RDN is de verwachting dat ziektebeelden gekenmerkt door een verhoogde sympathische activiteit, het meest gebaat zijn bij deze behandeling. Een voorbeeld van een dergelijk ziektebeeld is chronische nierfalen. Uit **hoofdstuk 6** blijkt dat het bloeddrukverlagende effect van RDN verwant is aan eGFR: dit is een parameter van de nierfunctie die beschikbaar is voor de dagelijkse klinische praktijk. Ten tweede constateerden we dat determinanten van het renine angiotensine aldosteron systeem (RAAS) gerelateerd waren aan een bloeddrukverlaging. In de momenteel beschikbare studies werden patiënten met een eGFR <30 ml/min/1.73m^2 over het algemeen uitgesloten. Het is derhalve nog niet mogelijk om onze bevindingen te extrapoleren naar deze populatie. Echter, op basis van het huidige kennis van de pathofysiologie van resistente hypertensie lijkt deze extrapolarisatie wel waarschijnlijk.

In de huidige beschikbare position papers en consensus documenten worden patiënten met multiple nierarteriën over het algemeen uitgesloten van behandeling. In **hoofdstuk 7** hebben we onderzocht wat de prevalentie van multipele nierslagaders is bij patiënten die verwezen zijn voor RDN. Daarnaast hebben we geanalyseerd of patiënten met multiple nierslagaders verschillend reageren op behandeling met RDN. We constateerden dat 34% van de patiënten verwezen voor RDN multipele nierarteriën had. Bovendien bleek dat de bloeddrukdaling bij patiënten met multipele nierarteriën, bij wie alle takken konden worden behandeld, vergelijkbaar was met het effect bij patiënten met solitaire nierarteriën. Patiënten met multipele nierarteriën, bij wie niet alle nierarteriën behandeld konden worden, hadden een minder uitgesproken daling van de bloeddruk. Op basis van deze resultaten en de hoge prevalentie van multipele nierarteriën bij patiënten met resistente hypertensie, lijkt het redelijk om patiënten met multipele nierarteriën niet uit te sluiten van behandeling. Om patiënten met een geschikte anatomie te identificeren hebben we een classificatie gemaakt voor een uniforme benadering van de renale anatomie.

Deel III De effecten van renale denervatie in hypertensieve patiënten

Na diverse studies met veelbelovende resultaten van renale denervatie toonde de Symplicity HTN-3 trial begin 2014 een minder uitgesproken bloeddrukverlaging, gecombineerd met een relevant placebo-effect. Net als andere centra die RDN uitvoeren, hebben wij de effecten van RDN in onze populatie geanalyseerd.

In tegenstelling tot andere centra hebben we geprobeerd om de omstandigheden waaronder de metingen werden uitgevoerd te standaardiseren. Dit deden we door de bloeddrukverlagende medicatie tijdelijk te stoppen tijdens de baseline en follow-up metingen (indien klinische verantwoord). De antihypertensiva kunnen de metingen namelijk verstoren. In **hoofdstuk 8** beschrijven we de bloeddrukdaling van de eerste 11 patiënten behandeld door RDN in het UMC Utrecht. We observeerden een gemiddelde daling van de spreekkamer systolische bloeddruk van 25±12 mmHg. **Hoofdstuk 9** richt zich op het effect van RDN op de sympathische activiteit gemeten met muscle sympathetic nerve activity (MSNA, een manier om sympathische activiteit te meten). MSNA wordt beschouwd als een van de weinig betrouwbare methoden om de centrale sympathische activiteit te kwantificeren. In deze observationele studie zagen we geen effect op MSNA, ondanks een bloeddrukverlaging bij de meeste patiënten. In **hoofdstuk 10** onderzochten we of renale denervatie een gunstig effect heeft op eindorgaanschade (EOD) als intermediair hard eindpunt. In deze observationele studie zagen we een lichte vermindering van de ambulante bloeddruk. Echter, RDN leidde niet tot een significant effect op eindorgaanschade twaalf maanden na de behandeling. Opmerkelijk was dat negen patiënten een afname van de linker ventrikelmassa lieten zien ondanks een gelijkblijvende (of hogere) bloeddruk. Mogelijk kan dit het gevolg zijn van een bloeddrukonafhankelijk effect van RDN. Het kan echter ook zijn dat de therapietrouw bij deze patiënten is verbeterd, wat vervolgens heeft geleid tot een afname van de linker ventrikelmassa.
Hoofdstuk 9 en 10 benadrukken dat zeer wisselende effecten van renale denervatie worden waargenomen. Bovendien identificeerden we een groter aantal “non-responders” en een minder uitgesproken afname van de bloeddruk dan eerdere studies.

Deel IV De effecten van renale denervatie op andere aandoeningen
Zoals gezegd is het waarschijnlijk dat ziektebeelden gekenmerkt door een sympathische hyperactiviteit gebaat zijn bij behandeling met renale denervatie. Naast resistente hypertensie zijn voorbeelden hiervan het metabool syndroom (MetS), hartfalen (HF) of het obstructief slaapapneu syndroom (OSAS). Gebaseerd op het concept van een verhoogde sympathische activiteit bij patiënten met het metabool syndroom startten wij de prospectieve DREAMS studie. In deze studie werden patiënten met het metabool syndroom, die grotendeels drug-naïef waren, behandeld met RDN. In **hoofdstuk 11** evalueerden we het effect van RDN op de insulinegevoeligheid en bloeddruk in de DREAMS studie, alsmede het effect van RDN op sympathische activiteit. We toonden aan dat RDN niet leidt tot een verbetering van de insulinegevoeligheid twaalf maanden na de behandeling; wel observeerden we een afname van de ambulante bloeddruk. Opmerkelijk genoeg zagen we geen verandering in de systemische sympathische activiteit. Naast de ambulante bloeddruk hebben we ook elke maand thuis bloeddrukmetingen verricht bij deze patiënten, die we analyseerden met behulp van het lineair mixed model (LMM, een statistische methode). Opvallend was dat dit LMM demonstreerde dat de thuisbloeddrukken stabiel bleven gedurende de twaalf maanden na RDN. Dit kan erop duiden dat de daling van de ambulante bloeddruk verklaard wordt door het “regression to the mean” fenomeen en dat er geen daadwerkelijke bloeddrukdaling heeft plaatsgevonden. **Hoofdstuk 12** is gericht op het effect van renale denervatie op vaatstijfheid. In deze substudie van de DREAMS studie constateerden we dat de centrale bloeddruk significant afnam na RDN. Echter, de parameters van vaatstijfheid (augmentatie index en pulse wave velocity) verminderden niet significant na RDN. Het is mogelijk dat deze substudie underpowered was om een significant verschil aan te tonen. Een mogelijke verklaring van onze resultaten is dat RDN wel verdere achteruitgang van vaatstijfheid heeft bewerkstelligd, aangezien de verwachting is dat deze zou stijgen in een populatie zoals de DREAMS patiënten. Dit weten we echter niet zeker door het gebrek aan een controle populatie. Ten slotte zou het ook kunnen zijn dat RDN simpelweg niet effectief is geweest in het verlagen van de vaatstijfheid.
Het is bekend dat sympathische hyperactiviteit betrokken is bij de pathofysiologie van HF met een verminderde linker ventrikel ejectiefractie. In **hoofdstuk 13** hebben we een systematische review

uitgevoerd om te onderzoeken of dit ook geldt voor hartfalen met een normale ejectiefractie (HFPEF). Op basis van de beschikbare literatuur concludeerden we dat er een relatie bestaat tussen sympathische hyperactiviteit en HFPEF. Echter, de huidige literatuur kan geen uitsluitsel geven of verhoogde sympathische activiteit leidt tot HFPEF, dan wel dat HFPEF resulteert in een verhoogde sympathische activiteit. Op basis van onze resultaten suggereren we dat er sprake is van een vicieuze cirkel waarin HFPEF en SNS elkaar aanjagen. Verstoring van deze vicieuze cirkel kan een mogelijke behandelingsoptie zijn voor deze aandoening, waarvoor momenteel geen (farmacologische) therapieën bewezen effectief zijn.

Conclusie en toekomstvisie

Concluderend kunnen we vaststellen dat het concept van renale denervatie nog steeds zeer veelbelovend is, hoewel dit proefschrift aantoont dat de huidige vorm van RDN niet altijd effectief is. Mogelijk wordt dit verklaard door een niet-adequate denervatie, dan wel door verkeerde patiëntenselectie. In het veld van renale denervatie moet men ervoor waken niet verblind te worden door de eerste, zeer bemoedigende studies. Het is waarschijnlijk dat een bloeddrukverlaging kan worden bewerkstelligd, maar wellicht in mindere mate dan door de eerste klinische studies is gesuggereerd. Daarnaast lijkt het waarschijnlijk dat renale denervatie een positieve rol kan spelen in andere aandoeningen geassocieerd met sympathische hyperactiviteit. Mijns inziens moeten we echter renale denervatie voorlopig alleen toepassen op het gebied van (“investigator driven”) studies, totdat meer bewijs is vergaard over het effect van RDN. Dit onderzoek moet zich richten op twee aspecten: de selectie van patiënten en een technische verbetering van de katheters.

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Changes in central hemodynamics and arterial stiffness after renal denervation in patients with metabolic syndrome

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Lieve **Lot**, in Utrecht oneindig veel sushi gemaakt, in Amsterdam vanuit de Kade “Boer zoekt vrouw”

kijken en wekelijks de Bastille in. Daarna bij elkaar om de hoek in de pijp waarbij je onze 3e huisgenoot was. Hoewel het nu net geen kruipafstand meer is hoop ik dat het badjas-ontbijt erin blijft met Pasen! Samen zijn we jut en jul organisatie voor Puk, het is daarom ook een fijn gevoel dat je mijn paranimf bent. Dank dat je naast mij wilt staan!

Lieve **Joris**, dat we een goed team zijn hebben we wel bewezen tijdens menig avondje Utrecht (dat wil zeggen: samen aan de bar hangen waarna ik je opving als jij weer eens slapend van je kruk dreigde te vallen). Nu hebben we dankzij jouw creativiteit samen een super kaft voor dit boekje kunnen maken. Ik hoop de vele successen van “studio Verloop” met je mee te mogen vieren!

Lieve **schoonouders** (in spé), een bezoekje aan het Bourgondische Brabant is altijd weer goed om even bij te tanken en te genieten van het leven. Dank voor jullie niet aflatende interesse en attentheid. Ik ben blij dat ik deel uit mag maken van jullie gezin.
Lieve **Caroline** en **Robert**, prachtig om te zien hoe jullie nu samen met Alexander een heus gezinnetje vormen. Robert, binnenkort eindelijk eens dat avondje oester-happy hour?

Hein en **Ernst**, lieve broertjes. Als jongste heb ik eigenlijk altijd enorm tegen jullie opgekeken (en doe dat eigenlijk nog steeds). Ik ben er trots op hoe jullie je leven inrichten en ben dankbaar dat we altijd voor elkaar klaar kunnen staan indien dat nodig is. Snel weer een weekendje “op stap met z’n 3-en”?
Schoonzusjes: lieve **Mijke**, ik kijk met bewondering toe hoe jij je carrière combineert met Alix (en straks ook “asterix”). Maar bovenal geniet ik van de momenten met *Melrose Place* en onze “zusjes”-momenten samen. Lieve **Juliette**, fijn om te zien hoe Ernst en jij elkaar aanvullen in carrière en hobby’s. Daarnaast heb ik bewondering voor je doorzettingsvermogen. Ik hoop dat we elkaar veel zullen zien in het Zwitserse.

Lieve **papa** en **mama**, wat was het fijn om op te groeien in zo’n warm en hecht gezin! Dankzij jullie hebben de jongens en ik alle mogelijkheden in het leven gekregen om ons te ontwikkelen zoals we zelf wilden, ik hoop dat jullie trots zijn op het resultaat. Ik weet zeker dat ik ook namens de jongens spreek als ik zeg dat wij intens dankbaar zijn voor jullie onvoorwaardelijke steun en liefde waar wij altijd op mogen rekenen.

Robert, liefste Yus, de afgelopen jaren heb jij dit traject zeer intensief meegemaakt en je trok voor mij aan de noodrem als dat nodig was (paklijst voor verrassingsweekendjes: Ik-telefoon zonder wifi). Ik bewonder je om het vermogen mij tot rust te brengen met je humor en relativeringsvermogen. Dank voor het feit dat je altijd eerlijk bent (ook als ik liever een leugentje om bestwil wil) en je oprechte geïnteresseerdheid in de mensen om je heen. Ik verheug me op onze toekomst samen! Rondje fietsen?

Curriculum vitae

Onder de Maleise zon werd Willemien Verloop op 26 maart 1985 geboren in Miri. Zij groeide op als jongste in een hecht gezin met twee oudere broers. Op haar 4e verhuisde het gezin naar Den Haag, waar zij opgegroeid is. Na het behalen van haar VWO diploma aan het Gymnasium Haganum in Den Haag is zij geneeskunde gaan studeren in Utrecht. Tijdens haar studietijd werd ze al snel geënthousiasmeerd door de fysiologie van orgaansystemen verantwoordelijk voor de circulatie.

De interesse voor wetenschappelijk onderzoek werd begin 2008 gelegd in het LUMC onder de motiverende begeleiding van Drs. C.J.W. Borleffs. Tijdens een keuze co-schap cardiologie in 2009 in het Meander MC in Amersfoort onder supervisie van Dr. P.J. Senden werd definitief de liefde voor de cardiologie geboren. Hierop volgde in 2010 het oudste co-schap cardiologie in het Antonius ziekenhuis in Nieuwegein onder leiding van Dr. W. Jaarsma. In het kader van de wetenschappelijke stage heeft zij tevens in het Antonius ziekenhuis onderzoek gedaan naar stenttrombose in diabetici onder leiding van Dr. J.M. ten Berg en Dr. J.W. van Werkum. Na het afronden van de studie geneeskunde is zij in 2010 gestart als ANIOS cardiologie in het Meander MC onder supervisie van Dr. P.J. Senden. Tegelijkertijd werd haar een promotie traject aangeboden in het UMC Utrecht. Daar heeft zij zich de afgelopen jaren verdiept in de nieuwe behandeling van renale denervatie onder leiding van Dr. M. Voskuil, Dr. W. Spiering en Prof. P.A. Doevendans. Sinds 1 september 2014 is zij gestart met haar vooropleiding interne geneeskunde in het OLVG in Amsterdam onder leiding van Dr. Y.F.C Smets. In 2016 zal zij haar opleiding tot cardioloog voortzetten in het UMC Utrecht onder leiding van Dr. J.H. Kirkels. Zij is verloofd met Robert Crutzen.