Distal ablation and directly observed medical therapy as potential protocol advancements for renal denervation for hypertension: a study evaluating invasive hemodynamic parameters to predict response to renal denervation

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A thesis submitted to the University of London for the degree of Doctor of Philosophy

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Submitted November 2016
Declaration of Originality
I confirm that the work in this Thesis is entirely my own. All other research that has been used when preparing this Thesis has been referenced appropriately.

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Abstract

Aims:

(1) Explore the physiological effect of renal denervation (RDN)

(2) Explore the efficacy of distal denervation on blood pressure (BP) reduction in patients undergoing directly observed anti-hypertensive therapy (DOT) to minimise measurement bias

(3) Evaluate the 6-month safety of distal denervation

Methods:

Patients with resistant hypertension were recruited and underwent assessment of drug compliance by assaying urinary drug levels. All subsequent measurements were recorded under DOT.

Pre-denervation, office and ambulatory BP were measured, and patients underwent bilateral renal angiography and invasive measurement of aortic and renal arterial pressure and blood flow velocity.

RDN was performed using the Symplicity Spyral catheter, denervating in the main renal arteries and each distal branch ≥3mm diameter.

Invasive and non-invasive measurements were repeated 6-months post denervation under DOT.

Results:

16 patients underwent denervation (age 63±12 years) with referral office SBP 180±18 mmHg. In total each patient received 22.6±5.0 ablations, 9.3±2.9 ablations in the main trunk and 13.3±4.8 ablations distally.
At 6-months follow-up, overall unblinded 24-hour SBP reduction was -5.1±7.5 mmHg (p=0.020), with DBP reduction -3.4±4.9 mmHg (p=0.018).

At 6-months follow-up an overall increase in renal blood flow velocity occurred at rest (1.91±3.51 cm/s, p=0.04) and under identical sedation states (1.81±3.44 cm/s, p=0.05). Patients with the largest reduction in ambulatory SBP at 6-months had the largest increase in renal blood flow acutely after RDN ($R^2=0.60$, $p<0.001$) and the largest decrease in renal resistance ($R^2=0.56$, $p<0.001$).

Quantitative vessel angiography showed no significant change in any main or distal renal artery dimensions at 6-months.

**Conclusion:**
This unblinded study of distal RDN showed a significant reduction in ambulatory systolic and diastolic BP with no safety concerns at 6-months. These exploratory results suggest that acute changes in renal hemodynamics may be predictive of blood pressure response at 6-months follow-up.
Dedication

I dedicate this thesis to my parents Julian and Lilian Finegold for always having faith in my abilities and to my uncle, David Glass, for his unwavering encouragement and enthusiasm. I am fortunate to have had such amazing role models and I hope that completion of this thesis will make them proud.
Acknowledgements

I owe gratitude to my supervisors Professor Darrel Francis, Dr Justin Davies and Dr Zachary Whinnett as well as Professor Jamil Mayet for their guidance throughout this period.

Thank you to my fellow PhD student, Dr Matt Shun-Shin and Dr Rasha Al-Lamee. Working with them made research an enjoyable and interactive experience. In particular, I am grateful to Matt for teaching me how to programme in R without which the processing and analysis of my data would have been much more laborious.

I would like to acknowledge the support given by all members of the cardiology team at Hammersmith Hospital and the International Centre for Circulatory Health.

Finally, my deepest gratitude is to the patients with hypertension who kindly gave their time to participate in my research trial. The trial was very involved and required each patient to make many trips to hospital and have two invasive procedures. Each patient was unwaveringly kind and supportive of the study and it was an honor to work with them.
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<tr>
<td>ABPM</td>
<td>Ambulatory blood pressure monitor</td>
</tr>
<tr>
<td>ACE-I</td>
<td>Angiotensin converting enzyme inhibitor</td>
</tr>
<tr>
<td>ACT</td>
<td>Activated clotting time</td>
</tr>
<tr>
<td>ARNA</td>
<td>Afferent renal nerve activity</td>
</tr>
<tr>
<td>BP</td>
<td>Blood pressure</td>
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<tr>
<td>CI</td>
<td>Confidence interval</td>
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<tr>
<td>CNS</td>
<td>Central nervous system</td>
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<tr>
<td>DBP</td>
<td>Diastolic blood pressure</td>
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<tr>
<td>DOT</td>
<td>Directly observed therapy</td>
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<tr>
<td>DRG</td>
<td>Dorsal root ganglia</td>
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<tr>
<td>ECG</td>
<td>Electrocardiogram</td>
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<tr>
<td>ERSNA</td>
<td>Efferent renal sympathetic nerve activity</td>
</tr>
<tr>
<td>FMD</td>
<td>Fibromuscular dysplasia</td>
</tr>
<tr>
<td>GFR</td>
<td>Glomerular filtration rate</td>
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<tr>
<td>HP LC-MS/MS</td>
<td>High-performance liquid chromatography-tandem mass spectrometry</td>
</tr>
<tr>
<td>HR</td>
<td>Heart rate</td>
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<tr>
<td>ICC</td>
<td>Intra-class Correlation Coefficient</td>
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<tr>
<td>IP3</td>
<td>Inositol triphosphate</td>
</tr>
<tr>
<td>IPV</td>
<td>Instantaneous peak velocity</td>
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<tr>
<td>MAP</td>
<td>Mean arterial pressure</td>
</tr>
<tr>
<td>MDRD</td>
<td>Modification of Diet in Renal Disease</td>
</tr>
<tr>
<td>MRI</td>
<td>Magnetic Resonance Imaging</td>
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<tr>
<td>NE</td>
<td>Norepinephrine</td>
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<tr>
<td>OCT</td>
<td>Optical Coherence Tomography</td>
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<td>PG</td>
<td>Prostaglandin</td>
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<tr>
<td>PKC</td>
<td>Protein kinase C</td>
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<td>PWV</td>
<td>Pulse wave velocity</td>
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<tr>
<td>QVA</td>
<td>Quantitative vessel analysis</td>
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<tr>
<td>RDN</td>
<td>Renal denervation</td>
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<tr>
<td>RRI</td>
<td>Renal Resistive Index</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
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<td>------------------------------</td>
</tr>
<tr>
<td>RSNA</td>
<td>Renal sympathetic nerve activity</td>
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<tr>
<td>RVR</td>
<td>Renal vascular resistance</td>
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<tr>
<td>SBP</td>
<td>Systolic blood pressure</td>
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<tr>
<td>SDD</td>
<td>Standard deviation of difference</td>
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1 Introduction
1.1 The global burden of hypertension

1.1.1 Epidemiology of hypertension
Hypertension is a major global public health burden affecting more than a quarter of adults in developed countries (1, 2). Poorly controlled blood pressure has an enormous impact on the risk of developing a major cardiovascular event (3). Globally, approximately half of all ischaemic heart disease (4) and two-thirds of strokes are attributable to uncontrolled blood pressure (5), resulting in 12.8% of total global deaths (5). In addition, hypertension is a significant risk factor for the development of end-stage renal disease (6).

The number of hypertensive individuals is predicted to grow in the future due to an ageing population. In higher-income regions, it is predicted that the number of hypertensive individuals will grow by 70 million people from 2000 to 2025 (7). In lower-income regions, the number is far greater, compounded also by increasing urbanization: here the number of cases will grow by more than 500 million over the same period. It is estimated that a reduction of 21 400 stroke deaths and 41 400 ischaemic heart disease deaths per year would be expected in the UK alone if adequate blood pressure reduction was achieved in the population (8). Owing to the significant impact of elevated blood pressure on public health, treatment of hypertension remains an important goal for health care policy makers worldwide.

1.1.2 Resistant Hypertension
Despite widespread prescribing of anti-hypertensive agents, there remains a significant proportion of patients (estimates ranging between 8.9% (9) (10, 11) – 47% (12) with uncontrolled hypertension in whom reduction of blood pressure to target values is not achieved. Uncontrolled hypertension, however, is not synonymous with resistant hypertension. Uncontrolled hypertension includes patients with poor blood
pressure control for many reasons, for example, inadequate treatment (medication choices, doses), and poor adherence to medication.

Resistant hypertension is defined separately (13) as blood pressure remaining above target (systolic/diastolic blood pressure \( \geq 140/90 \) mmHg) despite concurrent use of \( \geq 3 \) anti-hypertensive agents of different classes (ideally one of the 3 agents a diuretic and all agents at optimal doses), or patients whose blood pressure is controlled with use of \( \geq 4 \) agents. Resistant hypertension is more frequent in patients who are older, obese, male, African American or nonblack Hispanic (9), diabetic (14) and attend health care visits infrequently. Patients with resistant hypertension also have higher Framingham 10-year coronary risk scores (11).

Not all patients diagnosed with resistant hypertension are truly resistant to medical therapy: some are found to have pseudo-resistant hypertension. There are multiple causes of pseudo-resistant hypertension, including white-coat hypertension, poor medication adherence, secondary hypertension (15) and inappropriate drug prescribing (13).

The white-coat effect describes a cohort of patients with elevated office blood pressure readings in whom ambulatory blood pressure monitoring shows controlled blood pressure (16). It is estimated that the white coat effect averages 10-25 mmHg (17) (18) (19), and that approximately one-third of patients with suspected resistant hypertension show this significant white-coat effect (10, 20).
1.1.2.1 Prevalence of resistant hypertension

Owing to these factors the true prevalence of resistant hypertension is difficult to quantify. In the ALLHAT study, after 5 years follow-up of 33,357 patients with hypertension, 49% of ALLHAT participants were controlled on 1 or 2 medications (21). This implies that approximately 50% of participants would have needed 3 or more blood pressure medications to control blood pressure and therefore fulfill the AHA criteria of resistant hypertension. However, the results from ALLHAT may both underestimate the prevalence of treatment resistance, as patients with a history of difficult-to-treat hypertension were precluded from enrolling in ALLHAT, or overestimate the prevalence, due to the restricted antihypertensive regimens in the ALLHAT protocol. A further study (22) using data from the US National Health and Nutrition Examination Survey from 2003 through 2008, found that among US adults with hypertension, 8.9% met criteria for resistant hypertension. This represented 12.8% of the antihypertensive drug-treated population.

1.1.2.2 Management of patients with Resistant Hypertension

Management of patients with Resistant Hypertension should follow a number of steps:

Key Definitions

- Resistant hypertension - blood pressure remaining above target (systolic/diastolic blood pressure ≥140/90 mmHg) despite concurrent use of ≥ 3 anti-hypertensive agents of different classes, or patients whose blood pressure is controlled with use of ≥ 4 agents
- Pseudo-resistant hypertension - patients initially diagnosed with resistant hypertension who are subsequently found to not fulfil criteria of resistant hypertension. This can be for multiple reasons including poor medication adherence and secondary hypertension (15)
- White coat effect - a cohort of patients with elevated office blood pressure readings in whom ambulatory blood pressure monitoring shows controlled blood pressure.
1.1.2.2.1 Investigations to confirm diagnosis and identify secondary contributing factors
Initial steps should confirm the diagnosis and identify secondary contributing factors e.g. obstructive sleep apnoea, renal artery stenosis, renal parenchymal disease, heavy alcohol use, thyroid disorders and primary hyperaldosteronism. All these conditions will benefit from targeted therapy, which will also help with blood pressure control.

1.1.2.2.2 Lifestyle changes
Lifestyle changes, such as weight loss, regular exercise, a low-salt diet and decreased alcohol intake, should be included in the therapeutic protocol.

1.1.2.2.3 Antihypertensive drug therapy
International guidelines (23) (24) suggest physicians should follow a treatment pathway for management of patients with resistant hypertension, typically A + C + D, where “A” is an angiotensin-converting-enzyme (ACE) inhibitor or an angiotensin II receptor blocker, “C” is a calcium channel blocker and “D” is a thiazide or thiazide-like diuretic.

If patients continue to have uncontrolled blood pressure on maximal doses of 3 antihypertensive agents, then the next treatment strategy is to add in a fourth treatment. The recent PATHWAY-2 double-blind, placebo-controlled, crossover trial rotated patients through 12 weeks of once-daily treatment with spironolactone, β-blockers, α-blockers, or placebo, in addition to their three baseline BP drugs. This study reported that Spironolactone was the most effective blood-pressure lowering treatment in patients with uncontrolled blood pressure on 3 anti-hypertensive medication, when compared with doxazosin and bisoprolol.

1.1.2.2.3.1 Natriuretic peptide agonists
Human recombinant atrial and brain natriuretic peptides are currently used for the treatment of heart failure. In patients with heart failure they elicit beneficial
hemodynamic effects, including arterial and venous dilatation, increased sodium excretion, and suppression of the renin–angiotensin–system. Sacubitril/valsartan (LCZ696) which combines inhibition of neprilysin (the enzyme responsible for degradation of the natriuretic peptides) and angiotensin-receptor blockade, leads to lower BP compared to ARB therapy alone (25). Therefore, natriuretic peptide agonists might be a future target for anti-hypertensive therapy.

1.1.2.2.3.2 Device based therapy
In recent years there has been extensive research on device based interventional management of resistant hypertension, including use of catheter based renal denervation as discussed in this Thesis.

Several novel interventional therapies stimulating baroreceptors, including baroreflex activation therapy and baroreceptor stenting, remain investigational at this point. In 1958, Carlsen et al showed that electrical stimulation of the carotid sinus nerve decreased blood pressure in patients undergoing neck surgery (26). Carotid sinus baroreceptors detect an increase in blood pressure and send afferent nerve impulses to the central nervous system, which increase parasympathetic tone and decrease sympathetic outflow, decreasing blood pressure.

Iliac arteriovenous anastomosis is another method that has been shown to reduce blood pressure in patients with uncontrolled hypertension in a preliminary study. In 2015, results from a prospective, randomised, controlled trial where patients were allocated in a 1:1 ratio to undergo implantation of an arteriovenous coupler device plus current pharmaceutical treatment or to maintain current treatment alone, showed that arteriovenous anastomosis was associated with significantly reduced blood pressure and hypertensive complications. However, the long-term safety of this device has not yet been assessed.
1.1.2.3 Long term outcome of patients with Resistant Hypertension

The long-term outcomes of patients with resistant hypertension are poor as persistently elevated blood pressure leads to increased risk of cardiovascular mortality(27) (22) (28), including ischaemic heart disease, stroke, peripheral arterial disease and renal disease. In a large community cohort of patients with incident hypertension, 1.9% developed resistant hypertension within 1.5 years from initial treatment, and were almost 50% more likely to experience a cardiovascular event over 3.8 years follow-up compared to patients without resistant hypertension (14).

Analysis of 14684 patients in the Antihypertensive and Lipid-Lowering Treatment to Prevent Heart Attack Trial (ALLHAT) (29) found the following multivariable adjusted hazard ratios comparing participants with versus without apparent treatment resistant hypertension: coronary heart disease (1.44 [1.18-1.76]), stroke (1.57 [1.18-2.08]), heart failure (1.88 [1.52-2.34]), peripheral artery disease (1.23 [0.85-1.79]), and end-stage renal disease (1.95 [1.11-3.41]). In addition, the hazard ratio for all-cause mortality was (1.30 [1.11-1.52]).

1.1.3 Assessing medication adherence

One cause of pseudo-resistance is poor medication adherence. Studies have shown that approximately 40-50% of patients with newly diagnosed hypertension discontinue medication within the first year of treatment (30). Poor adherence with medication is not confined to patients with hypertension but contributes to many poorly controlled disease states (31). For example, despite cardiovascular disease being the main cause of death worldwide, adherence with primary prevention
cardiovascular medication is poor with patients prescribed lipid-lowering therapy remaining without filled prescription for over a third of the year (32). A large cohort study (33) assessed 2-year adherence rates for patients who received at least one statin prescription between January 1994 and December 1998 (22379 patients with acute coronary syndrome, 36106 with coronary artery disease, and 85020 with statin for primary prevention). The authors found that two-year adherence rates were only 40.1% for acute coronary syndrome patients, 36.1% for chronic coronary artery disease patients, and 25.4% for primary prevention patients. This study suggests that many patients initiating statin therapy may receive limited benefit due to premature discontinuation of statins.

Diagnosing poor medication adherence is critical for both patients and health care providers. For the patient, those who adhere to antihypertensive treatment are found to have a 37% reduced risk of cardiovascular outcomes than those who experience at least 1 episode of treatment discontinuation (34). For healthcare providers, patients with poor adherence and therefore uncontrolled blood pressure often are referred to specialist centres. Commonly, clinicians may then order many further investigations to establish a cause of poor blood pressure control, leading to considerable expense.

In addition, individual patient adherence can have an impact on population health. For example, small decreases in individual adherence for patients on anti-retroviral therapy for HIV have been shown to reduce the ability to achieve an undetectable viral load at a population level (35).

1.1.3.1 Patient characteristics associated with medication non-adherence

Patient characteristics associated with medication adherence were assessed in a large retrospective study, which used ICD-9 codes to assess adults with primary or secondary diagnoses for any of eight conditions (depression, hypertension,
hyperlipidemia, diabetes, asthma or chronic obstructive pulmonary disease, multiple sclerosis, cancer, or osteoporosis) (36). Electronic pharmacy data was then obtained for 128 medications used to treat these conditions and medication possession ratios calculated. This study found that for patients with only one condition, medication adherence was higher in males, Caucasians, older patients, and those living in areas with higher education rates and higher income. For the whole group of patients with one and more than one comorbidity, adherence was higher in patients with fewer comorbidities. Patient adherence was lowest in patients with diabetes (51%) and asthma (33%).

1.1.3.2 Adherence with anti-hypertensive therapy
Poor adherence with anti-hypertensive therapy contributes to approximately 10-20% of patients diagnosed with resistant hypertension (37). Factors influencing adherence with prescribed medication include which anti-hypertensive is prescribed in the first instance, with improved adherence if patients start with a dihydropyridine calcium antagonist or an angiotensin converting enzyme inhibitor (ACE-I) compared to those starting with diuretics or beta-blockers (38). Patients also showed improved adherence with medication if they were initially treated in tertiary hospital care than by general practitioners (38) (39). Adherence is affected by dosing frequency: reducing the number of daily doses has been found to be effective at increasing adherence (40). Finally, the number of medications prescribed to treat hypertension also affects adherence, with the likelihood of blood pressure remaining uncontrolled increasing from 3.6% of those needing ≥4 classes of antihypertensive medications to >40% of participants taking ≥5 classes (27).
Measurement of medication adherence

It is therefore critical when assessing a patient with resistant hypertension to be able to assess medication adherence accurately (41). Current clinical guidelines (13) acknowledge the importance of checking for medication adherence, and suggest that this should be performed by patient self-reporting in clinic visits, alongside discussion of medication side effects, costs and other factors which might limit adherence. However, this approach will only identify the patients who are willing to acknowledge non-adherence (42).

Another option is measure the rate of prescription filling. Between July-September 2008 one study assessed 10 349 139 index prescriptions filled by 5 249 380 patients in one large US pharmacy (43). Overall, 3.27% of index prescriptions were abandoned. Other options to measure adherence include electronic adherence monitoring e.g. electronic recording of medication box opening (44, 45) (46). However these methods are still subjective; picking up a prescription refill or opening a medication box does not ensure that the patient has taken the medication.

Recent advances have concentrated on detecting medication adherence through serum or urine assays of prescribed antihypertensive drugs (47) (48). These methods use high-performance liquid chromatography-tandem mass spectrometry (HP LC-MS/MS) to analyse spot urine or serum samples to detect the presence of medication. A study (48) has shown that in a cohort of 208 hypertensive patients, overall 10.1% were totally non-adherent to medication and 14.9% partially non-adherent. Within this cohort, the highest prevalence of partial and total non-adherence was among follow-up patients with inadequate blood pressure control (28.8%) and those referred for consideration of renal denervation (23.5%), respectively. These findings highlight
the importance of assessing for medication adherence in patients with uncontrolled blood pressure.

1.1.3.4 Improving medication non-adherence
To improve patient adherence with medication it is key to understand that adherence is a cluster of many stages (31). There are many steps before a patient is able to take a pill, which are required for a patient to remain adherent with a certain medication e.g. attending a doctor’s appointment, picking up the medication prescription, paying for the prescription and finally taking the medication on a daily basis. Each individual may not be equally adherent with all of the required steps. For example, environmental conditions that promote appointment keeping (such as availability of transport, clinic waiting times (49)) differ from those that promote pill taking (adverse effects, number of medications taken(27)).

There are many interventions that might improve patient adherence(50) which can be split into improving medication adherence for patients requiring short and long term treatment. Patients requiring short-term treatment should be counseled about the importance of adherence and given written instructions for taking the medicine with reminder packaging(51). Improving adherence for patients requiring long-term therapy is more challenging and should include instructional material, simplifying medication regimes (e.g. controlled release dosage forms), reminders for medication and synching medication to daily events(51). Physician time constraints also limit the ability of physicians to provide all preventive services recommended by the US Preventive Services Task Force (USPSTF), at the recommended frequency (52). Therefore, separate behavioral counseling consultations outside of main clinic appointments may be useful. These methods are in general time consuming and
labour-intensive (51) and newer innovative approaches are being trialed in patients with different co-morbidities, such as automated adherence interventions by interactive voice response, e-mail, and text messaging (53).
1.2 The neural control of renal function

Since the 1850s, the importance of the sympathetic nervous system in the control of hypertension has been established (54). In 1851, Claude Bernard showed that section of the cervical sympathetic nervous system caused an increase in skin temperature. Following on from this work, in 1852, Charles Brown-Sequard demonstrated that sympathetic nerve stimulation leads to vasoconstriction and elevated blood pressure. Since this point, the sympathetic nervous system has been categorized as the “pressor nerves”.

Within the sympathetic nervous system are two types of nerve fibers: the efferent nervous system, which are fibers from the neuraxis to the kidney that use norepinephrine as the primary neurotransmitter, and the afferent nervous system, fibers running from the kidney to the neuraxis which contain substance P and calcitonin gene-related peptide as primary neurotransmitters. The afferent and efferent nerves interact to form a complex feedback system which controls volume homeostasis and therefore blood pressure control, Figure 1-1.
Figure 1-1. Schematic summary of renorenal reflex response to stimulation of renal mechanoreceptors or chemoreceptors

DRG (dorsal root ganglia), CNS (central nervous system), ERNA (efferent renal nerve activity), ARNA (afferent renal nerve activity)

Reproduced with kind permission from G DiBona et al. (55)
1.2.1 Neural control of the renal circulation: the efferent nervous system

The renal nerves enter the kidney in association with the renal artery and vein in the adventitia of the blood vessel wall in the renal cortex and outer medulla (56). The sympathetic nerves innervate all parts of the renal vasculature and nephrons, with the greatest density of innervation along the thick ascending limbs and distal convoluted tubules (57) (58).

1.2.1.1 Mediators of the efferent nervous system

Efferent renal nerve activity is considered exclusively sympathetic. The renal postganglionic sympathetic neurons are controlled by preganglionic autonomic neurons originating in the medulla oblongata and thoracic and lumbar segments of the spinal cord. The postganglionic nerves release norepinephrine (59) into the synaptic cleft on the renal vasculature. Since this discovery by Von Euler in 1948, norepinephrine release rate has been used as a measure of sympathetic activity (60). Norepinephrine in turn stimulates $\alpha_1$-adrenoreceptors on the renal vasculature, which triggers a signaling cascade, resulting in smooth muscle contraction via activation of inositol triphosphate (IP3) and Protein Kinase C (PKC) (61). Circulating norepinephrine is also released which overflows from the synaptic cleft into the renal interstitium and acts on adrenoreceptors on the renal vasculature.

Norepinephrine also causes renin secretion from the juxtaglomerular cells (62), which are in direct apposition with sympathetic nerve fibres (63). Alterations in efferent renal sympathetic nerve activity are directly related to renin secretion with graded increases in renal sympathetic nerve stimulation producing graded increase in renin secretion (64). This reflex mechanism is dependent on renal beta 1-adrenoceptors and is partly dependent on intact renal prostaglandin synthesis (65). Renin in turn
circulates in the blood stream and breaks down angiotensinogen released from the liver into angiotensin I. Angiotensin I is further broken down by angiotensin-converting enzyme into angiotensin II which then facilitates aldosterone secretion from the adrenal glands, Figure 1-2.

**Figure 1-2.** *Figure to illustrate the major effects of increased renal sympathetic nerve activity on juxtaglomerular granular cells, tubules, and arterial vessels*
1.2.1.2 **Response to efferent renal sympathetic activation is dependent on stimulation frequency**

The response to efferent sympathetic activation varies with stimulation frequency, Figure 1-3. Under normal physiological conditions, basal efferent renal sympathetic nerve activity (RSNA) is low. Low basal efferent RSNA (low level stimulation frequencies <1 Hz) is sufficient to cause a decrease in urinary sodium excretion and an increase in renal tubular sodium and water reabsorption without a change in renal perfusion pressure, glomerular filtration rate (GFR), renal blood flow, or intrarenal distribution of blood flow (64) (66). This response is blocked with renal adrenoreceptor blockade (67) but not with administration of a peptide angiotensin II receptor antagonist, which indicates that low frequency renal sympathetic nerve stimulation increases renal tubular sodium reabsorption via activation of α-adrenoreceptors exclusively. After renal denervation of animals with low efferent sympathetic nerve activity, the response consists of an increase in urinary sodium excretion and a decrease in renin secretion with no change in renal blood flow or glomerular filtration rate.

Conversely, in dogs with increased renal sympathetic nerve activity (68), high stimulation renal nerve activation also produces a decrease in renal blood flow with a decrease in GFR and urinary sodium excretion(69) and renal vasoconstriction. High level stimulation of 5-7 Hz is shown to result in complete renal vasoconstriction(55). Similarly, after renal denervation in rats with high sympathetic tone (congestive heart failure or hypertension), an increase in basal renal blood flow velocity is shown (70) (71).
Figure 1-3 Relationship of frequency of renal sympathetic nerve stimulation with response of renin secretion rate (increases), urinary sodium excretion (decreases), and renal blood flow (decreases)

Reproduced with kind permission from G DiBona et al. (55)
1.2.1.3 Reflex inhibition of the efferent renal sympathetic nervous system by the left atrial volume receptor reflex

Similar to low dose direct electrical stimulation of the renal sympathetic nerves, reflex increases in efferent renal sympathetic nerve activity (ERSNA) cause antidiuresis and antinatriuresis without alteration in renal blood flow. Conversely, inhibition of renal sympathetic nervous activity, by stimulation of left atrial stretch receptors through balloon inflation above the mitral valve, have been shown to cause the opposite effect: a decrease in efferent renal sympathetic nerve activity induces an increase in urinary flow rate in anaesthetised dogs\(^{(72)}\). This diuretic response is blocked after vagotomy or cardiac denervation, indicating that the diuretic response has an afferent pathway in the vagus nerve\(^{(73)}\).

In animal studies of dogs that had either cardiac (afferent) or renal (efferent) denervation, reflex inhibition of ERSNA by left atrial receptor stimulation failed to cause an increase in urinary flow rate and sodium excretion \(^{(74)}\). Therefore, these results suggest that reflex changes in RSNA originating from atrial receptors are also involved in eliciting natriuresis.

1.2.2 Neural control of the renal circulation: the afferent nervous system

The afferent sensory nerves are predominately located in the renal pelvic wall from where they integrate at a supraspinal level within cell bodies from T6 to L4 \(^{(75)}\). Within the spinal cord, the afferent nerves project to the ipsilateral dorsal horn where they synapse with neurons that project to sites within the central nervous system that are involved in cardiovascular regulation including the hypothalamus.

The afferent and efferent nervous systems interact through the excitatory and inhibitory reno-renal reflexes. Furthermore, the afferent nervous system is also
influenced by direct stimulation through mechanoreceptor and chemoreceptor activation, as well as dietary salt intake.

1.2.2.1 Stimulation of the afferent nervous system: Interaction of the afferent and efferent nervous system forming an inhibitory renorenal reflex

The afferent nerves form a renorenal reflex through their interaction with the efferent nervous system (76). Activation of the efferent nervous system activates the afferent nervous system through release of norepinephrine, which stimulates $\alpha_1$ and $\alpha_2$ adrenoreceptors on the renal pelvic sensory nerve fibers, modulating afferent nerve activity independently of hemodynamic changes (77). Activation of the efferent nervous system thus directly activates the afferent nervous system, which in turn inhibits the efferent nervous system through the inhibitory renorenal reflex, increasing urinary flow rate and urinary sodium excretion. This inhibitory renorenal reflex mechanism is key in maintaining efferent renal sympathetic activity at a normal low level to ensure renal sodium excretion. This inhibitory reflex has been shown to be impaired in hypertensive rats, contributing to their high level of efferent activity (78).

This reflex is terminated after renal arterial occlusion which causes ischaemic injury to the renal nerves (79). Similarly, renal denervation in normotensive rats destroys this reflex and therefore abolishes contralateral urinary sodium excretion due to an increase in contralateral efferent renal sympathetic nerve activity (80). This suggests that the afferent renal nerves exert tonic inhibition of ERSNA that is removed once unilateral renal denervation occurred.

This reflex has also been shown to be activated after renal pelvic administration of substance P (81) and blocked by substance P receptor blockade and prostaglandin
(PG) synthesis inhibition (82), indicating the importance of these two substances in the afferent renal nervous system.

### 1.2.2.2 Stimulation of afferent nervous system through mechanoreceptors and chemoreceptors

The renal afferent nervous system can also be activated directly through renal mechanoreceptors and chemoreceptors. Renal mechanoreceptors are activated by changes in ureteric pressure from changes in urinary flow rate which act as a marker of fluid status. An increase in ureteral pressure causes an increase in ipsilateral afferent renal nerve activity and a reflex decrease in contralateral efferent renal nerve activity (and thus an increase in urinary flow rate and urinary sodium excretion as described) (83).

Activation of the chemosensitive renal nerves elicits in rats a similar response, however differential blockade with pelvic lignocaine suggests that sensory mechanoreceptors are located in an anatomically different area to chemoreceptors (83).

### 1.2.2.3 Interaction of the afferent and efferent nervous system forming an excitatory renorenal reflex

There is also an excitatory renorenal reflex, in which activation of the afferent nerves of one kidney elicits an increase in the efferent nerves of the contralateral kidney (84). In patients with advanced renal disease, it is postulated that there is a shift from the inhibitory to excitatory renorenal reflex, leading to increased water and sodium retention and thus increased arterial blood pressure commonly seen in end-stage renal disease. Studies in dialysis patients with and without their native diseased kidneys shows increased arterial blood pressure and muscle sympathetic nerve activity in
patients with their diseased kidneys compared to those who have had nephrectomy (85). This was not correlated with plasma renin or norepinephrine levels, supporting the theory that in advanced renal disease, overactivity of the afferent nervous system is a key mechanism in the aetiology of hypertension.

1.2.2.4 Modulation of the afferent nervous system by dietary salt

The responsiveness of renal afferent and efferent nerves has been found to be increased by dietary salt, thus producing physiologically appropriate responses to maintain sodium balance. Experiments have shown that rats fed high salt diets have greater increases in ipsilateral afferent renal nerve activity and greater reductions in contralateral ESRNA to those rats fed a low salt diet (86).

1.2.3 Assessment of human sympathetic nervous system activity

1.2.3.1 Urine and plasma assays of norepinephrine

Von Euler first characterized the sympathetic nervous system transmitter as norepinephrine in 1948 (87), fuelling subsequent research into the use of norepinephrine to quantify the activity of the sympathetic nervous system. Initially, activity was assessed using static urine (88) and plasma (89) assays of norepinephrine. These approaches were limited, however, as the rate of norepinephrine synthesis and release does not correlate with its concentration in the urine or plasma due to variable plasma and urine clearance of norepinephrine (90).
1.2.3.2 Muscle microneurographic electrophysiology methods

Hagbarth devised electrophysiological methods for measuring nerve firing in sympathetic nerves distributed to skeletal muscle and skin (91). This has been used to study sympathetic nervous function in humans, but is limited to studying only the sympathetic nerves to skin and skeletal muscles and not nerves innervating internal organs. Muscle sympathetic nerve activity (microneurography) is performed by obtaining multifiber recordings of muscle sympathetic nerve activity from the peroneal nerve posterior to the fibular head with microelectrodes. Neural activity is quantified by counting the number of sympathetic pulse-synchronous impulse bursts in the mean voltage neurogram (burst incidence).

Initial studies conducted by Wallin et al (92) showed that between different subjects there were marked differences in mean burst incidence, and there was a tendency for increasing values with increasing age. They reported no difference in neural activity between hypertensive and normotensive individuals after accounting for age. However, these studies involved patients with moderate-to-severe hypertension who were not age matched nor on controlled sodium intake diets.

Subsequently, Anderson et al (93) recorded microneurography in 15 normotensive and 12 borderline hypertensive age-matched men on controlled sodium diets to assess if borderline hypertensive individuals have elevated sympathetic nerve activity. They found significant elevation of sympathetic nerve activity in borderline hypertensive individuals on both low and high sodium diets.

1.2.3.3 Urinary excretion of norepinephrine

Subsequent research focused on assessing sympathetic nervous system activity from dynamic measures of norepinephrine synthesis or release. Total urinary excretion of
norepinephrine can be used as a measure of norepinephrine synthesis as it is excreted in the urine (94). One major drawback to this approach is that rates of norepinephrine synthesis and release do not exactly coincide; some amount of intraneuronal metabolism of norepinephrine occurs before its release (95). In addition, not only norepinephrine produced in the sympathetic nervous system is excreted in the urine; norepinephrine from the adrenal medulla is also excreted as well as norepinephrine metabolites from the central nervous system. These factors make the use of urinary excretion of norepinephrine an inaccurate marker of its synthesis in the sympathetic nervous system alone.

1.2.3.4  Norepinephrine spillover

In contrast, norepinephrine in plasma is derived largely by norepinephrine release from sympathetic nerves, with only a very small contribution from the adrenal medulla and none from the central nervous system (96). To avoid the concern, as mentioned previously, that plasma norepinephrine concentration is dependent on the rate at which norepinephrine is removed from the circulation, the concept of norepinephrine spillover was developed. The norepinephrine spillover rate gives the rate at which released norepinephrine enters plasma: in humans this is approximately 20% of the total turnover of norepinephrine in sympathetic nerves (94). This can be measured by radiolabeled noradrenaline.

Total norepinephrine spillover is reduced in the presence of low sympathetic nervous activity e.g. in cases of sympathetic nerve failure (97) or administration of clonidine (98), a sympathetic nervous system suppressant. In contrast, norepinephrine spillover is increased in the presence of high sympathetic nervous system activity e.g. in patients with hypertension (99), cirrhosis(100) and congestive heart failure(101).
1.2.4 Sympathetic over activation in patients with hypertension

In patients with hypertension there is increased cardiac, renal (102) and peripheral (93) sympathetic nervous system activity(55). The inhibitory renorenal reflex is suppressed in hypertensive rat models (103), which leads to increased ERSNA and therefore water and sodium retention. In addition, there is some evidence that renal inflammation from hypertension will separately cause activation of the excitatory renorenal reflex. This is indirectly supported by the finding (104) that in patients with treatment resistant hypertension, after denervation there is a sustained decrease in muscle sympathetic nerve activity, suggesting that denervation interrupted excitatory reflexes originating in the kidneys. Suppression of the inhibitory renorenal reflex and activation of the excitatory renorenal reflex will then cause a cycle of efferent nerve activation leading to further high blood pressure and thus further inflammation.
1.3 Targeting the renal sympathetic nervous system as a treatment strategy for hypertension

1.3.1 Surgical sympathectomy: historical perspective
Surgical sympathectomy to treat hypertension was performed from 1935 onwards. The initial approach was to surgically ligate as many sympathetic nerves as possible through intraspinal section of the lower six thoracic and first two lumbar anterior spinal nerve roots (105). The results showed a decrease in blood pressure that persisted for 1-2 years, however the authors noted, “the serious danger in the operation is the occurrence of a lesion of the cord giving rise to paresis or paralysis of the lower extremities, bladder and rectum.”

Supradiaphragmatic extrapleural splanchnicectomy was subsequently performed in 700 patients by resecting a long section of the greater splanchnic nerves above the diaphragm and the lesser splanchnic nerves (106) (107). Again, a reduction in blood pressure was noted, however again with a high operative mortality and disabling side effects in those that survived.

Grimson (108) subsequently performed an extensive operation consisting of total thoracic and partial to total lumbar sympathectomy and coeliac ganglionectomy. It required a 3-stage technique and 3 operation sites: 2 thoracic and 1 abdominal (illustrated in Figure 1-4 from the original paper). They performed this in 11 patients. Two patients passed away from the procedure however in the remaining 9 the authors commented that “the lowering of the blood pressure with the patient standing, sitting, or walking about has been more marked and is present in all patients”. Reassuringly, the authors described that the side effects of this operation were less disabling than previous approaches: “This extensive sympathectomy has been demonstrated to be compatible with a relatively normal existence. The patients dress more warmly in cold
weather and notice excess perspiration during warm weather in those areas still capable of sweating.” Other side effects described included postural and postprandial hypotension, as well as syncope and sexual dysfunction.

With the advent of pharmacological therapy for hypertension in the form of ganglion blockers in the 1950s, surgical sympathectomy for the treatment of hypertension ceased.
Figure 1-4. Illustration of Grimson’s surgical sympathectomy operation. Diagram from the original article by Grimson et al illustrating the position of the patient during the thoracic operation and the location of the two thoracic incisions.
1.3.2 Evolution of catheter based renal denervation

Although surgical sympathectomy, targeting a non-renal selective portion of the sympathetic nervous system, showed a reduction in blood pressure, its side effect profile was unacceptable.

Howard Levin and Mark Gelfand in the USA first conceived the concept of Catheter based renal denervation for treatment of essential hypertension in the early 2000s. The theoretical background of this procedure was based on three research observations. The first observation has already been discussed, which is that there is activation of sympathetic outflow in patients with essential hypertension.

The second observation came from the results of two studies which showed a reduction of sympathetic nervous activation after nephrectomy \(^{85, 109}\). These studies highlighted the central importance of the kidneys in sympathetic nervous system control and that denervation might be able to be target the renal sympathetic system alone.

The third observation came from anatomical research which showed that postganglionic renal sympathetic nerves pass into the kidneys via the outer adventitia of the renal arteries, therefore within reach of radiofrequency ablation from within the lumen.

These three findings helped to fuel the concept of catheter-based renal denervation as a method for reducing renal sympathetic activity.

1.3.2.1 Catheter based renal sympathetic denervation trials: discrepancy of blood pressure reduction

There were many outstanding queries about this novel treatment that needed to be answered. Although encouraging, surgical sympathectomy was of course unblinded, and therefore the extent of blood pressure reduction was not easily quantifiable.
Furthermore, whether adequate denervation reduction was achievable through a catheter based approach also needed to be assessed.

Since 2007, over 100 clinical trials have been performed assessing the impact of catheter-based renal denervation on blood pressure reduction. A number of unblinded observational trials and randomized, controlled trials produced initially exciting results.

The Symplicity HTN-1 trial (110) enrolled 50 unblinded patients of which 45 underwent renal denervation and showed reduction in office systolic/diastolic blood pressure of -14/-10, -21/-10, -22/-11, -24/-11, and -27/-17 mm Hg at 1, 3, 6, 9, and 12 months, respectively. As these results were so encouraging, Symplicity HTN-1 continued to enroll 153 patients and showed at 36 months in 88 patients completing follow-up a persistent BP reduction of -32/-14.4mmHg(111). In addition, denervation was free of complications in 97% of patients.

Following on from this, Symplicity HTN-2 evaluated 106 patients randomised in a 1:1 fashion to receive denervation plus medical therapy or medical therapy alone. Again, encouraging results were seen with a significant reduction in office blood pressure in the denervation arm of -32/-12mmHg at 6 months (112) and -28/-10 mmHg at 1 year (113). The details of Symplicity HTN-1 and HTN-2 trials are summarized in Table 1-1.
Table 1-1. Details of Symplicity HTN-1, HTN-2 and HTN-3 clinical trials

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<th>Blinding performed</th>
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<th>No. patients in control arm (office BP)</th>
<th>No. patients in active arm (ambulatory BP)</th>
<th>No. patients in control arm (ambulatory BP)</th>
<th>Baseline office SBP active arm (mmHg)</th>
<th>Baseline office SBP control arm (mmHg)</th>
<th>Change in ambulatory BP in active arm (mmHg)</th>
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<td>180.2</td>
<td>-6.75</td>
<td>-4.79</td>
<td>5.1</td>
<td>5.1</td>
<td>5.1</td>
<td>6</td>
</tr>
</tbody>
</table>
1.3.2.2 Criticism of clinical renal denervation trials

Several concerns were raised about the design of these clinical trials. First, none of the trials up to this point had included blinded end-points. Although Symplicity HTN-2 was a randomised controlled study, there was no sham control procedure, which is known to be important to accurately evaluate efficacy of a new procedure (114).

Second, there was no mandated use of ambulatory blood pressure monitoring either as an entry criteria or as an endpoint. Without the use of ambulatory blood pressure monitoring as an inclusion criteria, this might lead to patients being included with pseudo-resistant hypertension. In addition, only using office blood pressure as an endpoint would likely increase the expected effect size (115).

Third, the reduction in office SBP of >10mmHg shown in Symplicity HTN-1 and HTN-2 was not replicable in a number of smaller studies (116) (117), which showed substantial heterogeneity of blood pressure response to RDN. These findings raised concerns over the true effect size of denervation.

Fourth, several concerns were raised surrounding the patients enrolled in Symplicity HTN-1 and 2. In particular, there was no ‘per protocol’ exclusion of secondary causes of hypertension. In addition, there was no assessment of adherence to medication in either trial. Finally, many patients were on non-optimized hypertensive medications and doses in these trials.

The next step was therefore to proceed to a sham- controlled trial, which would be expected to reduce, but not eliminate, the reduction in blood pressure (118). The results of Symplicity HTN-3 (119), the first sham-controlled RDN trial, were disappointing as the trial failed to reach its targeted reduction in blood pressure. Symplicity HTN-3 included 535 patients with severe resistant hypertension who were randomly assigned in a 2:1 ratio to undergo renal denervation using the Symplicity
Flex catheter (Medtronic, MN, USA) or a sham procedure (renal angiogram only). Patients were blinded to which arm of the trial they were in. Overall there was only a decrease of $-2.39$ mm Hg in ambulatory blood pressure ($p=0.26$) between the two arms.

### 1.3.3 Insights from previous renal denervation trials

Despite the disappointing blood pressure reduction results from Symplicity HTN-3, there are several important learning points for future trial design:

#### 1.3.3.1 Changes in medication regime within the trial period

In Symplicity HTN-3, a substantial decrease in blood pressure was seen in the *sham* patient group, more than can be expected by natural variability alone. Medication adherence within Symplicity HTN-3 was assessed using only patient self-reported medication diaries, so the full extent of change in adherence is not known. In addition, during the trial, many patients (38% in RDN arm, 42% in sham arm) underwent medication regime changes (both changes in dose and class of antihypertensive medication) between randomization and 6-month follow up (120). Therefore, the change in ambulatory blood pressure in the sham arm may be due to a combination of change in medication adherence and alteration in anti-hypertensive prescription within the course of the trial.

#### 1.3.3.2 Discrepancy in blood pressure reduction when measured using office versus ambulatory blood pressure

Symplicity HTN-3 assessed its primary outcome with ambulatory blood pressure reduction, compared to previous trials that measured office blood pressure only. In
anti-hypertensive drug trials without randomisation or blinding, meta-analysis (118) shows blood pressure reductions are 5.6 mm Hg larger with office measurements than with ambulatory blood pressure monitoring. It has been claimed that office and ambulatory blood pressure reductions differ in anti-hypertensive drug trials. However, this would not seem logical as ambulatory drug trials are averages of multiple repeated blood pressure measurements.

Further analysis actually shows that these differences between office and ambulatory blood pressure measurements depend on trial design in anti-hypertensive drug trials. Anti-hypertensive drug trials that use randomisation and blinding show identical office and ambulatory blood pressure reduction (118).

There are other differences between office and ambulatory blood pressure measurements which make ambulatory blood pressure a preferred end-point (121):

- Office blood pressures measurements are known to have poor test-retest reproducibility (122), which can lead to noisy measurements
- With office blood pressure measurements, a medical practitioner tends to take multiple measurements and then average the measurements. This leads to observed bias in measurements e.g. terminal digit preference (123) with zero being recorded 44.6% of the time for systolic BP (124).
- Office pressure drops may be larger than ambulatory drops in unblinded trials as operators will be biased to what stage of the trial the patient is in, and therefore either overestimate baseline office pressure or underestimate final pressures (125). In comparison, ambulatory blood pressure monitoring does not allow this user bias to occur.
- Overestimation of baseline blood pressure may occur because of the effect of
regression to the mean (126) (127). In hypertension trials this is commonly seen as patients tend to be enrolled in trials on a day when their blood pressure is above its average. Since ambulatory blood pressure averages multiple recordings, its variation is therefore smaller.

All renal denervation trials prior to Symplicity-3 were performed without blinding. Therefore, it would be expected that the 30mmHg drop in office systolic blood pressure in previous trials of renal denervation would be lower once randomization and blinding were instituted to reduce bias in Symplicity HTN-3. It was not expected though that the efficacy would be reduced to quite such a large extent.

### 1.3.3.3 Procedural analysis- number and location of ablations

Symplicity HTN-3 utilised the Symplicity Flex catheter for denervation. This catheter had a single electrode, each ablation taking 2 minutes. This therefore discouraged a large number of ablations due to procedural length. Post hoc analysis (120) of Symplicity HTN-3 indicated that greater reductions in blood pressure were identified with a higher number of renal artery ablations (at least 12 ablations per patient), thus suggesting that insufficient denervation may explain the disappointing results in Symplicity HTN-3. In addition, delivery of ablations in a four-quadrant pattern to either one or both renal arteries revealed greater reductions in blood pressure reduction.

Recent pre-clinical data has shown that denervation lowers renal norepinephrine more significantly when performed distally rather than proximally (128) (129). There is a histological rationale for this in humans. Although renal sympathetic nerve fibres are less numerous around the distal branches, the fibres are closer to the artery lumen in man (130), and a greater proportion of them are efferent (131). During catheter-based
radiofrequency ablation, the greatest injury is expected to be to the nerves closest to the arterial lumen. For example, a recent study reported that renal denervation injured 50% of nerves when performed distally but only 33% of nerves when performed proximally (129).

Together, these findings support the hypothesis that increasing the number of ablations and performing ablations in distal branches may improve the efficacy of denervation in achieving blood pressure reduction. No studies in humans yet have assessed the effect of distal renal artery denervation although recent studies in pigs have shown promising preliminary results (128).

1.3.3.4 Lack of an intraprocedural marker of efficacy

In clinical cardiology, many procedures are performed on a daily basis, for example, pacemaker implantation and stent insertion. All of these procedures have direct intraprocedural markers of efficacy. For example with a pacemaker, it is possible to test pacing thresholds and lead parameters directly after implantation. Similarly with coronary angioplasty, angiography and IVUS can be performed to ensure a stent is well deployed.

Currently there is a large amount of clinical uncertainty about the efficacy of renal denervation in achieving blood pressure reduction (132). Failure of blood pressure reduction might be for a number of reasons including procedural failure. Currently the primary endpoint in denervation procedures is a measurable change in blood pressure recorded a set time duration following denervation. Blood pressure measurements, as discussed, are noisy, prone to bias error, and cannot give immediate intra-procedural feedback.
Procedural failure could be ameliorated by developing a method to assess intraprocedural feedback acutely to assess ensure catheter ablation has actually caused denervation. Renal vein norepinephrine spillover is currently the gold standard for assessing change in renal sympathetic nerve activity after denervation. Previous catheter based denervation trials have shown a mean reduction in renal noradrenaline spillover of 47% only (110), suggesting incomplete efferent denervation. Although several markers along with renal vein norepinephrine spillover (110) have been found to correlate with denervation efficacy, such as baroreflex function (133), these are not feasible as acute intraprocedural clinical feedback markers. For example, measurement of renal vein norepinephrine spillover requires catheterization of the renal vein and ingestion of radioisotopes.

An alternative approach may be to assess change in acute renal haemodynamics before and immediately after ablation. If an acute haemodynamic variable was found to predict long-term reduction in blood pressure, then this may be useful in the future as an intra-procedural marker of efficacy of denervation.
1.4 Renal denervation as a treatment strategy for other disease states

Sympathetic over-activity has been found to play an important role in the pathophysiology of other disease states as well as hypertension. For example, sympathetic over-activation is associated with insulin resistance, sleep apnoea, heart failure, cardiac arrhythmia, nephrotic syndrome and liver cirrhosis. Renal denervation has also been proposed as a treatment strategy for these other disease states. The progress up to this point in these disease states is outline below. In general, research into the use of renal denervation in these other disease states is at an early stage, with no blinded randomised-trials completed at this time.

1.4.1 Insulin resistance

Interest in this topic stems from the observations made by Huggett et al (134), who noted that patients with diabetes have high resting sympathetic nerve activity. They measured resting muscle sympathetic nerve activity as the mean frequency of multi-unit bursts in 68 subjects with hypertension and diabetes, diabetes only, hypertension only and normal individuals. The results of this study showed that muscle sympathetic nerve activity was greater in patients with hypertension and diabetes (97+/−3.8 impulses/100 beats) than those with hypertension only (69+/−3.4 impulses/100 beats) and those with diabetes only (78+/−4.1 impulses/100 beats). However, all these results were significantly greater than patients with neither hypertension nor diabetes (53+/−3.3 impulses/100 beats), despite similar baseline characteristics. This study suggested that patients with diabetes, as well as patients with hypertension, had central sympathetic over-activity.

This observation prompted further studies exploring the potential effect of denervation on metabolic syndrome. Preliminary studies have suggested that
denervation may help reduce insulin resistance. Mahfoud et al (135) studied 50 patients prospectively and showed reduced fasting glucose (from 118±3.4 to 108±3.8 mg/dL, p=0.039) and serum insulin (from 20.8±3.0 to 9.3±2.5 µIU/mL, p=0.006) in patients treated with renal denervation compared to control subjects who showed no significant change in markers.

These results and other small studies sparked considerable interest in the use of denervation for insulin resistance, prompting further trials. The DREAMS study (136) (Denervation of the Renal Arteries in Metabolic Syndrome) was designed to investigate the effects of RDN on insulin sensitivity in patients with metabolic syndrome. Twenty-nine patients treated with renal denervation showed no change in median insulin sensitivity (measured using the Simple Index assessing the oral glucose tolerance test) at 6- and 12-month follow-up (p=0.60 and p=0.77, respectively).

In conclusion, the efficacy of renal denervation in improving insulin sensitivity currently still remains unclear, and has not yet been tested in a blinded randomised control trial.

1.4.2 Heart failure
Heart failure is associated with increased renal norepinephrine spillover, indicating efferent renal sympathetic over-activity (101). Furthermore, anti-adrenergic agents e.g. beta-blockers, reduce mortality in patients with chronic heart failure (137) (138) (139).

In pre-clinical models, heart failure rats who underwent renal denervation showed increased basal renal blood flow and improved autoregulation of renal blood flow (140). Indirect clinical evidence from trials investigating renal denervation for
resistant hypertension suggested that denervation might be beneficial for patients with hypertension due to an observed reduction in left ventricular mass index, and an increase in lateral tissue Doppler (141).

The REACH pilot study (142) (Renal Artery Denervation in Chronic Heart Failure) assessed renal denervation in 7 patients with New York Heart Association class III to IV heart failure and left ventricle ejection fraction 43±15% without hypertension. The patients showed improvement in their 6-minute walk test (221±33m to 249±34m, p=0.03). These results were used to design the REACH trial, a randomised blinded trial with a sham arm, which is currently on-going and will provide clarity on whether renal denervation will be beneficial for this patient group.

1.4.3 Obstructive sleep apnoea
Obstructive sleep apnoea has been suggested to have an additive effect on sympathetic over-activation in patients with metabolic syndrome as recent studies suggests that patients with the metabolic syndrome and OSA had higher sympathetic activity than patients with the metabolic syndrome alone (143) (144). Current evidence assessing whether renal denervation might be a potential treatment for sleep apnoea has been inconclusive and limited. A prospective unblinded study by Witkowski et al (145) in 10 patients found that denervation decreased sleep apnoea. However, any impact of denervation on apnoea will not be understood without performing randomised blinded clinical trials.

1.4.4 Atrial fibrillation
A meta-analysis of denervation in patients with resistant hypertension suggests a decrease in atrial fibrillation relapse in patients treated with renal denervation plus pulmonary vein isolation (PVI) compared with pulmonary vein isolation alone (146). At 12 months, 63% of the PVI with denervation had no atrial fibrillation compared to 41% in the PVI only group. There are ongoing trials such as the ERADICATE-AF trial currently assessing the efficacy and safety of denervation for the management of AF.
1.5 Meta-analysis assessing the magnitude and variability of the effect size from renal denervation

1.5.1 Introduction

As described previously in this Chapter, there is currently inconsistency in the outcome of renal denervation trials in terms of blood pressure reduction. One potential cause of this inconsistency described previously relates to bias in blood pressure measurement, which can be assessed as the standard deviation of the effect size. Bias in the measurement of effect size will lead to more noisy measurements, and therefore a larger variability or standard deviation in the measured effect size. Bias within renal denervation trials can occur from many causes (147). First, studies select patients on the basis of their blood pressure being higher than a certain threshold. This selection process may tend to select patients when their blood pressures are above their individual long-term averages. Second, in an unblinded trial where doctors measure office blood pressure reduction, if a doctor is faced with no fall in blood pressure after an intervention, they may tend to re-measure blood pressure until a lower value is recorded. This tendency is therefore removed when outcome is measured by automatic ambulatory blood pressure monitors.

The standard deviation of the effect size is a key factor of trial design for several reasons:

1) The standard deviation of the effect size determines the ability of a study to detect effect size: narrow between-individual variation increases the ability of a study to detect small effect sizes.
2) The standard deviation is a parameter which determines the sample size of a study required: the smaller the standard deviation, the fewer patients that need to be enrolled.

**1.5.2 Aim of meta-analysis**

Based on these observations I conducted a meta-analysis to assess the impact of renal denervation trial design on the magnitude and variability of the effect size from renal denervation. Measurement bias is assessed as the published standard deviation of effect size with trial design.

**1.5.3 Methods**

**1.5.3.1 Data sources and search strategy**

I systematically identified published renal denervation studies including 5 or more patients and measuring office and/or ambulatory blood pressures. The studies were identified using the search terms “renal AND denervation” on MEDLINE and EMBASE databases (January 2009 - December 2015). The search criterion were extended from a previous meta-analysis performed by my group (147). However, the data was reanalyzed to assess Controlled trials were not eligible for inclusion if the standard deviation of blood pressure change was not reported. If the raw data was in the study, the standard deviation was computed where possible. In addition, trials were not eligible for inclusion if the control arm received an alternative intervention.

Studies were categorised by:

(a) design (randomised/ non-randomised)

(b) type of data collected (office/ ambulatory blood pressure).
Each trial could contribute data to more than one category.

1.5.3.2 Data abstraction

From each study the following parameters were extracted from the active arm:

- Number of patients (for both office and ambulatory pressure monitoring)
- Baseline and final office blood pressure
- Standard deviation of office blood pressure change
- Baseline and final ambulatory blood pressure
- Standard deviation of ambulatory blood pressure change
- Mean or median time to follow up

Studies were then categorised by whether they used office or ambulatory blood pressure and whether they were randomized or unrandomised (no control group).

1.5.3.3 Statistical analysis

Data analysis was performed using R (148) and Comprehensive Meta-analysis software. Effect estimates and 95\% confidence intervals (CI) were calculated and assessed by a fixed and random-effects model. There was no difference in output between both models. The $I^2$ statistic was calculated to assess heterogeneity.
Records identified from database search (n = 1013) → 654 studies excluded which did not meet inclusion criteria

Abstracts screened (n = 359) → 204 studies further studies excluded which did not meet inclusion criteria

Full text articles assessed for eligibility (n = 155) → 99 studies excluded due to lack of reporting of standard deviation of pressure change

Studies included in meta-analysis (n = 56)

*Figure 1-5. Selection of studies for inclusion in the meta-analysis*
1.5.4 Results

1.5.4.1 Studies included
The studies were identified using the search terms “renal AND denervation” on MEDLINE and EMBASE databases. This search strategy identified a total of 56 studies meeting the inclusion and exclusion criteria between January 2009 - December 2015 (Figure 1-5). These included 30 trials measuring office blood pressure changes, 8 trials measuring ambulatory blood pressure changes and 18 trials measuring both office and ambulatory blood pressure. Baseline characteristics are shown in Table 1-2, organized by blood pressure measurement and presence of randomisation. All the details of the trials included are shown in Appendix 1.

1.5.4.2 Blood pressure reduction is greater when measured using office than ambulatory blood pressure and is not affected by randomisation
Table 1-2, Figure 1-6 illustrates the results of the meta-analysis. Figure 1-6 shows the results for office blood pressure (left hand diagram) and ambulatory blood pressure (right hand diagram). Each red dot represents the effect size for office blood pressure and each blue dot the effect size for ambulatory blood pressure for an individual trial. The area of each dot represents the number of patients in each trial. The black diamonds indicate the group mean of the standard deviations, with 95% confidence intervals. The published trials are categorized by whether they were randomised (left-hand panel) or non-randomised (centre panel).
As discussed previously in this Chapter, across the 4 categories of trial design, systolic blood pressure reduction was greater in randomised and non-randomised trials that measured outcome with office (-21.0 ± 2.6 mmHg and -25.8 ± 5.4 mmHg respectively) versus ambulatory blood pressure (-11.6 ± 2.4 mmHg and -7.5 ± 1.25
mmHg respectively), p<0.001 for both randomised and non-randomised trials respectively.

However, use of randomisation did not significantly affect the magnitude of blood pressure reduction for either office (p=0.2781) or ambulatory blood pressure reduction (p=0.108).

1.5.4.3 **The Standard deviation of effect is greater when measured using office than ambulatory blood pressure and is not affected by randomisation**

Figure 1-7 shows the results for the standard deviation of effect size for office blood pressure and ambulatory blood pressure. This meta-analysis shows that trials measuring office blood pressures show more variability in effect size between patients than trials measuring ambulatory blood pressure for both non-randomised trials (standard deviation of effect size 20.7 ± 7.3mmHg office BP, 12.8 ± 4.8mmHg ambulatory BP, p<0.001) and randomized trials (standard deviation of effect size 21.6 ± 2.5mmHg office BP, 13.6 ± 2.6mmHg ambulatory BP, p=0.003).

Randomization within a study, however, was not found to reduce the variability in effect size whether the trial end-point was office blood pressure (p=0.804) or ambulatory blood pressure (p=0.789).
**Table 1-2. Results of meta-analysis of published trials**

<table>
<thead>
<tr>
<th></th>
<th>Non randomised office</th>
<th>Randomised office</th>
<th>Non randomised ambulatory</th>
<th>Randomised ambulatory</th>
<th>p value between non-randomised and randomised measures</th>
<th>p value between non-randomised and ambulatory measures</th>
<th>p value between randomised and ambulatory measures</th>
<th>p value between non-randomised office and ambulatory measures</th>
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</thead>
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<tr>
<td>Trials</td>
<td>40</td>
<td>8</td>
<td>20</td>
<td>6</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Patients</td>
<td>1548</td>
<td>660</td>
<td>567</td>
<td>381</td>
<td>-</td>
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<td>-</td>
</tr>
<tr>
<td>Mean baseline SBP (mmHg)</td>
<td>174</td>
<td>176</td>
<td>154</td>
<td>152</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Age</td>
<td>60</td>
<td>58</td>
<td>60</td>
<td>60</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Mean follow-up (months)</td>
<td>7.4</td>
<td>6.5</td>
<td>5.6</td>
<td>6</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Mean change in SBP in active arm (±95% CI), mmHg</td>
<td>-21.0 (-23.6 to -18.4)</td>
<td>-25.8 (-31.2 to -20.4)</td>
<td>-11.6 (-14.0 to -9.1)</td>
<td>-7.5 (-8.8 to -6.3)</td>
<td>0.31</td>
<td>0.108</td>
<td>&lt;0.000</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Mean standard deviation of effect size in active arm (±95% CI), mmHg</td>
<td>20.4 (18.1 to 22.7)</td>
<td>21.1 (19.4 to 22.9)</td>
<td>125 (10.4 to 14.7)</td>
<td>14.5 (12.4 to 16.8)</td>
<td>0.801</td>
<td>0.336</td>
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<td>0.001</td>
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<td>I² (%)</td>
<td>96.4</td>
<td>0</td>
<td>98.2</td>
<td>96.3</td>
<td>-</td>
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</tbody>
</table>
Figure 1-6. Change in blood pressure for trials included in meta-analysis

The left hand diagram indicates the results for trials measuring office blood pressure and the right hand diagram for trials measuring ambulatory blood pressure. Each red dot represents the effect size for office blood pressure and each blue dot the effect size for ambulatory blood pressure for an individual trial. The area of each dot represents the number of patients in each trial. The black diamonds indicate the group mean, with 95% confidence intervals. The published trials are categorized by whether they were randomised (left-hand panel) or non-randomised (centre panel).
Figure 1-7. Standard deviation of change in blood pressure for trials included in meta-analysis

The left hand diagram indicates the results for trials measuring office blood pressure and the right hand diagram for trials measuring ambulatory blood pressure. Each red dot represents the standard deviation of effect size for office blood pressure and each blue dot the standard deviation of effect size for ambulatory blood pressure for an individual trial. The area of each dot represents the number of patients in each trial. The black diamonds indicate the group mean of the standard deviations, with 95% confidence intervals. The published trials are categorized by whether they were randomised (left-hand panel) or non-randomised (centre panel).
1.5.5 Discussion
This meta-analysis shows that the magnitude and variability in effect size is influenced by trial design. The magnitude and variability in effect size is increased in denervation trials that use office blood pressure to measure effect size. However, randomization within a study does not reduce the magnitude or variability in effect size.

1.5.5.1 Magnitude and variability in effect size is influenced by choice of blood pressure measurement end-point
In this meta-analysis, choice of method of measurement of blood pressure end-point strongly influenced the magnitude and standard deviation of the effect size. When office blood pressure is the study end-point, a medical practitioner needs to manually record each measurement and then average the measurements. This can lead to unintentional bias in measurements e.g. terminal digit preference (123, 124), as well as overestimation of baseline office pressure or underestimation of final pressures (125). In comparison, ambulatory blood pressure monitoring does not allow this user bias to occur as all measurements are recorded in the monitor and subsequently downloaded. Although user bias might be expected to reduce the standard deviation of the effect size, as users might unintentionally bias outcomes to seem more positive, this meta-analysis implies this is not the fact, and that blinding of measurements e.g. through the use of ambulatory blood pressure monitors, is an important step in trial design.
1.5.5.2 Magnitude and variability in effect size is not influenced by randomisation alone

Surprisingly, randomisation was not found to reduce the magnitude or standard deviation of effect size. Randomisation is commonly perceived to be a bias-resistant method for trials seeking to assess the impact of an intervention. These results may be due to the disproportionately higher number of studies without randomisation than with randomisation included in this meta-analysis. However, similar results were seen in a recent meta-analysis (147) which assessed the impact of randomisation on magnitude of effect size and concluded that randomisation alone did not reduce bias. This was hypothesized to be due to two remaining sources of bias. First, as these trials were not blinded, research staff were aware of treatment allocation and therefore reported larger drops in blood pressure in the active arm than control arm. However, this would not alone explain why this same pattern of bias was seen in ambulatory as well as office blood pressure trials. This is explained by the second source of bias: as patients were also not blinded in the active arm they may have been more likely to increase adherence to anti-hypertensive medications regimes whilst in the active trial arm. One way to address this second source of bias would be to conduct a trial with patients participating in directly observed anti-hypertensive therapy, to ensure no changes in medication adherence within the trial period.

1.5.6 Study Limitations

This meta-analysis uses data from multiple heterogeneous renal denervation trials with a variety of trial designs, including inclusion criteria and blood pressure measurement end-points. This may affect both the net effect on blood pressure, as well as the standard deviation of blood pressure response.
In addition, the majority of renal denervation studies published and therefore included in this meta-analysis are non-randomised case-control studies with a minority of randomised trials. This might potentially affect the results by having unequal numbers of trials in each trial-design arm in Figures 1-6 and 1-7.

1.5.7 Conclusion

This meta-analysis highlights the importance of measuring ambulatory versus office blood pressure as an outcome measure to minimise measurement noise. However, in comparison, use of randomisation in trial design was not found to significantly affect measurement bias. Narrow between-individual variation increases the ability of a study to detect small effect sizes. As a consequence, this may also reduce the sample size required for future trials, minimizing the expense of running a future clinical trial. The results of this meta-analysis were instrumental in designing the study outlined further in my Thesis. My study measured ambulatory blood pressure as the primary outcome to determine effect size. Furthermore, although not blinded, in this Thesis I assess the impact of directly-observed anti-hypertensive therapy on the variability of effect size.
2 Aims and objectives
2.1 Aims

My research in Chapter 1 helped me frame some current issues in the field of renal denervation. One of my key findings was that the basic physiological effects of denervation in humans has been minimally studied compared to pre-clinical studies, and that current research was focusing predominantly on outcome trials rather than mechanistic studies. Furthermore, the majority of results in Chapter 1 discussing acute haemodynamic changes after renal denervation were from pre-clinical unblinded studies. Therefore, direct translation into humans cannot be extrapolated from these studies. Potentially, if the mechanism of physiological changes which occur after denervation in humans were further assessed, this might help fuel future studies aimed to improve the efficacy of the treatment.

My meta-analysis in Chapter 1 outlining the effect of trial design on the magnitude and variability of effect size in previous denervation trials informed me of the need to meticulously design my study. One aspect I have chosen to focus on is the potential impact of improving medication adherence on the variability of effect size.

Therefore, based on my findings from my background research described in Chapter 1, I defined the following key Aims for my thesis:

2.1.1 **Aim 1** to assess the immediate and 6-month effects of bilateral renal denervation on invasively measured aortic and renal haemodynamics

In Chapter 1 the physiological changes of denervation on invasive renal haemodynamics in pre-clinical studies have been discussed. One of the key underlying questions in clinical studies is whether effective denervation is currently occurring, and whether there might be an intra-procedural marker of efficacy in the future.
Therefore, my first objective was to assess the immediate and 6-month effects of bilateral renal denervation on invasively measured aortic and renal haemodynamics (blood pressure, flow, resistance and wave speed). In particular, I aimed to investigate whether a similar pattern of change in haemodynamics as shown in pre-clinical studies occurs in humans after denervation.

Invasive renal haemodynamics have not been studied previously in humans to my knowledge. Therefore, the first stage in this Aim will be to assess the accuracy and repeatability of these measurements, both acutely and at 6-months follow-up duration. This objective necessitated designing my study as an unblinded proof-of-concept trial where each patient received renal denervation.

2.1.2 Aim 2- to explore the efficacy of distal renal denervation on blood pressure reduction whilst minimizing the impact of drug compliance by measuring urine anti-hypertensive levels at baseline and 6-month follow up visits

Recent preclinical studies have suggested that denervation performed in distal branches may increase the reduction in norepinephrine post denervation, and this may translate into improved efficacy in terms of blood pressure reduction. At the time of design of this trial, no study had assessed the impact of distal denervation on blood pressure reduction in humans.

However, as this trial was designed as an unblinded non-randomised trial, this prevented a thorough assessment of the efficacy of distal denervation on blood pressure reduction. Therefore, my second Aim was exploratory to investigate, but not accurately quantify, the impact of distal denervation on blood pressure reduction. As shown in previous denervation trials discussed in Chapter 1, I expected my unblinded trial to increase any blood pressure reduction measured.
Furthermore, to measure bias-minimised efficacy data on blood pressure reduction, I also designed the trial to measure urine anti-hypertensive levels at baseline and 6-month follow up. This would allow any changes in blood pressure observed to be more accurately interpreted as to whether they were due to the denervation therapy or due to changes in drug compliance.

2.1.3 Aim 3- to assess the safety of distal renal denervation in humans at 6-month follow up
At the time of trial design, distal renal denervation had not been performed previously in humans. Therefore, I designed the trial to assess the safety of performing denervation in distal branches of the renal arteries >3mm diameter at two time-points: immediately after denervation and at 6-months follow-up.

2.1.4 Aim 4- To assess the impact of using directly observed anti-hypertensive therapy on the variability of blood pressure reduction from denervation
From the results of my meta-analysis discussed in Chapter 1, I designed the trial to assess the impact of using directly observed anti-hypertensive therapy on the variability of blood pressure reduction from denervation. These results would add to the body of knowledge researchers are accumulating elucidating how to design trials minimising measurement noise.

2.1.5 Outcome measures
At the time of the procedure and at 6-months follow up to measure:

1. Invasive aortic and renal pressure, flow and resistance
2. Aortic and renal wave intensity
3. Non-invasive 24-hour blood pressure monitoring
4. Non-invasive office blood pressure
5. Non-invasive renal function
6. Drug compliance assessed by urine antihypertensive drug analysis

### 2.2 Funding and conflicts of interest

This study was supported via an unrestricted educational grant from Foundation for Circulatory Health. I was also supported by an individual grant from the British Heart Foundation (FS/14/25/30676). Dr Justin Davies, the Principal Investigator, is a consultant for Volcano Corporation and Medtronic Corporation.

### 2.3 Ethics

The experimental protocol was reviewed and approved by the City Road and Hampstead NRES ethics committee, REC reference number 14/LO/0026.

### 2.4 Sample size calculation

This was a proof-of-concept study, designed to use high precision measurements of renal artery blood pressure and flow to assess the impact of denervation. To my knowledge, renal pressure and flow velocity and wave speed have never been assessed before invasively in humans. Therefore, an accurate power calculation was not possible at the start of the trial.

Funding was requested and granted for 20 patients to undergo the study. This number was chosen due to two previous observations from research within my research group.
First, previous invasive work performed by my group (149) in aortic pressure and reservoir calculation demonstrated that statistical significance can be reached in its correlation with wave speed in 15 patients.

Second, my group has shown that differences in wave-intensity analysis of coronary arteries in patients undergoing TAVI were significantly shown in 11 patients (150), reflecting the sensitivity of this modality.

18 patients were consented for the trial, of which 16 patients completed the trial (further details in Chapters 4 and 5). The trial concluded at this point due to difficulties with recruitment within the given time frame.
3 Methods and Study Protocol
3.1 Study population

Patients with severe resistant hypertension were recruited into the trial. Patients were required to have an office systolic blood pressure greater than or equal to 160mmHg (as an average of three measurements of office blood pressure), and to be taking maximally tolerated doses of at least 3 antihypertensive medications. Alternatively, patients fulfilling the same blood pressure criteria but without optimal pharmacological treatment due to medication intolerance were enrolled into the trial, if they had been reviewed by a hypertension specialist doctor.

Exclusion criteria were:

- Estimated glomerular filtration rate (eGFR) <45 mL/min/1.73m²
- Secondary cause of hypertension
- Prior renal artery intervention
- Significant stenotic valvular heart disease
- Myocardial infarction, unstable angina or stroke in the preceding 6 months

Between April 2014 and December 2015, 18 patients were enrolled into the trial. All patients were referred from specialist hypertension clinics (Cardiology, Clinical Pharmacology) and not from primary care, and had been investigated and prescribed medication according to current guideline recommendations (151).

3.2 Outline of Study protocol

Figure 3-1 shows an outline of the study protocol, which is explained in more detail below. Each patient attended for 5 visits in total.
### 3.2.1 Screening visit

Before enrollment in the trial patients were thoroughly assessed. A detailed drug history was taken of previous anti-hypertensive drug prescription. A full medical history was taken and prior investigations reviewed to ensure that all secondary causes of hypertension had been excluded. If secondary causes of hypertension had not been excluded at this point, then all necessary investigations were performed prior to trial enrollment.

In addition, prior to enrolment all patients underwent non-invasive imaging (ultrasound or MRI) of the renal arteries to assess for renal artery stenosis. Patients were only consented for the trial if there was no evidence of renal artery stenosis on non-invasive imaging.

### 3.2.2 Visit 1, Day 1: Non-invasive assessment

Patients were asked not to take their usual medication on the morning prior to clinic review. Patients were requested to provide a urine sample when they reached the clinic, which was stored at -80°C before being sent for mass spectrometry at the University of Leicester for assessment of drug metabolomics. These results were used as a baseline indicator of medication adherence prior to the trial. Patients were informed that these urine samples assessed their medication levels, but were not told explicitly that this was to check their medication adherence.

Patients were also asked to bring their prescribed medication to the first visit. After the urine sample was taken, medication was documented and administered by the study doctor and swallowed by the patient under direct observation (directly observed anti-hypertensive therapy). Patients remained in the hospital for 2 hours after directly observed antihypertensive therapy to ensure there were no significant episodes of
hypotension. All subsequent non-invasive assessments were performed after the directly observed therapy.

Office blood pressure was measured 3 times over 5 minutes in the sitting position using the Omron automatic blood pressure monitor, and average systolic and diastolic pressure calculated.

Blood tests were performed to check renal function prior to renal denervation.

24-hour ambulatory blood pressure monitors (ABPM) were fitted the day prior to the procedure using the Mobil-O-Graph system.

### 3.2.3 Visit 2, Day 2: Renal denervation

Repeat directly observed anti-hypertensive therapy was performed prior to admission to the cardiac catheterization laboratory for renal angiography, invasive haemodynamic measurements and bilateral renal artery denervation.

The full procedure in the cardiac catheterization laboratory is described later in this Chapter. In summary, after femoral access was gained, bilateral renal angiography was performed to ensure there was no evidence of renal arterial disease that would prevent denervation. Subsequently, invasive measurement of aortic and renal artery blood pressure and flow were performed with the patient in the resting state using the ComboWire. An individualized dose of intravenous sedation was then given per patient, and repeat measurements made when the patient was fully sedated. Renal artery sympathetic denervation was then performed in both renal arteries using the Symplicity Spyral system. Post denervation, measurement of aortic and renal artery blood pressure and flow were repeated.

Anti-platelet therapy was not given specifically to patients post procedure however patients already taking anti-platelet therapy continued their current regime.
3.2.4 6-month follow-up period

Within the 6-months follow-up period, participants were asked not to alter their anti-hypertensive medication regime. Participants and their General Practitioners were asked to contact the trial team if any medication changes were being considered.

3.2.5 Visit 3, Day 1: 6 months non-invasive follow up

The same non-invasive protocol was performed at 6-months follow up. Initially, urine samples were taken as a marker of drug compliance within the 6 months follow-up period, and then all subsequent investigations performed under directly observed anti-hypertensive therapy. Repeat office BP, repeat blood tests, and 24-hour ABPM were performed.

3.2.6 Visit 4, Day 2: 6 months invasive follow up

Directly observed anti-hypertensive therapy was again performed prior to admission to the cardiac catheterization laboratory for repeat renal artery angiography and invasive haemodynamic measurements. Invasive haemodynamic measurement of renal artery and aortic blood pressure and flow were initially assessed at rest. Each patient’s individual dose of sedation given in the initial visit was then given at the 6-month visit, and measurement of aortic and renal artery blood pressure and flow repeated once the patient was sedated.
**Figure 3-1. Outline of study protocol**

**ACT= Activated Clotting Time**

### Day 1
Non-invasive study visit

**Non-invasive study visit**
- Urine taken for liquid chromatography-tandem mass spectrometry
- Direct observation of anti-hypertensive medication (DOT)
- Subsequent measurements taken after DOT:
  - 3x consecutive seated office blood pressure measurements within 5 minutes
  - 24-hour ambulatory blood pressure monitor
- Blood tests for renal function

### Day 2
Invasive study visit

**Invasive study visit**
- Direct observation of anti-hypertensive medication
- Subsequent procedure after DOT in cardiac catheterisation lab:
  - Invasive access gained right femoral artery
  - Intravenous heparin administered- ACT levels monitored throughout procedure
- 6Fr Pigtail catheter advanced in aorta to point of renal artery bifurcation - non-selective angiogram (assess location of renal arteries/ any accessory arteries)
- Intra-arterial nitroglycerin (300mcg) given prior to bilateral selective invasive renal angiogram with 6Fr Judkins right catheter
- Invasive measurements of BP/flow velocity using Combowire presedation (2x 30-second recordings, separated in time by 30 seconds)
  - Aortic arch
  - Aorta at level of renal arteries
  - Left and right renal artery sequentially

**Individual dose of intravenous sedation (morphine and midazolam)**
- Repeat invasive measurements of BP/flow velocity in the right renal artery with patient under conscious sedation
- Bilateral renal denervation using Symplicity Spyral Catheter
- Intra-arterial nitroglycerin (300mcg) given prior to repeat bilateral selective invasive renal angiogram post denervation
- Repeat invasive measurements of blood pressure/flow velocity using Combowire (1-minute recordings):
  - Aortic arch
  - Aorta at level of renal arteries
  - Left and right renal artery sequentially
Day 1
Non-invasive study visit

Non-invasive study visit
Urine taken for liquid chromatography-tandem mass spectrometry
Direct observation of anti-hypertensive medication (DOT)

Subsequent measurements taken after DOT:
3x consecutive seated office blood pressure measurements within 5 minutes
24- hour ambulatory blood pressure monitor
Blood tests for renal function

Day 2
Invasive study visit

Invasive study visit
Direct observation of anti-hypertensive medication
Subsequent procedure after DOT in cardiac catheterisation lab:
Invasive access gained right femoral artery
Intravenous heparin administered- ACT levels monitored throughout procedure
Previous non-selective renal angiogram from baseline visit reviewed to assess the location of each renal artery
Intra-arterial nitroglycerin (300mcg) given prior to bilateral selective invasive renal angiogram with 6Fr Judkins right catheter
Invasive measurements of BP/flow velocity using Combowire pre-sedation (2x 30-second recordings, separated in time by 30 seconds)
- Aortic arch
- Aorta at level of renal arteries
- Left and right renal artery sequentially

Identical dose of intravenous sedation given for each patient as baseline procedure
Repeat invasive measurements of blood pressure/flow velocity using Combowire with patient under conscious sedation (1minute recordings):
- Aortic arch
- Aorta at level of renal arteries
- Left and right renal artery sequentially
3.2.7 My personal involvement

I performed every aspect of running and coordinating the trial as described above including recruitment and patient screening. I conducted each non-invasive visit, including the screening visit. I performed all non-invasive measurements including blood and urine analysis, as well as blood pressure assessment.

This was a very intensive trial for the patients. It required five hospital visits, as well as two invasive procedures. To ensure that I had 100% trial completion I liaised with the patients enrolled in this study regularly in the 6-month period between the two visits.

During the invasive procedure two physicians were required in the cardiac catheterization lab: one physician to be scrubbed and perform the denervation and the other to remain unscrubbed to perform all data collection on the Combomap and Symplicity Generator machines. As distal denervation is currently an experimental procedure, my supervisor Dr Justin Davies, who has extensive experience performing renal denervation, performed the denervation. Simultaneously I was in the catheterization laboratory coordinating the procedure to ensure all stages were followed sequentially. In addition, I carried out all data collection during the procedure. This included continuous monitoring and adjustment of parameters on the Combomap machine which is required every time the Combowire is adjusted to ensure adequate data quality.

Furthermore, during the invasive procedure, I was responsible for making all adjustments on the Symplicity Generator machine. The machine allows individual manipulation of each of the four electrodes on the catheter. Therefore, in discussion with Dr Davies, each time the catheter was relocated we would assess vessel diameter
on the radiographs and whether that area had been treated, and then decide which electrodes to activate for each ablation.
3.3 Non-invasive data acquisition protocol

3.3.1 Minimising medication interactions and assessing for drug compliance

All measurements within the study period were performed under directly observed anti-hypertensive therapy. This involved the study doctor checking the medication and dose of each tablet, and watching every participant take every tablet within all four study visits.

Two urinary samples were taken prior to observed anti-hypertensive therapy at baseline and 6-month follow up visits. The samples were frozen at -80°Celsius within our clinic and then sent to the Department of Chemical Pathology, University Hospitals of Leicester NHS Trust. The samples underwent assessment of antihypertensive drug therapy using high-performance liquid chromatography-tandem mass spectrometry (HP LC-MS/MS) urine analysis.

3.3.2 Measurement of adherence

This was performed as previously described in publications from the University of Leicester (48). It is a robust method that has been adapted from techniques routinely used in forensic laboratories (152) (153). The method is a qualitative test that detects 40 of the most common antihypertensive medications. Briefly, 5-10ml of plain urine is collected in a standard container without preservative. Urine samples are batch analysed after extraction from -80 °C storage. Samples are separated using the Agilent Technologies 1290 High Pressure Liquid Chromatograph and identified by linking the HPLC to the Agilent Technologies 6460 Triple Quadrupole Mass Spectrometer (Santa Clara, USA). Samples are run after solvent extraction in positive or negative mode.
based on the polarity of the analyte of interest (48). The tandem mass spectrometer is run in random acquisition mode. Each analyte is identified by the mass to charge ratio (m/z) of the product ions – at least two for each analyte. Each sample is analysed in duplicate.

### 3.3.3 Measurement of office blood pressure

Change in non-invasive office blood pressure pre renal denervation and at 6-month follow-up was assessed.

Office blood pressure measurements were taken with the Omron automatic blood pressure monitor. Three seated blood pressure measurements were taken 5 minutes apart and the average taken. For each patient, the appropriate cuff size was determined at baseline by measuring each patient’s left upper arm circumference, and the cuff size recorded and used for all further visits. The same arm was also used for each blood pressure measurement at each visit.

The protocol stated that only the first 3 office blood pressure recordings were to be documented, even if the clinical researcher felt one or more values were unexpected.

### 3.3.4 Measurement of ambulatory blood pressure

Twenty-four hour ambulatory blood pressure monitoring was performed using the Mobil-O-Graph system (I.E.M. GmbH, Stolberg, Germany). At the first visit, cuff size was determined by measurement of each patient’s arm and then the same cuff size used at 6-month follow-up. The cuff was also placed on the same arm at baseline and 6-month follow-up. The ABPM was set to check blood pressure every 30 minutes throughout the day (7am to 9:59pm) and then hourly overnight. Patients were given
instructions that when they felt the blood pressure cuff start to inflate, they should try
to stop their current activities and rest their arm until inflation finished.

Patients were classified as (154):

- Dippers = 10-20% decline in night to day BP
- Non-dippers = <10% decline in BP at night
- Reverse dippers = night BP greater than day BP

The proportion of patients in each category was calculated at baseline and 6-month
visit.

3.3.5 Non-invasive measurement of central haemodynamics

The Mobil-O-Graph system (I.E.M. GmbH, Stolberg, Germany) includes the
ARCSolver application (155), which facilitates pulse wave analysis of central blood
pressure estimates from the brachial blood pressure cuff.

Pulse wave analysis is used to generate a corresponding central ascending aortic
wave. The software then calculates:

- Augmentation pressure- difference between second and first systolic peaks
- AIX at 75bpm- augmentation pressure as percentage of aortic pulse pressure,
corrected for a heart rate of 75bpm (156)
- Aortic pulse wave velocity
- Cardiac output
3.3.6 Renal function monitoring

Blood tests were monitored pre-denervation at the baseline visit and at 6-month follow-up. Estimated Glomerular filtration rate was calculated using the modified MDRD (Modification of Diet in Renal Disease) equation.
3.4 Invasive data acquisition protocol

3.4.1 Renal artery angiography

Patients were admitted to the cardiac catheterization laboratory. All patients had undergone previous non-invasive imaging of the renal arteries to ensure that renal artery stenosis was not present prior to the procedure.

3.4.1.1 Percutaneous access
Access sites were cleaned with topical chlorhexidine, and the area was draped to maintain a sterile field. Local anaesthesia was injected and access gained at the right femoral artery by a 7Fr arterial sheath.

3.4.1.2 Materials
ECG leads were placed on the chest and limbs to ensure continuous electrocardiographic monitoring. Blood pressure was transduced and monitored continuously via femoral transducer.

An initial dose of heparin was given dependant on the patient’s body weight, and the Activated Clotting Time (ACT) monitored at 30 minute intervals, with additional heparin given as required to maintain adequate ACT levels.

3.4.1.3 Repeat invasive assessment of renal artery vasculature
The first stage in the procedure was to perform bilateral invasive renal angiography, to further ensure there was no renal artery stenosis or significant vascular disease present pre-denervation that might not have been evident on non-invasive imaging.
A 6Fr Pigtail catheter was inserted into the aorta and then advanced to the point of renal artery bifurcation. A bolus of contrast was given and a non-selective angiogram performed to assess the location of each renal artery and any accessory arteries (Figure 3-2). A 6Fr Judkins right catheter was then introduced into each renal artery selectively and 300mcg of intravenous nitroglycerin given into each renal artery. Further angiography of each renal artery was then performed (Figure 3-3). If there was any evidence of renal artery stenosis on angiography the procedure was terminated at this point and the patient excluded from the trial.

Renal artery angiography was performed three times in each renal artery per patient: pre-denervation, immediately post denervation and at 6-month follow up (Figure 3-4). In each patient the same dose of intravenous nitroglycerin (300mcg) was given before each angiographic image.
A non-selective angiogram has been taken to assess the location of each renal artery and to check for the presence of any accessory arteries.
Figure 3-3. Selective right renal angiogram image at level of renal arteries
A subsequent selective right renal angiogram was performed by advancing a 6Fr Judkins right catheter into the ostium of the right renal artery. 300mcg of intra-arterial nitrates were given prior to the angiogram.
Figure 3-4. Time points in the trial at which renal artery angiography was performed

Angiography was performed three times in each renal artery per patient: pre-denervation, immediately post denervation and at 6-month follow up
3.4.2 Assessment of renal and aortic haemodynamics

3.4.2.1 Materials
Aortic and renal pressure and flow velocity were measured simultaneously with a dual sensor-equipped Doppler flow and pressure guide wire (Combowire, Volcano Corporation, San Diego, CA, USA). The diameter of this wire is 0.0014”. The Doppler transducer is situated at the tip of the distal end of the wire and uses pulse wave Doppler to measure blood flow velocity. The pressure transducer is situated back from the distal end of the wire (Figure 3-5). The proximal portion of the wire is attached to the ComboMap console.

Each wire is calibrated prior to its use. The instantaneous and simultaneous Doppler flow and pressure signals are displayed on the ComboMap graphical interface.

3.4.2.2 Methods
After exclusion of renal artery stenosis, a 6Fr Judkins right catheter was then advanced into the aortic arch and a Combowire introduced into the aorta at the level of the aortic arch. Simultaneous recordings of pressure and flow velocity were recorded for at least 30 seconds. A repeat recording of at least 30 seconds was then made after a 30 second break to assess test-retest repeatability. This was then repeated at the aorta at the level of the renal arteries, and the left and right renal arteries in the resting state.

The patient was then given a dose of intravenous sedation (combination of midazolam and morphine), according to their body weight until full sedation was achieved. Only once full sedation was achieved was the Combowire placed again in the aorta and both renal arteries and all measurements repeated for at least 30 seconds at each location.
At the baseline visit, after the measurements in the sedated state were taken, renal sympathetic denervation was then performed as detailed below. After renal denervation, the Combowire was re-positioned in the aorta and both renal arteries and repeat 30-second measurements recorded.

At the 6-month follow up visit, the same dose of sedation was given for each patient as during the baseline visit to ensure that measurements were made under identical sedated conditions.
Figure 3-5. Schematic illustration of the Combowire

The Doppler transducer is located at the distal end of the wire, with the pressure transducer located proximal to this. The proximal end connects to the ComboMap. Picture reproduced with kind permission from Volcano Therapeutics Corp.
3.4.2.3 **Alignment of pressure and velocity wires within the renal arteries**

To study the relationship between pressure and flow velocity at different time points, it was essential to ensure that all recordings were made consistently with the pressure and flow transducers aligned at the same position within the artery.

The renal arteries were intubated with a 6Fr Judkins right diagnostic coronary catheter. The Combowire was then introduced just beyond the entry of the main renal artery (Figure 3-6). The Combowire was directed to ensure that it was in the same laminar plane as renal blood flow, as slight deviations in orientation could make significant changes to measurement of renal flow. Fluoroscopy was used to guide the positioning of the Combowire, document each position, and ensure that the Combowire was placed in the same position in each artery for subsequent measurements.

Renal denervation was not performed within 2 diameters width of the ostium of the main renal artery trunk where the Combowire was situated as after renal artery denervation it is common to see denervation notches that represent acute oedema, which might have affected subsequent Combowire measurements. All measurements were made under resting conditions without pharmacological provocation.
Figure 3-6. Fluoroscopic image showing the alignment of the Combowire in the right renal artery.

A Judkins Right catheter is shown intubating the right renal artery. The Combowire is placed just beyond the ostium of the main renal artery and directed to ensure that it was on the same laminar plane as renal blood flow.

This angiographic image was taken and stored and used to ensure that the Combowire was placed in the same configuration for subsequent data collection.
3.4.2.4 Alignment of pressure and velocity wires within the aorta

A similar principle was used for the acquisition of simultaneous pressure and velocity measurements in the aorta at the level of the aortic arch and at the level of the renal arteries. At each position, the Combowire was advanced just outside the lumen of the Judkins right catheter to ensure that the catheter provided support for the wire in the more turbulent aortic flow (Figure 3-7). Again, all measurement positions were recorded using fluoroscopy to try to ensure that subsequent measurements were made at the same position.
Figure 3-7. Angiogram image illustrating ComboWire position in the aortic arch

A Judkins Right catheter is shown in the aortic arch. The ComboWire has been introduced into the aorta at the level of the aortic arch and simultaneous recordings of pressure and flow velocity taken for at least 30 seconds.
3.4.2.5 Optimising the velocity signal
To optimise the velocity signal of the Combowire a number of steps were performed. The first step was to ensure that the flow transducer was within the same plane as the renal blood flow. This was achieved by advancing the ComboWire tip just outside the Judkins right catheter, and making small rotational movements until a full laminar flow pattern was shown on the ComboMap screen (Figure 3-8).

Second, the ComboMap automatically traces a Doppler envelope around the flow velocity recording (Figure 3-8). Using the ComboMap a number of steps can be made to optimise the Doppler envelope drawing, which include changing the velocity scaling, direction of tracking and the instantaneous peak velocity (IPV) threshold:

- **IPV threshold**: The IPV threshold alters the Doppler signal intensity. Adjustment of the IPV threshold allows optimisation of the tracking algorithm. Baseline IPV threshold was set at 2.

- **Direction**: Baseline direction of tracking was set to anterograde for aortic flow and retrograde for renal flow.

- **Selection of velocity scale**: The velocity scale was adjusted to ensure good tracking by adjusting the top limit of the velocity scale to just above the peak velocity of the Doppler envelope.

3.4.2.6 Repeatability assessment
Repeatability of pressure and flow recordings were assessed in both renal arteries and in the aorta for each patient at rest prior to sedation and denervation. Thirty-second recordings of renal pressure and flow velocity were made with the ComboWire, and then repeated at a separate point in time.
The ComboWire initially was orientated in the renal artery and aorta as described previously and 30-second recordings of simultaneous pressure and flow were recorded. Then a 1-minute break in recordings was taken during which the catheter remained in location either in the renal artery or aorta, but the guidewire was not held in one orientation, which allowed the guidewire to shift position within the arterial flow. After the 1-minute interval the guidewire was then re-positioned using the radiographs taken previously to indicate previous orientation, and matching the appearance of the pressure and flow signal on the ComboMap machine to the previous form. At this point, once myself and Dr Davies felt that a similar location had been achieved, a repeat 30-second recording was taken.
Figure 3-8. Picture of the ComboMap interface

The picture shows simultaneous pressure (middle panel) and flow velocity recordings (bottom panel) with electrocardiography (top panel). VTI flow velocity is automatically traced around the flow signal.
3.4.3 Catheter based renal sympathetic denervation

3.4.3.1 Materials
Patients underwent renal sympathetic denervation using the Symplicity Spyral catheter (Symplicity, Medtronic Inc., Minneapolis, MN) system. The Symplicity Spyral catheter is a 6 French catheter with 4 electrodes in a circumferential configuration, which delivers low-level radiofrequency energy circumferentially to the renal artery wall from within the vascular lumen. This causes thermal injury to the renal nerves within, and external to, the renal artery wall. The multi-electrode design allows four ablations to occur simultaneously within 60 seconds. The helical design allows the catheter to be advanced to deliver ablations to a range of renal arteries shaped 3-8mm in diameter. The Spyral Catheter was attached into the Symplicity Generator. The generator simultaneously displays impedance measurements and the temperature achieved for each renal artery ablation. It has a touchscreen interface which allows the user to control the number of electrodes activated (Figure 3-9)

3.4.3.2 Methods
A Judkins right catheter was placed via femoral artery access into each renal artery and a 0.0014” Hi-Torque balance middleweight elite coronary angioplasty Guide Wire (Abbott Vascular) advanced into the distal branches of the renal arteries. The Symplicity Spyral catheter was then advanced over the Guide wire, Figure 3-10). Fluoroscopic guidance was used to document where each denervation ablation was performed. Denervation was performed distally beyond the main renal artery bifurcation in any artery branches >3mm diameter. The diameter of each renal artery branch was measured from the renal angiogram. Ablations were performed from the
distal to proximal end of the artery, whilst monitoring impedance and temperature
during the ablations using the Symplicity generator system (Figure 3-9).
Figure 3-9. Symplicity Spyral catheter and generator

The left hand diagram shows the Spyral catheter (Symplicity, Medtronic Inc., Minneapolis, MN), which is a 6-French catheter with 4 electrodes in a circumferential configuration. In the middle diagram the end of the catheter is magnified showing the four electrodes that simultaneously deliver radiofrequency energy circumferentially to the renal artery wall from within the vascular lumen. The Symplicity Generator is shown in the right hand diagram. The screen shows continuous feedback of impedance changes and temperature of ablation for each renal artery electrode simultaneously.
Figure 3-10. Angiogram image illustrating Symplicity Spyral catheter within the right renal artery

The four electrodes are visible in a corkscrew configuration that delivers radiofrequency energy to the renal artery wall simultaneously.
3.5 Data analysis

3.5.1 Non-invasive measurements

3.5.1.1 Office and ambulatory blood pressure
Change in office SBP and DBP were compared from baseline to 6 months after renal denervation.
Change in 24-hour ambulatory systolic and diastolic blood pressure was also assessed from baseline pre-denervation to 6-months after denervation.
Variability of ambulatory blood pressure and heart rate in each visit was defined as the standard deviation.
Between visit differences were compared using confidence intervals and paired t-tests. For categorical variables, I present the counts and percentages.

3.5.1.2 Medication adherence
Adherence to medication is shown as a percentage (%) for each patient of the total number of medications detected in their urine sample:

\[ \text{Adherence} = \frac{\text{Number of anti-hypertensive medications detected in urine}}{\text{Number of anti-hypertensive medications prescribed}} \times 100\% \]

The strength of association between variables was expressed as \( R^2 \).

3.5.1.3 Renal function
Blood tests were performed at baseline pre-renal artery denervation and repeated at 6-months follow up after renal artery denervation. Serum creatinine was measured and estimated glomerular filtration rate calculated using the modified MDRD (Modification of Diet in
Renal Disease) equation. Changes in eGFR and serum creatinine were compared from baseline to 6 months after renal denervation using the paired students t-test.

### 3.5.2 Invasive haemodynamic data analysis

Invasive pressure and flow recordings were extracted from data storage (ComboMap, Volcano Corporation) and processed offline using a custom software package with Matlab (Mathworks, Inc., Natick, Massachusetts), which has been developed by our group previously (157) (Figure 3-11).

The blood pressure and Doppler velocity recordings were filtered using a Savitzky-Golay filter and assembled with the ECG, which was used for timing.

#### 3.5.2.1 Blood pressure

The automated Matlab algorithm detects the foot of each pressure waveform in the 30-second recordings of raw invasive data. An average is then calculated by aligning each trace around this point. The maximum, minimum and mean blood pressure and pulse pressure are automatically calculated.

#### 3.5.2.2 Velocity flow data

The automated Matlab algorithm calculates the maximum and mean velocity (Vmax, Vmean), and velocity-time integral (VTI) from the 30-second recordings of invasive flow data.

#### 3.5.2.3 Aortic and renal artery wave speed

The single point approach (158) was used to measure wave speed within the aorta and renal arteries using a formula derived from the water hammer equation (159), where \( \rho \) is the
density of blood, and \( dP \) and \( dU \) are the changes in pressure and velocity over one sampling period. Further details are discussed in Chapter 4.

\[
c = \frac{1}{\rho} \frac{\sum dP^2}{\sum dU^2}
\]

3.5.2.4 Vascular resistance

Vascular resistance was calculated for both the aorta and renal arteries. In the aorta, vascular resistance was calculated as:

\[
Vascular\ resistance = \frac{Mean\ aortic\ blood\ pressure}{Cardiac\ output}
\]

In the renal arteries, vascular resistance was calculated as:

\[
Renal\ vascular\ resistance = \frac{Mean\ renal\ blood\ pressure\ (mmHg)}{Renal\ blood\ flow\ velocity \times Cross\ sectional\ area\ of\ main\ renal\ artery}
\]
**Figure 3-11. Matlab programme**

Screenshot of Matlab programme which calculated average flow and pressure data from the raw 30-second recordings.
3.5.3 Safety measurements

3.5.3.1 Quantitative vessel analysis (QVA)

Renal artery angiography was performed three times in each renal artery per patient: pre-denervation, immediately post denervation and at 6-month follow up (Figure 3-4). In each patient the same dose of intravenous nitroglycerin was given before each angiographic picture.

All single plane renal angiograms were collected at trial end and anonymised to the patient and procedural time point. Quantitative angiographic analysis was performed offline using dedicated software (Medcon Software Informer). Angiographic images were calibrated using the 6Fr catheter.

Each assessor measured in the main renal artery trunk and in each branch >3mm: length, proximal and distal lumen diameter, minimal lumen diameter and area. The presence of calcification, notches, spasm or dissection was also qualitatively assessed. This was performed in the left and right renal arteries of each patient at 3 separate time points.

Three blinded assessors performed independent measurements. None of the assessors had been involved with the data acquisition of the study. Operators were provided with the renal artery angiograms as movies and advised to pause the movies where the image showed the best opacification of the renal arteries. They were not provided with preselected images.

5.1.1.1 Cross-sectional area of main renal arterial trunk

Angiogram images of each patients’ left and right renal artery trunks were anonymised retrospectively. The cross-sectional area of each main renal artery was estimated for each renal artery for each patient at rest pre-denervation. This was done by the three blinded assessors, who were not involved in the study, as described above.
3.5.4 Statistical analysis

Statistical analysis was performed using the statistical environment “R” (148). Data are presented as mean ± SD. A p-value of <0.05 was considered statistically significant. Before parametric testing, baseline variables were checked for normality using the Shapiro-Wilk normality test (160) (161). The results of the Shapiro-Wilk normality test for each baseline variable are shown in Table 3-1.
Table 3-1. Shapiro-Wilk p values are shown for baseline haemodynamic variables to assess for normality

A p-value >0.05 implies a normal distribution

<table>
<thead>
<tr>
<th>Baseline haemodynamic variable</th>
<th>Shapiro-Wilk p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient age</td>
<td>0.797</td>
</tr>
<tr>
<td>Referral SBP</td>
<td>0.299</td>
</tr>
<tr>
<td>Referral DBP</td>
<td>0.340</td>
</tr>
<tr>
<td>Baseline Office SBP</td>
<td>0.933</td>
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<tr>
<td>Baseline Office DBP</td>
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<tr>
<td>Baseline ABPM SBP</td>
<td>0.472</td>
</tr>
<tr>
<td>Baseline ABPM DBP</td>
<td>0.173</td>
</tr>
<tr>
<td>Baseline ABPM Pulse pressure</td>
<td>0.465</td>
</tr>
<tr>
<td>Baseline aortic resistance</td>
<td>0.564</td>
</tr>
<tr>
<td>Baseline renal resistance</td>
<td>0.436</td>
</tr>
<tr>
<td>Baseline aortic BP</td>
<td>0.176</td>
</tr>
<tr>
<td>Baseline renal BP</td>
<td>0.091</td>
</tr>
<tr>
<td>Baseline aortic wave speed</td>
<td>0.089</td>
</tr>
<tr>
<td>Baseline renal wave speed</td>
<td>0.322</td>
</tr>
</tbody>
</table>
3.5.4.1 Change in non-invasive variables
Pre and 6-month post renal denervation non-invasive variables (renal function, office and ambulatory blood pressure) were compared with the paired students t-test.

3.5.4.2 Test-retest repeatability
The test-retest repeatability of measurements was calculated as the standard deviation of the difference between two readings. The results were assessed using a Bland–Altman plot and presented with the 95% Limits of Agreement (162). The correlation between the two optima was expressed as $R^2$.

3.5.4.3 Change in invasive variable
Mean and peak renal and aortic blood pressure and flow, and renal and aortic wave speed were compared at baseline visit pre-sedation, post sedation and post-denervation, and at 6 months pre-sedation and post sedation. Differences were analysed using a 2-tailed paired t-test.

3.5.4.4 Linear regression analysis
The relationship between the change in ambulatory systolic blood pressure at 6-months and the baseline variables was assessed using linear regression. Univariate regression was performed with each baseline variable (such as office SBP, ambulatory SBP). The beta-coefficient and its 95% confidence intervals and p-value are presented, along with the $R^2$ for the fit of the model.

For each regression, a quad of diagnostic plots was generated (residuals vs. fitted values, normal Q-Q plot of standardized residuals, scale-location plot, and residual leverage) to
assess the linearity of relationship between predictor and outcome variables, normal
distribution of residuals, homoscedasticity, and influential outliers.

3.5.4.5 **Multivariate regression model: predictors of reduction in 6-month ambulatory systolic blood pressure**

In Chapter 6 a multivariate regression model is performed to assess the statistical
independence of significant univariate predictors. The multivariate model was obtained by
performing backward elimination of nonsignificant baseline variables. The outcome variable
is change in 6-month ambulatory systolic blood pressure. The exposure variables, after
elimination, were baseline pre-denervation renal wave speed and baseline pre-denervation
renal vascular resistance. Results are presented as the beta-coefficients, their 95% confidence
intervals, p-values, intercept, and the $R^2$ of the overall model fit.

3.5.4.6 **Safety data analysis**

Changes in quantitative analysis of the renal angiograms were assessed between time points
using the ANOVA test.

Inter-observer variability between measurements acquired from the three assessors were
compared using the intra-class correlation coefficient (ICC). ICC is given as “ICC (95% confidence interval)” A p-value < 0.05 was considered statistically significant.
4 Validation of non-invasive and invasive measurements with repeatability assessment
4.1 Introduction

4.1.1 Measurement of aortic arterial stiffness

Aortic arterial stiffness increases with age (163), and in patients with hypertension, diabetes mellitus (164), atherosclerosis (165), and renal disease (166). Despite these conditions being risk factors for atherosclerosis, aortic stiffness is also an independent risk factor for cardiovascular disease (167), cardiovascular mortality (168) (169) and all-cause mortality (170).

There are different options to measure arterial stiffness (171) which are outlined below.

4.1.1.1 Measurement of invasive wave speed using the carotid-femoral foot-to-foot approach

Aortic pulse wave velocity (PWV) measured non-invasively is widely regarded as the gold standard in the assessment of aortic arterial stiffness (172) (173) (174). Aortic pulse wave velocity is calculated by measuring the time taken (δt) for a pressure wave to travel between two points a known distance apart (δd). The wave speed is calculated as:

\[ \text{Pulse wave velocity} = \frac{\delta d}{\delta t} \]

The pressure curves at two sites are acquired either simultaneously or with one transducer moving between two positions and gating of the ECG R-wave. \( \delta d \) is calculated over a length of the arterial tree, most commonly the aorta from the carotid to the femoral level.

The main drawback of this approach is that errors in estimating the distance travelled by the pressure wave can introduce inaccuracies into the calculation (175) (176).
4.1.1.2 Measurement of invasive wave speed using pulse wave analysis to approximate the foot-to-foot approach

An alternative approach is using pulse wave analysis to calculate aortic pulse-wave velocity. This relies on the principle that the arterial pressure waveform is composed of a forward wave generated by ventricular contraction of the heart and a reflected wave returning from the peripheries to the heart (177). With elastic arteries, the reflected wave tends to arrive back slower at the central arteries during diastole. With increasing arterial stiffness, reflected waves return earlier to the central arteries, augmenting the systolic pressure and adding to the forward wave.

Using pulse wave analysis, the reflected wave in the ascending aorta is derived, and then travel time ($\delta t$) is calculated by comparing the backward and forward wave (178). To calculate $\delta d$, initially it was assumed that the reflection site between the forward and backward wave is located at the lower abdominal aorta, and therefore $\delta d$ was twice this length. However, subsequent research showed that PWV obtained using this method was different to the carotid-femoral-derived PWV (179). These calculations are based on the simplification that reflection takes place at one “real” location in the lower aorta. Several researchers tried to identify a principal reflection location, and arrived at varying conclusions (159) (180). Initially it was found by researchers that reflection timing was increased with age (179), whereas other researchers found it was decreased with age (181). A subsequent meta-analysis found that reflection timings changes little with age (182). One potential explanation is that there is no single dominant reflection site, but instead multiple reflection sites along the aorta, for which the contributions are attenuated with distance (177).
4.1.1.3 **Measurement of invasive wave speed using the single point approach**

An alternative approach to measure arterial stiffness is using central pulse wave analysis using the single point approach (158). Pulse wave analysis with the single point approach uses simultaneously acquired pressure and velocity data from a single position within a vessel to ascertain the wave speed, eliminating the necessity of estimating distance travelled by the pressure waveform. This approach uses a formula derived from the water hammer equation (159), where $\rho$ is the density of blood, and $dP$ and $dU$ are the changes in pressure and velocity over one sampling period:

$$c = \frac{1}{\rho} \sqrt{\frac{\sum dP^2}{\sum dU^2}}$$

This has been validated invasively in vitro (183) and in vivo (184) against the foot-to-foot method, as well as by my research group (158).

Another benefit of this approach is that it allows wave speed to be calculated in small vessels e.g. the coronary arteries and the renal arteries. Furthermore, it has been shown that the time resolution of the single-point method is sufficient for identification of acute changes in the coronary arteries e.g. change in wave speed after administration of isosorbide dinitrate.

4.1.2 **Non-invasive aortic wave speed measured using the Mobil-O-Graph system**

In this study I used the Mobil-O-Graph system (I.E.M. GmbH, Stolberg, Germany) to measure each patient’s ambulatory blood pressure, as discussed in Chapter 3. The Mobil-O-Graph has in-built ARCSolver technology (Austrian Institute of Technology, Vienna, Austria) (155) which automatically performs pulse wave analysis on the central blood
pressure estimates from brachial blood pressure (cuff) readings to measure aortic pulse wave velocity.

The Mobil-O-Graph performs two inflations for each set of haemodynamic measurements. The first inflation obtains conventional systolic and diastolic blood pressure recordings. The machine then re-inflates a second time to the diastolic blood pressure level to measure pulse waves. The software then uses a transfer function to compute central estimates of blood pressure readings, which has previously been reported and published (185).

The majority of previous research on aortic pulse wave velocity uses either the invasive gold standard measurement using the foot-to-foot approach or carotid-femoral pulse wave velocity with tonometry using single-point measurement of aortic wave speed. These two methods have been compared and validated against each other (175). To use the Mobil-O-Graph automated measure of aortic pulse wave velocity further in my thesis, it is important to validate aortic pulse wave measurements measured with the Mobil-O-Graph 24h PWA Monitor against the invasive gold standard measurements.

4.1.3 Test-retest repeatability versus reproducibility

When the accuracy of a measurement method is assessed, two measures of accuracy should be considered: repeatability and reproducibility (186). Repeatability describes comparing measurements that are obtained with the same method under identical test conditions in the same laboratory using the same equipment and same operators. Reproducibility, however, describes comparing independent measurements that are obtained with the same method under identical test conditions in different laboratories with using different equipment.

In this study we assess the repeatability of invasive measurements as all measurements were made in the same laboratory using the same equipment and operators. It is critical to assess the test-retest repeatability of all measurements, to assess their validity before using them
further within this thesis. To assess change in invasive haemodynamics within a 6-month follow up period will require very accurate test-retest repeatability: i.e. narrow error bars within individuals (187). Wide error bars cause two harms. First, I may presume that changes in haemodynamics are due to renal denervation treatment, and not purely a factor of measurement error. Second, true changes in haemodynamics may not be obvious in noisy measurements, and hence remain undetected.
4.2 Aims

In this Chapter I assess and validate the measurements used throughout my thesis.

My aims are:

1) To validate the use of the ComboWire to measure blood pressure and flow velocity in the renal arteries, including test-retest repeatability assessment

2) To assess the repeatability of my measurement of renal wave speed using the single point approach

3) To compare renal wave speed measurements between both renal arteries

4) To assess the repeatability of my measurements of invasive aortic blood pressure using the ComboWire in the ascending aorta

5) To assess the repeatability of measurement of non-invasive blood pressure using the Mobil-O-Graph

6) To compare invasive aortic haemodynamic measurements with those measured non-invasively using an ambulatory blood pressure machine

7) To assess whether measurement of aortic stiffness using pulse wave analysis produces comparable results when estimated from non-invasive measurement estimates of central BP using a brachial blood pressure cuff or invasively in the aorta
4.3 Results

4.3.1 Characteristics of study participants at baseline and at 6 months

A total of 22 patients fulfilling inclusion criteria were referred by their clinicians to discuss participation in this trial. Of these, four declined to participate. Of the remaining 18 who enrolled, two were excluded after the time of consenting. One of these was excluded because of significant right renal artery stenosis found on renal angiography. The other exclusion was because of symptomatic hypotension which occurred when directly observed anti-hypertensive therapy was applied, with systolic office blood pressure dropping from 170mmHg to 80mmHg.

The baseline characteristics of all 18 patients consented into the trial are shown in Table 4-1. None of my patient population had arrhythmias which might have affected the non-invasive pulse recordings.
Table 4-1. Baseline characteristics of the study population.

Values are shown as mean ± SD

<table>
<thead>
<tr>
<th></th>
<th>Patients undergoing renal denervation (n=16)</th>
<th>Patients excluded from trial (n=2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>63.2 ± 12.0</td>
<td>68.3 ± 4.5</td>
</tr>
<tr>
<td>Male</td>
<td>12 (75%)</td>
<td>2 (100)</td>
</tr>
<tr>
<td>BMI (Kg/m²)</td>
<td>30.5 ± 5.3</td>
<td>26.5 ± 5.3</td>
</tr>
<tr>
<td>Smoking status- no. (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current</td>
<td>3 (18.8)</td>
<td>0</td>
</tr>
<tr>
<td>Ex-smoker</td>
<td>6 (37.5)</td>
<td>1 (50)</td>
</tr>
<tr>
<td>Never smoked</td>
<td>7 (43.8)</td>
<td>1 (50)</td>
</tr>
<tr>
<td>Race - no. (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>White Caucasian</td>
<td>11 (68.8)</td>
<td>1 (50)</td>
</tr>
<tr>
<td>Afro-Caribbean</td>
<td>3 (18.8)</td>
<td>0</td>
</tr>
<tr>
<td>South Asian</td>
<td>2 (12.5)</td>
<td>1 (50)</td>
</tr>
<tr>
<td>Medical History- no. (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ischaemic heart disease</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>Diabetes Mellitus Type 2</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>Obstructive sleep apnoea</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Stroke</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>TIA</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Atrial fibrillation</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Referral Office SBP (mmHg)</td>
<td>180.1 ± 17.8</td>
<td>201.0 ± 55.2</td>
</tr>
<tr>
<td>Referral Office DBP (mmHg)</td>
<td>107.9 ± 38.5</td>
<td>89.0 ± 9.9</td>
</tr>
<tr>
<td>Average number of anti-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>hypertensives</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ACE-I/ ATI blocker</td>
<td>14 (87.5)</td>
<td>2 (100)</td>
</tr>
<tr>
<td>Beta-Blockers</td>
<td>14 (87.5)</td>
<td>1 (50)</td>
</tr>
<tr>
<td>Calcium Channel Blockers</td>
<td>10 (62.5)</td>
<td>2 (100)</td>
</tr>
<tr>
<td>Aldosterone antagonists</td>
<td>10 (62.5)</td>
<td>1 (50)</td>
</tr>
<tr>
<td>Thiazide diuretics</td>
<td>6 (37.5)</td>
<td>1 (50)</td>
</tr>
<tr>
<td>Loop diuretics</td>
<td>2 (12.5)</td>
<td>0</td>
</tr>
<tr>
<td>Office SBP at study visit (mmHg)</td>
<td>172.3±23.0</td>
<td>168.2 ± 26.2</td>
</tr>
<tr>
<td>Office DBP at study visit (mmHg)</td>
<td>97.7±14.4</td>
<td>83.3 ± 10.8</td>
</tr>
</tbody>
</table>
4.3.2 Test-retest repeatability of invasive measurement of renal blood flow and systolic blood pressure

Repeatability of invasive measurement of renal blood flow velocity and systolic blood pressure was assessed in each patient. Recordings of 30 seconds were made in each renal artery (Test 1) and then repeated after a 1-minute interval (Test 2). During the 1-minute interval the catheter remained in the renal artery but the guidewire was not held in one orientation which allowed the guidewire to shift position within the arterial flow. After the 1-minute interval the guidewire was then re-positioned using the radiographs taken previously to indicate previous orientation.

The results are shown in Figure 4-1. Repeatability of haemodynamic recordings was assessed using Bland-Altman plots, and expressed with the mean bias and 95% limits of agreement. The repeatability for renal blood flow velocity was 0.02 m/s with 95% limit of agreement -0.06 to 0.10 m/s. The repeatability for renal arterial blood pressure was surprisingly worse (-0.70 mmHg with 95% limit of agreement -18.8 to 17.4 mmHg).
Figure 4-1. Repeatability of measurements of renal systolic blood pressure and flow velocity

The repeatability for renal blood flow velocity was 0.02m/s with 95% limit of agreement -0.06 to 0.10m/s ($R^2=0.89$, $p<0.001$). The repeatability for renal arterial blood pressure -0.70 mmHg with 95% limit of agreement -18.8 to 17.4 mmHg ($R^2=0.96$, $p<0.001$).
4.3.3 Test-retest repeatability of invasive renal wave speed

The repeatability of renal artery wave speed was assessed in both renal arteries. Recordings of 30 seconds were made sequentially in each renal artery (Test 1) and then repeated after a 1-minute interval (Test 2).

The repeatability for left renal artery wave speed was -0.44 m/s with 95% limit of agreement -4.61 to 3.73 m/s. The repeatability for right renal artery wave speed was -0.89 m/s with 95% limit of agreement -3.96 to 2.19 m/s, Figure 4-2.
Figure 4-2. Repeatability of measurement of renal wave speed

The repeatability for right renal artery wave speed was -0.89m/s with 95% limit of agreement -3.96 to 2.19m/s ($R^2=0.92$, $p<0.001$). The repeatability for left renal artery wave speed was -0.44m/s with 95% limit of agreement -4.61 to 3.73m/s ($R^2=0.86$, $p<0.001$).
4.3.4 Test-retest repeatability of invasive and non-invasive measurement of aortic systolic blood pressure

In each patient two repeat 30-second recordings of invasive aortic pressure were recorded after a 30 second break in measurements. The test-retest repeatability of invasive measurements is shown in Table 4-2, and correlation in Figure 4-3. There was excellent test-retest repeatability for all invasive measurements: SDD 5.7 mmHg for invasive SBP and 3.1mmHg for invasive DBP respectively.

Three non-invasive measurements were made within 30 minutes using the Mobil-O-Graph, Table 4-2. Repeatability was much poorer for non-invasive BP (SDD 13.5mmHg and 10.8mmHg for SBP and DBP respectively), however this is also likely to reflect the time delay between readings and therefore natural variation in blood pressure readings.

4.3.5 Test-retest repeatability of invasive and non-invasive measurement of aortic wave speed

Invasive aortic wave speed was calculated for all 16 patients. However, non-invasive aortic wave speed was only calculated for 14 patients: one patient refused ambulatory blood pressure monitoring and one patient’s device failed to calculate PWV due to the patient’s relative bradycardia interrupting with the algorithmic calculation (average pulse rate 45-50 bpm, sinus bradycardia). The results illustrated in this Chapter compare invasive and non-invasive aortic wave speed for these 14 patients.

Repeatability was excellent for both invasive and non-invasive aortic wave speed ($R^2 = 0.98$ and 0.97 respectively).
Table 4-2. Repeatability of invasive and non-invasive measurements of blood pressure and wave speed

<table>
<thead>
<tr>
<th></th>
<th>Average first measurement</th>
<th>Average second measurement</th>
<th>Average third measurement</th>
<th>Standard deviation of difference</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Systolic BP (n=15)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Invasive aortic systolic BP (mmHg)</td>
<td>161.4</td>
<td>162.6</td>
<td>5.7</td>
<td>0.96</td>
<td></td>
</tr>
<tr>
<td>Non-invasive systolic BP (mmHg)</td>
<td>147.1</td>
<td>148.3</td>
<td>139.4</td>
<td>13.5</td>
<td>0.55</td>
</tr>
<tr>
<td><strong>Diastolic BP (n=15)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Invasive aortic diastolic BP (mmHg)</td>
<td>84.5</td>
<td>83.3</td>
<td>3.1</td>
<td>0.95</td>
<td></td>
</tr>
<tr>
<td>Non-invasive diastolic BP (mmHg)</td>
<td>94.4</td>
<td>95.3</td>
<td>90.1</td>
<td>10.8</td>
<td>0.54</td>
</tr>
<tr>
<td><strong>Aortic wave speed (n=14)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Invasive aortic wavespeed (m/s)</td>
<td>14.2</td>
<td>14.3</td>
<td>1.1</td>
<td>0.98</td>
<td></td>
</tr>
<tr>
<td>Aortic wavespeed estimated from brachial BP readings (m/s)</td>
<td>9.5</td>
<td>9.5</td>
<td>9.3</td>
<td>0.38</td>
<td>0.97</td>
</tr>
</tbody>
</table>
Figure 4-3. Repeatability between invasive blood pressure and wave speed recording

The repeatability for invasive aortic wave speed was -0.12 m/s with 95% limit of agreement -2.20 to 1.96 m/s. The repeatability for invasive aortic SBP was -1.22 mmHg with 95% limit of agreement -12.39 to 9.95 mmHg. The repeatability for invasive aortic DBP was 1.24 mmHg with 95% limit of agreement -4.88 to 7.36 mmHg.
4.3.6 Comparison of non-invasive and invasively assessed aortic blood pressure

Non-invasive measurement of systolic blood pressure was on average lower than those measured invasively, Table 4-3. Conversely, non-invasive measurements of diastolic blood pressure were on average higher than those measured invasively.

Correlation between invasive and non-invasive blood pressure measurements is shown in Table 4-4. The correlations between invasive and non-invasive readings were improved using central estimation of blood pressure for both systolic and diastolic measurements (SDD 24.2 mmHg and 10.7 mmHg respectively) compared to when central estimation of blood pressure was not used (SDD 27.0 mmHg and 12.4 mmHg respectively). This is shown in Figure 4-4.
**Table 4-3. Invasive and non-invasive measurements of blood pressure and aortic pulse wave velocity.**

Values are shown as mean ± SD

<table>
<thead>
<tr>
<th></th>
<th>Invasive measurements</th>
<th>Non-invasive measurements</th>
<th>Central estimate of non-invasive measurement</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Systolic BP (mmHg)</strong></td>
<td>161.4 ± 28.8</td>
<td>132.1 ± 20.4</td>
<td>144.9 ± 16.9</td>
</tr>
<tr>
<td><strong>Diastolic BP (mmHg)</strong></td>
<td>84.5 ± 12.7</td>
<td>88.5 ± 13.8</td>
<td>92.2 ± 13.7</td>
</tr>
<tr>
<td><strong>Aortic pulse wave velocity (m/s)</strong></td>
<td>14.2 ± 6.6</td>
<td>-</td>
<td>9.5 ± 2.4</td>
</tr>
</tbody>
</table>
### Table 4-4. Comparison of systolic and diastolic blood pressure determined using invasive and non-invasive measurements

<table>
<thead>
<tr>
<th></th>
<th>Mean absolute difference between invasive and non-invasive measurements</th>
<th>Standard deviation of difference between invasive and non-invasive measurements</th>
</tr>
</thead>
<tbody>
<tr>
<td>Invasive SBP and Brachial SBP (mmHg)</td>
<td>29.3</td>
<td>27.0</td>
</tr>
<tr>
<td>Invasive DBP and Brachial DBP (mmHg)</td>
<td>4.0</td>
<td>12.4</td>
</tr>
<tr>
<td>Invasive SBP and Brachial cSBP (mmHg)</td>
<td>16.4</td>
<td>24.2</td>
</tr>
<tr>
<td>Invasive DBP and Brachial cDBP (mmHg)</td>
<td>7.7</td>
<td>10.7</td>
</tr>
<tr>
<td>Aortic pulse wave velocity (m/s)</td>
<td>4.7</td>
<td>5.0</td>
</tr>
</tbody>
</table>
Figure 4-4. Correlation of systolic and diastolic blood pressure determined using invasive and non-invasive measurements
4.3.7 Comparison of non-invasive and invasively assessed aortic pulse wave velocity

Mean aortic PWV was 9.5 ± 2.4 m/s estimated from non-invasive brachial BP readings and 14.2 ± 6.6 m/s measured invasively, Table 4-3. The correlation between invasively measured aortic wave speed and non-invasively estimated aortic wave speed is shown in Figure 4-5 (R² = 0.70, p<0.001). This was further compared using the Bland-Altman method, Figure 4-6. The mean bias was 4.69 m/s with 95% limits of agreement -5.17 to 14.55 m/s, Table 4-4.
Figure 4-5. Correlation of aortic pulse wave velocity measured invasively and non-invasively, $R^2 = 0.70$

Note: 14 data points are shown as one patient refused ambulatory blood pressure monitoring and one patient’s device failed to calculate PWV due to patient’s relative bradycardia interrupting with the algorithmic calculation.
Figure 4-6. Bland-Altman plot showing the correlation of measurements for aortic wave speed measured invasively and non-invasively.

The mean bias was 4.69 m/s with 95% limits of agreement -5.17 to 14.55 m/s.
4.4 Discussion

4.4.1 Use of the ComboWire in the renal arteries to measure renal arterial blood pressure and flow velocity

This study aims to detect any changes in renal haemodynamics at 3 time points: pre-renal denervation, immediately after denervation and at 6-months follow-up. It is critical, therefore, to ensure that measurement of renal haemodynamics is accurate and repeatable when measured invasively with the ComboWire.

There were several potential steps in my methodology that required optimisation to ensure a repeatable assessment of renal haemodynamics:

1) Ensuring that the ComboWire sensor was positioned in same location in renal arteries
2) Ensuring that the ComboWire tip was placed in laminar flow in renal arterial blood stream
3) Ensuring that the ComboWire was correctly calibrated
4) Ensuring identical pharmacological conditions at both visits, including pre-procedural hypertensive medication given under directly observed anti-hypertensive therapy, and intra-procedure sedation medication and doses

My study methodology described in Chapter 3 aimed to minimise these potential biases to ensure accurate repeatable measurements. The outcome, as shown in this Chapter, is that repeatability was improved when measuring renal artery flow velocity than blood pressure.

The Bland-Altman plot in Figure 4-1 illustrated that in certain patients there was a surprisingly large absolute difference in repeat measurements for renal arterial blood pressure which were taken within a few minutes apart. This might be for a number of reasons. First, before these measurements were made, intra-arterial nitrates were given to enable accurate angiography. It is possible that if the measurements were taken too close in time to the nitrates then this might affect certainly the first reading. This should be an
unlikely cause though as the intra-arterial nitrates were given at the start of the procedure to ensure vessel patency. After this, measurements were taken in the aortic arch. Therefore, there should have been an adequate length of time between administration of nitrates and recording renal blood pressure measurements to prevent any interactions. Second, arrhythmia could explain this large variability, however none of the patients had arrhythmia recorded during their procedures. Third, this might reflect normal biological variability between repeat measurements in the renal arteries. It is possible that changes in sympathetic tone affect the variability of renal arterial blood pressure to a great extent than in the more rigid aorta. This, to my knowledge, has never been assessed formally in humans but could easily be measured by performing repeat invasive renal blood pressure measurements over a longer time period in both normal and hypertensive individuals. This would allow the extent of the impact from natural biological variability to be assessed.

Furthermore, I would expect, the reproducibility of this test to be lower, especially in the hands of operators without extensive experience using the Combowire. This could be improved by training operators first on using the Combowire and the technician collecting data on the Combomap.

4.4.2 Repeatability of invasive and non-invasive measurements of aortic blood pressure

There was a smaller standard deviation of difference between repeat measurements of invasive blood pressure than for non-invasive blood pressure readings. This is likely due to the difference in methodology of acquisition of invasive and non-invasive blood pressure. Repeat invasive measurements were performed within a short time period in the cardiac catheterisation laboratory: simultaneous readings of aortic pressure and flow velocity were recorded for 30 seconds, then a break of 30 seconds was taken, followed by repeat
recordings of 30 seconds duration. In comparison, repeat measurements of non-invasive blood pressure occurred within a longer time period (30 minutes). Therefore, the larger standard deviation of difference between repeat non-invasive blood pressure readings is likely due to natural variability between blood pressure recordings rather than measurement noise.

4.4.3 Repeatability of invasive and non-invasive measurements of aortic wave speed

There was good repeatability, however, for repeat measurements of aortic wave speed whether measured invasively or estimated from non-invasive brachial blood pressure measurements. This suggests that non-invasive estimates of central aortic wave speed show less natural variability than blood pressure measurements.

4.4.4 Validation of brachial Cuff-based ambulatory blood pressure monitor for estimating aortic pulse wave velocity

The Mobil-O-Graph monitor uses ARCSolver software which applies a transfer function on the brachial wave form to reconstruct a central aortic pulse wave. This allows the aortic pulse wave velocity to be computed. This has been validated against a tonometric system for measurements of AIx and SBP(155).

In this study I validated measurement of aortic wave speed by the Mobil-O-Graph against invasive aortic wave speed measured using the single point approach, and found that central aortic wave speed measurements in this cohort were consistently higher than those computed from the brachial artery. This suggests that there is a difference in the methods used to calculate aortic wave speed: potentially a difference in transfer functions between both approaches. Unfortunately, however, the transfer function details for the ARCSolver
software is not freely available and therefore I was not able to directly compare with the transfer function used in our software.

However, my findings are out of keeping with recent reports (189) that showed a much smaller mean difference between brachial derived values and intra-aortic values (0.43±1.24 vs 4.7±5.0 in this study). This is unlikely to be due, however, to measurement noise in my invasive data set as there was excellent repeatability between invasive recordings in this data set, validating this method of data acquisition.

There are two possible reasons for this discrepancy. First, in this previous report by Hametner(189), the methodology reports a degree of manual intra-procedural measurements: “The foot-point of the pressure wave was identified manually using the method of intersecting tangents. The interobserver variability in the manual measurements in our laboratory is adequate, as reported previously (190).”

Second, in Hametner’s experiment, there was a time-delay of one day between invasive and non-invasive measurement of aortic pulse wave velocity. It is possible that these two factors may have introduced a degree of measurement bias into the data set. In contrast, all the analysis in my study was done after the procedure, preventing this same bias from occurring. According to published recommendations (188) for the validation of non-invasive devices for PWV measurements, these non-invasive wave speed results show insufficient correlation with invasive results to be used further in my thesis.

4.4.5 Correlation between invasive and non-invasive measures of blood pressure is improved when central estimates are used

The Mobil-O-Graph 24h Monitor has previously been validated for blood pressure measurement according to British Hypertension Standards(191). My results showed that the correlation between invasively measured aortic pressure and non-invasively measured aortic
pressure was improved when central estimates are used. However, even with central estimation in my sample, there was still a large discrepancy between invasively measured aortic pressure and that measured non-invasively. This may be due to the time delay between invasive and non-invasive measurements, rather than a difference in measurements. My results are in keeping with a previous experiment comparing invasive measurements with those measured non-invasively using the SphygmoCor cuff (192) which also showed a large discrepancy between invasively measured aortic pressure and that estimated from non-invasive brachial pressure waveforms. These results are also in keeping with previous studies that showed when invasively and non-invasively measured aortic blood pressures are compared, SBPs are underestimated and DBPs overestimated with oscillometric measurements (192–194).
4.5 Study limitations
In my study three ambulatory blood pressure readings over 30 minutes were averaged to increase accuracy of readings. This will, however, introduce a time delay between invasive and non-invasive measurements that may explain the discrepancy between these results and previous reports.

Non-invasive measurements were made outside the cardiac laboratory. In contrast, invasive aortic measurements were made after a femoral puncture with patients supine in the cardiac catheterisation laboratory. Although all measures were taken to relax patients, any pain response or sympathetic activation from stress response from being in the laboratory may well have led to the elevation in invasive blood pressure recorded.

Finally, the discrepancy between non-invasive estimates of wave speed using a brachial blood pressure cuff and invasive measurements of aortic wave speed may simply be due to use of a different transfer factor. However, the details of the transfer factor used within the Mobil-O-Graph system are not available to further assess this.
4.6 Conclusions
In this study I validated my methodology for measuring invasive renal and aortic blood
pressure and aortic wave speed using the Combowire which showed excellent test-retest
repeatability.

In addition, I found that non-invasive estimates of wave speed using a brachial blood
pressure cuff were consistently lower and showed poor correlation with invasive gold
standard aortic wave speed. This suggests a degree of methodological difference. My
approach using invasive aortic wave speed has been validated against the foot-to-foot
approach (158) (184), and therefore in future chapters I will continue to use only invasive
measurements of aortic wave speed to ensure accuracy.

Finally, there was a discrepancy between invasive measurements of blood pressure and those
measured non-invasively, which is likely in part due to the time difference between invasive
and non-invasive measurements. In my continuing trial I therefore opt to continue using both
non-invasive and invasive measurements of blood pressure.
5 Distal ablation and directly observed medical therapy as potential protocol advancements for renal denervation for hypertension: impact on blood pressure reduction
5.1 Introduction

5.1.1 Discrepancy about efficacy of renal denervation

There has been considerable discrepancy about the true efficacy of renal denervation on blood pressure reduction, as described in Chapter 1. The results of Symplicity HTN-3 (119), the first placebo-controlled RDN trial, showed a disappointing non-significant effect on ambulatory blood pressure. Although the results of the trials so far have been extensively debated, they do provide several important learning points, which have been incorporated into my trial design.

5.1.1.1 Measuring medication adherence

As discussed in Chapter 1, poor adherence with medication is known to contribute to poor control of many diseases (33) (31, 32) (195). Approximately 40% of patients with a new diagnosis of hypertension discontinue their medication within the first year of treatment (30). In resistant hypertension, poor adherence with anti-hypertensive therapy is common and has a reported prevalence of 10-25% (37) (34) (48). There are multiple methods of assessing adherence to medication, as discussed in Chapter 1. One method is by measuring blood pressure after directly observed anti-hypertensive tablet feed and comparing to referral blood pressure measurement. One study found that only approximately 40% of patients were truly resistant after tablet feed (196). Another option is to measure urine or plasma drug metabolites.

It is critically important to include medication adherence within a trial protocol, as changes in medication adherence throughout a trial could cause a change in blood pressure that would otherwise falsely be attributed to the intervention. Medication adherence within Symplicity HTN-3 (120) was assessed using patient self-reported medication diaries. This method of adherence reporting is known to have poor diagnostic ability and poor correlation with blood
pressure (197) (198). Therefore, the substantial decrease in blood pressure in the placebo
group could be caused by either an unrecognised change in medication adherence or
increased anti-hypertensive prescription.

5.1.1.2 Standardizing pharmacotherapy within the trial period
Post-hoc analysis of the Symplicity HTN-3 trial showed that approximately 39% of patients
changed their medication regime within the trial period (120). It is critical when estimating
an effect size from renal denervation to ensure that medication is identical at follow-up to the
baseline assessment, otherwise any change in blood pressure may again falsely be attributed
to the treatment rather than to the medication. There are concerns that this might not be
ethical (199), as patients with resistant hypertension might have high rates of adverse events
from medication and therefore need medication alterations within a follow-up period.
However, meta-analysis of 25 randomised controlled trials of antihypertensive therapy
versus placebo showed a minimal rate of adverse events in the control arm, indicating that
patients can maintain an anti-hypertensive regime (200) (201). One potential confounding
factor to this might be if patients experience a large reduction in blood pressure post renal
denervation, which might necessitate a change in medication dose.

5.1.1.3 Assessing blood pressure reduction using ambulatory blood pressure versus office
blood pressure measurements
The primary outcome of Symplicity HTN-3 was change in ambulatory blood pressure,
compared to previous trials that measured change in office blood pressure. In anti-
hypertensive drug trials without randomisation or blinding, meta-analysis (118) shows blood
pressure reduction is 5.6 mm Hg larger with office measurements than ambulatory blood
pressure monitoring. Once blinding is implemented, however, this difference disappears.
This suggests that the discrepancy in unblinded office and ambulatory BP data is because of
unequal handling of data when clinicians are unblinded to treatment allocation, rather than a
difference in office and ambulatory blood pressure response.

5.1.1.4 Anatomical considerations: denervating distal branches
Previously, renal denervation technique has focused on delivering radiofrequency energy to
the main trunk of the renal artery, avoiding distal branches. Recent studies assessing the
distribution of the renal sympathetic nervous system in human tissue suggest that the mean
distance from renal artery lumen to nerve is least in the distal segments of the renal arteries,
although the density of nerve fibres is lower in distal segments with a predominance of
efferent nerve fibres (131). A recent pre-clinical study (128) assessing the effect of distal
renal artery denervation in pigs has shown a greater reduction of norepinephrine tissue
content when the main artery and distal branches were treated by renal denervation.

5.1.1.5 Anatomical considerations: depth of denervation
Previous devices deliver energy to a depth of 3-4mm. This may be insufficient to reach
deeper nerves and achieve denervation. Although in a healthy population, 90.5% of detected
nerves have been found to be located within 2mm of the lumen, hypertensive patients have
increased vascular hyperplasia, increasing the distance of nerves from the lumen (202). A
case report that provides a histopathological evaluation of the effects of denervation on
perivascular nerves of the renal arteries highlights this (203). In this case-report, a large
portion of nerves were not damaged after denervation, with nerve bundles ranging from 1mm
to 4mm distance from the lumen. The newer Symplicity Spyral catheters increase lesion
depth to 2.15±0.02 mm, and the EnlighHTN system (St Jude Medical, USA) to 2.32±0.02
mm (204).
5.1.1.6 Anatomical considerations: increasing the number of ablations

As discussed in Chapter 1, post hoc analysis of Symplicity HTN-3(120) indicated that a greater reduction in blood pressure was achievable in patients who had more ablations performed. Together, all these findings suggest the need to explore renal denervation targeting distal nerves and using a larger numbers of circumferential ablations.
5.2 Aims

- To assess reduction in ambulatory 24-hour blood pressure when renal denervation was performed using the Symplicity Spyral catheter to ensure ablations were performed in a four-quadrant pattern.

- To assess reduction in ambulatory 24-hour blood pressure when renal denervation was performed in both the main renal artery and distal renal artery branches >3mm.

- To measure bias-resistant efficacy data on ambulatory blood pressure reduction by performing all measurements under directly observed anti-hypertensive therapy.

- To evaluate the prevalence of antihypertensive treatment non-adherence in patients referred for renal denervation using objective urine screening by HPLC – MS/MS.

- To assess whether patients’ treatment adherence is affected within a 6-month period of an intensive blood pressure trial by measuring treatment non-adherence at baseline and at 6-months follow-up after renal denervation.
5.3 Results

5.3.1 Characteristics of study participants at baseline and at 6 months

The baseline characteristics of all 18 patients consented into the trial are shown in Table 4-1. My protocol specified that enrollment eligibility was dependent upon the blood pressure indicated by the referrer when the patient self-reported full adherence with medical therapy. If I later recorded a lower blood pressure after consent and enrollment, for example after directly observed therapy, I did not use this to automatically exclude the patient.

In 4 patients, after directly observed anti-hypertensive therapy, their subsequent (pre-denervation) ambulatory systolic blood pressure was < 130mmHg. These 4 patients had a mean referral office SBP of 179 ± 15mmHg. All four had had 24-hour ABPM by their referring clinicians (although this was not an inclusion criterion) which showed a mean ambulatory referral SBP of 166 ± 27 mmHg. These four patients were prescribed on average 5 ± 2.2 anti-hypertensive medications.

Patients were prescribed on average 5 anti-hypertensive medications. One patient was on no anti-hypertensive therapy, having been thoroughly assessed by a clinical pharmacologist and reported intolerance to all 7 anti-hypertensives that they had tried. Each patient’s medication and dose is shown in Figure 5-1. At 6 months, each patient was still taking the identical anti-hypertensive regime at identical doses as they had been taking at baseline.
Patients are shown in order of reducing number of daily antihypertensives prescribed

5.3.2 Procedural details

In total 16 patients underwent an average of 22.6 ± 5.0 ablations, comprising one main branch and at least two distal branch ablations per kidney, Table 5-1. The average number of ablations per kidney was 9.3 ± 2.9 ablations in the main trunk and 13.3 ± 4.8 ablations distally.
Table 5-1. Procedural details of denervation performed on each of the 16 patients within the trial.

On average, 23 denervation were performed per patient, with the majority of denervations in the distal branches.

Values are shown as mean ± SD

<table>
<thead>
<tr>
<th>Details of renal denervation (n=16)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Left Kidney</strong></td>
</tr>
<tr>
<td>Total number of ablations left renal artery</td>
</tr>
<tr>
<td>Average number of ablations left main renal artery</td>
</tr>
<tr>
<td>Average number of ablations distal branches left renal artery</td>
</tr>
<tr>
<td><strong>Right Kidney</strong></td>
</tr>
<tr>
<td>Total number of ablations right renal artery</td>
</tr>
<tr>
<td>Average number of ablations right main renal artery</td>
</tr>
<tr>
<td>Average number of ablations distal branches right renal artery</td>
</tr>
</tbody>
</table>
5.3.3 Reduction of non-invasive office blood pressure 6-months after renal denervation

In 16 patients who had renal denervation and completed 6-month follow up, average office systolic blood pressure was 172.3±23.0mmHg pre-denervation and 162.4±26.0mmHg 6-months after denervation (p=0.009). Average office diastolic blood pressure was 97.7±14.4mmHg pre-denervation and 96.0±13.6 mmHg 6-months after denervation (p=0.478), Figure 5-2, Table 5-2.

5.3.4 Reduction of non-invasive ambulatory blood pressure 6-months after renal denervation

All ambulatory blood pressure monitoring was performed after directly observed anti-hypertensive therapy to ensure patients were on identical medication regimes when the monitor was fitted at baseline and 6-months follow-up. After giving consent, one patient refused all ambulatory blood pressure monitors scheduled because, the patient stated, this had in the past caused extensive bruising of the arm. The patient agreed to have all other processes and had already formally enrolled and therefore was retained in the trial. This patient was patient 04 in Figure 5-1 who was on no medical therapy due to multiple intolerances.

The average change in ambulatory blood pressure in 15 patients completing the trial is illustrated in Figure 5-3, Table 5-2. Baseline ambulatory 24-hour systolic blood pressure significantly decreased by 5.1±7.5mmHg from 142.8±17.9mmHg pre-denervation to 137.7±18.4mmHg 6-months after denervation (p=0.020). Baseline ambulatory 24-hour diastolic blood pressure significantly decreased by 3.4±4.9mmHg from 87.9±12.8mmHg pre-denervation to 84.5±11.2mmHg 6-months after denervation (p=0.018). 24-h Holter heart rate remained unchanged 6 months after RDN (62.9±12.2 pre-denervation and 64.1 ± 14.4 post denervation, p=0.921).
Variability of blood pressure and heart rate, defined as the standard deviation over the values recorded over 24 hours, is shown in Table 5-2. There was no significant change in variability in any parameter at 6-months follow-up.
Overall there was a reduction in SBP of 9.9 ± 13.3 mmHg and DBP of 1.7 ± 9.5 mmHg (p=0.009 and 0.48 respectively).

*Figure 5-2. Change in office blood pressure 6-months after distal renal denervation*

Overall there was a reduction in SBP of 9.9 ± 13.3 mmHg and DBP of 1.7 ± 9.5 mmHg (p=0.009 and 0.48 respectively).
Overall there was a reduction in ambulatory SBP of 5.1±7.5mmHg and DBP of 3.4±4.9mmHg (p=0.020 and 0.018 respectively).

Figure 5-3. Change in ambulatory blood pressure within the 6-months follow up period
**Table 5-2. Analysis of ambulatory blood pressure and heart rate variability**

Values in table represent mean ± SD

<table>
<thead>
<tr>
<th></th>
<th>Baseline pre-denervation</th>
<th>6-months post-denervation</th>
<th>Change within 6 months</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Office BP (n=16)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>172.3±23.0</td>
<td>162.4±26.0</td>
<td>-9.9±13.3</td>
<td>0.009</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>97.7±14.4</td>
<td>96.0±13.6</td>
<td>-1.7±9.5</td>
<td>0.478</td>
</tr>
<tr>
<td><strong>Ambulatory measurements (n=15)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24 hour SBP (mmHg)</td>
<td>142.8±17.9</td>
<td>137.7±18.4</td>
<td>-5.1±7.5</td>
<td>0.020</td>
</tr>
<tr>
<td>24 hour DBP (mmHg)</td>
<td>87.9±12.8</td>
<td>84.5±11.2</td>
<td>-3.4±4.9</td>
<td>0.018</td>
</tr>
<tr>
<td>24 hour MAP (mmHg)</td>
<td>113.1±14.6</td>
<td>108.7±14.1</td>
<td>-4.3±14.1</td>
<td>0.010</td>
</tr>
<tr>
<td>24 hour HR (bpm)</td>
<td>67.0±11.2</td>
<td>67.2±13.3</td>
<td>0.16±6.2</td>
<td>0.921</td>
</tr>
<tr>
<td>Day SBP (mmHg)</td>
<td>143.5±16.9</td>
<td>139.1±19.3</td>
<td>-4.3±9.8</td>
<td>0.108</td>
</tr>
<tr>
<td>Day DBP (mmHg)</td>
<td>90.0±12.7</td>
<td>87.1±11.8</td>
<td>-2.9±6.1</td>
<td>0.091</td>
</tr>
<tr>
<td>Day MAP (mmHg)</td>
<td>114.5±14.1</td>
<td>110.9±14.7</td>
<td>-3.6±7.5</td>
<td>0.085</td>
</tr>
<tr>
<td>Day HR (bpm)</td>
<td>69.4±11.7</td>
<td>69.2±13.4</td>
<td>-0.21±7.9</td>
<td>0.919</td>
</tr>
<tr>
<td>Night SBP (mmHg)</td>
<td>141.7±21.7</td>
<td>135.8±18.4</td>
<td>-5.9±10.8</td>
<td>0.052</td>
</tr>
<tr>
<td>Night DBP (mmHg)</td>
<td>84.4±14.8</td>
<td>80.9±11.1</td>
<td>-3.6±8.0</td>
<td>0.107</td>
</tr>
<tr>
<td>Night MAP (mmHg)</td>
<td>110.7±17.4</td>
<td>106.0±13.4</td>
<td>-4.8±8.4</td>
<td>0.045</td>
</tr>
<tr>
<td>Night HR (bpm)</td>
<td>62.9±12.2</td>
<td>64.1±14.4</td>
<td>1.24±6.5</td>
<td>0.472</td>
</tr>
<tr>
<td><strong>Standard deviation of Ambulatory measurements (n=15)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24 hour SBP (mmHg)</td>
<td>16.2±2.9</td>
<td>16.0±5.9</td>
<td>-0.19±4.6</td>
<td>0.872</td>
</tr>
<tr>
<td>24 hour DBP (mmHg)</td>
<td>13.5±2.6</td>
<td>11.8±3.2</td>
<td>-1.71±3.4</td>
<td>0.071</td>
</tr>
<tr>
<td>24 hour MAP (mmHg)</td>
<td>13.6±2.1</td>
<td>12.6±4.0</td>
<td>-1.04±2.9</td>
<td>0.189</td>
</tr>
<tr>
<td>24 hour HR (bpm)</td>
<td>8.5±3.6</td>
<td>8.5±3.2</td>
<td>-0.04±3.2</td>
<td>0.958</td>
</tr>
<tr>
<td>Day SBP (mmHg)</td>
<td>15.8±3.5</td>
<td>16.2±7.2</td>
<td>0.4±5.3</td>
<td>0.768</td>
</tr>
<tr>
<td>Day DBP (mmHg)</td>
<td>13.0±4.4</td>
<td>11.3±3.8</td>
<td>-1.74±5.3</td>
<td>0.225</td>
</tr>
<tr>
<td>Day MAP (mmHg)</td>
<td>13.2±3.5</td>
<td>12.5±4.8</td>
<td>-0.75±3.7</td>
<td>0.450</td>
</tr>
<tr>
<td>Day HR (bpm)</td>
<td>7.7±3.5</td>
<td>8.1±2.9</td>
<td>0.38±3.2</td>
<td>0.642</td>
</tr>
<tr>
<td>Night SBP (mmHg)</td>
<td>14.5±5.6</td>
<td>13.5±6.0</td>
<td>-1.08±7.4</td>
<td>0.584</td>
</tr>
<tr>
<td>Night DBP (mmHg)</td>
<td>10.2±4.4</td>
<td>11.2±4.2</td>
<td>0.96±4.3</td>
<td>0.408</td>
</tr>
<tr>
<td>Night MAP (mmHg)</td>
<td>11.2±3.3</td>
<td>11.1±4.8</td>
<td>-0.14±4.7</td>
<td>0.912</td>
</tr>
<tr>
<td>Night HR (bpm)</td>
<td>6.6±3.1</td>
<td>6.2±3.0</td>
<td>-0.38±3.1</td>
<td>0.646</td>
</tr>
<tr>
<td><strong>Dipper status (n=15)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dipper, %</td>
<td>2 (13.3)</td>
<td>2 (13.3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-dipper, %</td>
<td>6 (40)</td>
<td>7 (46.7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reverse dipper, %</td>
<td>7 (46.7)</td>
<td>6 (40)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
5.3.5 Correlation of ABPM blood pressure reduction with baseline characteristics

The correlation of 6-month reduction in ambulatory systolic blood pressure with baseline characteristics is shown in Table 5-3. No baseline characteristic was found to be a predictor of ambulatory blood pressure response at 6-month follow up.
Table 5-3. The correlation of 6-month reduction in ambulatory systolic blood pressure with baseline characteristics

<table>
<thead>
<tr>
<th>Correlation of reduction in 6-month SBP ABPM</th>
<th>$R^2$</th>
<th>Beta coefficient</th>
<th>95% confidence interval</th>
<th>p value</th>
<th>Intercept</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient age</td>
<td>0.012</td>
<td>0.062</td>
<td>-0.24 to 0.36</td>
<td>0.694</td>
<td>-8.82</td>
</tr>
<tr>
<td>Referral SBP</td>
<td>0.096</td>
<td>0.123</td>
<td>-0.09 to 0.34</td>
<td>0.280</td>
<td>-26.7</td>
</tr>
<tr>
<td>Referral DBP</td>
<td>0.001</td>
<td>0.014</td>
<td>-0.24 to 0.26</td>
<td>0.914</td>
<td>-5.82</td>
</tr>
<tr>
<td>Baseline Office SBP</td>
<td>0.020</td>
<td>-0.046</td>
<td>-0.22 to 0.13</td>
<td>0.612</td>
<td>2.83</td>
</tr>
<tr>
<td>Baseline Office DBP</td>
<td>0.072</td>
<td>-0.135</td>
<td>-0.40 to 0.13</td>
<td>0.334</td>
<td>8.26</td>
</tr>
<tr>
<td>Baseline ABPM SBP</td>
<td>0.022</td>
<td>-0.062</td>
<td>-0.29 to 0.16</td>
<td>0.595</td>
<td>3.87</td>
</tr>
<tr>
<td>Baseline ABPM DBP</td>
<td>0.081</td>
<td>-0.165</td>
<td>-0.47 to 0.14</td>
<td>0.305</td>
<td>9.52</td>
</tr>
</tbody>
</table>
5.3.6 Change in invasive blood pressure

Baseline invasive blood pressure was measured for each patient using a Combowire in the renal arteries before and after sedation. The same measurements were made at 6-months follow-up. Each patient’s 6-month follow-up sedation dosage was identical to that used for their baseline visit, as shown in Figure 5-1.

The change in blood pressure between baseline and 6-month follow up for each modality of blood pressure measurement is shown as a waterfall diagram in Figure 5-4. As evident in the Figure, the change in blood pressure was similar between baseline and 6-month visits for ambulatory blood pressure (reduction in BP of -5mmHg), and pre and post sedation renal invasive blood pressure (reduction in BP of -3 and -7mmHg respectively). In contrast, however, the reduction in Office BP was far greater (-10mmHg).
Figure 5-4. The cascade of falling blood pressure within the 6-month trial period
5.3.7 Assessment of medication adherence before the trial and at trial completion

All 16 patients had a urine sample assessed for adherence to anti-hypertensive medication at the baseline visit ("entry adherence") and the 6-month visit. This sample was taken immediately before the directly observed antihypertensive therapy at the baseline and 6-month visits. A sample was taken for each of the 15 patients taking antihypertensive medication, all of whom self-reported 100% adherence with prescribed antihypertensive medication regime.

On their entry sample, 9 patients (60%) were adherent to all prescribed antihypertensive medications of which 8 remained adherent at 6-months follow-up.

On their entry sample, 2 patients (13%) were non-adherent to all prescribed antihypertensive medications of which 1 remained non-adherent to all medication at 6-months follow-up.

The change in anti-hypertensive adherence at 6 months is shown in Figure 5-5. At 6-months, adherence was broadly similar to entry adherence.

Baseline adherence to antihypertensive medication was shown to be significantly negatively correlated with baseline office SBP ($R^2=0.46$, $p=0.0053$). No other baseline blood pressure measurement showed a significant correlation with baseline adherence: office DBP ($R^2= 0.24$, $p=0.063$), ABPM SBP ($R^2= 0.051$, $p=0.41$), ABPM DBP ($R^2= 0.066$, $p=0.36$))

As outlined earlier, one patient was excluded from the trial prior to denervation due to symptomatic hypotension after directly-observed anti-hypertensive therapy. This patient was prescribed 6 anti-hypertensives at maximum dose. Urine adherence testing was taken prior to direct tablet feed which detected only 1 out of 6 anti-hypertensive medications.
Figure 5-5. Assessment of medication adherence before the trial and at trial completion

This figure shows the change in medication adherence at entry pre denervation and at 6-months follow up. Data points are shown for the 15 patients taking antihypertensive medication. One patient was on no therapy having been found to be intolerant of 7 anti-hypertensives and is not shown in the Figure above.
5.3.8 Correlation of 6-month systolic ambulatory blood pressure reduction with patients’ medication adherence

Figure 5-6 shows for each patient their ambulatory systolic blood pressure reduction at 6-months along with their baseline and 6-month medication adherence. Change in ambulatory systolic blood pressure at 6-months was not correlated with adherence to medication at baseline visit ($R^2 = 0.054$, $p=0.41$) or 6-months follow-up ($R^2 = 0.016$, $p=0.66$).

As illustrated in Figure 5-6, there were 2 patients who had higher medication adherence at 6-months follow-up then at the baseline visit. These two patients had a reduction in ambulatory systolic blood pressure of -19.6mmHg and -5.5mmHg respectively. One patient showed reduced anti-hypertensive medication adherence at 6-months follow-up. This patient had an ambulatory blood pressure reduction of 7.8mmHg at 6-months follow-up.

There were four patients who had an increase in ambulatory blood pressure $\geq 0$mmHg at 6-months, all of whom had identical medication adherence at the baseline and 6-months visit.
Figure 5-6. Correlation of 6-month ambulatory systolic blood pressure reduction with patients’ medication adherence

This figure shows 6-month change in ambulatory systolic blood pressure for each patient along with baseline (left panel) and 6-month (right panel) anti-hypertensive medication adherence. Only two patients increased medication adherence between the baseline and 6-month visit; these patients had reduction in 6-month ambulatory blood pressure of -19.6 and -5.5 mmHg respectively. One patient reduced medication compliance between baseline and 6-month visit; this patient had a reduction in 6-month ambulatory blood pressure of -7.8 mmHg.
5.3.9 Blood pressure reduction in patients 100% compliant with medication

Subgroup analysis of the 9 patients who were 100% compliant with all prescribed anti-hypertensive medication at baseline and 6-month visit is shown in Table 5-4, Figure 5-7. These 9 patients had the following characteristics: age 65.2 ± 15.6 years, 67% male, average number of antihypertensive agents 4.6, referral office systolic blood pressure 176.5 mmHg, referral office diastolic blood pressure 91.5 mmHg. Baseline office SBP showed no significant change (-5.2±14.2 mmHg, p=0.31) 6-months after denervation. Baseline ambulatory 24-hour systolic blood pressure and diastolic blood pressure also showed no significant change 6-months after denervation (-3.0±6.6 mmHg, p=0.21 and -3.4±4.9 mmHg, p=0.07 respectively). 24-hour Holter heart rate also remained unchanged 6 months after RDN ((-1.3±5.9 bpm, p=0.52).
Table 5-4. Analysis of ambulatory blood pressure and heart rate variability in 9 patients who showed 100% compliance with prescribed antihypertensive therapy at baseline and 6-month visit

Values in table represent mean ± SD

<table>
<thead>
<tr>
<th></th>
<th>Baseline pre-denervation</th>
<th>6-months post-denervation</th>
<th>Change within 6 months</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Office BP (n=16)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>163.9±18.6</td>
<td>158.8±26.7</td>
<td>-5.2±14.2</td>
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<tr>
<td>DBP (mmHg)</td>
<td>94.6±13.9</td>
<td>94.0±14.7</td>
<td>-0.6±10.3</td>
<td>0.860</td>
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<tr>
<td><strong>Ambulatory measurements (n=15)</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>24 hour SBP (mmHg)</td>
<td>143.6±18.0</td>
<td>140.6±18.7</td>
<td>-3.0±6.6</td>
<td>0.209</td>
</tr>
<tr>
<td>24 hour DBP (mmHg)</td>
<td>87.0±12.6</td>
<td>83.6±11.2</td>
<td>-3.4±4.9</td>
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</tr>
<tr>
<td>24 hour MAP (mmHg)</td>
<td>113.0±14.3</td>
<td>109.7±13.9</td>
<td>-3.3±4.8</td>
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</tr>
<tr>
<td>24 hour HR (bpm)</td>
<td>67.1±10.5</td>
<td>65.8±10.7</td>
<td>-1.3±5.9</td>
<td>0.518</td>
</tr>
</tbody>
</table>
Figure 5-7. Change in ambulatory blood pressure within the 6-months follow up period in patients who were 100% adherent to antihypertensive medication

Overall there was no significant change in ambulatory SBP (-3.0±6.6mmHg, p=0.21) and ambulatory DBP (-3.4±4.9mmHg, p=0.07)
5.4 Discussion

This human study of distal renal denervation used HPLC-tandem mass spectrometry to assess medication adherence and used directly observed anti-hypertensive therapy prior to all measurements. It measured unblinded blood pressure in four settings: office, ambulatory, invasive before sedation, and invasive after sedation. However, as this trial was designed as a proof-of-concept study, it was not powered appropriately to assess efficacy of distal renal denervation on blood pressure reduction. Instead, the significant reduction of 10mmHg in office systolic blood pressure, 5mmHg reduction in ambulatory systolic and 3mmHg reduction in ambulatory diastolic blood pressure should be considered exploratory. Furthermore, the blood pressure changes reported need to be interpreted in the context of the lack of blinding in this study. In Chapter 1, analysis of previous renal denervation trials showed that previous unblinded studies show a larger efficacy in blood pressure reduction than blinded studies. Therefore, despite many measures taken to reduce bias in this study, it is likely that if the study had been blinded, this reduction in blood pressure would have been reduced.

In addition, I found that between-individual standard deviation in effect size of renal denervation was 13mmHg for office blood pressure and 8mmHg for ambulatory blood pressure. Narrow between-individual variation increases the ability of a study to detect small effect sizes. No non-invasive baseline characteristic was found to be a predictor of blood pressure response.

5.4.1 Efficacy of renal sympathetic denervation using distal denervation in branches

My study protocol was informed by recent pre-clinical data that showed greater reduction in renal norepinephrine when denervation is performed distally rather than
proximally (128) (129). Although renal sympathetic nerve fibres are less numerous around the distal branches, the fibres are closer to the artery lumen (129, 130) and a greater proportion of them are efferent (131). During catheter-based radiofrequency ablation, the greatest injury is expected to be to the nerves close to the arterial lumen. For example, a recent study reported that renal denervation injured 50% of nerves when performed distally but only 33% of nerves when performed proximally (129).

I found a significant reduction of 10mmHg in office systolic blood pressure, 5mmHg reduction in ambulatory systolic blood pressure and 3mmHg reduction in ambulatory diastolic blood pressure. This is in keeping with a recent randomised unblinded trial (205) that also used an intensive protocol of antihypertensive prescription throughout the trial period, and a recent unblinded study which also used directly observed antihypertensive therapy (206).

My results showed a greater drop in 24-hour nocturnal systolic and diastolic blood pressure than day blood pressure. While it is possible that this reflects merely the play of chance, it is consistent with the isolated nocturnal efficacy reported in a pooled analysis of ambulatory data from Symplicity HTN-3 and HTN-Japan (207).

Isolated nocturnal reduction should not be assumed to be trivial, since nocturnal hypertension has an even stronger association with cardiovascular events (208) (209) than does daytime BP.

5.4.2 Office versus ambulatory effects

There was greater reduction in office blood pressure than ambulatory. In the past this pattern has been attributed to a lesser effect of denervation on nocturnal pressures, but my data and that of Symplicity HTN-3 and HTN-Japan (207) indicate greater rather than less nocturnal efficacy. Whilst initially thought to be a feature present in drug
trials, detailed assessment reveals it is a feature of unblinded trials (where those
documenting the office pressure know whether the patient is receiving the trial
medication) and not a feature of blinded trials (118).
Ambulatory blood pressure has the advantage of reducing unintentional bias arising
from the clinical habit of discarding seemingly inappropriate values. It also reduces
the impact of random variation by providing an average of multiple values. For this
and other reasons it is therefore a preferred end-point (121).

5.4.3 Ambulatory blood pressure reduction is decreased when full
medication adherence is confirmed
In the subgroup analysis of patients who were 100% adherent to hypertension
medication at baseline and at 6-month visit, systolic and diastolic ambulatory blood
pressure reduction was less than in the general cohort. This implies that some of the
ambulatory blood pressure reduction shown in this trial may be due to changes in
medication adherence, rather than due to the denervation treatment. Although this
should have been controlled for by directly observed anti-hypertensive therapy, as
DOT was performed for only one day prior to non-invasive measurements and two
days prior to invasive measurements, it is possible that some medication may need
longer periods of observed administration to get to a steady-state level if patients had
been non-compliant with medication.
This subgroup analysis might explain the disparity between blood pressure reduction
in this trial and other previous denervation trials e.g. Symplicity HTN-1 and 2. As
previously discussed in Chapter 1, these previous trials, which showed far larger
reduction in blood pressure at 6-months, did not quantitatively and objectively assess
for medication adherence within the trial periods.
5.4.4 Confirming resistant hypertension

Many patients clinically considered to have resistant hypertension turn out, on urine screening, to be taking few or none of their prescribed medications (41). Directly observing patients taking medication is a simple step to confirming medication resistance. In my study, one patient suffered a large drop in systolic BP from 170 to 80 mmHg after directly observed therapy and had to be withdrawn from the study. This patient had 1 out of 6 anti-hypertensives present on urine testing. The remaining patients had a mean drop of 12 mmHg from referral office pressure (without directly observed therapy) to trial baseline office pressure (with directly observed therapy). This is despite all patients reporting complete adherence at trial entry.

Current clinical guidelines (13) recommend checking medication adherence by asking the patient whether they are adherent. Our data suggests this alone might be inadequate, and that this should be accompanied by directly observing patients taking medication. Realising the importance of assessing medication adherence, previous workers have explored many methods to assess adherence. Prior work has sought to assess adherence using many approaches, including checking records of pharmacy prescription refills and electronic compliance monitoring which electronically records medication box openings and closures (44, 45) (46). However, despite going beyond subjective self-reports from patients, they still do not confirm that the medication has been swallowed. Confirmation of medication intake can be obtained through high-performance liquid chromatography-tandem mass spectrometry (HP LC-MS/MS) of spot urine or serum samples (47) (48).

In this Chapter I illustrate medication adherence at baseline and 6-month visit. In my study, adherence was high on the urine samples taken at the start of each assessment and only 3 patients showed a change in medication adherence within the 6-months
follow up. In addition, patients underwent directly observed therapy during each assessment before any blood pressure measurements. Therefore, the combination of these attenuates the potential effect of increased adherence to artifactually expand the observed blood pressure effect (210).

5.4.5 Directly observed anti-hypertensive therapy - potential limitations of study protocol

The purpose of performing directly observed anti-hypertensive therapy was to ensure that each patient was on identical medication and dosing regimes before measurements were taken at baseline and 6-month visits. This was to ensure that any change in blood pressure was not due to changes in medication adherence.

In my study, patients received 2 episodes of directly observed anti-hypertensive therapy prior to invasive measurements but only 1 episode prior to all non-invasive measurements being recorded.

It is known, however, that some medications might require more than one dose to stabilize plasma levels if the patient had been non-compliant prior to clinic review, depending on the medication pharmacokinetics (211). Therefore, it is possible that changes in medication adherence might still have influenced the blood pressure effect size as well as the standard deviation of overall effect size in this study. This would be especially relevant in two situations:

1. If patients were taking anti-hypertensive medication requiring multiple doses to attain steady state plasma drug concentrations. For example, pharmacokinetic studies of amlodipine show that oral administration resulted in steady state plasma drug concentration being reached after seven doses (212). Conversely, pharmacokinetic studies of Lisinopril in healthy volunteers showed that steady state was attained after the second daily dose (213).
2. Patients in whom a change in medication compliance occurred between baseline and 6-month visits. If patients maintained the same medication adherence between visits, then even if they were 100% non-adherent then as long as DOT occurred at each visit, medication adherence should not effect the treatment effect size. However, patients who altered their medication compliance between visits (3 patients in this study) could still have different plasma levels of anti-hypertensives when effect measurements were made, especially if prescribed medication requiring multiple doses to attain steady state plasma drug concentrations.

Furthermore, 4 patients took twice-daily doses of anti-hypertensive therapy, as shown in Figure 5-1. These patients would go home with their ambulatory blood pressure monitor and be asked to take their medication themselves at home. Their medication compliance was always checked the subsequent day by assessing their self-reported compliance. Therefore, it is not possible to fully be confident that all patients had taken identical medication regimes in the period prior to measurement recording as 4 patients had unwitnessed medicine consumption episodes. This is potentially another source of bias in my study as patients may have altered adherence to their evening medication between baseline and 6-month visits. Several studies (214) (215) (216) (217) (218) have investigated the impact of adherence with medication dosing schedules and found that adherence is improved when medication is given less frequently i.e. once vs twice daily dosing.
5.4.6 The impact of directly observed anti-hypertensive therapy on standard deviation of effect size: Comparison of meta-analysis with current study results

The results of this study, using directly observed therapy, showed a below average variability of 13mmHg for office blood pressure and 8mmHg for ambulatory blood pressure, compared to both the mean of the randomized and non-randomised published trials, for both office and ambulatory blood pressure which were shown in Figure 1-6.

Comparing my results to those of my meta-analysis, I have shown that using directly observed anti-hypertensive therapy prior to all measurements can reduce the between-individual variation in effect size. In addition, I found in the meta-analysis that the between-individual variation in effect size is not minimised when randomisation alone is used in trial design, however is minimised when ambulatory versus office blood pressure is used as an endpoint.

These observations may be valuable for future drug and denervation trials aiming to assess the impact of an intervention on blood pressure. By including directly observed anti-hypertensive therapy in trial design, researchers may be able to further reduce between-individual variation in effect size, thereby reducing the sample size required as well as increasing the ability of a study to detect small effect sizes.
5.5 Study limitations

This was a proof-of-concept study, designed to use high precision measurements to enable a reliable estimate of effect size of distal renal artery denervation. The study was not randomised or blinded and therefore there is a risk of bias (147). Several steps were taken to minimise the magnitude of these biases (219). First, to avoid the temptation to discard seemingly inappropriate BP values, the protocol pre-specified that office blood pressures were only allowed to be taken 3 times and these first 3 numbers used even if they seemed out of keeping with clinical expectation (121). Second, every patient underwent ambulatory blood pressure monitoring and directly observed antihypertensive therapy. Third, invasive measurement of blood pressure was performed both at rest and under sedation. For each patient the exact same dose of sedation was given at baseline and 6-month follow up visit to ensure consistent conditions when measuring blood pressure. However, despite these measures, the change in blood pressure results reported in this section need to be interpreted with caution as implementation of randomisation and blinding would likely reduce the magnitude of this effect size, as discussed in Chapter 1.

This study included patients who are routinely seen in hypertension clinics. In particular, patients were included who fit blood pressure criteria but who were not taking three antihypertensives as long as they had been seen by a hypertension specialist, thoroughly assessed and had been deemed intolerant of multiple medical therapies.

Patients were followed up for 6 months. It is possible that further blood pressure reduction may occur with increasing time as seen in the follow up to the Symplicity HTN-2 trial (220). However, this pattern was not evident in Symplicity HTN-3, a blinded sham-controlled trial (221).

Enrollment of patients in our study was pre-specified based on office SBP and not 24-hour ambulatory SBP. This may, however, lead to inclusion of patients with e.g. white
coat hypertension, who may have a different pathophysiology as compared to patients with “true” resistant hypertension. In our study, 6 out of 16 patients had SBP <140mmHg on ambulatory 24-hour ABPM. However, this reduction in SBP may not only be attributable to a white coat effect, and may also be due to changes in medication adherence as all ABPMs were performed after directly observed antihypertensive therapy. If only patients with ambulatory SBP>140mmHg had been included in our analysis, then although mean reduction in ambulatory SBP was not significantly altered (5.1mmHg to 5.4mmHg reduction), the standard deviation of reduction is reduced (7.4 mmHg to 3.8mmHg). This illustrates a potential limitation of our inclusion criteria, that patients with white coat hypertension as well as poor medication adherence were included that might have different physiological pathways for hypertension.

My study population was recruited prior to publication of the PATHWAY-2 study and included patients taking maximally tolerated doses of at least 3 antihypertensive medications, in comparison to the PATHWAY-2 study, which challenges the convention of defining resistant hypertension to patients taking 4 antihypertensive medications including spironolactone. In our study, 10 out of 18 patients would have been included using these PATHWAY-2 criteria, of which 9 would have undergone denervation and had a reduction in ambulatory SBP at 6-months of -3.0mmHg.
5.6 Conclusions

In this unblinded study distal renal denervation was performed to assess efficacy in blood pressure reduction. A significant reduction of 10mmHg in office systolic blood pressure, 5mmHg reduction in ambulatory systolic blood pressure and 3mmHg reduction in ambulatory diastolic blood pressure was shown, however, the blood pressure reduction measured is likely to have been reduced if blinding was included in my trial design.

In this study, the results showed that directly observed anti-hypertensive therapy prior to all measurements can reduce the between-individual variation in effect size. This may be valuable for future drug and denervation trials aiming to assess impact of an intervention on blood pressure. The impact of this observation will be assessed further in Chapter 8.
6 Systematic evaluation of hemodynamic parameters to predict responders to renal denervation
6.1 Introduction

The kidney is the central homeostatic organ that regulates blood pressure and volume control within the human body. Myogenic and tubuloglomerular feedback mechanisms in the kidney maintain a constant renal blood flow despite variations in renal arterial pressure. In addition, alterations in efferent renal sympathetic nerve activity influence renal blood flow.

Animal models show that the physiological response to renal denervation, in particular change in renal blood flow, varies depending on resting activity of the renal sympathetic nervous system. Under normal physiological conditions, basal efferent renal sympathetic nerve activity is low. Studies have shown that low efferent renal sympathetic nerve stimulation (stimulation frequencies <1 Hz) is sufficient to cause a 13-26% (64) decrease in urinary sodium excretion, an increase in renal tubular sodium and water reabsorption but no change in renal perfusion pressure, renal blood flow velocity or glomerular filtration rate (222). After renal denervation of animals with low efferent renal sympathetic nerve activity, an increase in urinary sodium excretion and a decrease in renin secretion are shown with no change in renal blood flow or glomerular filtration rate. Conversely, high stimulation renal nerve activation (68) produces a decrease in urinary sodium excretion with an associated decrease in renal blood flow (69). Similarly, after renal denervation in rats with high sympathetic tone (congestive heart failure or hypertension), an increase in basal renal blood flow was seen (70) (71).

Currently there is uncertainty about the ability to translate animal models of renal denervation into blood pressure reduction in humans (132). This may be for a number of reasons including procedural failure in humans, change in medication adherence interacting with human trial results, or participants with blood pressure elevation not due to renal sympathetic overactivity. Procedural failure could be ameliorated by developing a
method to provide immediate intraprocedural feedback, which would ensure that catheter ablation has produced sufficient renal denervation. Several markers have been found to correlate with renal denervation efficacy such as baroreflex function (133), and the gold standard renal vein norepinephrine spillover (110). However neither of these is feasible as an immediate intraprocedural assessment to determine acutely within the procedure whether renal sympathetic denervation has occurred.

An alternative viewpoint is that it is not procedural failure that determines the success or failure of renal denervation, but rather, that certain physiological states may be indicative of patients who are more or less likely to respond. For example, if patients with baseline haemodynamic characteristics that were known to correlate with response to renal denervation were able to be pre-specified, then this might improve the efficacy of renal denervation. This is a similar approach compared to pre-specification of patients who will respond to cardiac resynchronization therapy using the morphology and width of QRS complex on baseline electrocardiogram (ECG).
6.2 Aims

1) To measure invasive blood pressure and Doppler flow velocity in the renal arteries and aorta at baseline and 6 months follow-up after denervation in a carefully controlled directly observed therapy and sedation protocol to reduce confounders

2) To assess acute and 6-month change in parameters of microvascular tone (resistance) and arterial stiffness (wave speed) following renal denervation

3) To explore how baseline parameters are related to any observed changes in ambulatory blood pressure to detect whether any baseline parameters may predict change in blood pressure
6.3 Results

6.3.1 Baseline renal haemodynamic assessment

6.3.1.1 Measurement of renal artery wave speed
In all subjects, renal artery wave speed was derived in both the left and right renal arteries. There was strong correlation between the renal artery wave speed in the left renal artery and the right renal artery at rest at the baseline visit ($R^2=0.76$, $p<0.001$), in patients with no angiographic evidence of calcification or renal artery stenosis. The repeatability of renal artery wave speed was also assessed in both renal arteries and results have been discussed in Chapter 4.

6.3.1.2 Comparison of renal and aortic wave speed
Overall mean aortic wave speed derived from the aortic arch in all 16 patients was $15.4\pm8.4$ m/s. There was no significant difference between mean aortic wave speed than mean renal wave speed ($3.2\pm4$ m/s, $p=0.12$).
I tested the relationship between individual patient’s aortic and renal artery wave speed. There was a weak correlation between baseline renal and aortic wave speed as shown in Figure 6-1, ($R^2= 0.17$).
In addition, unlike aortic wave speed, which correlates with patient age ($R^2= 0.43$, $p=0.006$), renal artery wave speed did not correlate with patient age ($R^2=0.09$, $p= 0.774$), Figure 6-2.
Figure 6-1. Correlation between wave speed in the renal arteries and the aorta

Overall there was a weak correlation between wave speed in the renal arteries and the aorta ($R^2=0.17$).
Figure 6-2. Correlation between wave speed in the renal arteries and the aorta and baseline age

Unlike aortic wave speed, which correlates with patient age ($R^2 = 0.43$, $p=0.006$), renal artery wave speed did not correlate with patient age ($R^2 = 0.09$, $p = 0.774$)
6.3.1.3 Correlation of invasive aortic and renal wave speed with non-invasive aortic pulse wave velocity measured using non-invasive brachial blood pressure

I tested the correlation between invasive wave speed (aortic and renal) and aortic pulse wave velocity measured using non-invasive brachial ambulatory blood pressure. Non-invasive estimate of aortic pulse wave velocity (PWV) was calculated as the mean of all aortic PWV recordings within 24 hours.

Baseline invasive aortic wave speed correlated well with baseline non-invasive aortic pulse wave velocity ($R^2=0.70$). However, baseline invasive renal wave speed did not correlate well with baseline non-invasive aortic pulse wave velocity ($R^2=0.09$), as shown in Figure 6-3.
Figure 6-3. Correlation between invasive baseline wave speed in the renal arteries and the aorta with aortic pulse wave velocity measured non-invasively

Overall there was a strong correlation between wave speed in the aorta measured invasively and non-invasively ($R^2=0.70$). However, the correlation between non-invasive aortic PWV and renal artery wave speed was much weaker ($R^2=0.09$).

Note: 14 data points are shown as one patient refused ambulatory blood pressure monitoring and one patient’s device failed to calculate PWV due to patient’s relative bradycardia interrupting with the algorithmic calculation.
### 6.3.1.4 Correlation of renal wave speed with other renal haemodynamic indices

The relationship between renal wave speed and other renal haemodynamic indices was compared. There was a strong positive correlation between baseline renal wave speed, baseline renal vascular resistance \( R^2 = 0.38, \ p=0.01 \) and renal artery blood pressure \( R^2=0.52, \ p=0.0016 \), Figure 6-4.
There was a positive correlation between renal artery wave speed and renal vascular resistance ($R^2 = 0.38$, $p=0.01$) and renal blood pressure ($R^2=0.52$, $p=0.0016$).

**Figure 6-4. Correlation between wave speed in the renal arteries and renal vascular resistance and renal blood pressure**
6.3.2 Change in invasive aortic and renal haemodynamics acutely after denervation and at 6-months follow up

Table 6-1 illustrates the overall changes in renal and aortic artery blood flow, blood pressure and vascular resistance throughout the trial. Pre-denervation, when intravenous sedation was given a significant decrease in renal blood pressure (-15 ± 14 mmHg, p<0.001) was seen with a corresponding non-significant change in renal vascular resistance (-0.73 ± 1.65 mmHg/min/ml, p=0.097), and renal blood flow (-0.48 ± 1.12 ml/min, p=0.11).

At six-months follow up there was an overall significant increase in renal blood flow both at rest (1.91 ± 3.51 cm/s, p=0.04) and under identical sedation states (1.81 ± 3.44 cm/s, p=0.05). This was despite renal perfusion pressure (measured invasively) being the same or lower following renal denervation. Renal vascular resistance was unchanged at both rest (-0.86 ± 2.50 mmHg/min/ml, p=0.46) and under identical sedation (-0.32 ± 3.10 mmHg/min/ml, p=0.68).

In the aorta, at 6-months follow up, there was no-significant change in rest invasive BP (-4 ± 19 mmHg, p=0.36). In the aorta, blood flow velocity did not significantly alter either acutely post denervation (-0.02 ± 0.14 cm/s, p=0.64) or at 6-months follow up. However, a significant decrease in aortic vascular resistance did occur at 6-months follow up (-0.80 ± 1.25 mmHg/min/ml, p=0.049), however not acutely after denervation (-0.82 ± 1.67 mmHg/min/ml, p=0.14).

Table 6-1 illustrates the overall changes in renal wave speed throughout the trial. Pre-denervation, when intravenous sedation was given a significant decrease in renal wave speed was shown (-1.64 ± 2.33 m/s, p=0.013) which further decreased after denervation (-1.53 ± 2.81 m/s, p=0.046). At 6-months follow up, renal wave speed was similar under sedation (9.3 ± 4.8 m/s) to immediately following the renal
denervation procedure (8.7 ± 3.3 m/s), Figure 6-5. This was despite additional sedation being given at the time of renal denervation procedure.
Table 6-1. Overall changes in renal and aortic blood flow, blood pressure, vascular resistance and wave speed throughout the trial.

Note: Results shown for all 16 patients within the trial

<table>
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<th></th>
<th>Baseline visit</th>
<th>6-month follow up</th>
</tr>
</thead>
<tbody>
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<td>Resting state</td>
<td>Post sedation</td>
</tr>
<tr>
<td>Renal Arteries</td>
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<tr>
<td>Systolic blood pressure (mmHg)</td>
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<td>136 ± 30</td>
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<td>Renal artery blood flow (ml/min)</td>
<td>13.3 ± 3.9</td>
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<td>Vascular resistance (mmHg/min/ml)</td>
<td>8.9 ± 4.1</td>
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<td>Wave speed (m/s)</td>
<td>11.8 ± 4.5</td>
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<tr>
<td>Heart rate (bpm)</td>
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<td>67 ± 13</td>
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<tr>
<td>Aorta</td>
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<td></td>
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<tr>
<td>Systolic blood pressure (mmHg)</td>
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<td>Blood flow velocity (cm/s)</td>
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<tr>
<td>Vascular resistance (mmHg/min/ml)</td>
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<td>Wave speed (m/s)</td>
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<td>-</td>
</tr>
<tr>
<td>Heart rate (bpm)</td>
<td>64 ± 12</td>
<td>-</td>
</tr>
</tbody>
</table>
Figure 6-5. Renal wave speed in individual patients at baseline visit immediately after denervation and at 6-months follow-up after sedation

At 6-months follow up, renal wave speed was similar under sedation (9.3 ± 4.8 m/s) to immediately following the renal denervation procedure (8.7 ± 3.3 m/s), p=0.55
6.3.3 Acute change in renal haemodynamics after denervation correlates with long-term blood pressure reduction

Although overall there was no significant change in renal blood flow velocity immediately post renal denervation, at 6-months follow-up there was an overall significant increase in renal blood flow both at rest and under identical sedation states. Furthermore, immediately after denervation, individual patients showed a variable response in acute change in renal haemodynamics after renal denervation, and these changes correlated with individual 6 month ambulatory blood pressure response. Patients who showed the largest increase in renal blood flow velocity acutely after renal denervation had the largest reduction in ambulatory systolic blood pressure at 6-months \( R^2=0.60, \, p<0.001 \), Figure 6-6. Similarly, patients who showed the largest decrease in renal vascular resistance acutely after renal denervation had the largest reduction in ambulatory systolic blood pressure at 6-months \( R^2=0.56, \, p<0.001 \). These findings suggest that acute changes in renal hemodynamics may be predictive of blood pressure response at 6-months follow-up.
Figure 6-6. Correlation between change in renal blood flow and renal vascular resistance acutely after denervation with 6-month change in ambulatory SBP

Patients who showed the largest reduction in ambulatory systolic blood pressure at 6-months had the largest increase in renal blood flow acutely after renal denervation ($R^2=0.60$, $p<0.001$) and the largest decrease in renal resistance acutely after renal denervation ($R^2=0.56$, $p<0.001$).
6.3.4 Predictors of response to renal denervation: correlation of baseline haemodynamic markers with ambulatory blood pressure reduction at 6-months

Univariate predictors of reduction in 6-month ambulatory systolic blood pressure were assessed (Table 6-2). Renal wave speed and renal vascular resistance were found to predict reduction in ambulatory blood pressure at 6-months. Patients with higher baseline resting renal vascular resistance showed a greater reduction in ambulatory systolic blood pressure ($R^2 = 0.50, p=0.003$). Similarly, and even more markedly, higher renal wave speed was associated with a greater reduction in ambulatory systolic blood pressure ($R^2 = 0.59, p<0.001$), Figure 6-7. This pattern was not seen with baseline aortic resistance ($R^2 = 0.06, p=0.36$) or baseline aortic wave speed ($R^2 = 0.02, p=0.66$).

A multivariable regression model was obtained by using a multinomial logistic regression analysis with backward elimination of nonsignificant ($p<0.05$) variables, as described in Chapter 3. For this overall model, $R^2$ was 0.656, $F(2,12)=11.42$, ($p=0.0017$). Multivariable analysis (Table 6-3) showed that only renal artery wave speed ($p = 0.04$) remained an independent predictor of reduction in ambulatory systolic blood pressure at 6-months.
Table 6-2. Univariate predictors of reduction in 6-month ambulatory systolic blood pressure.

<table>
<thead>
<tr>
<th>Regression of baseline parameter with 6-month change in ambulatory SBP</th>
<th>Beta coefficient</th>
<th>95% confidence interval</th>
<th>p value</th>
<th>$R^2$</th>
<th>Intercept</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline aortic resistance</td>
<td>-0.0001</td>
<td>-0.00038 to 0.00018</td>
<td>0.36</td>
<td>0.06</td>
<td>-0.14</td>
</tr>
<tr>
<td>Baseline renal resistance</td>
<td>-0.0005</td>
<td>-0.0008 to -0.0002</td>
<td>0.003</td>
<td>0.50</td>
<td>7.36</td>
</tr>
<tr>
<td>Baseline aortic BP</td>
<td>-0.0720</td>
<td>-0.211 to 0.067</td>
<td>0.33</td>
<td>0.07</td>
<td>6.47</td>
</tr>
<tr>
<td>Baseline renal BP</td>
<td>-0.0879</td>
<td>-0.235 to 0.059</td>
<td>0.26</td>
<td>0.10</td>
<td>8.09</td>
</tr>
<tr>
<td>Baseline aortic wave speed</td>
<td>-0.1442</td>
<td>-0.771 to 0.483</td>
<td>0.66</td>
<td>0.02</td>
<td>-2.97</td>
</tr>
<tr>
<td>Baseline renal wave speed</td>
<td>-1.3070</td>
<td>-1.902 to -0.712</td>
<td>&lt;0.001</td>
<td>0.59</td>
<td>9.98</td>
</tr>
</tbody>
</table>
Table 6-3. Multivariate predictors of reduction in 6-month ambulatory systolic blood pressure

Only univariate predictors of reduction in ambulatory systolic blood pressure with p value <0.05 were included in the multivariate analysis i.e. baseline renal wave speed and renal resistance.

<table>
<thead>
<tr>
<th>Regression of baseline invasive haemodynamic parameter with 6-month change in ambulatory SBP</th>
<th>Beta coefficient</th>
<th>95% confidence interval</th>
<th>t value</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline renal wave speed</td>
<td>-1.87</td>
<td>-0.11 to -3.63</td>
<td>-2.10</td>
<td>0.04</td>
</tr>
<tr>
<td>Baseline renal resistance</td>
<td>0.00026</td>
<td>0.00102 to -0.0005</td>
<td>0.68</td>
<td>0.51</td>
</tr>
</tbody>
</table>
Figure 6-7. Correlation between baseline (pre-denervation) wave speed in the renal arteries and 6-month change in ambulatory systolic blood pressure

There was a strong positive correlation between baseline wave speed in the renal arteries and change in ambulatory systolic blood pressure at 6-month follow up ($R^2=0.59 \ p<0.001$)


6.4 Discussion

The main findings of my exploratory proof-of-concept study are that when pharmacological therapies are administered under direct observation, and compliance ensured with urine adherence testing:

1. Baseline renal artery wave speed is suggested to predict the success of renal denervation at 6 months
2. Renal artery wave speed is similar at 6 months to immediately following the renal denervation procedure
3. Renal artery wave speed is independent of aortic invasive wave speed
4. Unlike aortic wave speed, renal artery wave speed is not correlated with baseline patient age

This study was designed to explore changes in haemodynamics acutely and at 6-months after denervation. It was not blinded and this will interfere with my results. Also, as it was designed as an exploratory proof-of-concept study it was not powered sufficiently to prove associations. Therefore, all results discussed should be viewed as exploratory at this point.

6.4.1 Increase in renal blood flow and reduction in aortic vascular resistance at 6-months follow up after denervation

There are two physiological mechanisms that underpin the acute renal haemodynamic changes seen after renal denervation: autoregulation and the renal sympathetic nervous system. In the situation of low basal renal sympathetic activity, a decrease in blood pressure triggers mainly compensatory autoregulatory mechanisms, causing a compensatory decrease in renal vascular resistance via the myogenic mechanism to maintain a stable renal blood flow velocity. Conversely, in patients with high renal sympathetic nerve activity, reduction of
sympathetic tone (70) through renal denervation or a drop in blood pressure will result in an increased renal blood flow velocity as well as a greater reduction in renal vascular resistance (71).

In my study, pre-denervation, following sedation, there was a negligible decrease in renal blood flow velocity with a small decrease in renal vascular resistance and blood pressure. However, 6-months after renal denervation, overall there was an increase in renal blood flow velocity both at rest and under identical sedation regimes. This implies after renal denervation there was a change in renal haemodynamics which is mostly likely due to reduction in efferent renal sympathetic neural vasoconstrictor tone affecting both the renal artery (as evidenced by a fall in wave speed), and also the intrarenal microvasculature (as evidenced by a fall in renal resistance).

These findings are in keeping with those of Tsioufis et al (71) who showed in a swine study a significant increase in renal blood flow one month post denervation compared to pre-denervation, and those of DiBona et al who showed a similar increase in renal blood flow post denervation in congestive heart failure and spontaneously hypertensive rats (70). However, in previous research denervating Sprague Dawley rats (which have normal sympathetic activity), there was no change in either renal blood flow or renal vascular resistance after denervation(223). This implies not a discrepancy in results, but that renal sympathetic nerves have a more important role on renal haemodynamics in the presence of augmented renal sympathetic nerve activity.

Drug trials of angiotensin-converting-enzyme inhibitors have shown that benefit from these medications is independent of their blood pressure lowering effect (225). It is possible, therefore, that renal denervation may similarly have further benefits aside from an anti-hypertensive effect by improving renal perfusion through and increasing renal blood flow velocity. This may be particularly important in conditions of elevated sympathetic tone such
as heart failure, where vasodilators reduce pressure at the expense of renal perfusion. If sympathetic modulation of the renal vasculature meant that renal blood flow could be preserved, it may be possible to avoid the characteristic fall in glomerular filtration rate which accompanies commencement of heart failure therapies.

At 6 months follow up there was also an overall decrease in aortic vascular resistance in the patient group. The reduction in ambulatory blood pressure at 6 months may be due to a corresponding decrease in peripheral sympathetic nerve activity (104) contributing to a decrease in peripheral vascular resistance. Alternatively, the decrease in aortic vascular resistance at 6 months may contribute to a decrease in overall peripheral resistance (224). Changes in invasive haemodynamics at 6-months might be for a number of other reasons not related to renal denervation treatment. For example, the impact of treatment compliance on haemodynamic measurements should also be considered. As mentioned in Chapter 5, invasive measurements were taken after only 2 episodes of directly observed anti-hypertensive therapy. In patients taking medication with a longer half-life, it might take longer to reach steady-state plasma concentrations. Therefore, if medication adherence was different prior to the baseline and 6-month visits, then changes in treatment adherence might still be responsible for the change in renal haemodynamics described. Secondly, the impact of other unrelated factors needs to be considered. For example, patients were starved for at least 2-hours prior to entering the cardiac catheterisation laboratory. In general, we always would aim to maintain hydration for patients with an intravenous drip. However, between different visits it is likely that patients’ hydration status was different, and this could have a large impact on their invasive haemodynamic measurements.
6.4.2 Change in renal haemodynamics correlates with 6-month reduction in ambulatory systolic blood pressure

There was considerable intra-individual change in renal vascular resistance and blood flow velocity acutely after renal denervation and these correlated with reduction in ambulatory SBP at 6 months. There may be two reasons for this. Either these patients had a higher baseline renal sympathetic vasoconstrictor tone and therefore showed the greatest change in haemodynamics acutely, as well as the greatest reduction in ambulatory SBP. Alternatively, these patients may have had more complete denervation resulting in these changes. Either way it is clear from the data, that both sedation and renal denervation alter renal haemodynamic parameters in a repeatable stepwise manner. The fall in renal wave speed with sedation prior to renal denervation was -1.64 m/s, which was almost the same (-1.23 m/s) at follow-up 6 months later. This is despite the renal wave speed starting from a lower value. In fact at six months the renal wave speed following sedation was almost identical to that found immediately after acute renal denervation. This is despite additional sedation being given for the renal denervation procedure, suggesting a modification of renal vascular tone.

6.4.3 Renal artery wave speed may be a proxy of baseline sympathetic nerve activity

In this study I measured invasive renal artery wave speed using a ComboWire and assessed its characteristics. My findings suggest that, despite variation in the length, diameter, number of branches and tortuosity between the renal arteries, there was a close relationship between renal artery wave speed measured in the left and right renal arteries in patients without angiographic evidence of obstructive renal artery disease.
My results also found a good relationship between aortic wave speed and age, which is similar to findings of several other studies (163, 226). However, I did not find a relationship between wave speed in the renal arteries and patient age.

In addition, this study showed that although invasive aortic wave speed correlated well with baseline non-invasive aortic pulse wave velocity, renal wave speed did not correlate well with non-invasive aortic pulse. In combination with the results in Chapter 4, where there was shown to be good test-retest repeatability implying good measurement precision of invasive aortic and renal wave speed and non-invasive aortic pulse wave velocity, these results suggest that the difference between renal and aortic wave speed is unlikely to be due to measurement error, but may illustrate a difference in physiology between the renal and aortic vascular beds. It is generally thought wave speeds of different vascular beds (e.g. the coronary arteries and the aorta) are closely related (158). My study suggests that wave speed in the renal arteries and in the aorta may only be modestly related.

These findings suggest that both autonomic and structural factors may influence different vascular beds in different proportions. Specifically, renal artery wave speed may be more dependent on sympathetic tone regulation, where high renal artery wave speed may indicate high baseline renal sympathetic activity. This is in contrast to the aorta where wave speed may be more influenced by structural parameters and markers of degenerative calcification, and loss of elastic-collagen matrix.

Renal wave speed has not been investigated in humans prior to this study, and even data in humans on invasive renal vascular resistance has been limited due to the difficulties in acquiring simultaneous pressure and flow velocity recordings prior to the ComboWire.

Although there is currently no outcome data to illustrate how changes in renal wave speed might show beneficial effects beside blood pressure reduction, there is outcome data for renal
resistive index (RRI). The dimensionless RRI is a method to assess the compliance of vessels and is calculated using the following formula:

\[
\text{Renal resistive index} = \frac{\text{Peak systolic velocity (Vmax)} - \text{End diastolic velocity (Vmin)}}{\text{Peak systolic velocity (Vmax)}}
\]

This approach has previously been validated against non-invasive Doppler estimation of the renal resistive index (159).

Research in hypertensive patients has shown that a high RRI is associated with an increased risk of cardiovascular and renal end points in hypertensive patients (227) (228) (229). Therefore, it is possible that a reduction in renal artery wave speed through denervation may have similar protective properties as a reduction in RRI as both appear to be markers of vascular compliance. A reduction in RRI has been shown to reduce the incidence of albuminuria (230).

### 6.4.4 Baseline renal artery wave speed predicts response to ambulatory blood pressure

I explored baseline haemodynamic variables as predictors of response to renal denervation. This study was not powered appropriately to identify predictors of response and therefore the results need to be interpreted cautiously. Despite renal artery wave speed showing a positive correlation with renal blood pressure and vascular resistance, renal wave speed remains significant in the multivariate model even after adjustment for baseline resting blood pressure, suggesting it is not just a consequence of the “regression to the mean” statistical phenomenon or a consequence of a decrease in pressure-volume loading.

It has been shown that patients with renal hypertension have a higher degree of sympathetic nerve activation and higher renal vascular resistance than patients with hypertension and no renal disease (85). Therefore, high renal artery wave speed in our patient cohort may indicate a higher baseline renal sympathetic nerve activity, explaining the greater reduction in
ambulatory blood pressure 6 months after renal denervation. At 6-months follow-up a non-significant drop in renal artery wave speed is shown. This may be potentially related to incomplete denervation, or may be due to differences in overall physiology post denervation. My findings suggest that it might be possible to use baseline renal wave speed to predict responders to renal denervation therapy, and that that baseline renal wave speed magnitude predicts the magnitude of blood pressure reduction. For example, if only patients with high baseline renal artery wave speed had been denervated (>10m/s), then 10 patients would have been included and at 6-months there would have been a reduction in ambulatory SBP of 7.6mmHg. Therefore, using a renal wave-speed cut-off point may be possible to reduce the likelihood of non-responders and increase the magnitude of blood pressure reduction. Of course, the exact cut-off would have to be extensively assessed in a much larger patient sample.

To further validate this association would require this study to be repeated in a blinded fashion with appropriate powering of the study population so that any associations found were not just due to the possibility of chance. If this association was then to be proven, it might be possible to use this technique prior to renal denervation to identify likely responders. As renal wave speed is similar in the left and right renal artery our findings suggest that measurement would only be required in a single kidney. This simple approach might increase the mean effect size of blood pressure reduction, and decrease the variability of response.
6.5 Study limitations

This study was designed to assess invasive renal haemodynamic changes acutely after denervation and at 6 months. I did not design the study to include a sham control arm, as it would have been ethically challenging to ask patients to come for two invasive angiography procedures in the cardiac catheterization laboratory without receiving any treatment or intervention in the sham arm. This, however, has the disadvantage that I do not have a control arm to compare our findings. I tried to minimise the confounding from a lack of sham by using directly observed therapy with urine compliance testing repeatedly for every patient on every visit.

Leading on from the point above, in this Chapter I have used correlation to compare changes in 6-month ambulatory systolic blood pressure with baseline invasive haemodynamics. I chose to base this correlation on change in ambulatory and not office SBP as the automatic measurement of ambulatory SBP minimises bias as shown in Chapter 1. However, as all blood pressure measurements in this trial are unblinded, the correlations need to be interpreted with caution.

Furthermore, this trial was not powered appropriately to identify predictors of response to renal denervation, as it was designed to be an exploratory human study illustrating invasive haemodynamic changes after renal denervation. Therefore, it is possible that these correlations are due to statistical under powering, and my results should be considered exploratory only and would need to be validated in a blinded trial with appropriate study participant powering.

A limitation of this study is that measurements of wave speed were carried out sequentially and not simultaneously in the aorta and renal arteries. This protocol was designed for patient safety, to prevent multiple arterial punctures as well as excess instrumentation within the aorta. The protocol was designed, however, to ensure that all measurements were made
sequentially with minimal time intervals between repeat measurements to try to minimise this limitation.

In this study renal resistance was calculated as a ratio of renal artery blood pressure and blood flow velocity. This is a simplification, as it doesn’t include venous pressure in the calculation. This simplification was to prevent an extra venous puncture in the study protocol.

Finally, in this study we did not use other modalities to assess baseline sympathetic activity e.g. muscle sympathetic nerve activity (microneurography) (93) or renal vein norepinephrine spillover. Therefore, it is not possible to validate the theory that elevated renal artery wave speed may suggest high resting sympathetic nerve activity. This may be a worthwhile experiment for future researchers.
6.6 Conclusions

Invasive renal wave speed is independent of invasive aortic wave speed, is reduced acutely after renal denervation and remains reduced at 6-months follow up. Invasive renal artery wave speed is closely correlated with renal vascular resistance and may be a marker of sympathetic nerve activity. When pharmacological therapies are directly administered, my study suggests that renal artery wave speed may predict the success of renal denervation at 6 months.

In addition, acutely after catheter based renal denervation there was an increase in renal blood flow velocity with a corresponding significant decrease in aortic vascular resistance. Renal blood flow velocity was significantly increased at 6 months follow up. Renal blood flow and resistance parameters may be therefore useful as peri-procedural markers of successful renal denervation.
7 Long-term Safety of Renal Denervation
7.1 Introduction

Catheter based renal denervation is a novel technique which aims to denervate the renal sympathetic nerves through the lumen of the renal artery to reduce systemic blood pressure (BP). Although damage to the renal sympathetic nerves is desirable through this approach, the safety of applying radiofrequency energy to the vascular lumen as well as the surrounding anatomical structures needs to be thoroughly assessed. Owing to this risk, the procedure is contraindicated in patients with significant renal arterial calcification and stenosis (231).

7.1.1 Short-term safety data

7.1.1.1 Pre-clinical studies

Short-term safety data in pre-clinical studies immediately after denervation shows local tissue damage. Pre-clinical swine studies (232) performed optical coherence tomography (OCT) and histological assessment directly after denervation and at 10-day follow-up. They found that denervation of the vessel wall induced transmural tissue coagulation and loss of endothelium resulting in local thrombus formation which was detectable both on histological assessment as well as OCT immediately after denervation. At 10 days follow-up, however, the luminal surface was almost completely re-endothelialized. Histological assessment at 10 day follow-up showed replacement of mural wall damage with fibrotic tissue and vasculogenesis in the adventitial layer. There was no derangement of blood parameters of renal function outside the normal physiological range at any point.

7.1.1.2 Clinical studies

Similarly, Templin et al (233) assessed renal arteries with OCT immediately after denervation in a cohort of 32 patients with treatment resistant hypertension. They showed vasospasm (42%) and vessel wall oedema (96%) with some evidence of localised dissection
Similar to pre-clinical studies, there was evidence of activation of coagulation pathways and intraluminal thrombus formation (67%) with increased thrombus formation per renal artery significantly higher after the procedure. The significance of this localized tissue damage immediately after denervation, however, is unclear (235) as longer-term clinical safety data is scarce.

7.1.2 Long-term safety data

7.1.2.1 Pre-clinical studies

Long-term pre-clinical studies (236) in swine performed renal angiography before and 6 months after renal denervation and examined the renal vessels histologically. They found minimal vascular damage at 6-months with only minor fibrosis of 10-25% of the arterial media and adventitia but with no inflammation, no stenosis or thrombosis and no damage to the kidney or bladder histologically. This suggests potential recovery of the acute renal vascular injury seen post ablation. Histology analysis at multiple time points post renal sympathetic denervation has further substantiated this by showing that renal artery and peri-arterial soft tissue injury is greatest in the subacute phase, and least in the chronic phase (237).

7.1.2.2 Clinical studies

In humans, long-term safety studies have focused on using non-invasive imaging to assess long-term vascular integrity (238) (112) (230). Although there have been a few cases of renal artery stenosis reported (239) (240) (241) (242), overall the intervention appears to be safe when ablation is performed in the main renal arterial trunk. Reported rates of vascular complications range from 0.3% (119) to 2.6% (111) in initial reported study outcomes. All of these reported outcomes are for clinical studies, which have performed ablation in the
main renal arterial trunk. Current European Society of Cardiology guidance recommends annual renal artery imaging (ultrasound, MRI/CT) during follow-up beginning 6 months after denervation (231).

7.1.3 Distal renal artery denervation
Recent data suggests that the efficacy of renal denervation may be improved by performing catheter based renal denervation in distal renal arterial branches. Distal renal artery ablation aims to improve nerve denervation by targeting distal targets, where the mean distance from renal artery lumen to nerve is least with a predominance of efferent nerve fibres (131). Sakakura et al examined 10,329 nerves from 20 (12 hypertensive and 8 non-hypertensive) human autopsy subjects, and assessed the peri-arterial renal nerve anatomy. They found that although the mean number of nerves in the proximal and middle segments was higher than in the distal segment, the mean nerve distance to arterial lumen was greatest in proximal segments, followed by middle segments, and least in distal segments. Furthermore, nerve anatomy was not different in hypertensive patients to non-hypertensive patients.

A recent pre-clinical study in swine (128) has assessed this by examining the impact of ablation location on norepinephrine tissue content and axon density in renal tissue histology. They compared outcome when ablation was performed in the main renal artery versus the distal arterial branches, and the main arteries and branches combined. The results of this study showed that targeted ablation of the distal renal arterial branches or the distal segment of the main renal artery significantly reduced renal norepinephrine content and axon density compared to conventional treatment of only the main renal artery. Combination ablation of both the main artery and distal branches produced the greatest reduction in renal norepinephrine and axon density. In addition, this study found that distal ablation could be performed with minimal acute procedural complications or long-term sequela in swine. Although encouraging, it is necessary to see whether these surrogate markers of tissue
norepinephrine and axon density will translate to improved and sustained blood pressure reduction in humans. Furthermore, ablation in young swine is likely to have a different safety profile to distal ablation in often atherosclerotic renal arteries of human patients with resistant hypertension. Therefore, the short and long-term safety of distal denervation will need to be assessed in a hypertensive human population.
7.2 Aims

The aim of this prospective study was to evaluate the safety of the multi-electrode radiofrequency Symplicity Spyral ablation catheter performing distal denervation by performing invasive renal arterial angiography immediately pre procedure, immediately post procedure (to assess short term vascular integrity) and at 6-months follow up.
7.3 Results

7.3.1 Change in renal function 6-months after renal denervation

Pre-denervation, baseline creatinine for the 16 patients who underwent denervation was 95.9 ± 25.4μmol/l with estimated glomerular filtration rate 66.8 ±14.6ml/min/1.73m². At 6-months follow up, renal function was unchanged with baseline creatinine 89.4+/- 22.2umol/l, p=0.124 and estimated glomerular filtration rate 71.0 ±13.7 ml/min/1.73m², p=0.134, Figure 7-1.
Figure 7-1. Renal function remained unchanged at 6 months

At 6-months follow up, renal function was unchanged with baseline creatinine 95.9+/-25.4 vs 89.4+/-22.2umol/l, p=0.124. At 6-months follow-up the estimated glomerular filtration rate was also unchanged 66.8+/-14.6 vs 71.0 ±13.7 ml/min/1.73m², p=0.134
7.3.2 Quantitative vessel angiography analysis

Angiographic analysis of the right and left renal artery angiograms was performed at 3 time points for each patient. An example of a patient’s repeat angiograms in the right and left renal arteries are shown in Figure 7-2. Three independent assessors analysed each anonymised angiogram, blinded to the time point. The average results of all three assessors are shown in Figure 7-3, Table 7-1 for the main renal artery trunk and for branches >3mm.

In the main trunk acutely post-denervation there was no significant change from baseline in the length (38.09±12.19mm pre-denervation, 37.6±11.61mm acute post denervation, p=0.93), proximal artery diameter, (5.72±0.94mm pre-denervation, 5.64±0.99mm acute post denervation, p=0.72), distal artery diameter (5.64±0.62mm pre-denervation, 5.62±0.98mm acute post denervation, p=0.98), minimal lumen diameter (5.26 ± 0.88 mm pre-denervation, 5.10 ± 0.79 mm acute post denervation, p=0.11) or area (21.82 ± 7.34 mm² pre-denervation, 20.61 ± 6.23 mm² acute post denervation, p=0.20).

In the main trunk at 6-months follow up, there was no significant change from baseline in the length (38.09±12.19mm pre-denervation, 36.99±12.89mm 6-months, p=0.70), proximal artery diameter (5.72±0.94mm pre-denervation, 5.56±0.96mm 6-months, p=0.21), distal artery diameter (5.64±0.62mm pre-denervation, 5.48±1.05mm 6-months, p=0.20), minimal lumen diameter (5.26 ± 0.88mm pre-denervation, 5.09±0.91mm 6-months, p=0.08) or area (21.82 ±7.34mm² pre-denervation, 20.67±7.11mm² 6-months, p= 0.23).

Pre-denervation, on average there were 2.89 ± 0.98 branches off the main trunk, with 2.39 ± 0.55 branches >3mm in diameter. There was no significant evidence of calcification in either the main trunk or branches for any patient recorded by each assessor.

In the branches, acutely after denervation there was no significant change from baseline in proximal artery diameter (4.09±0.84mm pre-denervation, 4.05±0.94mm acute post denervation, p=0.99), distal artery diameter (4.00±0.86mm pre-denervation, 3.98±0.88mm
acute post denervation, p=0.96), minimal lumen diameter (3.82±0.82mm pre-denervation, 3.77±0.90mm acute post denervation, p=0.99) or area (11.99±5.47mm² pre-denervation, 11.82±5.91mm² acute post denervation, p=0.88).

In the branches, 6-months after denervation there was no significant change from baseline in proximal artery diameter (4.09±0.84mm pre-denervation, 4.03±0.95mm 6-months, p=0.76), distal artery diameter (4.00±0.86mm pre-denervation, 3.96±0.96mm 6-months, p=0.91), minimal lumen diameter (3.82±0.82mm pre-denervation, 3.74±0.95mm 6-months, p=0.91) or area (11.99±5.47mm² pre-denervation, 11.52±5.81mm² 6-months, p=0.99). The minimal lumen diameter for each patient in the trial pre-denervation, acutely post denervation and at 6-months follow up in the main renal artery and the branches is shown in Figure 7-4.

Two patients had an accessory artery described by all three observers (mean proximal diameter 1.86mm). As both accessory arteries were <3mm proximal diameter, neither artery was treated with renal denervation.

Results of the qualitative assessment of vascular integrity by each assessor showed no evidence of dissection, notches or spasm in any angiograms after denervation.
Figure 7-2. Renal angiograms pre and post denervation, and at 6 months follow up for an example patient
Figure 7-3. Quantitative vessel angiographic results for the main renal artery trunk and branches>3mm, averaged across all 3 assessors and averaged across left and right renal arteries

Mean values are shown with standard deviation of measurements as error bars.
Table 7-1. Quantitative angiographic results for the main renal artery trunk and branches>3mm, averaged across all 3 assessors.

Values are shown as mean ± SD (2 decimal places)

<table>
<thead>
<tr>
<th></th>
<th>Pre denervation</th>
<th>Acute post denervation</th>
<th>6 months follow up</th>
<th>Δ pre denervation to acute post denervation</th>
<th>p value</th>
<th>Δ pre denervation to 6 months follow-up</th>
<th>p value</th>
<th>Δ acute post denervation to 6 months follow-up</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Main vessel (mm)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Length</td>
<td>38.09 ± 12.19</td>
<td>37.60 ± 11.61</td>
<td>36.99 ± 12.89</td>
<td>-0.5</td>
<td>0.93</td>
<td>-1.10</td>
<td>0.70</td>
<td>-0.61</td>
<td>0.90</td>
</tr>
<tr>
<td>Proximal diameter</td>
<td>5.72 ± 0.94</td>
<td>5.64 ± 0.99</td>
<td>5.56 ± 0.96</td>
<td>-0.07</td>
<td>0.72</td>
<td>-0.16</td>
<td>0.21</td>
<td>-0.09</td>
<td>0.62</td>
</tr>
<tr>
<td>Distal diameter</td>
<td>5.64 ± 0.62</td>
<td>5.62 ± 0.98</td>
<td>5.48 ± 1.05</td>
<td>-0.02</td>
<td>0.98</td>
<td>-0.16</td>
<td>0.20</td>
<td>-0.14</td>
<td>0.28</td>
</tr>
<tr>
<td>Minimal lumen diameter</td>
<td>5.26 ± 0.88</td>
<td>5.10 ± 0.79</td>
<td>5.09 ± 0.91</td>
<td>-0.16</td>
<td>0.11</td>
<td>-0.17</td>
<td>0.08</td>
<td>-0.01</td>
<td>0.99</td>
</tr>
<tr>
<td>Minimal lumen area</td>
<td>21.82 ± 7.34</td>
<td>20.61 ± 6.23</td>
<td>20.67 ± 7.11</td>
<td>-1.21</td>
<td>0.20</td>
<td>-1.16</td>
<td>0.23</td>
<td>0.05</td>
<td>0.99</td>
</tr>
<tr>
<td>Number of branches</td>
<td>2.89 ± 0.98</td>
<td>2.88 ± 0.95</td>
<td>2.89 ± 0.97</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of branches &gt;3mm diameter</td>
<td>2.39 ± 0.55</td>
<td>2.39 ± 0.55</td>
<td>2.37 ± 0.53</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Branches&gt;3mm diameter (mm)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proximal diameter</td>
<td>4.09 ± 0.84</td>
<td>4.05 ± 0.94</td>
<td>4.03 ± 0.95</td>
<td>-0.02</td>
<td>0.99</td>
<td>-0.08</td>
<td>0.76</td>
<td>-0.07</td>
<td>0.85</td>
</tr>
<tr>
<td>Distal diameter</td>
<td>4.00 ± 0.86</td>
<td>3.98 ± 0.88</td>
<td>3.96 ± 0.96</td>
<td>0.03</td>
<td>0.96</td>
<td>-0.05</td>
<td>0.91</td>
<td>-0.08</td>
<td>0.77</td>
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<tr>
<td>Minimal lumen diameter</td>
<td>3.82 ± 0.82</td>
<td>3.77 ± 0.90</td>
<td>3.74 ± 0.95</td>
<td>-0.01</td>
<td>0.99</td>
<td>-0.04</td>
<td>0.91</td>
<td>-0.03</td>
<td>0.95</td>
</tr>
<tr>
<td>Minimal lumen area</td>
<td>11.99 ± 5.47</td>
<td>11.82 ± 5.91</td>
<td>11.52 ± 5.81</td>
<td>0.35</td>
<td>0.88</td>
<td>-0.11</td>
<td>0.99</td>
<td>-0.46</td>
<td>0.80</td>
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</tbody>
</table>
Figure 7-4. Minimal lumen diameter for each patient in the trial pre-denervation, acutely post denervation and at 6-months follow up in the main renal artery (left hand graph) and the branches (right hand graph)

The average of all measurements for all patients at each time point is shown in red
7.3.3 Inter-observer variability of quantitative analysis of renal angiograms
The inter-observer variability for each of the quantitative angiographic measures was measured using the intra-class correlation coefficient. Results are shown in Table 7-2, Figure 7-5. When sub analysis was performed, interobserver agreement differed according to lesion measured, range (0.31 to 0.70). On average, ICC measurements were lower in the branches (0.37) than in the main trunk of the renal artery (0.63), p<0.001.
**Figure 7-5. Figure illustrating the intraclass correlation coefficient for each quantitative vessel measurement performed.**

On average, ICC measurements were lower in the branches (0.37) than in the main trunk of the renal artery (0.63), p<0.001.
<table>
<thead>
<tr>
<th>Table 7-2. Interobserver variability measured using intra-class coefficients for each quantitative variable assessed.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ICC</strong></td>
</tr>
<tr>
<td><strong>Main vessel</strong></td>
</tr>
<tr>
<td>Length</td>
</tr>
<tr>
<td>Proximal diameter</td>
</tr>
<tr>
<td>Distal diameter</td>
</tr>
<tr>
<td>MLD</td>
</tr>
<tr>
<td>MLA</td>
</tr>
<tr>
<td>Number of branches</td>
</tr>
<tr>
<td>Number branches &gt;3mm</td>
</tr>
<tr>
<td><strong>Branches&gt;3mm</strong></td>
</tr>
<tr>
<td>Proximal diameter</td>
</tr>
<tr>
<td>Distal diameter</td>
</tr>
<tr>
<td>MLD</td>
</tr>
<tr>
<td>MLA</td>
</tr>
</tbody>
</table>
7.4 Discussion

This study prospectively evaluated the vascular integrity of the renal arteries after distal renal artery denervation acutely and at 6-months follow up using invasive renal angiography. The main findings of this study are that in patients undergoing distal vessel renal artery denervation (1) I found no significant difference in post procedural vessel dimension, (2) and no evidence of denovo stenosis, dissection or worsening of preexisting atheroma either acutely after denervation or at 6 months follow-up, and (3) there was no deterioration in renal function at 6 months.

7.4.1 No significant change in renal artery dimensions acutely after denervation and at 6-months

In my study I found no significant change in renal artery vessel dimensions either acutely after denervation or at 6-months follow up, measured by three blinded independent assessors. This was seen for all vessel dimensions in both the main renal artery trunk, as well as in the distal artery branches. There was no evidence of change in minimal lumen diameter either, and no evidence of denovo renal artery stenosis or extension of any atheromatous segment. These findings were consistent in the main trunk and in daughter branches, and correlated with the report given by the operator for each procedure. These results indicate no evidence of long-term renal artery disease when denervation is performed in the distal branches, and correlate with the results of Mahfoud discussed earlier in healthy swine (128). I believe that my study population represents a typical resistant hypertension cohort and therefore these safety results should be representative when denervation is performed in experienced hands.
7.4.2 Assessment of reno-vascular injury
All three observers reported no evidence of dissection either acutely after denervation or at 6-months follow up evident on angiography. It is possible, however, that some vascular damage occurred, especially acutely after denervation, that was not evident through angiography alone (235) (233), but may have been visible with the use of Optical Coherence Tomography (OCT). Previous studies have used OCT to assess for renal vascular injury that might not be evident with angiography (233).

OCT can be challenging, especially in the main renal artery where the vessel diameter can often prevent adequate visualization of the vessel wall. However, as I found no concerns with the long-term 6-month angiography analysis or renal function, the clinical importance of any vascular injury that would have been visualized with OCT is likely to be small. For example, Symplicity HTN-1 (110) assessed the long-term safety of denervation in the main renal artery trunk. Renal angiographic studies were performed immediately after the procedure and then short-term (14–30 days) follow-up angiograms were performed in 18 patients and magnetic resonance angiograms in 14 treated patients at 6-months follow-up. In none of these patients in whom invasive angiography or non-invasive imaging showed reassuring short-term safety results were there subsequently any longer-term safety consideration at 36-month follow-up (111). Similarly, it is unlikely that any subsequent safety considerations should be seen in my cohort after 6-months follow-up. Therefore any clinical benefit from performing OCT directly after denervation is likely to be minimal.

7.4.3 Other safety concerns
Previous clinical trials and trial Registry data of catheter based renal denervation have found that the main safety risk acutely reported at the time of the procedure was
vascular damage to the femoral artery access site (112). In my study we had no cases of vascular damage, however this is a risk that all patients were consented for. The Symplicity Spyral is a 6Fr catheter that can fit through a 7Fr access site. However, some other catheters that are currently being trialed, for example, St Jude’s EnligHTN 8Fr catheter, are wider and will require wider femoral access, increasing the risk of vascular complications.

7.4.4 Inter observer variability
In my study three blinded cardiologists each performed measurements on renal angiograms from 16 different patients, in the left and right renal angiograms, at three separate time points, totaling 96 separate angiogram images. For each angiogram at least 9 separate measurements were performed on each angiogram (total number dependent on number of branches >3mm), producing a minimum total of 864 measurements per assessor.

There is always inherent measurement variability when quantitative vessel angiography is performed. In general, the intra class correlation coefficients comparing measurements between assessors were quite low in my data set, especially in the distal branches. This is concerning as accurate measurement of renal artery diameter is important for distal renal artery denervation to ensure that denervation is only performed in branches >3mm diameter.

Operators were provided all angiograms as angiogram movies and advised to pause the movies on images showing the best opacification of the renal arteries. They were not provided with preselected images. If I had provided preselected images than this is likely to have improved the interobserver variability, however this would not have been representative of “real-life” where operators need to pause renal angiogram
images under time pressure in the cardiac catheterization laboratory to assess renal artery dimensions and vascular integrity.

As mentioned, intra-class correlation coefficient values were higher in the main trunk than the distal branches, indicating that vessel size is an important factor in interobserver measurement variability. This may illustrate a challenge for clinicians who may want to perform distal renal denervation in the future as these results illustrate that using quantitative vessel angiography alone may not allow practitioners to accurately assess distal vessel dimension. In this situation, OCT may become a useful future tool as it might allow more accurate assessment of distal arterial diameter through three-dimensional intra-luminal assessment of vessel size. However, again the reliability of this approach would need to be tested before being used as a clinical tool.

Within the main trunk, the length of the main vessel showed lower interobserver agreement values than the other measurements within the main trunk. Each observer was asked to measure the length of the main vessel from the ostium of the renal artery to the point of bifurcation of the first branch. I hypothesise that this reduction in ICC, which implies increased measurement variability, may illustrate that operators find it more difficult to pick the start and end of a bifurcation than to measure a diameter.

**7.4.5 Risk versus reward balance**

Before agreeing to a procedure, the risk-reward balance should be assessed by every patient. In my thesis up to this point I have concentrated on the theoretical risk of damage to renal arteries from renal denervation, in particular due to distal rather than main branch denervation. However, there are other potential risks associated with this
procedure. Complications related to the denervation itself include that patients may experience pain during radiofrequency treatment or intra-procedural bradycardia. Other complications relate to the femoral access including femoral artery pseudoaneurysm, bruising, bleeding or infection at the catheter site insertion point. Overall, the large Symplicity HTN-1, HTN-2 and HTN-3 trials showed an acceptable safety profile. In Symplicity HTN-3 there were 1.4% major adverse events in the denervation group versus 0.6% in the sham procedure group (95% CI 0.9 to 2.5). The two most common adverse events within the 6 months post treatment in the denervation group were hypertensive crises in 2.6% of patients and myocardial infarction in 1.7% of patients. These risks need to be balanced against the potential benefits of denervation. At the current time this is not possible as the inconclusive evidence means it is impossible to assure reduction in blood pressure after the procedure. Until benefit from this treatment is known then denervation should not be a clinical option. However, if benefit in the future is documented then physicians could advise patients that sustained reduction in blood pressure from denervation would reduce their cardiovascular risk. For some patients this might be an acceptable risk-benefit balance, especially for those who are not adherent with medication. However, many other patients would not deem the potential risks associated with the procedure unacceptable, especially when there are options for further pharmacological treatments. In this study, the majority of patients were not on maximal doses of anti-hypertensives and 39% (7 out of 18 patients) were not prescribed an aldosterone antagonist, showing that there were still options to improve blood pressure control using pharmacological measures.
7.5 Study Limitations

In this study we used the Symplicity Spyral catheter. Previous research has shown variation in acute vascular injury depending on the catheter used, with higher rates of acute vascular injury immediately post denervation if a balloon catheter is used (235). Therefore, the reassuring safety data I have presented in this Thesis is specific to the catheter and technique as described. Furthermore, there are new approaches for renal denervation e.g. externally delivered focused denervation. All of these techniques will require individual assessment of long-term vascular safety.

A further limitation of this study is that a small number of patients were studied, and therefore these results should be confirmed in a larger population. In view of the large number of patients with resistant hypertension in whom renal denervation might be helpful, it is very important to continue close follow-up of patients treated with RDN, in particular in registries with regular and reliable imaging follow-up.

Finally, I have only studied distal renal denervation safety up to 6-months follow-up period. It is possible that further safety concerns may be evident with prolonged follow-up periods, although this wasn’t apparent in post-trial follow-up for the Symplicity trials (111) (220).
7.6 Conclusions

In this study I have prospectively assessed the short and long term vascular safety of performing renal denervation in the distal renal artery branches using the Symplicity Spyral system. I found no significant difference in post procedural vessel dimension either acutely after denervation or at 6 months follow-up with no deterioration in renal function at 6 months. These findings suggest that renal sympathetic denervation may be a safe tool for the treatment of resistant hypertension, if its efficacy in blood pressure reduction is proven.
8 Synthesis
Hypertension is a major global public health burden affecting more than a quarter of adults in developed countries. Patients with resistant hypertension (uncontrolled hypertension despite pharmacological therapy) represent a subset of hypertensive patients in whom resting renal sympathetic activity is elevated. Catheter-based renal denervation has therefore been suggested as a potential treatment option for patients with resistant hypertension.

8.1.1 Validation of methodology
The first stage in my methodology was to validate the invasive and non-invasive measurements used. In Chapter 4 I validated the measurements used in this Thesis, in particular the test-retest repeatability of these measurements. In particular, in this experiment I have used invasive assessments of renal arterial blood flow velocity and blood pressure at multiple time points to analyse the haemodynamic changes that occur after renal denervation. These renal measurements have not been previously studied invasively in humans. Therefore, it was essential to assess the validity of these measurements prior to interpreting the results of the experiment. Fortunately, the test re-retest repeatability of the invasive measurements were adequate. This was an important initial step as wide error bars would have prevented my ability to differentiate between true changes in haemodynamics after denervation and measurement noise.

8.1.2 Efficacy of distal renal denervation
In Chapter 5 I studied the change in non-invasive blood pressure measurements 6-months after distal renal denervation. However, as this trial was designed as a proof-of-concept study, it was not powered appropriately to assess efficacy of distal renal
denervation on blood pressure reduction. Instead, the significant reduction of 10mmHg in office systolic blood pressure, 5mmHg reduction in ambulatory systolic blood pressure and 3mmHg reduction in ambulatory diastolic blood pressure should be considered exploratory only. Furthermore, the blood pressure changes reported need to be interpreted in the context of the lack of blinding in this study. I would expect that the efficacy of blood pressure reduction may be less than this if a sham arm had been included in the trial. These results are similar to the blood pressure reduction found in a recent study (205) which also used a very rigorous protocol of uptitration of medical therapy in each arm.

My results showed a similar blood pressure reduction whether blood pressure was measured invasively or non-invasively. This suggests remarkable consistency in measurements, when measurements are performed after directly observed anti-hypertensive therapy to ensure that no changes in medication adherence confounded the results.

In Chapter 5 I also studied how anti-hypertensive medication adherence changed between baseline and 6-month visit. This was performed through measuring drug detection in urine assays. In my study, adherence was high on the urine samples taken at the start of both the baseline and 6-month assessment, and only 3 patients showed a change in medication adherence within the 6-months follow up. I hypothesize that the high medication adherence might be due to inevitable pre-selection of a dedicated patient group for the study, who were concerned with their health and therefore agreed to enter this very intensive study.

I found no correlation between medication adherence and change in 6-month ambulatory blood pressure. This might be due to all ambulatory blood pressure measurements being performed after patients had undergone directly-observed
therapy. This therefore aimed to ensure that patients had identical medication intake prior to blood pressure efficacy measurements.

8.1.3 Systematic evaluation of hemodynamic parameters to predict hemodynamic responders to renal denervation

In Chapter 6 I assessed the change in invasive renal haemodynamics immediately after denervation and at 6-months follow up. I also studied invasive renal wave speed, which I found was independent of invasive aortic wave speed and independent of patient age. These observations I hypothesize might illustrate a difference in physiology between the renal and aortic vascular beds.

I found that 6-months after renal denervation overall there was an increase in renal blood flow velocity and a decrease in aortic vascular resistance. These results are similar to the physiological changes witnessed in previous animal studies. In addition, there was an acute decrease in renal wave speed which was maintained at 6-months follow-up. I hypothesize that the haemodynamic changes after renal denervation are mostly likely due to reduction in efferent renal sympathetic neural vasoconstrictor tone effecting both the renal artery (as evidenced by a fall in wave speed), and also the intrarenal microvasculature (as evidenced by a fall in renal resistance). Correlating acute change in renal blood flow velocity and renal vascular resistance with 6-month change in ambulatory systolic blood pressure indicates that renal blood flow and resistance parameters may be useful as peri-procedural markers of successful renal denervation. Furthermore, my results suggest that when pharmacological therapies are directly administered, renal artery wave speed was the only invasive haemodynamic marker that predicted the success of renal denervation at 6 months. This trial, however, was not powered appropriately to ‘prove’ a correlation with blood pressure reduction and therefore these results should be considered exploratory only.
8.1.4 Safety of distal renal denervation

In Chapter 7 I went on to study the short and long-term vascular safety of distal artery renal denervation using invasive angiography. This was important as although the safety of main trunk denervation has been studied, distal denervation safety has not yet been assessed.

Three blinded investigators found no significant change in any measurement of artery diameter acutely after denervation or at 6-month follow up, either within the main vessel or distal branches. In addition, renal function was stable in all patients at 6-month follow up. These results are very reassuring for future researchers investigating renal denervation as they suggest that this procedure has a satisfactory long term vascular safety profile.

8.1.5 Reducing the variation in effect size with directly observed anti-hypertensive therapy

In Chapter 1 I performed a meta-analysis assessing the impact of trial design on the effect size and standard deviation of effect size within previous renal denervation trials. The results of this meta-analysis demonstrated that the between-individual variation in effect size is not minimised when randomisation alone is used in trial design, however is minimised when ambulatory versus office blood pressure is used as an endpoint.

In Chapter 5 I showed that using directly observed anti-hypertensive therapy prior to all measurements in my trial resulted in a between-individual variation in effect size from renal denervation of 13mmHg for office blood pressure and 8mmHg for ambulatory blood pressure. This was a smaller standard deviation of effect size for ambulatory and office blood pressure than other studies in my meta-analysis. My
results therefore suggest that using directly observed anti-hypertensive therapy prior to all measurements can further reduce the between-individual variation in effect size, which may be of importance to researchers designing future drug or device blood pressure trials.

8.2 Suggestions for future work

8.2.1 Suggestions for future trial design: minimizing confounding from changes in medication adherence

In order to prevent changes in medication adherence confounding efficacy results from distal denervation, I performed all blood pressure measurements after directly observed antihypertensive therapy. In my study I also stated that no medication changes were allowed through the 6-month trial period, and ensured this was the case by checking medication and doses at each visit.

One of the most striking findings was the reduction in size of the standard deviation of blood pressure reduction (effect size). This is an important finding for future researchers assessing the efficacy of a treatment on blood pressure reduction, whether pharmacological or device based. By reducing the standard deviation of the effect size this will both reduce measurement noise which can obscure results, and also decrease the future sample size required in trials.

8.2.1.1 Option 1- performing measurements after directly observed anti-hypertensive therapy

There are two options therefore for future trials. The first would be to use a similar protocol as used in this study, which involves directly observing all medical therapy. In my experience this was a difficult task. Despite providing clear written instructions many participants would forget to bring medication with them. This would then
necessitate waiting for the hospital pharmacy to fill an emergency prescription to ensure that all medication was taken as prescribed in the trial. In addition, to ensure that the dose of medication was not altered within the trial period, I had to ask patients to not bring home organized dossette boxes with them, where medication is pre-organized but not labelled, but instead medication in original packaging to confirm doses. This added another layer of complexity, especially for elderly patients who organize their medication at the beginning of the week.

Furthermore, there is a level of risk associated with directly observed therapy. As described in the study, one patient who was consented into the trial and reported that he was 100% compliant with medication had a hypotensive episode after he swallowed all prescribed pills in clinic. His urine adherence assay detected only 1 out of 6 anti-hypertensive medications. I realized the risk associated with this step in my study design and ensured that all study visits were performed in our research institute on the grounds of St Mary’s and Hammersmith Hospital with emergency services available. Fortunately, I was never required to use any hospital services. I also always advised all patients that they would be observed after taking medication in clinic and to expect that each clinic visit would last at least 3 hours, to ensure that no patient would have an unsupervised hypotensive episode. However, these methodology aspects may not be feasible in a large multi-centre trial.

I performed two days of directly observed therapy at baseline and 6-months. However, if patients were non-compliant with medication, for certain medications with variable absorption, it might take more than 2 days of therapy before adequate plasma levels are achieved. Therefore, if directly observed therapy was to be used in future trials then this would need to be assessed to decide the required duration of directly observed anti-hypertensive therapy.
8.2.1.2 Option 2- performing measurements “off” anti-hypertensive medication

Another alternative would be to perform all measurements ‘off’ medication. This would involve stopping all patients’ anti-hypertensive medication and ensuring there was a wash out period prior to blood pressure measurements being taken.

Confirmation of complete washout of antihypertensive therapy should also be confirmed with urine metabolomic testing. Any patients whose urine assays showed the presence of any anti-hypertensive therapy should be excluded from the trial results.

The main disadvantage to this protocol would be the chance of patients developing rebound hypertension when stopping anti-hypertensive medication. Rebound hypertension may put patients at risk of cerebrovascular accidents and other hypertensive emergencies, therefore raising significant safety concerns. However, anti-hypertensive studies have used this “off medication” strategy previously. Two large meta-analyses (243) (244) have assessed the risk of this strategy in >90,000 patients and have found that there was no additional risk to trial patients when taken off their medication for a limited time period in a study where patients are well-monitored with careful patient selection.

However, one consideration when taking patients off medication in denervation trials versus drug trials is that taking patients off all anti-hypertensive therapy is likely to increase their blood pressure at the time of the procedure, and therefore might increase the procedural risk of renal denervation, and therefore patients would need to have treatment re-started prior to the intervention. This would therefore increase the number of visits in the trial which might increase the likelihood of patients dropping out from the trial(245).
8.2.2 Future work: use of invasive renal haemodynamics as a peri-procedural feedback mechanism

As described, there was an increase in renal artery blood flow at 6-months follow up after fenervation. Change in renal blood flow immediately post denervation also correlated with reduction in ambulatory blood pressure at 6-months follow up. This indicates that change in renal blood flow may be a peri-procedural marker of efficacy of denervation, however as the trial was underpowered to prove an association this observation is exploratory only at this stage.

A future study may want to study this observation further. First, it would be interesting to see whether the change in renal flow after denervation is a function of the number or distribution of ablations. This could be assessed by performing a set number of ablations e.g. 3 ablations in 1 kidney, and measuring change in renal blood flow at this point. Further ablations could then be performed in a step-wise fashion and change in renal blood flow re-assessed after each ablation. The ablations should be performed separately in the main trunk and distal branches, to try to assess which ablations cause the greatest increase in renal blood flow. In some patients, despite multiple ablations in the main trunk or distal branches it may not be possible to cause an increase in renal blood flow, due to a currently unknown baseline characteristic.

Second, if it was found that renal blood flow was affected by the number or location of ablations, it would be interesting to assess this in terms of improving the efficacy of denervation. In this study investigators would perform denervation whilst measuring renal blood flow and only finish denervation when a certain blood flow increase had been achieved. Efficacy of ambulatory blood pressure reduction should then be re-assessed to see whether using renal blood flow as a peri-procedural marker may increase blood pressure reduction after denervation.
8.2.3 Future work: speculation on renal artery wave speed as a measure of baseline renal sympathetic tone

Based on previous work, I used the single-point approach to measure wave speed within the renal arteries. I found this measure was consistent within the left and right renal arteries. In addition, I found that renal artery wave speed did not correlate either with patient age or invasive aortic wave speed. These findings suggest that the tone in the renal artery is derived from more than anatomical structure, and may be based on another phenomenon. I hypothesise that renal artery wave speed may be a marker of sympathetic tone in the renal vascular bed.

This could be assessed further by future researchers. A pre-clinical study might measure invasive renal wave speed, for example in normal and hypertensive/heart failure rats with elevated sympathetic tone, and correlate wave speed with renal norepinephrine assays. In a clinical study, sympathetic tone could be assessed using either muscle microneurographic studies or renal norepinephrine levels, and renal artery wave speed could be correlated with these measures.

8.2.4 Future work: speculation on use of renal artery wave speed as a predictor of blood pressure reduction after renal denervation

In the discussion of Chapter 6, I conclude that patients who showed the greatest blood pressure reduction at 6-months follow-up had the highest renal vascular resistance as well as the highest renal wave speed at baseline. By assessing baseline renal wave speed, it may be possible to pre-specify patients who will show the greatest blood pressure reduction from renal denervation.

This needs to be validated in a blinded fashion with a sham arm in a future. This is essential to prevent any confusion around the magnitude of the effect size, as was seen in the previous renal enervation trials described in Chapter 1. For example, a future trial might investigate this prospectively by measuring renal artery wave speed...
pre-denervation, and then correlating wave speed with the 6-month response to
denervation. Furthermore, the trial will need to be is powered appropriately to detect
whether renal artery wave speed is a predictor of blood pressure reduction.
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Appendix 1


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Appendix 2

---Original Message-----
From: judith finegold On Behalf Of Judith Finegold
Sent: Sunday, August 21, 2016 09:02
To: Dibona, Gerald F <gerald-dibona@uiowa.edu>
Subject: Request for permission to use diagrams in thesis

Dear Gerry
Just to give you an update on the papers - they are still going back and forth with journals at the moment so no real progress but I will let you know when that changes. In the meantime I’m finishing my thesis and aiming to submit in November which will be exciting! Can I ask permission to use the following 2 diagrams in my thesis - of course acknowledging and crediting your paper appropriately.

I hope that you are well
Many thanks
Judy
Appendix 3

Awards and Publications arising from work undertaken

Papers under consideration


Presentations
