The Physiological Changes That Occur Post Endovascular Renal Denervation in Dialysis Patients

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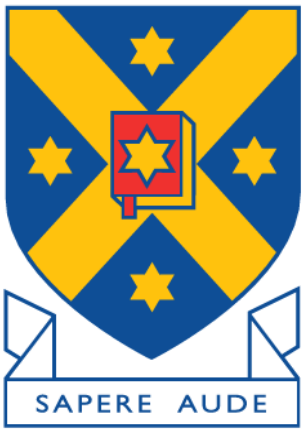

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Te Whare Wānanga o Otāgo
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A thesis submitted for the degree of
Master of Medical Science

University of Otago

October 2017

ABSTRACT

Sympathetic neural activation is markedly increased in end-stage kidney disease (ESKD). Catheter-based renal denervation (RDN) reduces sympathetic over-activity and blood pressure in resistant hypertension. The effect of renal denervation on sympathetic neural activation and left ventricular mass was investigated in patients with ESKD. Nine ESKD (six haemodialysis and three peritoneal dialysis) patients with a dialysis vintage of ≥11 months were treated with RDN (EnligHTN^{TM} system). Data were obtained on a non-dialysis day; at baseline, one (1M), three (3M) and twelve months (12M) post-RDN. At baseline, sympathetic neural activation measured by muscle sympathetic nervous activity (MSNA) and plasma norepinephrine concentrations were markedly elevated. Left ventricular hypertrophy (LVH) was evident in eight of the nine patients. At 12M post-RDN, blind analysis revealed that $MSNA_{frequency}$ (-12.2 bursts·min-1, 95% CI [-13.6, -10.7]) and LV mass (-27 $g \cdot m^{-2}$, 95% CI [-47, -8]) were reduced. Mean ambulatory BP (systolic: -24 mmHg, 95% CI [-42, -5] and diastolic: -13 mmHg, 95% CI [-22, -4]) was also reduced at 12M. Office BP was reduced as early as 1M (systolic: -25 mmHg, 95% CI [-45, -5] and diastolic: -13 mmHg, 95% CI [-24, -1]). Both ambulatory and office BP had clinically significant reductions in at least 50% of patients out to 12M. Catheter-based RDN significantly reduced MSNA and LV mass as well as systemic BP in this group of patients with ESKD.

ACKNOWLEDGEMENTS

This thesis was assisted by many people over a long period, people who gave their time willingly and with genuine support. I am greatly indebted to their input.

To Professor Rob Walker, you made all this possible, facilitating my placement in New Zealand, trusting someone you had never met with a project of this potential, welcoming me into your home in Wanaka, and supporting me through many personal and professional highs and lows. I cannot thank you enough for allowing me to complete this piece of work under your stewardship.

To Dr Luke Wilson, without your commitment, this project would never had seen the light of day. Tireless in your enthusiasm, your peer support, practical advice and determination to see things through to the end can only be admired.

To Associate Professors Gerry Wilkins and David Jardine, the mastery you both showed with the technical elements of denervation and MSNA recording were outstanding. Without your input, this research endeavour simply wouldn't have happened.

To Dr Ari Samaranayaka, although we have never met, I can only marvel at your statistical wizardry. Your ability turned a small project into a real proof-of-principle piece of research.

To Dr Chris Baldi, your invaluable advice and experience were key to a strong literature review, your humour and friendship made me feel welcome in a foreign land. To Dr John Schollum and Dr Tracey Putt, your quiet support and counsel were most welcome, and I certainly benefited from your experience, friendship and advice. To Fiona Roberts, cardiac sonographer, many thanks for your help, your expertise and your dry sense of humour.

I am extremely grateful to the Health Research Council of New Zealand for funding this study and to Otago University for granting the Tony Hocken Scholarship that provided a generous stipend in both 2012 and 2013. Grateful thanks must extend to St Jude Medical, Inc. and to Christchurch Heart Institute, who provided renal denervation equipment and plasma catecholamine analysis respectively. I also acknowledge the Department of Medicine, Otago University for their understanding in times of difficulty. That also extends to members of the DSM staff who kept me going, and kept me drinking tea!

To all the patients, a massive debt of gratitude. Without your sacrifice, this would have all been for nothing.

Lastly to Niki and my family, despite evidence to the contrary, you kept me sane throughout the darkest times. I am who I am only because of you.

GRANTS AWARDED

- University of Otago, Dunedin School of Medicine (Tony Hocken Scholarship) 2012/2013
- Australia and New Zealand Society of Nephrology (Travel Grant) 2013
- UK Renal Week (Amgen Bursary) 2014
- European Renal Association (Travel Grant) 2014

PRIZES AWARDED

- Best abstract by a young author, ERA-EDTA Congress 2014

PUBLICATIONS AND PEER-REVIEWED PRESENTATIONS ARISING

- Endovascular renal denervation in end-stage kidney disease patients: Cardiovascular protection – A proof-of-concept study. **Hoye NA**, Wilson LC, Wilkins GT, Jardine DL, Putt TL, Schollum JB, Samaranayaka A, Walker RJ. Kidney Int Reports 2017; 2: 856-865.
- Delayed but sustained blood pressure and sympathetic reduction one year after renal denervation in dialysis patients. **Hoye NA**, Baldi JC, Jardine DL, Schollum JB, Wilkins GT, Wilson LC, Walker RJ. Nephrol Dial Transplant 2016; 31 (Supplement 1): i53-i54.
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- Effect of renal ablation on blood pressure and muscle sympathetic nerve activity in chronic kidney disease patients. Wilson GA, **Hoye NA**, Wilson LC, Shoemaker JK, Schollum JB, Jardine DL, Walker RJ, Wilkins GT, Baldi JC. FASEB J 2014; 28 (Supplement 1): LB722.

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1.1 Chronic & End-Stage Kidney Disease

1.1.1 Definitions

In 2002 the National Kidney Foundation's Kidney Disease Outcomes Quality Initiative (KDOQI) introduced a model for the definition and classification of chronic kidney disease (CKD)[1]. Presence of kidney damage or glomerular filtration rate (GFR) < 60 ml/min per 1.73m^2 for ≥ 3 months was diagnostic of CKD, irrespective of cause. Kidney damage was defined as compromised kidney function, persistent microalbuminuria, proteinuria and/or haematuria, structural abnormalities or biopsy-proven chronic glomerulonephritis[1].

Five stages of CKD were proposed based upon the level of GFR and these were ratified the following year by the Kidney Disease: Improving Global Outcomes (KDIGO) group with minimal modification[2].The KDIGO group has reconvened and although the original definition of CKD is retained, an enhanced classification framework is proposed. This is to allow for better prognostication and management decisions. The 2012 guidelines[3] define CKD as "abnormalities of kidney structure or function, present for > 3 months with implications for health" and classify it "based on cause, GFR category and albuminuria category" as follows:

Figure 1: *Prognosis of CKD by GFR and Albuminuria Categories: KDIGO 2012*[3]

GFR categories (ml/min/ 1.73 m^2) Description and range				Persistent albuminuria categories Description and range		
				A1	A2	A3
				Normal to mildly increased	Moderately increased	Severely increased
				<30 mg/g <3 mg/mmol	30-300 mg/g 3-30 mg/mmol	>300 mg/g >30 mg/mmol
	G1	Normal or high	≥90			
	G2	Mildly decreased	60-89			
	G3a	Mildly to moderately decreased	45-59			
	G3b	Moderately to severely decreased	30-44			
	G4	Severely decreased	15-29			
	G5	Kidney failure	<15			

End-stage kidney disease (ESKD) therefore forms part of the clinical spectrum of CKD. Once GFR reaches < 15 ml/min/1.73m^2 patients are viewed to have established kidney failure, or ESKD.

1.1.2 Epidemiology

Chronic kidney disease is extremely common in Australasia. A very recent study of the Otago Southland population suggested that 12.8% have the disease (*manuscript in preparation*). This compares with a worldwide prevalence of 7.2% over the age of 30 years, and 23.4% - 35.8% in people over 65 years[4].

Age, hypertension and diabetes are strong predictors of renal dysfunction[5]. As the population becomes increasingly elderly, hypertensive and diabetic[6], the resultant burden of CKD is set to increase. Aside from the health implications, the financial sequelae of such an increase are massive. Renal disease currently consumes 5.7% of the Australian health care budget[5], a figure that does not include the cost of providing renal replacement therapy (RRT) for those with ESKD, currently estimated to rise to around $12 billion by 2020[7].

The prevalence of ESKD is increasing over and above that of CKD[8]. Current estimates place the New Zealand renal replacement population at 874 per million[9] with the population tripling since 1991[9]. This suggests a mechanism distinct from the natural progression of cases from CKD to ESKD. Increasingly liberal entry criteria for renal replacement are being used and patients are surviving co-incident morbidity such as infectious and cardiovascular disease[8]. We are in the midst of what has been viewed by some as a chronic kidney disease epidemic[10].

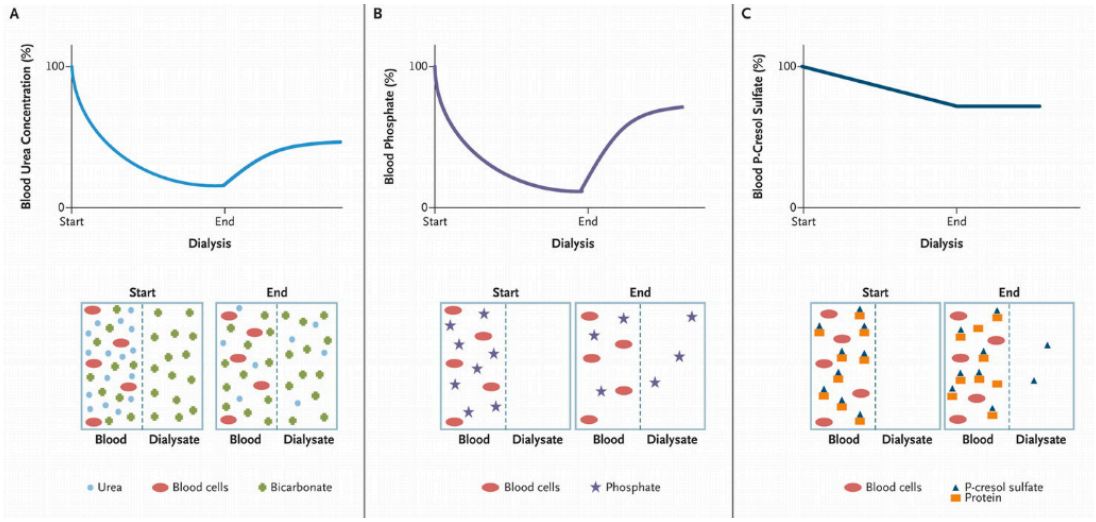
1.1.3 Treatment

Early detection and active management often can prevent or delay progression of CKD and associated adverse outcomes[11]. The specific goals of treatment for CKD are therefore to minimise any additional kidney damage, slow the rate of renal functional decline, reduce the risk of cardiovascular morbidity and manage the complications of CKD.

Once effective kidney function has been lost, patients are given the option of commencing renal replacement therapy or an active conservative care pathway. Renal replacement therapy encompasses both dialysis and renal transplantation. Transplantation (either living or deceased donor) remains the best treatment for ESKD, but is often deferred by necessity due to shortage of available organs. Dialysis provides an artificial replacement of kidney filtration although lacks the tubular and endocrine functions of the kidney. General indications to commence dialysis are often a combination of GFR < 10 ml/min, development of advanced uraemic symptoms, diuretic-resistant volume overload, hyperkalaemia or metabolic acidosis.

Dialysis is defined as the diffusion of molecules in solution across a semipermeable membrane along an electrochemical concentration gradient[12]. The primary goal of both haemo- and peritoneal dialysis is to restore the intracellular and extracellular fluid environment, thus mimicking normal kidney function. This is accomplished by the transport of solutes such as urea from the blood into the dialysate and by the transport of solutes such as bicarbonate from the dialysate into the blood. Solute concentration and molecular weight are the primary determinants of diffusion rates. Small molecules, such as urea, diffuse quickly, whereas compartmentalised and larger molecules, such as phosphate, β2-microglobulin, and albumin, and protein-bound solutes, such as p-cresol, diffuse much more slowly[12]:

Figure 2: *Patterns of Solute Removal with Haemodialysis*[12]



In addition to diffusion, solutes may pass through pores in a haemodialysis membrane by means of a convective process driven by hydrostatic or osmotic pressure gradients - a process called ultrafiltration[13].

Peritoneal dialysis (PD) is a less common yet still important variant of renal replacement therapy. In PD, solute and fluid exchanges take place between capillary blood and dialysis solution through the peritoneal membrane in the abdominal cavity. Small molecular weight solutes pass down a concentration gradient. Fluid transfer is achieved by osmosis and middle molecule transfer occurs via convection[14]. The removal of toxins by PD differs in many aspects from that by haemodialysis (HD), due to its continuous nature and the increased pore size of the peritoneal membrane.

1.1.4 Prognosis

CKD impacts prognosis at all stages[15] but it is ESKD in particular that substantially increases the risk of death, cardiovascular disease and specialised health care[16]. Dialysis patients have extraordinarily high mortality rates. The annual mortality rate for prevalent New Zealand dialysis patients in 2011 was 15.5 deaths per 100 patient years[17] with 5-year-survival rates worse than many solid organ cancers. Thirty-five percent of 319 dialysis deaths were due to cardiovascular causes[17]. Haemodialysis patients in particular have high cardiovascular risk; patients aged 15 to 30 years have 150 times greater risk of cardiovascular death than the general population, with over 1000 times greater risk of sudden cardiac death[18].

Sudden cardiac death (SCD) is an unexpected natural death due to cardiac aetiology preceded by a sudden loss of consciousness and is the single greatest cause of mortality in ESKD patients on dialysis[19]. Most such events are believed to be due to ventricular arrhythmias, that is ventricular tachycardia or ventricular fibrillation[20]. Although sudden cardiac death can occur in patients with structurally normal hearts, most occurs in patients with some form of underlying heart disease[21]. Acute myocardial ischaemia is believed to be the most common initiating event in the general population although other unique factors may contribute to the increased risk of SCD in ESKD; interstitial fibrosis due to chronic uraemia, microvascular disease or endothelial dysfunction, calcium/phosphate deposition and significant left ventricular hypertrophy have been implicated[22]. Additionally, an increased electrical instability may be seen due to fluid shifts, autonomic imbalance, chronic inflammation, acid/base disturbances, and electrolyte abnormalities[22]. The true risk of ventricular arrhythmias is likely due to a combination of these many interacting factors.

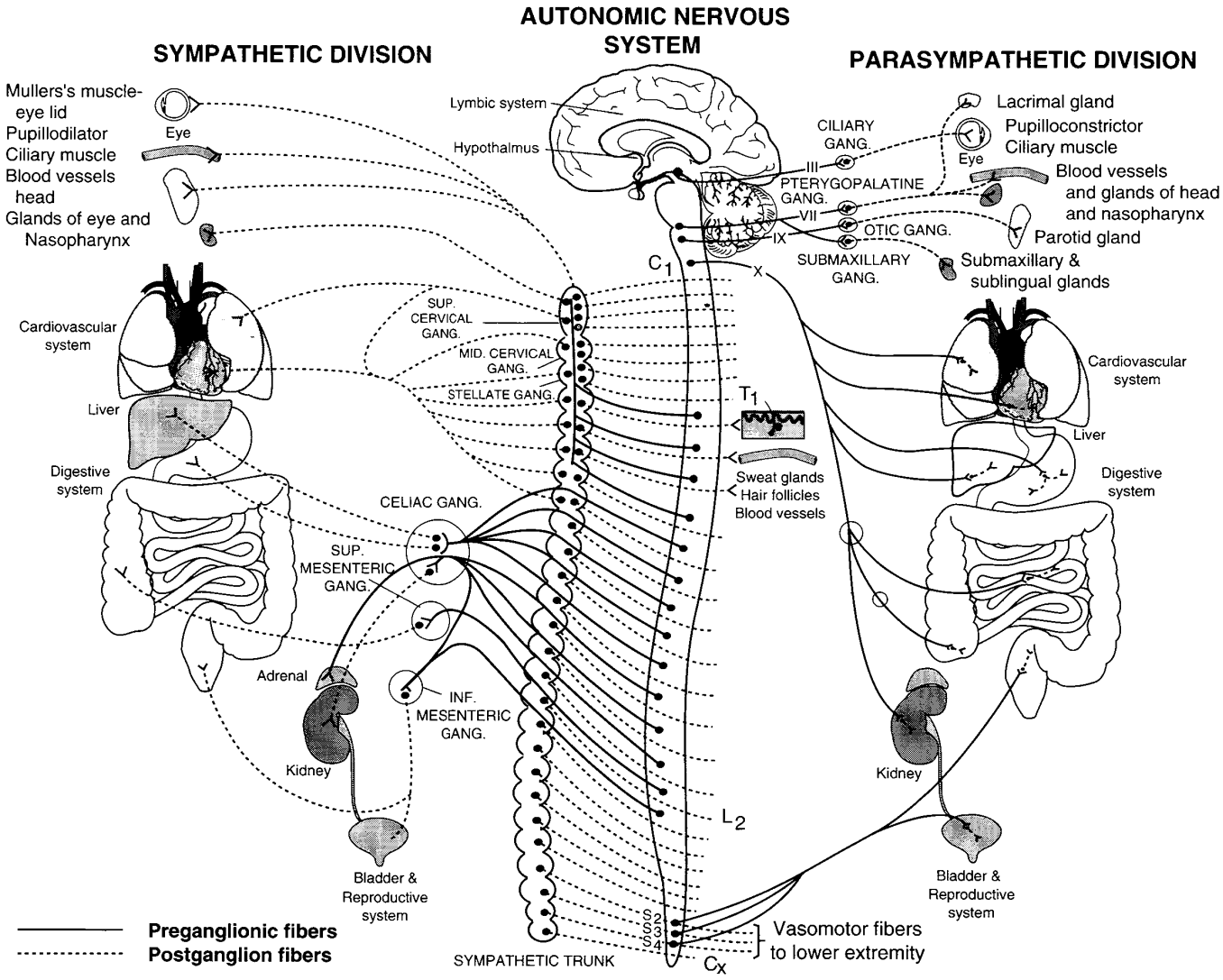
1.2 The Nervous System

The nervous system coordinates voluntary and involuntary actions and transmits signals between different parts of the body. It consists of two main parts, the central nervous system and the peripheral nervous system. The central nervous system comprises the brain and spinal cord. The peripheral nervous system represents all tissues distal to the spinal cord. It includes motor neurons mediating voluntary movement; the autonomic nervous system, comprising the sympathetic and parasympathetic nervous system and regulating involuntary functions; and the enteric nervous system, a semi-independent part that regulates gastrointestinal motility, fluid and electrolyte homeostasis.

1.2.1 The Autonomic Nervous System (from [23])

The autonomic nervous system is largely responsible for the regulation of visceral functions and the maintenance of homeostasis of the internal environment. It regulates visceral function primarily through its interaction with the endocrine system and via autonomic reflexes. The general anatomy of the efferent systems is illustrated over the page.

Figure 3: *The Efferent Systems of the Sympathetic and Parasympathetic Divisions of the Autonomic Nervous System*[23]



The cell bodies of efferent neurons in both divisions of the autonomic nervous system originate in the brainstem and spinal cord. Axons leave the central nervous system via cranial nerves or ventral roots to synapse upon specialized ganglia where second-order neurons give rise to axons that directly innervate smooth muscle and cardiac muscle and control glandular secretory function. This means there are both pre- and post-ganglionic fibres in the sympathetic and parasympathetic divisions.

Historically, the autonomic nervous system was considered a pure efferent or motor system. It has subsequently been shown that there are also afferent fibres within each division. These neurons have their cell bodies in the dorsal root ganglia and in certain cranial nerve somatic sensory ganglia and relay sensory information from the viscera back to autonomic nervous system centres[24]. Axons of these afferent fibres may travel in somatic peripheral nerves or in specialized autonomic nerves along with the autonomic efferent or motor fibres.

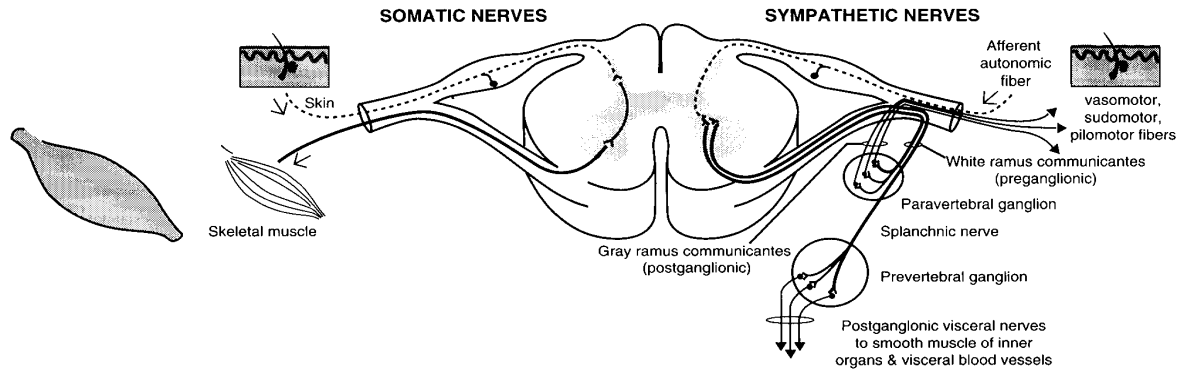
Higher central nervous system centres in the cerebral cortex, especially in the limbic system and hypothalamus, have important connections with the autonomic nervous system and participate in the integrated regulation of visceral function and homeostasis[25]. Most viscera and target organs of the autonomic nervous system have dual innervation from the sympathetic and parasympathetic nervous systems. Each system typically produces antagonistic effects. As such, a balance of activity between the sympathetic and parasympathetic nervous systems helps to regulate visceral and homeostatic mechanisms.

1.2.2 The Sympathetic Nervous System (from [23])

The cell bodies of the neurons that compose the efferent part of the sympathetic nervous system lie in the intermediolateral (IML) cell column of the spinal cord from the T1 to the L2 segments. As the outflow typically occurs between these segments, it has been referred to as the thoracolumbar system or thoracolumbar outflow.

The pre-ganglionic fibres that exit via the ventral root are small (2 - 5µm) myelinated fibres, the so-called white rami communicantes[26]. These fibres typically synapse on second-order neurons in the paravertebral ganglia (paired ganglia lying adjacent to the spine and extending from the cervical to the sacral segments).

Figure 4: *The Sympathetic Outflow from the Spinal Cord and the Course of the Sympathetic Fibres*[23]



There are usually three cervical ganglia, eleven thoracic ganglia, four lumbar ganglia and four or five sacral ganglia[27]. Some axons may synapse on neurons in adjacent ganglia at their segment of exit, but frequently axons pass up or down many segments before synapsing.

Each pre-ganglionic axon will typically synapse with many postganglionic neurons, permitting the sympathetic nervous system to produce a diffuse discharge. The postganglionic axons originating from the neurons in the paravertebral ganglia leave the ganglia as thin unmyelinated fibres, the grey rami communicantes (see above). Many of these fibres travel in segmental spinal nerves and are destined to innervate blood vessels, sweat glands, and hair follicles, whereas others will form plexuses that supply the thoracic, abdominal and pelvic viscera.

Some pre-ganglionic fibres do not synapse in the paravertebral ganglia but instead pass through the ganglia without synapsing and form splanchnic nerves. These nerves innervate three pre-vertebral ganglia, the coeliac and superior and inferior mesenteric ganglia. Second-order neurons in these ganglia send their post-ganglionic fibres into the hypogastric, splanchnic and mesenteric plexuses that innervate glands, blood vessels and smooth muscles of the abdominal and pelvic viscera.

The adrenal medulla is uniquely innervated. Pre-ganglionic fibres travelling in the splanchnic nerves directly innervate the chromaffin cells. This facilitates secretion of the sympathetic

neurotransmitters epinephrine and norepinephrine into the blood for a more diffuse and far-reaching sympathetic discharge effect.

The kidney receives its innervation from the renal plexus. It is formed from rami exiting the coeliac ganglion and plexus, the aortico-renal ganglion, the lower thoracic splanchnic nerves, the first lumbar splanchnic nerve and the aortic plexus. Both efferent and afferent pathways run in the renal plexus, which reaches the kidney via the outermost layer of the renal artery wall, the tunica adventitia.

1.2.2.1 Neurotransmitters & Receptors

The neurotransmitter for all pre-ganglionic fibres, both sympathetic and parasympathetic, is acetylcholine. Norepinephrine is the sympathetic transmitter at all post-ganglionic sites[23].

There are two distinct types of adrenoreceptor within the sympathetic nervous system, each receptor is identified by differing responsiveness to sympathomimetic amines. The alpha-adrenoreceptor is associated with most of the excitatory functions (e.g. vasoconstriction and stimulation of the uterus, ureter and dilator pupillae) and one important inhibitory function (intestinal relaxation)[28]. The beta-adrenoreceptor is associated with most of the inhibitory functions (e.g. vasodilatation and inhibition of the uterine and bronchial musculature) and one excitatory function (myocardial stimulation, both of contractility and heart rate)[28].

The alpha-receptors may be further divided into α-1 and α-2. The α-1 receptor is generally post-synaptic and excitatory and the α-2 receptor is principally a pre-synaptic inhibitory receptor. Although these general rules do apply, there are exceptions[29].

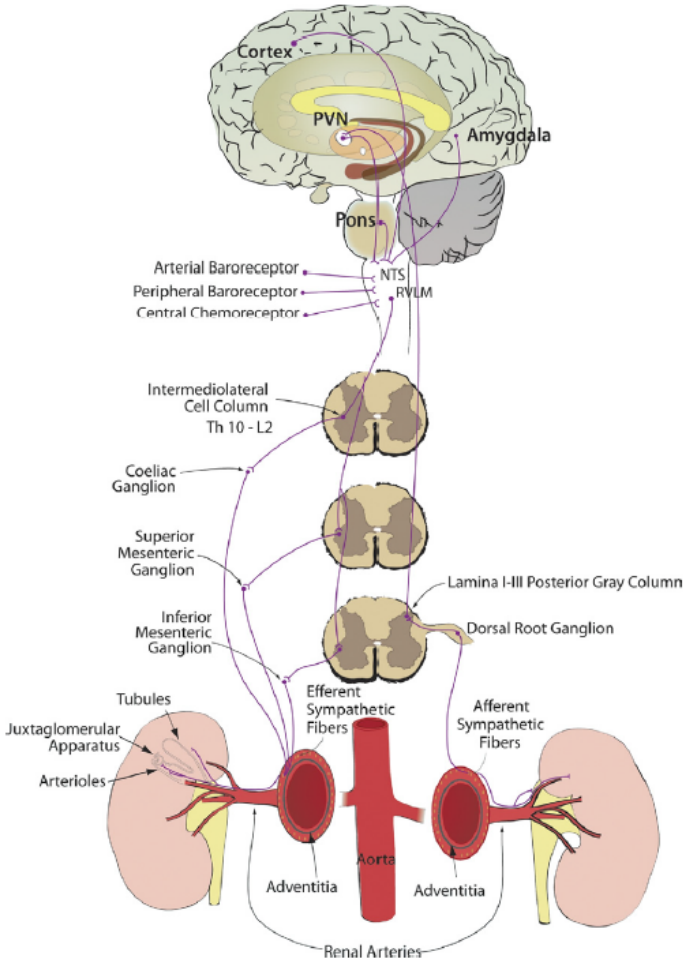
Alpha-1 receptors, when activated, set into motion a series of biochemical steps that result in increased synthesis of cyclic adenosine monophosphate (AMP) whereas α-2 receptors produce a respective reduction[29]. Alpha-2 receptors located primarily on the presynaptic sympathetic nerve terminals inhibit norepinephrine release and thus have an inhibitory impact on sympathetic function. These receptors may also stimulate platelet aggregation, inhibit lipolysis of fat, inhibit insulin release from the pancreas, and produce constriction of some vascular smooth muscle[23].

The α-1 receptors are responsible for relaxation of gastrointestinal smooth muscle, contraction of most other smooth muscle, secretory activity of salivary and sweat glands, positive inotropic effects on the heart, and metabolic effects such as glycogenolysis in fat and gluconeogenesis[23].

Beta-receptors may also be divided into two types, β-1 and β-2. These represent homologous proteins that both result in increased synthesis of cyclic AMP[30]. The β-1 receptor is important for generating a positive inotropic and chronotropic effect on the heart as well as lipolysis of fat. Beta-2 receptors are responsible for relaxation of the smooth muscle in bronchi, uterus, gut, bladder detrusor, spleen capsule, and vascular smooth muscle of skeletal muscle. Beta-2 receptors also result in amylase secretion from the salivary gland, gluconeogenesis, and glycogenolysis from the liver as well as glycogenolysis and increased lactate production from the skeletal muscle[29].

1.2.2.2 ***The Renal Sympathetic Nervous Supply***

Figure 5: *The Renal Sympathetic Nervous System*[31]
(NTS, solitary tract nucleus; PVN, paraventricular nucleus; RVLM, rostral ventrolateral medulla)

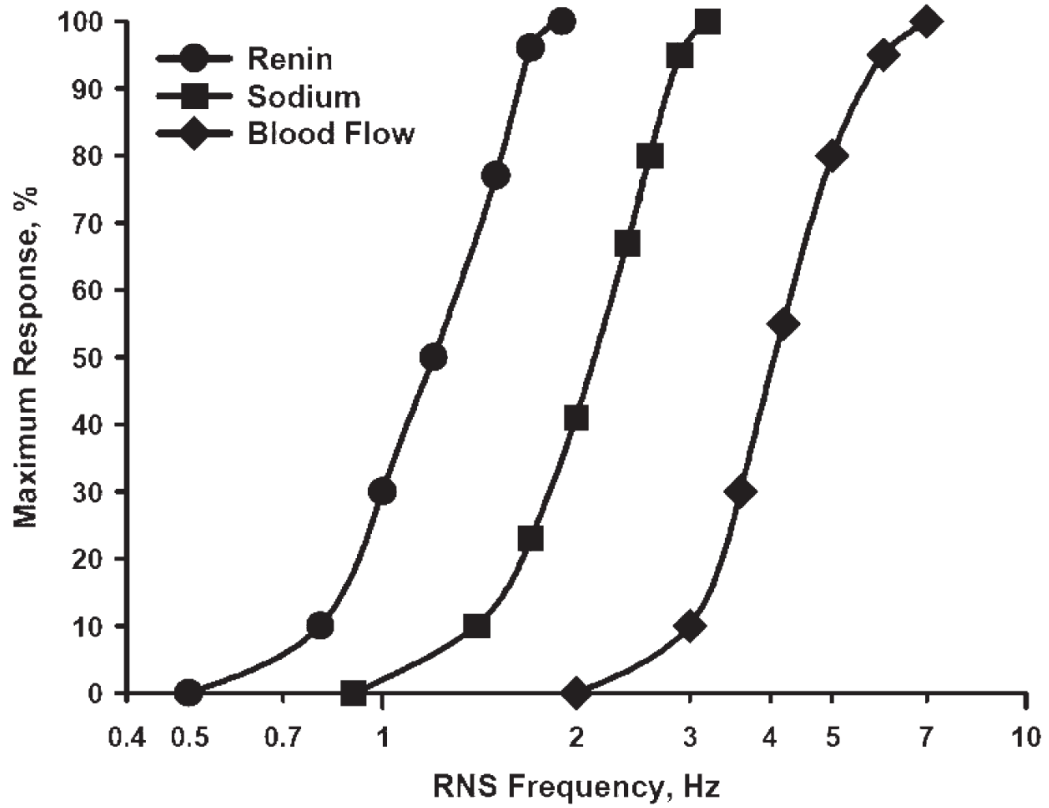


The renal sympathetic nervous supply has been particularly well characterised. Bernard noted that section of the greater splanchnic nerve (i.e. renal denervation) led to ipsilateral diuresis and stimulation of the cut end led to anti-diuresis[32]. This was the first suggestion that there was neural control of renal function as well as vascular tone. Sympathetic nerve stimulation also decreases renal blood flow whilst renal denervation leads to a respective increase[33]. These data supported the conclusion that sympathetic nerve fibres enter the renal hilum along the main renal artery and innervate the renal vasculature[34].

The advent of electron microscopy allowed Müller et al to demonstrate that intrarenal sympathetic nerve fibres also make direct contact with tubular epithelial basolateral membranes throughout the entire nephron[35]. This innervation is functional; increased sympathetic nerve activity leads to increases in renin secretion rate, renal tubular sodium reabsorption with anti-natriuresis as well as renal vasoconstriction[36]. Stimulation of fibres causes a graded response dependent on the intensity of the sympathetic signal[36]. At the lowest level, there is increased renin secretion mediated by β-1 adrenoreceptors located on the renin-containing juxtaglomerular granular cells[37]. At a slightly higher level of sympathetic

activity there is an additional anti-natriuresis. This is mediated by α-1b receptors located on the basolateral membrane of renal tubular epithelial cells in the proximal tubule, loop of Henle, distal tubule and the collecting duct[37]. At the highest level of renal sympathetic nerve activity, there is also a decrease in both renal blood flow and glomerular filtration rate (GFR). The renal vasoconstriction is mediated by α-1a receptors located on the vascular smooth muscle cells of the intrarenal resistance vasculature[37].

Figure 6: *The relationship between renal nerve stimulation (RNS) frequency and maximum response of renin secretion rate (increase), urinary sodium excretion (decrease) and renal blood flow (decrease)*[36]



At a cellular level, norepinephrine released from renal sympathetic nerve terminals binds to α-1a adrenoreceptors in the intrarenal resistance vasculature[37]. Subsequent activation of phospholipase C (PLC) hydrolyses phosphatidylinositol 4,5 bisphosphate (PIP_2) to inositol triphosphate (IP_3) and diacylglycerol (DAG)[37]. In turn, DAG can activate protein kinase C (PKC), resulting in vascular smooth muscle contraction[37]. The IP_3 released results in increased intracellular calcium concentration with subsequent activation of myosin light chain kinase also leading to vascular smooth muscle cell contraction[37].

In renal tubular segments, the activity of various transporters is increased by norepinephrine: Na^+/H^+ exchanger (NHE)-1, NHE-3 and sodium bicarbonate cotransporter (NBC) in the proximal tubule; Na-K-2Cl cotransporter (NKCC2) in the thick ascending limb of Henle's loop; and Na-K-ATPase throughout the nephron[37]. In renal tubular epithelial cells, α-1b adrenoreceptor stimulation activates multiple signalling pathways. Activation of $G_{q/11}$ engages PKC-dependent pathways, which increases the activity of basolateral NHE-1.

Activation of a PKC-independent mitogen activated protein kinase (MAPK) pathway increases the activity of apical NHE-3. Activation of the PLC-PIP_2-IP_3 pathway raises intracellular calcium concentration and increases the activity of calcineurin (protein phosphatase 2B), which dephosphorylates and thereby increases the activity of Na-K-ATPase[37].

In renin-containing juxtaglomerular granular cells, β-1 adrenoreceptor stimulation activates Gs, which increases adenylate cyclase and cyclic adenosine monophosphate (cAMP) concentration leading to protein kinase A (PKA) activation and exocytic renin release[37].

1.2.3 *Assessment of the Sympathetic Nervous System* (from [38])

1.2.3.1 Haemodynamic Measurement

Adrenergic activity can be inferred from the evaluation of the pressor and tachycardic responses to standardised stressful stimuli, such as the cold pressor test, isometric exercise, mental stress, stoop test, etc.[38] Usefully, these haemodynamic responses can be assessed in regional cardiovascular districts[38].
Although fruitful, this approach is invasive, has low within-subject reproducibility and poor correlation between the response and different stimuli. It must also be noted that the responses depend on the parasympathetic as well as the sympathetic nervous system[39], are regulated by cardiac adrenergic receptors[39] and display only a limited correlation with other adrenergic markers such as plasma norepinephrine or muscle sympathetic nerve activity.

1.2.3.2 Adrenergic and Ganglionic Pharmacological Blockade

The sympathetic cardiovascular component of a given intervention or condition can be estimated from the subtractive effect of blocking the adrenergic receptors located at the level of the heart and peripheral vessels[38]. Examples include evaluating the magnitude of the heart rate decrease induced by the beta-blocker propranolol and the blood pressure reduction and increase in regional blood flow induced by the alpha-blocker phentolamine[38]. As this approach depends on evaluation of heart rate, blood pressure and vascular resistances, the same limitations regarding concurrent parasympathetic nervous activity etc. apply. The same is true for infusion of ganglionic blockers such as trimetaphan.

1.2.3.3 Norepinephrine Measurement in Urine or Plasma

The activity of the adrenergic nervous system has been inferred previously from 24 hour urinary excretion of norepinephrine, epinephrine and their precursors or metabolites[40]. The approach provides a static picture of sympathetic function and is too sluggish to allow assessment of acute effects of adrenergic stimuli. It is also unclear to what extent urinary norepinephrine is derived from plasma or from the renal sympathetic nerves and clearly catecholamine excretion is dependent on renal function[41].

Plasma norepinephrine measurement has largely replaced urinary assessment and is currently the most commonly employed index of sympathetic activity in man. Despite this, the reproducibility and sensitivity of plasma norepinephrine values are lower than those of microneurographic measurements. Circulating norepinephrine represents only a minute fraction of the amount of adrenergic neurotransmitter secreted from nerve terminals[41,42]. Plasma levels of norepinephrine depend on secretion, tissue clearance and re-uptake processes[41,42] and therefore do not allow discrimination between central (increased secretion) or peripheral (reduced clearance) mechanisms.

1.2.3.4 Microneurography

Microneurography represents the only method so far available for directly recording efferent post-ganglionic muscle sympathetic nerve activity (MSNA), i.e. skeletal muscle vasomotor tone[43]. MSNA has been shown to closely correlate with global measures of sympathetic nerve activity such as total body norepinephrine spillover, and with regional (heart and kidney) norepinephrine spillover[44,45].

Sympathetic muscle fibre activity is characterized by bursts of discharges that are coupled to the cardiac rhythm[43]. Bursting occurs during systole and pauses occur during diastole[46]. In addition, the bursting appears to increase during reductions of arterial pressure, whereas the bursting is markedly reduced or abolished during elevations of blood pressure[47]. Furthermore, electrical stimulation of the carotid sinus nerve inhibits bursting[48]. These and other observations are consistent with an important role of the baroreceptor reflex in controlling the sympathetic vasoconstrictor fibres of skeletal muscle. Thus, the baroreceptor reflex can alter blood flow in skeletal muscle in such a way as to maintain blood pressure homeostasis[43,47].

The key feature of microneurography is the fact that it provides direct continuous evaluation of adrenergic activity to skeletal muscle circulation, allowing on-line dynamic assessment. Recording can be prolonged, allowing assessment of rapid changes in sympathetic activity to a variety of laboratory tests. Recordings from two remote sites are also remarkably similar, as is the reproducibility with time[49]. The procedure is safe, with only 10% experiencing a mild paraesthesia that usually dissipates within one week[50].

There are some drawbacks to the technique that must be noted. Firstly, burst amplitude cannot be easily compared between subjects. Secondly, muscle and skin regional vascular beds represent only a fraction of the peripheral circulation; hence their appropriateness to approximate cardiovascular adrenergic drive is questionable. Thirdly, the technique is invasive, complex and of doubtful significance in conditions characterized by sympathetic hypoactivity.

1.2.3.5 Plasma Norepinephrine Kinetics

Norepinephrine washout from an organ is proportional to the rate of electrical stimulation of its sympathetic nerves[51]. Plasma norepinephrine concentration however depends on the rate of removal from plasma, not just sympathetic tone and norepinephrine secretion[42]. "Net" neurotransmitter secretion can be studied clinically using radiotracer-derived measurements of the appearance rate of norepinephrine in plasma for the body as a whole (the so-called spillover rate)[52], thus negating the confounding effect of norepinephrine plasma clearance.

During constant-rate infusion of radio-labelled norepinephrine, and with regional catheterization, the *organ-specific* rate of spillover of norepinephrine to plasma can be determined by isotope dilution:

$$\text{Regional norepinephrine spillover} = [(C_V - C_A) + C_A \times E] \times PF$$

C_V = Concentration of norepinephrine in regional venous plasma
C_A = Concentration of norepinephrine in regional arterial plasma
E = fractional excretion of tritiated norepinephrine across the organ
PF = organ plasma flow

This method has greater analytical power than whole body spillover rate as sympathetic nervous system responses typically show regional differentiation; sympathetic outflow to some organs may be activated while that to other regions may be unchanged or inhibited[53]. Nevertheless, the use of tritiated norepinephrine has its own drawbacks; chiefly it is an invasive investigation with a radioactive substance. Although improved compared to whole body assessment, regional spillover remains an approximation and not a precise measurement; regional blood flow[54] and the exchange conductivity of the capillary and post-capillary venular bed[55] can still influence results.

1.2.3.6 Power Spectral Analysis of Heart Rate Variability

In this method, sophisticated mathematical techniques are used to identify super-imposed rhythms producing cyclical variations in heart rate. Underlying independent high-frequency (approximately 0.3Hz) and low-frequency (approximately 0.1Hz) rhythmic influences can be recognised. The autonomic nervous system provides the principal effector mechanism for this circulatory variability. The high-frequency (HF) component in heart rate variability is coupled with the respiratory cycle, determined primarily by vagal function and largely abolished by atropine[56]. Low frequency (LF) variability derives in part from the influence of the cardiac sympathetic nerves with an additional influence of the vagus[56].
Contrary to the views of many, LF variability does not strictly provide a measure of the rate of firing of the cardiac sympathetic nerves[38]. What is studied is primarily the baroreflex mechanisms and autonomic effector processes underlying circulatory rhythmicity. When a range of other stimuli produce sympathetic nervous activation, but for which the arterial and low-pressure baroreceptors are not engaged, an increase in low-frequency power is seen much less consistently, or not at all[38].

1.2.3.7 Imaging Techniques

Positron emission tomography and single photon emission computed tomography scanning have been used to visualise the sympathetic innervation of human organs. Primarily used in the heart, positron- or gamma-emitting probes are taken up into sympathetic neurons by the norepinephrine uptake transporter. Results suggest there is regional heterogeneity even in a healthy heart, with the atria and base of the ventricles showing greatest signal density[57].
In areas of recent myocardial infarction, norepinephrine uptake in the myocardium is regionally reduced due to ischaemic neuronal death in the infarct zone[58]. Widespread reduction of norepinephrine uptake is evident in the presence of global sympathetic denervation, in patients with pure autonomic failure, after heart transplantation and following administration of pharmacological inhibitors of the transporter, such as desipramine[57]. Notably in the period following transplantation, ingrowth of sympathetic fibres is also demonstrable[59].

Using these techniques to detect actual nerve firing and norepinephrine release is somewhat more difficult. The scanning agents [^{123}I]meta-iodobenzylguanidine (MIBG) and [^{11}C]hydroxyephedrine, unlike norepinephrine, are not stored in transmitter vesicles and are not subject to electrically-coupled release[57]. Also the metabolism and clearance of MIBG in the heart does not involve the same enzymes involved in catecholamine metabolism[57]. Wash-out of both tracers after initial uptake to the myocardium has been used as a marker of cardiac sympathetic activity. It is increased in some settings of sympathetic nervous activation (such as heart failure) but given the differences in the intraneural disposition and metabolism of norepinephrine and the tracers, what tracer wash-out actually signifies in terms of cardiac sympathetic function is not entirely clear. Aside from these concerns, the neuroimaging techniques require considerable financial and technical support, such as cyclotron use, which precludes their widespread use.

In summary, although technical improvements have allowed remarkable refinements in the assessment of human adrenergic function to be achieved, no technique so far available can be viewed as the “gold standard” with which the others might be compared. The “preferred” methods, i.e. microneurographic measurement of muscle sympathetic nerve activity and assessment of organ-specific norepinephrine spillover, despite their recognised merits, still suffer from some unavoidable intrinsic limitations. These disadvantages are minimised if these methods are seen as being complementary and used in combination, and coupled with the additional evaluation of end-organ cardiac and vascular adrenergic responses.

1.3 Hypertension

1.3.1 Definition

Systemic arterial hypertension is the condition of persistent, non-physiologic elevation of systemic blood pressure (BP). It is currently defined as a resting systolic BP (SBP) of 140 mmHg or greater, or diastolic BP (DBP) 90 mmHg or greater, or a condition for which a patient is receiving therapy for the indication of BP lowering[60].

1.3.2 Epidemiology

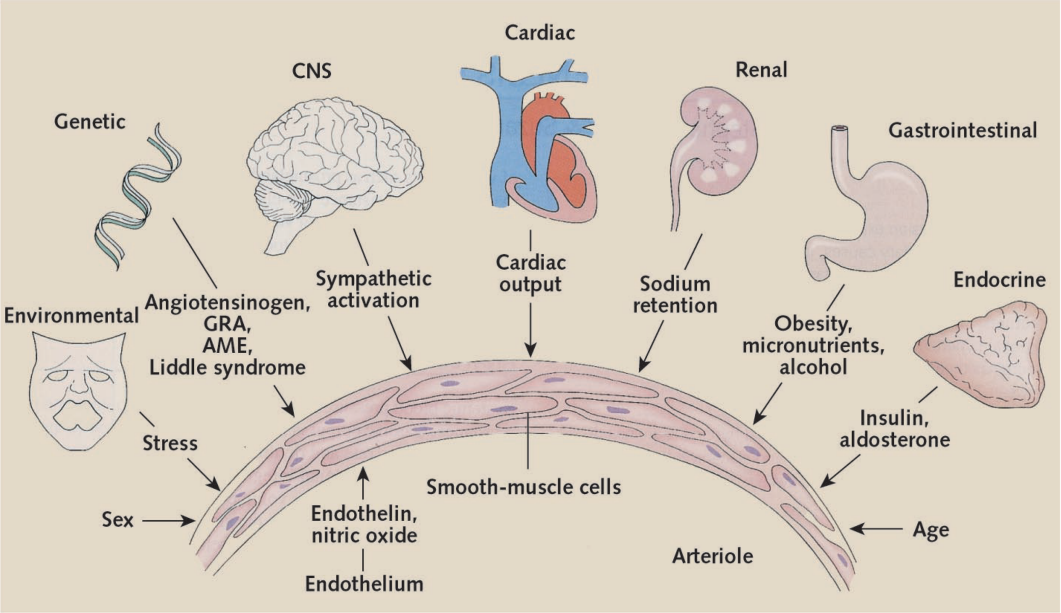
Worldwide prevalence estimates for hypertension may be as much as 1 billion individuals, and approximately 7.1 million deaths per year may be attributable to hypertension[61]. The World Health Organization reports that suboptimal BP (>115 mmHg SBP) is responsible for 62% of cerebrovascular disease and 49% of ischemic heart disease, with little variation by sex. In addition, suboptimal blood pressure is the number one attributable risk for death throughout the world[61].

Hypertension is unsurprisingly common in New Zealand, affecting at least 1 in 6 adults[62]. Pacific Islanders and those of Maori descent are most likely to suffer the disease[62]. Hypertension is also more common among people with lower family incomes, lower levels of education and among those living in more deprived areas[62].

1.3.3 Pathogenesis

Essential hypertension, or hypertension of unknown cause, accounts for more than 90% of cases of hypertension. Many pathophysiological factors have been implicated in the genesis of essential hypertension, although renal mechanisms probably play a primal role[63]. The implicated factors are illustrated below:

Figure 7: *Pathogenesis of Hypertension*[64]

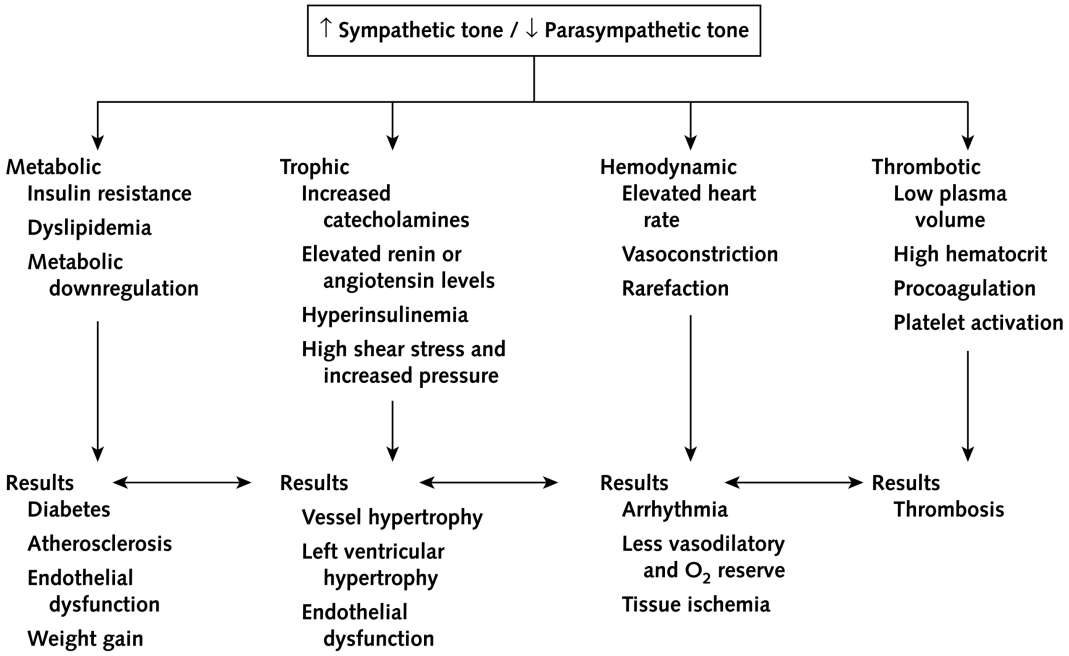


1.3.4 Hypertension & The Sympathetic Nervous System

Increased sympathetic nervous system activity increases blood pressure and contributes to the development and maintenance of hypertension through stimulation of the heart, peripheral vasculature and kidneys, causing increased cardiac output, increased vascular resistance, and fluid retention[65].

Autonomic imbalance (increased sympathetic tone accompanied by reduced parasympathetic tone) has been associated with many metabolic, haemodynamic, trophic and rheological abnormalities that result in cardiovascular morbidity and mortality[66]

Figure 8: *Role of the Sympathetic Nervous System in the Pathogenesis of Cardiovascular Diseases* (originally from[66], modified in [64] to the following)



Increased sympathetic tone increases diastolic blood pressure by causing vascular smooth muscle cell proliferation and vascular remodeling[64]. Norepinephrine spillover studies also suggest sympathetic cardiac stimulation is greater in young hypertensive patients than in normotensive controls, supporting the notion that increased cardiac sympathetic stimulation contributes to the development of hypertension[67].

The mechanisms of increased sympathetic nervous system activity in hypertension are complex and involve alterations in baroreflex and chemoreflex pathways at both peripheral and central levels. Arterial baroreceptors reset to a higher pressure in hypertensive patients[68]. There is also central resetting of the aortic baroreflex, resulting in suppression of sympathetic inhibition after activation of the aortic baroreceptor nerves[69]. The baroreflex resetting is at least partly mediated by a central action of angiotensin II[70]. Angiotensin II's role is complex; it also facilitates the pre-synaptic release of norepinephrine, inhibits synaptic

reuptake and enhances tissue response[71]. Furthermore, it stimulates the sympathetic ganglia and adrenal medulla effecting an increase in circulating epinephrine and norepinephrine[71].

Alongside angiotensin II, there are additional small-molecule mediators that suppress baroreceptor activity and contribute to exaggerated sympathetic drive. These include reactive oxygen species[72] and endothelin[73]. Finally, there is evidence of exaggerated chemoreflex function, leading to markedly enhanced sympathetic activation in response to stimuli such as apnoea and hypoxia[74]. This is clinically relevant in the setting of obstructive sleep apnoea where exaggerated sympathetic nerve activity is demonstrable alongside hypertension[75].

Chronic sympathetic stimulation induces vascular remodelling and left ventricular hypertrophy by the direct and indirect actions of norepinephrine. There is activation of norepinephrine's own receptors, as well as release of various trophic factors, including transforming growth factor-β (TGF-β), insulin-like growth factor 1, and fibroblast growth factors[66]. A positive correlation exists between circulating norepinephrine levels, left ventricular mass, and reduced radial artery compliance (an index of vascular hypertrophy)[76,77]. Thus, sympathetic mechanisms contribute to the development of target organ damage, as well as to the pathogenesis of hypertension.

1.3.5 Sympatholytic Therapy for Hypertension

1.3.5.1 Surgical Sympathectomy

Thoracolumbar sympathectomy was introduced in the 1930s for patients with severe hypertension. The aim was to recreate the vasodilatory effect seen after experimental nerve section to reduce peripheral vascular resistance and hence blood pressure. The procedure involved two stages carried out 10 days apart. A retropleural, retroperitoneal, transdiaphragmatic approach was employed through the beds of the 12th or the 11th and 12th ribs. The sympathetic trunks were removed from the eighth or ninth dorsal vertebrae through to the first or second lumbar vertebra inclusive, and the great splanchnic nerves were removed from the coeliac ganglion to the mid-thoracic level. The procedure improved mortality[78] (notably more so than best medical therapy at the time[78]) although the associated morbidity (severe orthostatic hypotension, impotence, urinary and faecal incontinence) reduced its universal acceptance. Ultimately it was to fall out of favour as pharmacological alternatives were developed.

1.3.5.2 Medical Therapy

Ganglion blockers represented the first pharmacological alternative. They compete with acetylcholine for sympathetic ganglionic cholinoceptive sites and prevent post-synaptic depolarization[79]. They have no effect on catecholamine neurotransmission at the post-ganglionic terminal or on peripheral arterioles. Increased peripheral pooling as a consequence of venous dilatation leads to decreased venous return to the heart and a fall in cardiac output; renal blood flow, GFR and cerebral blood flow are frequently diminished; skeletal muscle blood flow is unaltered, coronary flow is variable[80]. Side effects from non-specific ganglionic blockade are unfortunately numerous. Parasympathetic blockade leads to dry mucous membranes and skin and paralysis of accommodation; postural hypotension is common; constipation, paralytic ileus, urinary retention and impotence in males are also often reported[81]. Progressive pulmonary fibrosis is another potentially fatal adverse reaction[82].

The hexamethonium ganglion blockers were the first effective antihypertensive agents to be developed. Unreliable absorption from the gastrointestinal (GI) tract, exacerbated by treatment-induced impaired GI motility, meant oral therapy was unsatisfactory however. Subsequently, mecamylamine was developed; as a long-acting tertiary amine it was more reliably absorbed from the GI tract, although was variably excreted in the urine[83].

Rauwolfia alkaloids were the next sympatholytics to market. They inhibit chemical neurotransmission at post-ganglionic sympathetic nerve fibres, with resultant decrease in peripheral vascular resistance. In addition, they affect sympathetic discharge from vasomotor centres and the hypothalamus[84]. Side effects result from unopposed parasympathetic activity: bradycardia, excess salivation, nausea and diarrhoea, nasal congestion, gastric acid secretion and peptic ulcer formation. The central sympatholytic effects may also result in drowsiness, flesh and fluid weight gain, depressive states, Parkinsonian rigidity and occasionally frank psychosis[85]. The high frequency of depressive reactions coupled with the lower potency/slower onset of action limited the uptake of alkaloids.

Methyldopa acts as a competitive inhibitor in the biosynthetic pathway of catecholamines, reducing synthesis of dopamine and norepinephrine at adrenergic post-ganglionic nerve endings. It also forms the false neurotransmitter alpha-methyl-norepinephrine that fails to increase vasoconstriction[85]. Generally well tolerated, the most common side effect of methyldopa is somnolence. Sodium retention, constipation, abdominal cramps and postural hypotension also occur however, as does haemolytic anaemia[85].

Guanethidine also acts by disruption of the adrenergic post-ganglionic nerve terminals, thereby decreasing arteriolar vasoconstriction. It prevents the release of norepinephrine from the post-ganglionic nerve terminals and depleting the stores of norepinephrine at these terminals[81]. The clinical effects of guanethidine result from the sympatholytic effects on the heart and manifest as postural hypotension, often a treatment-limiting side effect. Other adverse reactions include diarrhoea, generalized muscle weakness, fluid retention, impotence and myocardial depression. Guanethidine is potent and has a notable side effect profile. Resistance to therapy has been observed in some patients. Caution must be used in combination with beta-blockade, in heart failure or renal insufficiency[81].

Monoamine oxidase inhibitors (MAOIs) act primarily on the sympathetic post-ganglionic nerve terminals. Inhibition of norepinephrine synthesis occurs due to accumulation of norepinephrine and false neurotransmitters result in attenuated signalling[81]. The effects develop after three to four weeks and GFR is slightly reduced. Several adverse reactions have been reported; hepatocellular necrosis, blood dyscrasias and optic atrophy. Symptomatic postural hypotension is a frequent and troublesome problem. Tyramine containing foods such as aged cheese can also cause serious reactions so for the most part MAOIs use is restricted[81].

Beta-adrenoreceptor-blocking agents were first described in 1958[86]; dichloroisoproterenol was potent but also possessed some native beta sympathomimetic activity. Pronethalol was therefore subsequently developed. On finding this was carcinogenic[87], propranolol became the next evolutionary step. It was a competitive beta-antagonist ten times less toxic than its predecessor that specifically blocked the positive inotropic and chronotropic effects of adrenergic stimuli[88]. It heralded one of the most important advances in cardiovascular pharmacotherapy with scores of variants developed. Beta-blockers became mainline treatment in hypertension, arrhythmia, thyrotoxicosis, hypertrophic cardiomyopathy, migraine and glaucoma[89]. Subsequent variants with selectivity for beta-1 (cardio-stimulation, lipolytic) and beta-2 (broncho- and vasodilatation) were developed[90], others with alpha-adrenergic partial antagonism were also introduced[91]. Increased beta-1 selectivity led to a lower incidence of bronchospasm with newer agents[92]. Once daily dosing was also feasible with the advent of atenolol[93].

Traditional beta-blockers reduce blood pressure via a direct reduction in cardiac output. Side effects occur in 5-10% of people with dizziness, fatigue, paraesthesia, depression and gastrointestinal disturbances commonly reported. More serious reactions including pulmonary oedema, hypotension, and heart block are fortunately rare[94]. Treatment with beta-adrenergic blockers affects diurnal sodium excretion; more is excreted at night and during the early morning hours[88]. Traditional agents can also lead to a loss of glycaemic control and dyslipidaemia. In contrast, vasodilating beta-blockers (nebivolol, labetalol and

carvedilol) lower blood pressure via a reduction of peripheral vascular resistance but have little or no effect on cardiac output. These agents can increase peripheral blood flow, which may result in improved tolerability and metabolic profiles[95].

Clonidine hydrochloride is a centrally acting sympatholytic. It has a depressive effect at the central nervous system vasomotor centre[96] resulting in a reduction of both peripheral vascular resistance and cardiac output[97]. It does not directly affect arteriolar walls nor does it influence adrenergic neurotransmission. The decrease in heart rate and stroke volume seen result from the central nervous system action of the drug rather than beta blockade of the myocardium[98]. A reduction in venous return occurs secondary to dilatation of the venous capacitance vessels, also contributing to the reduced cardiac output[99]. Renal plasma flow and GFR are preserved during long-term clonidine use[99] although propensity to sodium retention reduces the anti-hypertensive effect. Because of the negative inotropic effects, caution for use should be exercised in those with previous history of congestive heart failure or cardiomegaly. The related agent moxonidine has been shown to result in increased mortality in this population[100].

Veratrum alkaloids are also thought to cause central depression of sympathetic tone. This manifests as a reduction in peripheral vascular resistance although the mechanisms have not been completely clarified[81]. The resultant hypotension is more marked than the bradycardia that results from centrally-induced parasympathetic outflow. Cardiac output is maintained, as is renal blood flow and GFR unless the hypotensive effect is profound[81]. Unfortunately, intravenous veratrum has an extremely narrow therapeutic window before respiratory depression occurs. Severe nausea and vomiting are also frequent occurrences due to direct stimulation of the cholinergic nerves of the gut. Salivation, sweating, blurring of vision and mental confusion are not uncommon. More tolerable agents have unsurprisingly surpassed veratrum alkaloids, even for the management of hypertensive crises where they initially found favour.

Alpha-antagonists lower blood pressure by selectively blocking post-synaptic α_1-adrenoreceptors, which antagonizes catecholamine-induced constriction of the arterial and venous vascular beds[101]. The first generation of alpha-receptor antagonists, phentolamine and phenoxybenzamine, were of the nonselective variety; numerous therapy-limiting side effects resulted from their additional pre-synaptic blockade (orthostatic hypertension, tachycardia, dizziness, drowsiness, syncope, nausea and nasal congestion). In 1976, prazosin was first approved for use in the United States. It had the advantage of not interfering with the compensatory effects of α_2-adrenoreceptors, thus preventing tachycardia. First-dose syncope was an important side effect, as was salt and water overload with long-term use. Multiple daily dosing saw for the development of terazosin and doxazosin; related drugs with extended half-lives.

Alpha-blockers seem to induce a favourable lipid profile as well as reducing blood pressure. Despite this, in 2002 the doxazosin arm of the ALLHAT (Antihypertensive and Lipid-Lowering Treatment to Prevent Heart Attack Trial) study was stopped early due to an unexpectedly higher rate of cardiovascular disease, heart failure and stroke[102]. Alpha-blockers have also been linked to an increased risk of hip/femur fractures, a likely manifestation of the first-dose effect[103].

1.3.5.3 ***Endovascular Renal Denervation***

In the last decade, a novel procedure of endovascular renal denervation has been developed[104]. Unlike the traditional invasive surgical approach, this involves a percutaneous, catheter-based method whereby radiofrequency waves are applied to the endothelial surfaces of both renal arteries. The treatment causes controlled burns through to the tunica adventitia, the location of the afferent pre-ganglionic, and efferent post-ganglionic renal nervous supply. Nerve tissue is particularly sensitive to thermal injury. Post-ablation histology from pre-clinical swine studies reveals a pattern of nerve fibrosis, replacement of nerve fascicles with fibrous connective tissue, and thickening of the epineurium and perineurium[105]. In contrast, the renal arteries demonstrated fibrosis of 10-25% of the total media and underlying adventitia, with mild disruption of the external elastic lamina. Although thickened, the intima remained intact with complete endothelial coverage[105].

The first large clinical trial of endovascular renal denervation involved a case series of 45 patients with resistant hypertension[104]. This was defined as a blood pressure greater than 140/90 despite three anti-hypertensive medications (including a diuretic) at maximal tolerated dosage. A significant office-based blood pressure reduction at one-month follow-up of 14/10 mmHg was followed by a sustained response at 12 months of 27/17 mmHg. Procedural complications were limited to one renal arterial dissection due to catheter manipulation (treated successfully with renal artery stenting) and one femoral artery aneurysm.

The Symplicity-HTN 2 trial[106] then randomised 52 resistant hypertensive patients to catheter-based therapy in addition to conventional anti-hypertensive medications versus anti-hypertensive medications only. There was a significant difference in blood pressure from baseline to six month follow-up of 33/11 mmHg between treatment groups[106]. Following patient cross-over at six months, further analysis at 12 months[107] suggested the antihypertensive effect is maintained. A single femoral pseudoaneurysm occurred in the treatment group and was successfully treated with ultrasound-guided compression. Transient bradycardia arose in seven patients but no systemic side effects (such as postural hypotension or incontinence) have been reported.

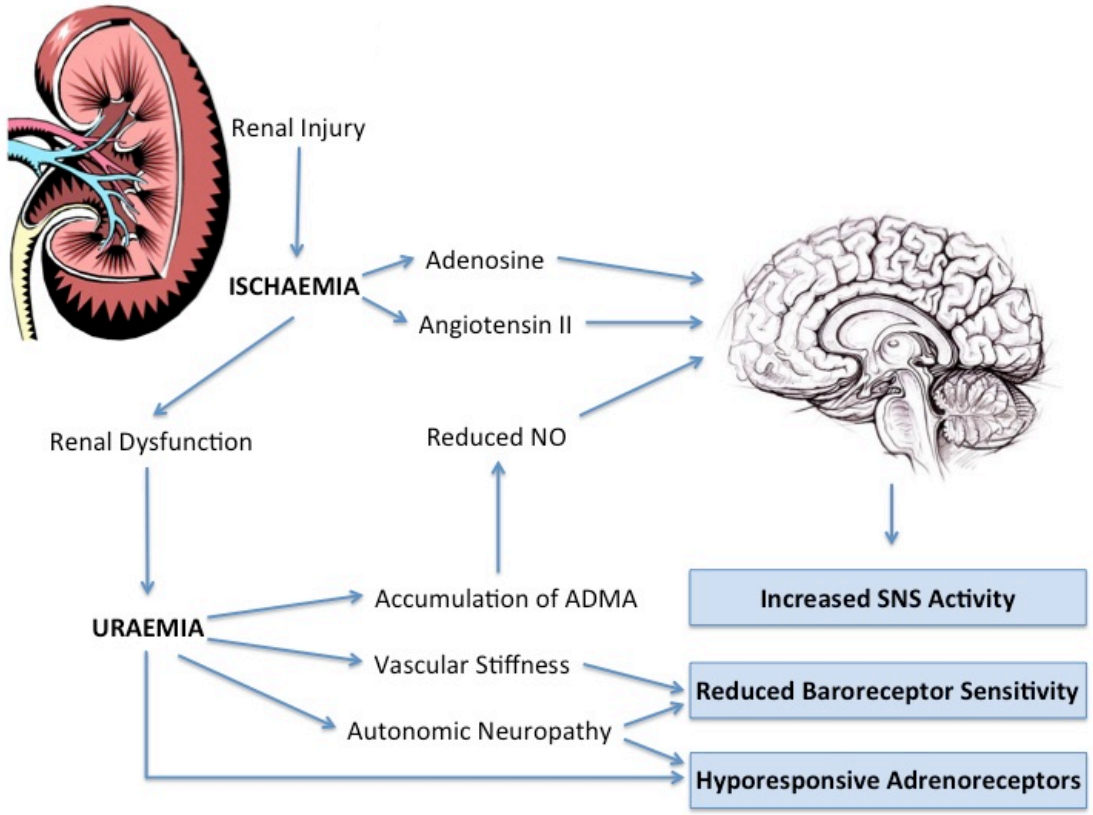
Although not universal[108], the suggestion that sympathetic hyperactivity associated with resistant hypertension is ameliorated by renal denervation[109,110] offers some compelling prospects for research. Other clinical conditions where sympathetic activity is elevated may also benefit from this novel technique over and above resultant changes in blood pressure control.

1.4 Sympathetic Hyperactivity in CKD

1.4.1 Pathophysiology

Chronic kidney disease is characterised by marked activation of the sympathetic nervous system, as evidenced by increased levels of circulating norepinephrine and an elevated number of sympathetic neural bursts recorded in the peroneal nerve via microneurography[111,112]. The residual kidneys are critically involved in the pathogenesis of the sympathetic hyperactivity (Figure 9). In fact, evidence indicates that the sympathetic hyperactivity originates in the diseased kidney; MSNA in bilaterally nephrectomised patients on dialysis is comparable with that of healthy controls and unilateral nephrectomy does not change MSNA[113].

Figure 9: *The Pathophysiology of Sympathoexcitation in Chronic Kidney Disease*[114]



Renal ischaemia is key to the pathogenesis. Ischaemia leads to sympathetic activation through the release of adenosine from proximal tubular cells[115]. Adenosine increases afferent renal nerve traffic, as can be shown during an adenosine infusion into the renal artery of uninephrectomised dogs[116]. In rats, induction of renal artery stenosis[117], partial renal ablation by arterial ligation[118] or intra-renal phenol injection[119] causes excitation of the renal afferent nerves, which results in neurogenic hypertension. Even a small injury in one kidney caused by intra-renal injection of phenol (an intervention that does not affect glomerular filtration rate but results in a local inflammatory response and scarring) leads to hypertension in association with an increased central sympathetic activity[120]. In these

animal models, dorsal rhizotomy (selective renal sympathetic denervation) results in a reduction or total prevention of hypertension. Additionally, in the phenol hypertension model, nephrectomy of the injured kidney several weeks after the induction of renal damage results in normalization of blood pressure[121]. From these experimental observations, renal injury can lead to sympathetic hyperactivity and hypertension and this hyperactivity is associated with activation of the renal afferent nerves.

Parallel activation of the renin-angiotensin system also occurs following ischaemic renal injury[113], resulting in increased peripheral and central sympathetic activity. Angiotensin II facilitates the pre-synaptic release of norepinephrine, inhibits its synaptic reuptake and enhances tissue response[71]. It also stimulates the sympathetic ganglia and adrenal medulla effecting an increase in circulating epinephrine and norepinephrine[71]. Lastly, Angiotensin II directly stimulates brainstem sympathetic signalling[71]. Aldosterone, acting via mineralocorticoid receptors, also increases sympathetic nerve activity by up-regulating the brain renin-angiotensin system components and induction of oxidative stress in the hypothalamus[122]. Consequentially there is up-regulation not only of peripheral, but also central sympathetic activity following ischaemic renal injury.

Although renal ischaemia is key, it does not represent the complete pathophysiological picture. Autonomic dysfunction is frequently observed in patients with significant chronic kidney disease[123]. This is accompanied by many cardiovascular disturbances including dysfunction of the baroreflex arc[124] and hyporesponsiveness of adrenergic receptors. It is thought that afferent baroreflex arc dysfunction (exacerbated by the stiff vessels induced by vascular hypertrophy and calcification[125]) leads to enhanced sympathetic outflow, elevated plasma norepinephrine levels[126] and resultant down-regulation of adrenoreceptors[127]. A coexistent defect in coupling between the beta-adrenoreceptor and the effector enzyme adenylyl cyclase[128] and reduced density and binding affinity of alpha receptors[127,129] has also been demonstrated. Baroreceptor and adrenoreceptor dysfunction leaves patients with an impaired ability to react to changes in blood pressure, particularly an acute hypotensive episode, despite their elevated sympathetic tone. This is particularly relevant to the response to ultrafiltration for the haemodialysis population[130,131].

Uraemia cannot solely be blamed for the sympathetic hyperactivity in ESKD patients, as activity is similarly increased in patients who have undergone renal transplantation[132]. Accumulation of asymmetric dimethyl-l-arginine with inhibition of endothelial nitric oxide synthase and hence less nitric oxide production is therefore postulated as a contributory risk factor[133]. Neuronal nitric oxide is a major component of the signal transduction pathway involved in the tonic restraint of central sympathetic outflow[134].

1.4.2 Clinical Significance

Sympathetic nerve activity is inversely correlated with estimated glomerular filtration rate (eGFR), implicating it as a causal agent in the progressive decline in kidney function seen in hypertensive CKD patients[111]. Furthermore, sympatholytic drug treatment attenuates albumin excretion in rats[135] and in patients with diabetic nephropathy[136]. Sympatholytic treatment has also been shown to prevent glomerulosclerosis in experimental hypertension[137]. Finally, selective renal sympathetic denervation improves experimental renal failure progression[138,139], an effect that is partially blood pressure independent[139]. These results would seem to indicate sympathetic hyperactivity is at least partially causal for the progression of CKD.

Pathological changes in sympathetic nervous activity also contribute to the higher incidence of sudden cardiac death in CKD and ESKD patients. Heart-rate variability (a marker of autonomic dysfunction) predicts ESKD- and CKD-related hospitalisation[140] as well as haemodialysis patient mortality[141]. Plasma norepinephrine predicts survival and incident cardiovascular events in patients with ESKD[142]. Lastly, MSNA associates with the composite of all-cause mortality and nonfatal cardiovascular events in CKD patients[143]. The mechanism for this relationship may well relate in part to left ventricular hypertrophy (LVH) and arrhythmogenesis[144]. LVH is an important, independent determinant of survival in patients receiving therapy for ESKD[145]. Sympathetic activity in CKD[146] and ESKD[147] patients correlates with left ventricular mass despite antihypertensive treatment. Endovascular renal denervation appears to reduce left ventricular mass and improves systolic and diastolic function[148,149] although there have been no studies with these outcomes in an ESKD population to date.

1.5 Endovascular Renal Denervation in CKD

Overall there are scant data concerning endovascular renal denervation in the CKD and dialysis populations. Hering et al[150] performed bilateral renal denervation in 15 patients with resistant hypertension and CKD stage 3 - 4. Mean reduction in office blood pressure was 33/19 mmHg at 12 months, night-time ambulatory blood pressure significantly decreased restoring a more physiological dipping profile and no deterioration in renal function was observed.

Kiuchi et al[151] performed denervation on 24 patients with resistant hypertension and CKD stages 2 - 4. They observed a marked reduction in office blood pressure at six months (51/20 mmHg). Ambulatory blood pressure also fell substantially (19/7 mmHg) and there was an improvement seen in both microalbuminuria and glomerular filtration rate. This is in keeping with pre-clinical studies where renal denervation has been shown to prevent glomerular hyperfiltration[152], and halt progression of renal disease[138]. Further prospective data should be sought in humans to corroborate these potentially important findings.

Similar procedures have been undertaken in ESKD patients. Although somewhat more technically challenging due to the smaller renal arteries induced by atrophic kidneys, procedures have been on the whole successful, efficacious and safe[153–155]. One case even had an observed blood pressure reduction of 76/51 mmHg at three months without report of systemic side effects[155]. The most substantial dataset thus far comes from Schlaich et al[156] who reported a case series of 12 ESKD patients, nine of whom were successfully denervated (three failed due to atrophic renal arteries). They reported a significant reduction in office systolic blood pressure although diastolic and ambulatory blood pressures were unchanged. Two of five patients had sympathetic nerve activity repeated post denervation, both demonstrated normalisation of hyperactivity. Clearly there is scope for further investigation in this population, especially if axial imaging or a renal angiogram can be obtained prior to the procedure[156].

Outside the benefits of improved blood pressure control and the potential effects on left ventricular mass and function, it appears renal denervation may also improve central arterial stiffness[157], central haemodynamics[157], baroreflex sensitivity[158] and arrhythmia frequency[159,160]. The rate of death from cardiovascular disease in younger patients on dialysis is 180 times greater than that for people in the general population of the same age, but the rate of cardiac arrest (or “sudden cardiac death”) is thousands of times greater[18]. Most of these events are believed to be due to ventricular arrhythmias[20]. By reducing such terminal events, renal denervation has great promise in reducing the incidence of sudden cardiac death in dialysis patients.

1.5.1 Further Clinical Relevance

Renal denervation has been demonstrated to disrupt afferent nerve signaling[109,110]. As well as the anti-hypertensive benefits this affords, afferent disruption has also been demonstrated to be advantageous for renal pain control. Autosomal dominant polycystic kidney disease (ADPKD) can be characterised by chronic and often severe abdominal, flank, or back pain. The enlarged cystic kidneys cause stretching of the capsule or traction on the renal pedicle, stimulating nociceptive afferent Aδ and C fibres[161]. A case study has been presented where a woman with ADPKD underwent renal denervation for resistant hypertension[161]. As well as a substantial decrease in blood pressure (office BP reduction of 44/34 at three months), she had incidental but immediate resolution of five-year chronic flank pain. A further case report demonstrated the analgesic potential of the procedure in haematuria loin pain syndrome[162], this time applied electively and unilaterally. Confirmation of these findings in prospective studies is needed.

It is not just patients with renal disease that may benefit from denervation. Sleep-disordered breathing, a common co-morbidity in dialysis patients[163], correlates with blood pressure and cardiovascular disease prevalence[164]. Obstructive sleep apnoea is also characterized by increased sympathetic activity[165] which is thought in part to be responsible for the pathophysiology. Ten patients with resistant hypertension and mild obstructive sleep apnoea underwent percutaneous catheter-based renal denervation. Six months later, eight out of the ten patients showed a reduction in apnoea-hypopnoea index (AHI) from 16.3 to 4.5 events per hour[166]. This was accompanied by significant decreases in blood pressure, plasma glucose concentration and HbA1c. The speculated mechanism for this change in AHI is an inhibition of the sympathetic nerve-mediated renal tubular sodium reabsorption throughout the nephron. As less fluid is reabsorbed, less fluid shifts from the legs to the neck with overnight recumbence and less apnoeic episodes result[167].

Changes in plasma glucose and HbA1c have been common findings post-denervation[168]. The significance of CKD in diabetes mellitus is well-established[169,170]. The specific effects of renal denervation on glucose metabolism has been studied by Mahfoud et al in 37 patients[171]. At both three and six months, patients exhibited significant decreases in systolic and diastolic blood pressure and fasting concentrations of glucose, insulin and c-peptide. The homeostasis model assessment-insulin resistance (HOMA) index was also significantly decreased. Insulin resistance links hand-in-hand with sympathetic hyperactivity; insulin resistance activates the sympathetic nervous system with the resultant overactivity inducing insulin resistance via regional haemodynamic and possibly more direct cellular effects[172]. Although there is a demonstrably bidirectional experimental relationship, observational data suggest sympathetic activation may in fact be the initial trigger[173]. Despite these encouraging data, it must be noted that Mahfoud's study was a retrospective analysis of Symplicity HTN-2 patients. Prospective data employing techniques such as the hyperinsulinaemic-euglycaemic clamp are needed before firm conclusions can be drawn in this area.

Mahfoud's group[174] have also reported reductions in blood pressure, renal resistive index and urinary albumin excretion following denervation, again without deleterious effects on glomerular filtration rate. Their study was run in parallel to the Symplicity trials so studied a resistant hypertensive population with normal (eGFR ≥ 45) renal function. As such, an extrapolation of data is needed but as markers of glomerular hyperfiltration, clear relevance to the CKD and particularly the diabetic nephropathy population is apparent.

1.5.2 *Unanswered Questions*

Endovascular renal denervation is a rapidly moving field of research. Technological advances have already yielded device progression. Multi-electrode baskets have been designed to reduce procedural time and increase procedural efficacy[175]. Radiofrequency ablation is also not the only option now, brachytherapy[176], chemical denervation[177,178], cryoablation[179] and intravascular[180] and extracorporeal[181] ultrasound have been developed as alternative techniques.

The US Food and Drug Administration asserts that there are several issues that need to be addressed before denervation can be considered outside a research environment. Despite these concerns, some have been keen to extol the wider application of the technique as first line therapy in essential hypertension[182] and make somewhat aspirational conclusions about cost-effectiveness[183]. One would remind readers of the analogy with renal angioplasty to treat hypertension in the setting of renal artery stenosis, which now has very few clinical indications[184].

Chief amongst concerns is that the long-term safety of the procedure is unproven. Registry data have been presented for patients enrolled in the early Symplicity studies out to three years post-denervation[185,186]. A reassuring (albeit manufacturer-funded) global registry exists for around 1000 patients at six months[187] although substantial published data past this point is so far lacking. Case reports have demonstrated late renal artery stenosis can occur[188] although with what frequency is yet to be established. Advanced imaging techniques also suggest the sparse pre-clinical studies do not tell the complete picture regarding the effects of ablation on the renal arteries. Demonstrable intimal damage with intraluminal thrombosis has been seen via optical coherence tomography post-procedure[189]. Dual anti-platelet therapy for between three and six months has consequently been proposed[189] with the risk of resultant renal ischaemia and micro-embolism unknown.

The efficacy of renal denervation is also unproven. Sham-procedure controls hadn't been utilised at the time of this literature review and little effort was made in the two Symplicity trials to ensure medication concordance prior to or during the study period[190]. Both call into question the validity of the blood pressure drops observed. Further to this, most studies of renal denervation have used office blood pressure as an outcome measure. By contrast, ambulatory blood pressure removes observer bias and measurement error, minimises the white coat effect, and has greater reproducibility[190]. Moreover, in a cohort of 109 treatment-resistant hypertensive patients followed up for 4.8 years, higher ambulatory blood pressure values predicted cardiovascular morbidity and mortality, whereas office blood pressure had no prognostic value[191].

The effect of possible sympathetic re-innervation remains unclear. Studies of renal transplantation suggest axonal regeneration of sympathetic nerves occurs as early as the fourth week post-surgical denervation[192], although the precise functional significance of this regrowth is less clear[193]. Most data suggest endovascular denervation remains efficacious over the medium term[185,186] although it has been reported that treatment failure occurring at 12 months was responsive to repeat denervation[194]. This suggests functional re-innervation may have occurred.

1.5.3 Symplicity HTN-3

The results of Symplicity HTN-3 were awaited with interest[195]. Such was the significance of the trial, and the implications it had for the field, the study has been retrospectively included in this literature review despite it being made public well after the commencement of this project.

A trial of 535 patients in 88 centres, Symplicity HTN-3 was prospective, single-blind, randomized and, for the first time with this technology, sham-controlled[196]. Patients with severe resistant hypertension were randomly assigned in a 2:1 ratio to undergo renal denervation or a sham procedure. The mean (±SD) change in office systolic blood pressure at 6 months was −14.13±23.93 mm Hg in the denervation group as compared with −11.74±25.94 mm Hg in the sham-procedure group (P<0.001 for both comparisons of the change from baseline), for a difference of −2.39 mm Hg (95% confidence interval [CI], −6.89 to 2.12; P = 0.26 for superiority with a margin of 5 mm Hg). The change in 24-hour ambulatory systolic blood pressure was −6.75±15.11 mm Hg in the denervation group and −4.79±17.25 mm Hg in the sham-procedure group, for a difference of −1.96 mm Hg (95% CI, −4.97 to 1.06; P = 0.98 for superiority with a margin of 2 mm Hg). There were no significant differences in safety between the two groups.

In sum, this blinded trial did not show a significant reduction of systolic blood pressure in patients with resistant hypertension 6 months after renal-artery denervation as compared with a sham control.

There have been many editorials[197] and commentaries[198] written about the trial, as well as post-hoc analysis[199], and it really has divided opinion in the denervation community. Irrespective of the findings of Symplicity HTN-3, I felt it appropriate to continue the study as the sympatholytic mechanism underlying renal denervation should confer prognostic benefit to the ESKD population, regardless of the presence or absence of a blood pressure change.

1.6 Summary & Rationale of the Study

Endovascular renal denervation offers a new and exciting therapeutic approach to resistant hypertension. It may also yield benefit as a tolerable sympatholytic and confer advantage over and above its blood pressure lowering effect. Sympathetic hyperactivity is linked both to chronic kidney disease progression and associated cardiovascular comorbidity. Applying renal denervation to a CKD population may slow the inexorable progression towards renal replacement therapy and facilitate an improvement in the dour cardiovascular prognosis patients suffer.

Dialysis patients have the highest level of sympathetic hyperactivity of all CKD patients. They also have the worst cardiovascular prognosis. It would be logical to apply this new technique to such a population with a view to improving cardiovascular outcomes. As this literature review has identified, there is scant data in this population, three case reports and one case series of nine patients. The latter was the only one to study mechanistic change (i.e. assessed sympathetic nerve activity) and that was in a mere two patients pre- and post-denervation. The complex physiological changes that occur in this population need to be fully described. This would inform the scientific community about the utility and safety of this new technique and potentially facilitate a larger study in which renal denervation can be employed in a head-to-head study with best medical care.

1.7 Aims

The aim of the study was to document the physiological changes that occur post-endovascular renal denervation in dialysis patients. Studies of the sympathetic nervous system were performed using MSNA and plasma catecholamine assays. Parallel recordings of both office and ambulatory blood pressure were implemented, along with echocardiographic assessments. Bioimpedance spectroscopy was utilised as a method for formalising volume status, a key component of a dialysis patient's blood pressure and a potential confounding factor.

1.8 Hypotheses

If dialysis patients follow a similar pattern to that demonstrated in a resistant hypertensive population with normal renal function, renal denervation should result in both efferent and afferent nerve section. Consequentially there should be a reduction in systemic MSNA and plasma norepinephrine. Blood pressure is hypothesised to reduce, a change that should occur independent of volume status. In view of reduced sympathetic tone, left ventricular mass is hypothesised to fall, a manifestation of both blood pressure reduction and reduced norepinephrine signalling.

2 GENERAL METHODS

2.1 Subjects, Ethics & Recruitment

In November 2012, all dialysis-dependent ESKD patients under the care of Southern District Health Board, New Zealand were screened. Inclusion criteria were:

i. Age over 18 years
ii. Office blood pressure > 140/90mmHg during a short-break, non-dialysis day for haemodialysis patients, despite antihypertensive treatment
iii. Intact native kidneys
iv. Dialysis therapy for at least three months
v. Clinical stability for at least three months, i.e. no evidence of fluid overload or myocardial ischaemia, no change in antihypertensive therapy and no change in dialysis prescription

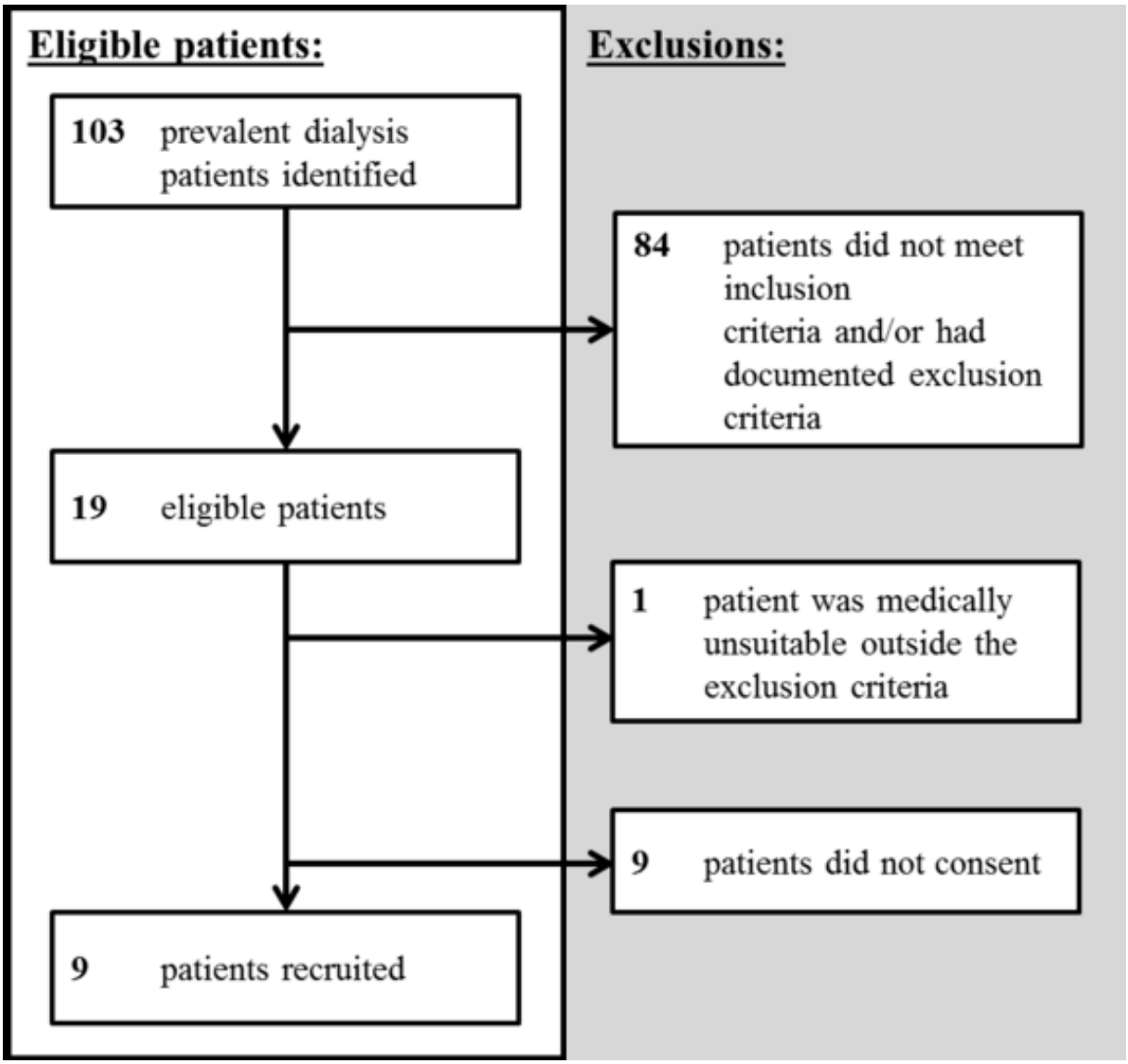
Exclusion criteria were:

i. Previous renal transplantation
ii. Significant renovascular abnormalities identified at the time of renal angiography (multiple main renal arteries or short length main renal arteries)
iii. Severe vascular disease
iv. Inability to provide consent

Nine ESKD (six haemodialysis and three peritoneal dialysis) patients were recruited into this proof-of-concept study.

2.2 Trial Profile

Figure 10: *Trial Profile*



This study was an investigator initiated and analysed study, independent to St. Jude Medical, Inc. The study was approved by the Lower South Health and Disability Ethics Committee, New Zealand (reference LRS/12/05/012), registered with the ANZCTR (ACTRN12613000562774) and complied with the Declaration of Helsinki. Patients gave written informed consent prior to participation.

2.3 Measures

All variables (unless stated) were assessed prior to (baseline), and at one month (1M), three months (3M), and twelve months (12M) post-RDN. At all time-points, measurements were taken on a short break non-dialysis day for haemodialysis patients.

2.3.1 Blood Pressure

Office BP was measured in triplicate using an automated BP system (Connex® ProBP™ 3400 series, WelchAllyn, NY) according to guidelines[60]. Ambulatory BP monitoring (Oscar2 Blood Pressure Monitoring System, SunTech Medical, Inc., Morrisville, NC) was performed every 20 minutes throughout the day and every 45 minutes at night, prior to offline analysis. This allowed for calculation of systolic and diastolic BP for the daytime, night-time and 24-hour periods, as well as nocturnal dipping status and the collection of ambulatory heart rate (AccuWin Pro, v3.4, SunTech Medical, Inc., Morrisville, NC). Patients lacking the physiologic decline in nocturnal SBP of at least 10% from daytime values were termed *non-dippers*, while those with normal diurnal BP variation were termed *dippers*[200,201].

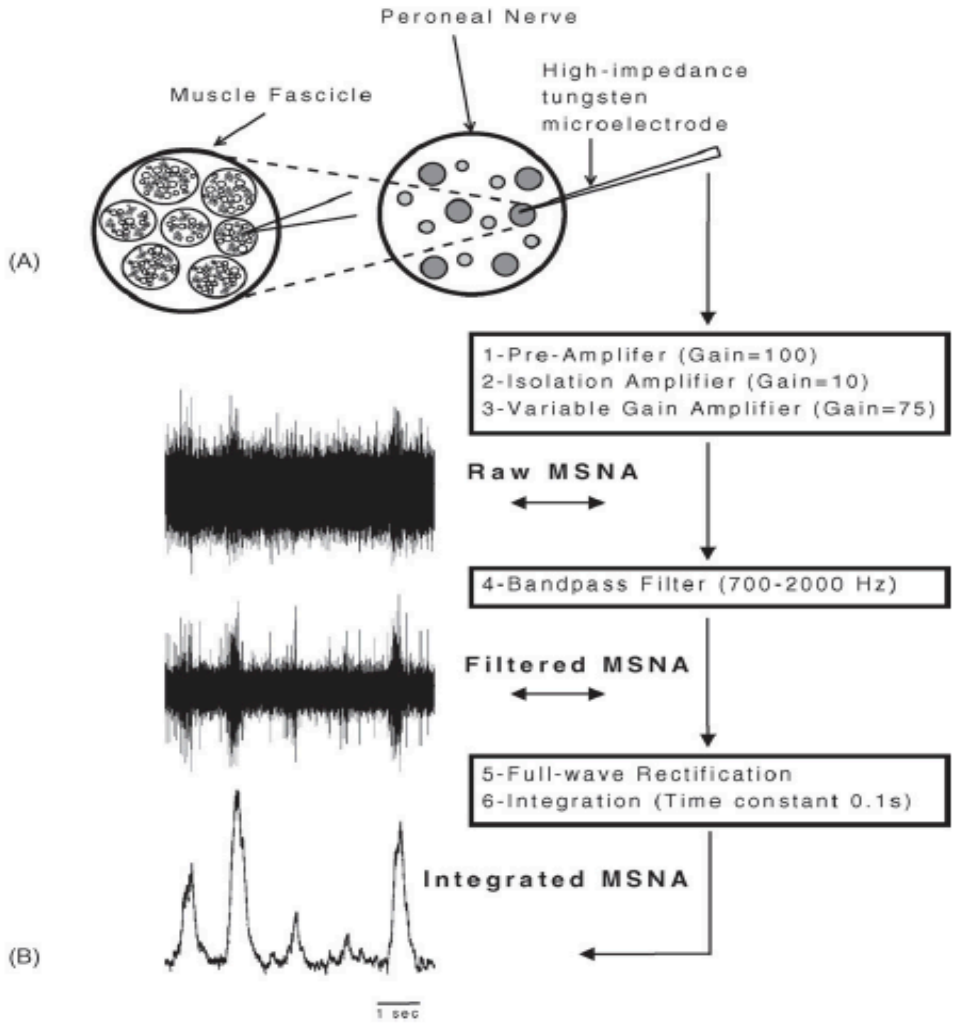
2.3.2 Microneurography

Multiunit MSNA was measured by a single investigator in the supine position using microneurography[47,202,203]. A tungsten microelectrode (200µm in diameter in the shaft, tapering to an uninsulated tip of 1 - 5µm) was inserted into the right peroneal nerve and manipulated until a satisfactory MSNA signal was obtained. A reference electrode was inserted subcutaneously 1 - 3 cm from the recording electrode. The electrodes were connected to a preamplifier with a gain of 100, an isolation amplifier with a gain of 10 and a variable gain amplifier with a gain of 75. The neural activity was then fed through a bandpass filter with a bandwidth of 700 - 2000 Hz and integrated with a time constant of 0.1s (Nerve Traffic Analyser 662C-4; Engineering Electronics Shop, University of Iowa, IA). The nerve signal was also routed to a loudspeaker and a computer for monitoring throughout the study. Beat-to-beat blood pressure (Finger-photoplethysmography; Finometer MIDI, Finapres Medical Systems, Enschede, the Netherlands) was simultaneously and continuously measured on the left side or contralateral side to patients' haemodialysis access. Respiratory rate (thoracic respiratory belt transducer, MTL1132; ADInstruments, Dunedin, New Zealand) and the heart's electrical activity (electrocardiography, lead II position; FE132; ADInstruments) were also continuously measured. Once a satisfactory signal had been obtained, the patient could rest for 15 minutes to reach steady-state, before 10 minutes of MSNA data was recorded during normal restful breathing. The data was sampled at 10 kHz with an analog-to-digital converter (PL3508/P; ADInstruments) and recorded via software (Labchart Pro v7.3; ADInstruments) for offline analysis by a blinded observer. Burst occurrence was confirmed by visually inspecting the corresponding raw neurogram and hence MSNA burst frequency ($MSNA_{frequency}$; bursts/min) and incidence ($MSNA_{incidence}$; bursts per 100 heart beats) were calculated.[47,202,203]

Figure 11: *Microneurography Recordings*[204]

(A) Schematic representation of a microelectrode inserted into a human peripheral nerve for sympathetic recordings. The nerve contains bundles of sympathetic nerves that target blood vessels in muscle or skin.

(B) Representative examples of the raw, filtered and integrated muscle sympathetic nerve activity.



There were three criteria for an acceptable recording of MSNA:

i. weak electrical stimulation (1-3 V; 0.2 msec; 1 Hz) through the electrode in the peroneal nerve elicited involuntary muscle contraction (muscle nerve fascicle) but not paraesthesia (cutaneous nerve fascicle).
ii. tapping or stretching the muscles or tendons supplied by the impaled fascicle elicited afferent mechanoreceptor discharges, whereas stroking skin in the distribution of the peroneal nerve did not evoke afferent discharges.
iii. the neurogram revealed spontaneous, intermittent, pulse-synchronous bursts that increased during held expiration and Phases 2 and 3 of a Valsalva manoeuver, characteristic of MSNA.

Evidence that such activity represents efferent sympathetic activity has derived from earlier studies and includes:

i. interruption of the activity by local nerve block proximal but not distal to the recording site
ii. elimination of the activity by ganglionic blockade
iii. conduction velocity approximating 1 m/s [43].

Neurograms that revealed spontaneous activity characteristic of cutaneous sympathetic activity were not accepted. Inadvertent contraction of the leg muscles adjacent to the recording electrode produces electromyographic activity, which causes a sudden rise in baseline noise level on the mean voltage neurogram and produces a characteristic repetitive firing that is evident on both the filtered neurogram and the audio display. These electromyographic artefacts were readily distinguished from sympathetic bursts.

2.3.3 Catecholamine Analysis

A blood sample was drawn from each patient into several Vacutainers (Becton, Dickinson & Company, New Jersey, USA) containing ethylenediaminetetraacetic (EDTA) acid solution. The suspensions were centrifuged at 1500g and 4^{o}C for 15 minutes before the plasma supernatants were removed and frozen at -80^{o}C. The frozen samples were couriered in a single batch to the Christchurch Cardioendocrine Research Group on dry ice for analysis.

For assay[205,206], the plasma was extracted on alumina and catecholamines were eluted with acetic acid. The extracted catecholamines were separated on a Beckman Ultrasphere 4.6 x 250mm 5µm reverse phase column using a Shimadzu HPLC system with an ESA Coulochem-II Electrochemical detector.

The reference range based on 45 healthy subjects selected at random from the Christchurch electoral roll is:

- Norepinephrine 470 – 3800 pmol/L
- Epinephrine <570 pmol/L[207].

2.3.4 Echocardiograph Recording

Echocardiography was performed at baseline, 3M and 12M in the left decubitus position. A Vivid E9 ultrasound machine with a M5S 1.5 - 4.6 MHz matrix array probe was used according to guidelines[208,209]. Analysis was performed using EchoPAC^{TM} (Version 112, GE Healthcare, Horten, Norway). Each representative value was obtained from the average of three measurements. All examinations were performed and reported by an experienced research cardiac technologist blinded to the denervation status of participants.

Left ventricular mass was calculated from LV linear dimensions using the Devereux formula[210,211]. LV mass was indexed to the body surface area and LVH was considered present when the LV mass exceeded 115 g/m^2 for men and 95 g/m^2 for women[210].

2.3.5 Body Composition Monitoring

Body composition monitor (BCM) measurements were performed using a portable whole body bioimpedance spectroscopy device, the BCM (Fresenius Medical Care, Bad Homburg, Germany). The BCM measures the impedance spectroscopy at 50 different frequencies between 5 kHz and 1 MHz and has previously been validated against all available gold-standard methods for fluid volume and body composition assessment[212]. Electrodes were attached to one hand and one foot at the ipsilateral side. Due to bio-physical reasons, bio-impedance spectroscopy does not measure sequestered fluid in the trunk[213]. Presence or absence of PD fluid in the abdomen does not therefore influence the readings of hydration status. For determination of weight, the weight adjusted for an empty abdomen was used. Extracellular water (ECW), intracellular water (ICW) and total body water (TBW) were determined from the measured impedance data following the model of Moissl et al[214]. Reproducibility of BCM derived parameters is high, with a coefficient of variation for the inter-observer variability ECW and TBW around 1.2%[215]. Therefore, only one BCM measurement was performed in each patient per visit.

2.4 Renal Denervation Procedure

Patients were taken to the catheterisation laboratory to undergo renal denervation. After administering conscious sedation and local anaesthesia, an 8-French guiding catheter sheath was inserted using fluoroscopic guidance to engage each main renal artery in turn. Intravenous heparin was administered (100IU/kg) although activated clotting time monitoring was not mandated. Images of the left and right main renal arteries were recorded using non-ionic contrast and the diameter and length of each of the main renal arteries measured. An appropriate basket size was subsequently chosen and the renal denervation catheter was inserted such that the catheter's tip was proximal to the bifurcation of one of the main renal arteries. The basket on the catheter was then opened with the impedance of each electrode on the basket monitored.

2.4.1 EnligHTN™ Renal Denervation System

The St Jude Medical EnligHTN™ renal denervation system used in this study consists of the following main components: the EnligHTN™ Ablation Catheter and EnligHTN™ Generator (Model ENL-T115 with Software 3.020). The EnligHTN™ Ablation Catheter (St Jude Medical, St Paul, MN, USA) was designed with an expandable electrode basket of four platinum–iridium ablation electrodes. The electrodes deliver low-level radiofrequency (RF) energy to the renal arterial wall. The distal segment of the ablation catheter is deflectable to assist basket positioning. The expandable feature of the basket and the deflectable distal catheter section establish good apposition between the ablation electrodes and the target ablation sites in the renal artery. Each electrode has a temperature sensor to monitor the temperature at the ablation site.

The EnligHTN™ RF Ablation Generator delivers RF energy to the EnligHTN™ Renal Artery Ablation Catheter using a proprietary algorithm. Each electrode on the ablation catheter has a corresponding display channel on the generator. The generator channels facilitate control and monitoring of the ablation process. It consists of four independent channels, which simultaneously monitor the temperature of each of the four ablation electrodes and adjusts the magnitude of the RF output power within the programmed maximum magnitude (6 W per electrode) to achieve and maintain the desired temperature (75°C) at each ablation site. The generator has built-in safety features, which include a self-test at power-up and automatic RF power shut-off if the measured tissue impedance is <50 Ohm or exceeds 400 Ohm or the temperature exceeds the setting by >5°C for >3 s or exceeds 80°C.

2.4.2 Renal Artery Ablation

There were two sizes of the EnligHTN™ Ablation Catheter available for use in the study. The small size basket is designed for renal artery diameters between 4 and 6 mm, and the large size basket is designed for renal artery diameters between 5.5 and 8 mm. After renal artery engagement and completion of a renal angiogram, the EnligHTN™ Ablation Catheter was inserted into the renal artery with the tip of the catheter positioned proximal to the bifurcation and the corresponding images recorded. The basket on the EnligHTN™ Ablation Catheter was then opened with the impedance of each electrode on the basket monitored. Renal artery denervation was commenced and performed simultaneously by all four electrodes with the impedance, temperature, and RF energy delivery monitored. The basket was then collapsed and pulled back a sufficient distance (~1 cm) to avoid lesion overlap, rotated ~45° and then expanded. Placement was confirmed under fluoroscopy and the ablation procedure was repeated. A minimum of four to maximum of eight ablation sites were performed in each main renal artery, with each simultaneous ablation lasting 60 seconds. In general, eight ablations were attempted per renal artery to achieve circumferential ablation. Images of the renal artery were taken using non-ionic contrast and checked for signs of renal artery irregularities (i.e. vasospasm, stenosis, or dissection). The renal artery ablation procedure was then repeated for the other renal artery, and the catheter was withdrawn. Finally, the sheath was removed and haemostasis achieved via insertion of an Angio-Seal^{TM} device (St Jude Medical, St Paul, MN, USA). Procedural data were recorded for each patient, including procedure duration and number of ablations delivered.

2.4.3 Post-Procedure and Pre-Discharge

Upon completion of the renal denervation procedure, the patient was moved to a recovery area and vital signs were monitored. BP was measured every 30 minutes during the first two hours post-procedure and then in four-hour intervals until discharge. Patients were discharged from the hospital on the following day (after inpatient haemodialysis if appropriate).

2.5 Statistical Analysis

Statistical analyses were done by a biostatistician using Stata® Statistical Software, version 13.1 (StataCorp LP, College Station, TX). The effect of RDN was explored by calculating each main dependent variable change from baseline to 1M, 3M, and 12M (i.e. change of interest = 1M – Baseline). The mean changes at each of these time-points were derived with associated 95% confidence intervals (CI) by bootstrapping[216] with 200 replicates. Bootstrapping is a resampling (i.e. a single replicate) procedure with replacement, where the mean is calculated from each resample. Thus 200 means were used to estimate the overall mean and CI. It provides a non-parametric way of estimating statistical quantities when other available formulas make inappropriate assumptions. Statistical significance was identified if the entire 95% CI were positive or negative (i.e. did not contain zero), at a level of $P < 0.05$. This approach was used due to the small sample size and to maximise the statistical power of the study with variations in the sample size during follow-up. In addition, the proportion of patients with a clinically significant reduction in office BP (defined as a ≥10 mm Hg systolic and ≥5 mm Hg diastolic reduction, respectively) and ambulatory BP (defined as a ≥5 mm Hg systolic and ≥2.5 mm Hg diastolic reduction, respectively) following RDN were calculated.

3 RESULTS

Patient demography and baseline characteristics for the study sample are described in Table 1. Causes of ESKD were glomerulonephritis (n = 4), polycystic kidney disease (n = 2), diabetes mellitus (n = 2), and of unknown origin (n = 1). The median dialysis vintage was 38 months (range: 11 – 110). All patients were on anti-hypertensive therapy with a median of 3 (range: 1 – 4) agents.

Table 1: *Baseline Characteristics of the Patients*

Variable	
Demographics:	
Age (y)	59 ± 9
Weight (kg)	74 ± 12
Height (cm)	172 ± 8
BMI ($kg \cdot m^{-2}$)	25 ± 2
Dialysis vintage (months)	38† (range: 11 – 110)
Male sex	8 (89%)
White ethnicity	8 (89%)
Anti-hypertensive drugs (n)	3† (range: 1 – 4)
Sympathetic nervous system activity:	
$MSNA_{frequency}$ ($bursts \cdot min^{-1}$; n = 7)	59 ± 12 (≤42*¥)
$MSNA_{incidence}$ (bursts·100 heart $beats^{-1}$; n = 7)	85 ± 9 (40 ± 22*)
Plasma epinephrine ($pmol \cdot L^{-1}$; n = 8)	211 ± 65 (360§)
Plasma norepinephrine ($pmol \cdot L^{-1}$)	5671 ± 3851 (3380§)
Echocardiography:	
LV mass ($g \cdot m^{-2}$)	169 ± 40
LV EDV (mL)	169 ± 59
LV ESV (mL)	103 ± 54
LV EF (%)	42 ± 15
GLS (%; n = 8)	-13 ± 4
TAPSE (mm)	20 ± 5
Diastolic Dysfunction Grade	2.0 ± 0.7
Left atrial diameter (mm)	45 ± 6
Indexed IVC size ($mm \cdot m^{-2}$)	11 ± 3
IVC collapsibility index	0.44 ± 0.20
Blood pressure:	
Ambulatory SBP (mm Hg)	173 ± 19
Ambulatory DBP (mm Hg)	92 ± 11
Day-time SBP (mm Hg)	173 ± 19
Day-time DBP (mm Hg)	91 ± 13
Night-time SBP (mm Hg)	175 ± 18
Night-time DBP (mm Hg)	91 ± 8
Office SBP (mm Hg)	179 ± 28
Office DBP (mm Hg)	90 ± 17
Heart rate ($beats \cdot min^{-1}$)	71 ± 9
Body composition:	
Total body water (L; n = 8)	43 ± 5
Overhydration (L; n = 8)	3.2 ± 1.7

Values are presented as mean ± SD from 9 patients (unless stated).

BMI; Body Mass Index, SBP; Systolic Blood Pressure, DBP; Diastolic Blood Pressure, MSNA; Multi-unit Muscle Sympathetic Nervous Activity, IVC; Inferior Vena Cava, TAPSE; Tricuspid Annular Plane Systolic Excursion, LV; Left Ventricle, EDV; End-diastolic Volume, ESV; End-systolic Volume, EF; Ejection Fraction, and GLS; Global Longitudinal Strain.

† Data expressed as median and range.

Normative value for MSNA in healthy male and female as previously described by *Narkiewicz et al. 2005[165] and *¥Hering et al. 2014[217]; Note as $MSNA_{frequency}$ data is separated for sex and by age therefore the highest normative value is presented. For $MSNA_{incidence}$ this approach was not presented, so the highest group mean ± SD is presented.

§ Upper limit of the normal range in 67 normotensive patients described by Lenders et al. 1995[218].

Individual patient measures at each time-point are presented in Table 2. At baseline six patients had all measures assessed. The two diabetic patients had unsuccessful MSNA measurement, therefore they were not reassessed in follow-up. Another patient was not assessed with BCM therefore this was not reassessed in follow-up. At 1M eight patients were fully assessed with their repeated baseline measures. One patient temporarily withdrew from the study due to a myocardial infarction, but returned to the study at 3M. At 3M seven patients were fully assessed with their repeated baseline measures. One patient did not consent to MSNA and another to echocardiographic measurements. At 12M three patients were fully assessed with their repeated baseline measures. One patient had an unsuccessful MSNA assessment. Another patient had died. The other four patients did not consent to all measures. Each patient is therefore presented with a unique figure symbol that is consistent across all figures and panels.

Table 2: *Measurements Completed for the Individual Patients Across the Study*

Patient	Baseline	1M	3M	12M
●	All assessments completed.	All assessments completed*.	All assessments completed.	All assessments completed.
■	BCM not completed. All other assessments complete.	All assessments completed*.	All assessments completed.	Did not consent to Ambulatory BP, MSNA, and blood sampling. All other assessments complete.
▲	All assessments completed.	Temporarily withdrew study consent to recover from MI.	Did not consent to MSNA. All other assessments complete.	Unsuccessful attempt at MSNA. All other assessments complete.
▼	Unsuccessful attempt at MSNA and not accessed in follow-up. All other assessments complete.	All other assessments complete*.	All other assessments complete.	*Died at 10 months (presumed cardiac event)*
◆	All assessments completed†.	All assessments completed*†.	All assessments completed†.	Did not consent to MSNA and blood sampling. All other assessments completed.
△	All assessments completed.	All assessments completed*.	All assessments completed.	All assessments completed†γ.
○	All assessments completed.	All assessments completed*.	All assessments completed.	All assessments completed.
□	All assessments completed.	All assessments completed*.	All assessments completed.	Did not consent to Ambulatory BP, BCM, MSNA, and blood sampling. All other assessments complete.
▽	Unsuccessful attempt at MSNA and not accessed in follow-up. All other assessments complete‡.	All other assessments complete*.	Did not consent to echocardiographic measures. All other assessments complete.	Did not consent to Ambulatory BP, BCM, and blood sampling. All other assessments complete.

BCM; Body composition monitoring.

*Echocardiographic measures were not assessed in any patient at 1M by experimental design as stated in the methods.

‡Global longitudinal strain not obtained.

† epinephrine was not detected.

γ norepinephrine was not detected.

3.1 Renal Denervation Procedure

All nine patients had a technically successful RDN. A total of 143 ablation sites out of 144 were completed to 60 s in this cohort, with 142 (eight in all but two vessels) performed with acceptable temperature rises and impedances. During all procedures, the catheter and electrodes functioned appropriately. A single renal artery was present in all the study patients; mean vessel diameter was 4.7 mm (SD: 1.5mm) as measured by renal angiography. Angio-Seal™ (St. Jude Medical, Inc., St Paul, MN) was deployed in all patients post-procedure.

3.2 Safety

One patient sustained a severe pseudoaneurysm post-procedure that needed surgical management. One week post-procedure, six of nine had palpable femoral haematomas despite the application of Angio-Seal™. One patient suffered a myocardial infarction four days post-RDN and another died in the period between 3M and 12M from dialysis-related complications.

3.3 Follow-up

The changes in the main dependent variables from baseline are presented in Figure 12.

3.3.1 Sympatholytic Effect:

In all patients marked sympathetic activation was evident at baseline, as indicated by elevated $MSNA_{frequency}$ and $MSNA_{incidence}$ as well as elevated plasma norepinephrine levels (Table 1). A clear mean reduction in $MSNA_{frequency}$ of 20% (SD: 5%) and $MSNA_{incidence}$ of 17% (SD: 8%) was seen in the three patients successfully measured at 12M (unsuccessful in one patient); these changes were not evident at 1M or 3M. Despite this reduction in MSNA, circulating plasma norepinephrine and epinephrine concentrations did not fall.

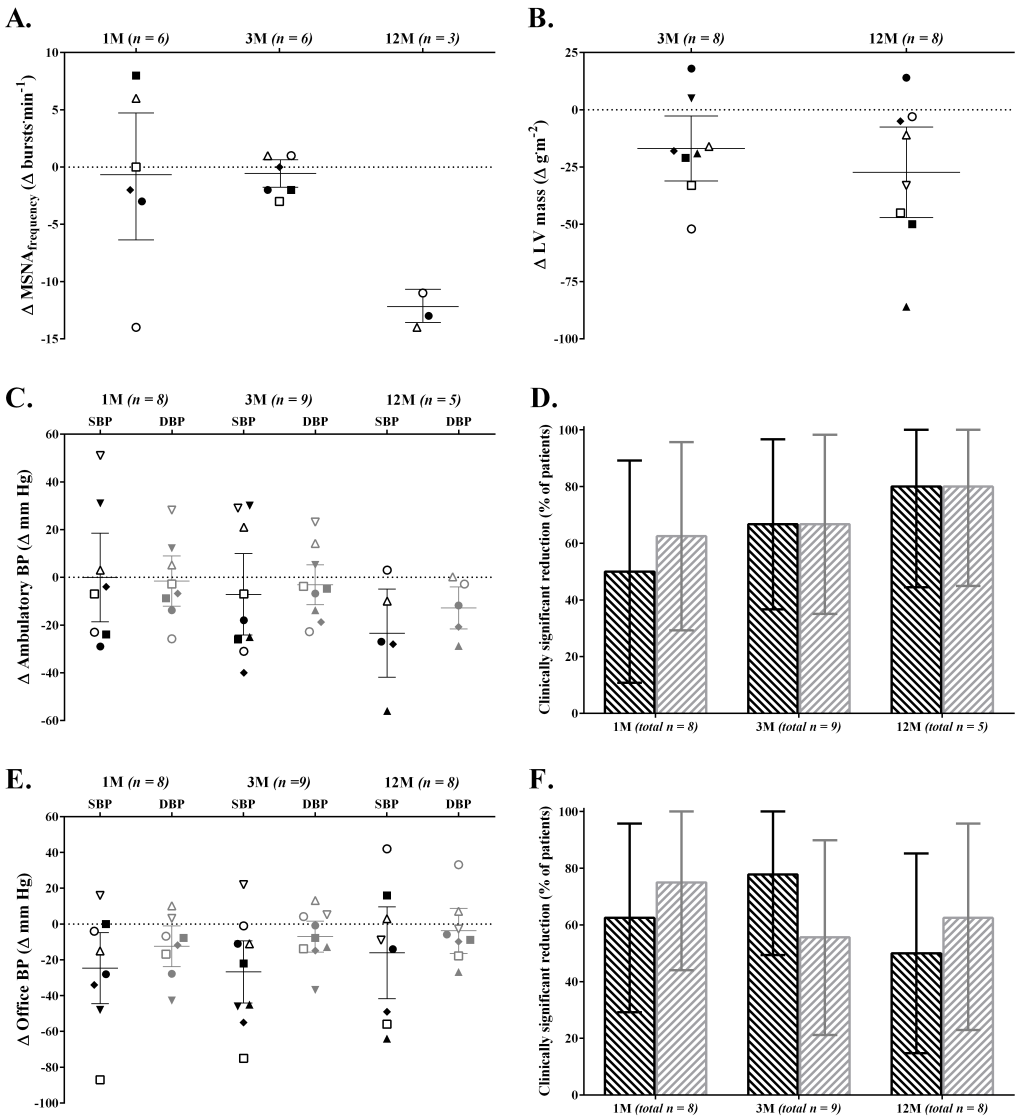
3.3.2 Left Ventricular Mass:

At baseline LV mass in eight out of nine patients met the American Society of Echocardiography 2015 guidelines criteria for LVH[210]. Following RDN mean LV mass was reduced by 8% (SD: 14%) at 3M and by 13% (SD: 19%) at 12M, indicating regression of LVH.

3.3.3 Blood Pressure:

All patients were hypertensive at baseline with no evidence of nocturnal dipping (Table 1). Anti-hypertensive therapy was kept constant after RDN, unless change was medically indicated (Table 4). Following RDN, mean ambulatory (24-hour period) BP demonstrated a gradual and evolving reduction which became significant at 12M, with systolic and diastolic BP both being reduced by 14% (SD: 13%). A parallel pattern in mean night-time BP was observed, with systolic and diastolic BP being reduced by 16% (SD: 11%) and by 15% (SD: 12%) respectively at 12M. Nocturnal dipping pattern improved from 0% of patients at baseline to 40% of patients at 12M. Mean office systolic and diastolic BP fell by 12% (SD: 16%) and by 13% (SD: 16%) respectively, as early as 1M, but this effect waned at 12M. In both ambulatory and office BP, clinically significant reductions were observed in at least 50% of patients out to 12M. Ambulatory heart rate remained unchanged.

Figure 12: *Changes in the Main Dependent Variables from Baseline*



(A) Change in muscle sympathetic nervous activity (MSNA) burst frequency.
(B) Change in left ventricular (LV) mass.
(C) Change in ambulatory blood pressure (BP); systolic BP ([SBP], black symbols and lines) and diastolic BP ([DBP], grey symbols and lines).
(D) Proportion of patients with a clinically significant reduction in ambulatory BP (i.e. ≥5 mm Hg systolic [black bars] and ≥2.5 mm Hg diastolic BP [grey bars] reduction, respectively).
(E) Change in office BP; SBP (black symbols and lines), and DBP (grey symbols and lines).
(F) Proportion of patients with a clinically significant reduction in office BP (i.e. ≥10 mm Hg systolic [black bars] and ≥5 mm Hg diastolic BP [grey bars] reduction, respectively).

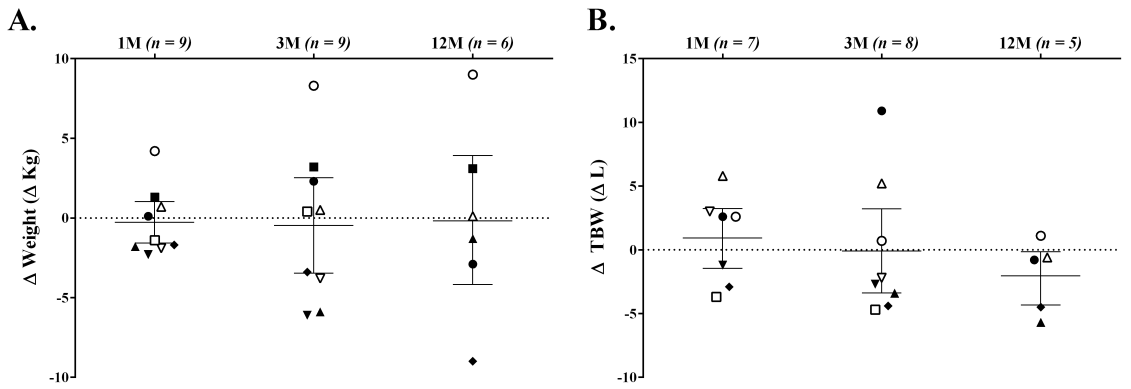
Except in (D) and (F), values are shown for individual patients, each represented by a unique symbol consistent across all panels, with mean and bootstrapped 95% confidence intervals overlaid and statistical significance identified if the entire 95% confidence interval was positive or negative (i.e. did not contain 0), at a level of $P < 0.05$.

1M, 1 month; 3M, 3 months; 12M, 12 months.

3.3.4 Volume Status:

At baseline, clinical measures of hypervolaemia were not overtly present, but BCM assessed mean fluid status as 3.2 L (SD: 1.7 L) over-hydrated, suggesting a degree of masked overhydration. Following RDN, individual body weights varied but as a group body weight did not change (Figure 13). Mean total body water as assessed by BCM was moderately reduced by 5% (SD: 6%) at 12M, but not at 1M or 3M. This occurred despite no change in dialysis ultrafiltration prescription or dry weight.

Figure 13: *Change in Volume Status from Baseline*



(A) Change in weight and (B) total body water (TBW) following renal denervation. Values are shown for individual patients, each represented by a unique symbol consistent across both panels, with means and bootstrapped 95% confidence intervals overlaid, and statistical significance identified if the entire 95% confidence interval was positive or negative (i.e. did not contain 0), at a level of $P < 0.05$.

1M, 1 month; 3M, 3 months; 12M, 12 months.

Assessment of inferior vena caval size gave a further assessment of volume status, most specifically the extracellular, intravascular compartment:

Table 3: *Change in Inferior Vena Caval Parameters*

Patient	Baseline				3M				12M			
	IVC_{Max} (mm)	IVC_{Min} (mm)	VCD (mm/m^2)	IVC-CI	IVC_{Max} (mm)	IVC_{Min} (mm)	VCD (mm/m^2)	IVC-CI	IVC_{Max} (mm)	IVC_{Min} (mm)	VCD (mm/m^2)	IVC-CI
●	17.00	8.00	8.20	0.53	11.00	6.00	5.25	0.46	15.00	5.00	7.40	0.67
■	20.00	13.00	12.85	0.35	22.00	16.00	13.81	0.27	19.00	11.00	11.99	0.42
▲	23.00	9.00	10.61	0.61	15.00	6.00	7.11	0.60	9.00	5.00	4.17	0.44
▼	24.00	22.70	12.75	0.05	17.00	8.00	9.34	0.53				
◆	14.00	6.00	7.82	0.57	9.00	4.00	5.13	0.56	11.00	7.00	6.47	0.36
△	20.00	6.00	11.57	0.70	19.00	14.00	10.95	0.26	18.00	11.00	10.27	0.39
○	25.00	14.00	12.66	0.44	21.00	7.00	10.19	0.67	21.00	12.00	10.11	0.43
□	18.00	9.00	10.18	0.50	16.00	9.00	9.02	0.44	10.00	4.00	5.61	0.60
▽	30.00	23.00	16.04	0.23					27.00	25.00	15.07	0.07
Mean	21.22	12.30	11.41	0.44	16.25	8.75	8.85	0.47	16.25	10.00	8.89	0.42
Standard Deviation	4.82	6.57	2.56	0.20	4.56	4.17	2.95	0.15	6.21	6.82	3.63	0.18
Change from Baseline					-4.97	-3.55	-2.56	0.03	-4.97	-2.30	-2.52	-0.02
Paired T-test (to baseline)					**0.01**	0.38	**0.01**	0.97	**0.02**	0.43	**0.01**	0.28

IVC, inferior vena cava; IVC_{Max}, maximum IVC diameter; IVC_{Min}, minimum IVC diameter; VCD, indexed IVC Size; IVC-CI, IVC collapsibility index

Statistically significant results are bolded

Table 4: *Prescribed Anti-Hypertensive Therapy*

Patient	Baseline		1M		3M		12M	
	Agent	**Dose**	**Agent**	**Dose**	**Agent**	**Dose**	**Agent**	**Dose**
●	Metoprolol Cilazapril Furosemide	47.5 mg (OD) 5 mg (OD) 250 mg (OD)	Metoprolol Cilazapril Furosemide	47.5 mg (OD) 5 mg (OD) 250 mg (OD)	Metoprolol Cilazapril Furosemide	47.5 mg (OD) 5 mg (OD) 250 mg (OD)	Metoprolol Furosemide	47.5 mg (OD) 250 mg (OD)
■	Metoprolol CR	47.5 mg (OD)	Metoprolol CR	47.5 mg (OD)	Metoprolol CR	47.5 mg (OD)	Metoprolol CR	47.5 mg (OD)
▲	Metoprolol Candesartan	95 mg (OD) 32 mg (OD)	Metoprolol Candesartan	95 mg (OD) 32 mg (OD)	Metoprolol Candesartan	95 mg (OD) 32 mg (OD)	Metoprolol Candesartan	95 mg (OD) 32 mg (OD)
▼	Carvedilol Furosemide Spironolactone Felodipine	25 mg (BD) 160 mg (BD) 25 mg (OD) 5 mg (OD)	Carvedilol Furosemide Spironolactone Felodipine	25 mg (BD) 160 mg (BD) 25 mg (OD) 5 mg (OD)	Carvedilol Furosemide Spironolactone Felodipine	25 mg (BD) 160 mg (BD) 25 mg (OD) 5 mg (OD)	*Died at 10 months* *(presumed cardiac event)*	
◆	Metoprolol Doxazosin Furosemide	47.5 mg (BD) 4 mg (OD) 250 mg (OD)	Metoprolol Doxazosin Furosemide	47.5 mg (BD) 4 mg (OD) 250 mg (OD)	Metoprolol Doxazosin Furosemide	47.5 mg (BD) 4 mg (OD) 250 mg (OD)	 Doxazosin	 4 mg (OD)
△	Metoprolol	95 mg (OD)	Metoprolol	95 mg (OD)	Metoprolol	95 mg (OD)	Metoprolol **Felodipine**	95 mg (OD) **5 mg (BD)**
○	Enalapril Furosemide	10 mg (BD) 40 mg (OD)	Enalapril Furosemide	10 mg (BD) 40 mg (OD)	Enalapril Furosemide	10 mg (BD) 40 mg (OD)	Enalapril Furosemide	10 mg (BD) 40 mg (OD)
□	Carvedilol Quinapril Losartan	25 mg (BD) 20 mg (BD) 12.5 mg (OD)	Carvedilol Quinapril Losartan	25 mg (BD) 20 mg (BD) 12.5 mg (OD)	Carvedilol Quinapril Losartan	25 mg (BD) 20 mg (BD) 12.5 mg (OD)	Carvedilol Quinapril Losartan	25 mg (BD) 20 mg (BD) 12.5 mg (OD)
▽	Carvedilol Cilazapril	12.5 mg (BD) 1 mg (OD)	Carvedilol Cilazapril	12.5 mg (BD) 1 mg (OD)	Carvedilol Cilazapril	12.5 mg (BD) 1 mg (OD)	Carvedilol Cilazapril	12.5 mg (BD) 1 mg (OD)

Additional therapy is bolded.

4 DISCUSSION

This clinical proof-of-concept study has demonstrated that endovascular RDN is feasible and potentially efficacious in dialysis-dependent ESKD. Denervation led to reductions in both MSNA and LV mass; ambulatory and office BP also improved over the duration of the study. These promising findings were observed despite the selection of an extremely high cardiovascular risk ESKD cohort.

4.1 Sympatholytic Effect

In our ESKD cohort, RDN had a promising sympatholytic effect. At baseline, MSNA was classified as moderately to extremely elevated[165,217]. It was significantly reduced by 12M in the three patients in whom repeat measurement was possible.

The direct prognostic benefit of lowering MSNA in clinical cohorts including ESKD is unknown. Reducing sympathetic burden in ESKD (albeit indicated by other measures) does lower all-cause and cardiovascular mortality[219]. An elevation in $MSNA_{frequency}$ of 10 bursts·min^{-1} in CKD patients was also independently associated with higher composite all-cause mortality and non-fatal cardiovascular events[143]. Therefore, the mean reduction in $MSNA_{frequency}$ of 12 bursts·min^{-1} observed in this study may well offer prognostic benefit.

A sympatholytic effect has been previously reported in ESKD, however it was observed in only one dialysis patient at 12M post-RDN[156]. The effect of RDN on MSNA is controversial. Although Schlaich and Prejbisz et al have demonstrated that reductions in MSNA can occur in different populations[109,156,220,221], three other groups in four studies suggest RDN does *not* change MSNA[108,158,222,223]. Seemingly neither baseline nor changes in MSNA correlate with change in blood pressure[108,158,222].The study by Hart et al.[158] showed that individuals with a change in MSNA at 6 months after RDN were not necessarily those with a change in systolic blood pressure. For example, one patient whose systolic blood pressure decreased most (44 mmHg) had an increase in MSNA (+28 bursts/100 heart beats)[158]. These studies indicate that RDN does not consistently decrease MSNA, and neither the baseline nor the change in MSNA is a good indicator of blood pressure response to RDN.

As would be predicted, enthusiasts have criticised the negative trials. It has been suggested[224] that the patients selected in the Brinkmann series did not truly have a neurogenic phenotype of resistant hypertension, given the more modest baseline blood pressure recordings (157±7/85±4 mmHg) and the lower MSNA levels (34±2 bursts/min) to that previously reported in this patient group[46,110,225]. Schlaich and the other rebutting authors propose that a less profound sympatholytic response would be expected given this phenotype[224].

Vink et al[222] denervated patients with a comparable baseline level of MSNA to the Brinkmann cohort (37±4 bursts/min), and their conclusions were similar. As the authors point out, a threshold MSNA for denervation efficacy may therefore exist, a threshold our cohort may well have crossed (baseline MSNA 59±12 bursts/min).

It is worthwhile to mention that all MSNA mentioned in both the above studies and indeed our own experimental work was multi-unit MSNA, which is routinely used and less challenging. Hering et al.[110] explored the more complex single-unit recording in the largest dataset yet recorded pre- and post-RDN. They compared the change in the single-unit MSNA data with the multi-unit MSNA data. The authors found that RDN moderately decreased multi-unit MSNA by 8%; whereas it substantially decreased all properties of single-unit MSNA including firing rates of individual vasoconstrictor fibres (a 37% reduction), firing probability (a 27% reduction), and multiple firing incidence of single units within a cardiac cycle (a 50%

reduction). The authors suggest that RDN could result in the substantial and rapid reduction in firing properties of single sympathetic vasoconstrictor fibres, this being more pronounced than multi-unit MSNA inhibition[110]. Whether the earlier changes in single-unit firing patterns may predict long-term blood pressure response to RDN needs to be further investigated.

4.2 Catecholamines

Despite the observed decrease in MSNA, plasma norepinephrine did not fall significantly post-RDN in our cohort. Plasma norepinephrine levels are an indicator of total body sympathetic activity but represent only a minute fraction of the amount of adrenergic neurotransmitter secreted from nerve terminals[41,42]. Plasma levels of norepinephrine depend on secretion, tissue clearance and re-uptake processes[41,42] and therefore do not allow discrimination between central (decreased secretion) or peripheral (increased clearance) mechanisms. This makes it difficult to demonstrate a significant fall in a sample of this size.

By contrast, although MSNA levels vary between individuals, they are remarkably consistent when measured in the same individual over time[49]. As such they offer a more reliable method of assessment of sympathetic activity, and can be more reactionary to changes in efferent tone.

Although we were unable to demonstrate a significant change in our population, plasma norepinephrine has been reported to fall post RDN in a larger resistant hypertensive population with normal renal function. Ezzahti et al studied seventeen patients and found that plasma norepinephrine decreased by 128pg/ml and 95pg/ml at six and 12 months after RSD respectively[226], indicating that RDN decreases plasma norepinephrine content, consistent with previously reported spillover data[104,109,156,220].

4.3 Regression of Left Ventricular Hypertrophy

The LV mass at baseline met criteria for defined LVH[210] in eight of our cohort. Following RDN, mean LV mass was reduced by 8% at 3M and by 13% at 12M. LVH is associated with elevated sympathetic signalling[147] and is an independent determinant of survival in ESKD[227–229]. Regression of LVH (by reducing LV mass in ESKD to the level seen in this study) has also been associated with reduced all-cause and cardiovascular mortality with hazard ratios of 0.78 and 0.72 respectively[230]. Regression of LVH post-RDN has not previously been demonstrated in ESKD, but has been observed after RDN in hypertensive patients[148,231] and in those suffering from CKD (stages 2 – 4;[149]).

RDN has demonstrated promising results in this study. One may postulate that reduced sympathetic activity leads to the observed reduction in LV mass seen in this group of high-risk dialysis patients. Heightened sympathetic activity is an independent risk factor for LVH in ESKD[146,147] and is reduced during standard hypertensive treatment[232]. Furthermore, a small but significant reduction in LV mass was observed in those classified as non-responders to RDN (systolic BP reduction < 10 mm Hg, see section 4.4). As such sympatho-inhibition might be a mechanism of LVH regression independent of BP reduction[231,233].

It is true that LV mass may be affected by hydration status[234], although LV mass had fallen prior to changes in total body water in our study. As such, it is unlikely that changing hydration status was the sole contributory factor behind the observed improvement in LVH.

4.4 Hypotensive Effects

In addition to the sympatholytic effects on LV mass, RDN reduced mean ambulatory and mean night-time systolic and diastolic BP at 12M with re-establishment of a nocturnal "dipping" pattern for two patients. Office systolic and diastolic BP were reduced as early as 1M but waned at 12M. Clinically significant reductions in ambulatory and office BP were observed in at least 50% of patients out to 12M. The re-establishment of nocturnal “dipping” has previously been observed in a CKD population[150] and may also confer prognostic benefit, as non-dipping vs. dipping has been associated with all-cause and cardiovascular mortality in ESKD[235]. Thus improved blood pressure control post-RDN may also contribute to a reduction in the substantial cardiovascular disease risk in these patients[235,236].

4.5 Volume Status

In ESKD, volume expansion is the major cause of hypertension in dialysis patients[237,238]. Volume overload leads to an elevation in BP via the combination of a rise in cardiac output and high systemic vascular resistance[239,240]. The removal of the excess sodium and reduction in target dry weight can result in the normalisation of BP in >60 percent of haemodialysis patients and in many peritoneal dialysis patients[240–248]. As such, ensuring equipoise in volume status was paramount to clarify if RDN has volume-independent effects in this population.

Clinical assessment was performed prior to RDN to confirm the accuracy of patients' dry weight, and then formal bioimpedance spectroscopy was undertaken to document volume status more precisely. At baseline, clinical measures of hypervolaemia were not overtly present, but BCM-assessed mean fluid status as 3.2 L (SD: 1.7 L) over-hydrated, suggesting a degree of masked overhydration. Following RDN, individual body weights varied but as a group body weight did not change (Figure 13). Mean total body water as assessed by BCM was moderately reduced by 5% (SD: 6%) at 12M, but not at 1M or 3M. This occurred despite no change in dialysis ultrafiltration prescription or dry weight.

4.6 Mechanistic Considerations

The complex and interlinking antihypertensive mechanisms of endovascular renal denervation remain unclear. The current presumption that a permanent loss of afferent or efferent renal nerves after RDN underlies the long-term reduction in blood pressure has been refuted by evidence to suggest almost complete anatomic and functional re-innervation of both afferent and efferent nerves as early as 5.5 months post endovascular RDN, with complete re-innervation seen by 11 months[249].

Further studies are required to determine whether RDN has prolonged actions that alter the control of renal blood flow, renin release, and sodium excretion by the re-innervated efferent renal nerves or whether the renal sensory afferent reflex is desensitised after re-innervation of the afferent renal nerves.

Although not a primary aim of this study, it is reasonable to discuss potential mechanistic clues regarding the pluripotent antihypertensive effect of denervation, albeit in an ESKD population who were oligo-anuric.

4.6.1 Baroreflex Resetting

Renal denervation effected a statistically significant sympatholytic response in our study, a novel result for this population. It is notable however that the response was delayed until 12 months of follow up, in contrast to a resistant hypertensive population in whom a reduction was seen by 3 months[110]. Although this may be sample size dependent, a mechanistic change may be manifesting itself with the delay, which is worthy of discussion.

The most systematic assessment of the temporal effects of denervation in a resistant hypertensive population was performed by Grassi et al[250]. They assessed clinic, ambulatory and beat-to-beat BP, MSNA, spontaneous baroreflex–MSNA sensitivity, and various humoral and metabolic variables before and 15 days, 1, 3, and 6 months after RDN. Amongst several results, they demonstrated a clear-cut improvement in baroreflex–MSNA control which achieved statistical significance at the 3rd and 6th months after RDN. Interestingly, these changes were significantly and directly correlated with the concomitant changes in MSNA values observed at the same time points. This suggests that the reduction in MSNA associated with renal denervation has a baroreflex origin, i.e. it is generated by an improved ability of this reflexogenic area to restrain sympathetic cardiovascular drive.

Increased baroreflex sensitivity post denervation is not a unique finding in the literature; Schlaich et al documented a similar change in their seminal paper from 2009[109]. Schiller[251] has reported analogous findings in rabbits and Hart et al[158] also subsequently reported an effect in both rats and humans following denervation. It was notable too that all patients in Hart's study were observed to have improved baroreflex sensitivity, irrespective of their blood pressure and sympatholytic response to denervation. Significantly, depressed baroreflex sensitivity is one clinically useful method for predicting the response to denervation[252], is known to be a feature of ESKD[124] and has been demonstrated to improve post denervation in an ESKD population[253]. Hart et al[158] postulate that the baroreflex may be more sensitive to changes in afferent input compared with the set point of blood pressure itself, hence accounting for the more consistent results observed post procedure. These results are particularly relevant to our dialysis population as acute hypotensive stimuli were more easily buffered post denervation - a key physiological response to ultrafiltration during haemodialysis[130,131].

Although our measures were indirect, one may suggest that with afferent renal nerve section, altered central storage of catecholamines or central integration of reflexes results[254], changing afferent inputs to the hypothalamus or medulla[255]. This could lead to central resetting of the baroreceptor, potentially manifesting the observed MSNA changes.

In addition to a central resetting of the baroreceptor, more direct vascular effects could lead to peripheral resetting post denervation. Aortic stiffness, ejection duration and aortic systolic pressure load significantly reduce post denervation[157]. Progressive remodelling of vessel tone therefore results, alongside improved cardiac work and consequently less ventricular hypertrophy[148,231,256]. There are also venous effects; demonstrably reduced caval diameter ensues, manifesting reduced low-

pressure baroreceptor stretch alongside the high-pressure changes (unpublished data, see Section 4.6.2).

Although demonstrable in some studies, improved baroreflex sensitivity has not universally been reported post denervation. Brinkmann et al[108] could find no such change in their human study. Once again though, patient selection and indeed efficacy of denervation (see section 4.7) remains questionable in this early trial.

4.6.2 Blood Volume Redistribution

The hallmark haemodynamic change in established hypertension is increased vascular resistance. However, in human subjects with established hypertension, total vascular capacitance and specifically venous capacitance are also reduced[257].

The splanchnic venous bed represents the most important active capacitance bed in the body and is densely innervated by the sympathetic nervous system[258]. Electrical stimulation of sympathetic nerves depolarises the venous smooth muscle cells more than arteriolar smooth muscle cells, and the contraction is greater and earlier in veins than in arteries[259]. Venoconstriction in the splanchnic circulation therefore results in a significant shift of blood towards the heart, increasing diastolic filling and hence cardiac output.

In established hypertension, central blood volume is near normal, but total blood volume is reduced[260–263]. Thus, peripheral to central redistribution of circulating blood appears to be an important aspect in the haemodynamics of sympathetic nervous system-mediated hypertension.

We assessed the effects of denervation on blood volume indirectly by recording inferior vena cava (IVC) diameter and calculating both VCD (diameter of the IVC expressed as an index to body surface area) and IVC collapsibility index (IVC-CI). VCD has been suggested to be pressure and volume dependent in an ESKD population whereas IVC-CI is merely pressure related[264]. There was a significant reduction in VCD but no change in IVC-CI at three months post denervation; a change that persisted at 12 months. This would support the notion that there had been a reduction in central venous volume, although right-sided pressures were maintained.

One may conclude that endovascular renal denervation may exert a hypotensive effect partially via increased venous capacitance. Whether this associates with the reset baroreflex post denervation remains to be established and would be a valuable line of future enquiry.

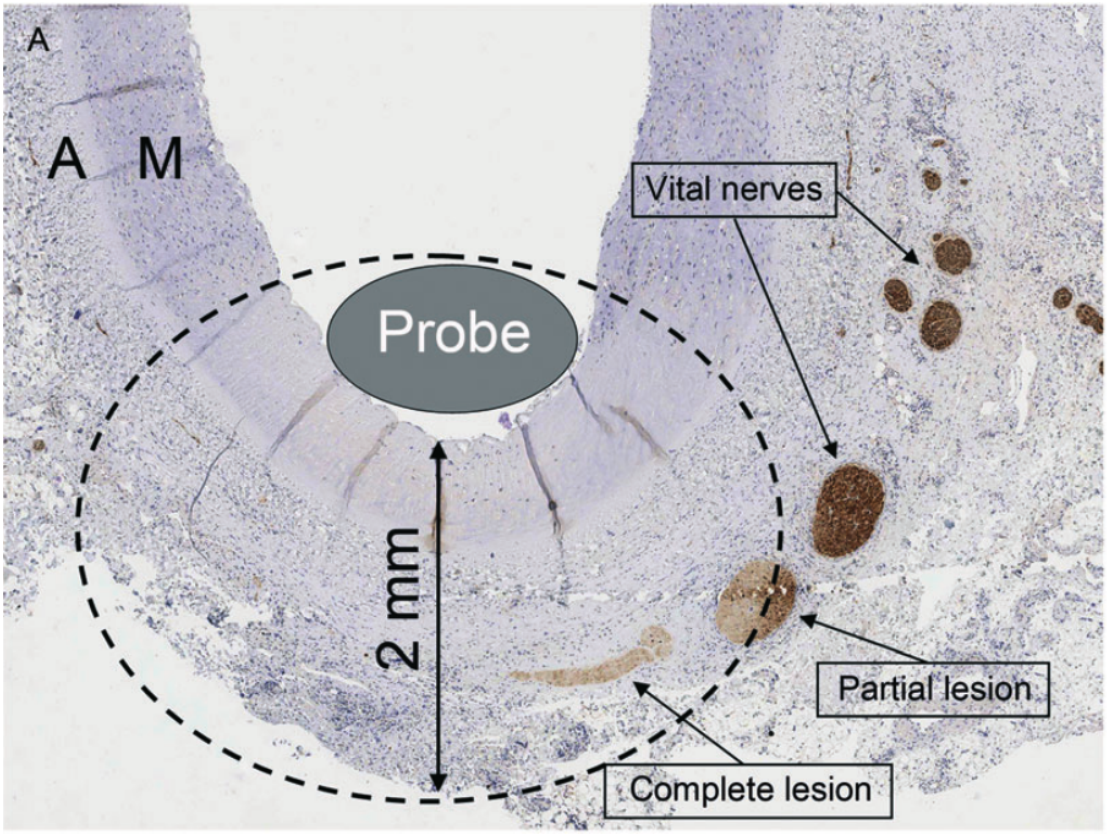
4.7 Procedural Considerations

An especially challenging aspect of RDN therapy is that no practical and immediate measure of procedural success exists. Although some authors have ingeniously employed an absence of rising blood pressure following electrical stimulation of the renal nerves[265], most are denervating blind.

Based on early experience, catheter-based RDN was expected to result in ~50% reduction in renal norepinephrine spillover[104]. However, subsequent investigations regarding the effect of RDN on norepinephrine spillover reported more modest and highly variable declines in sympathetic activity[217,266].

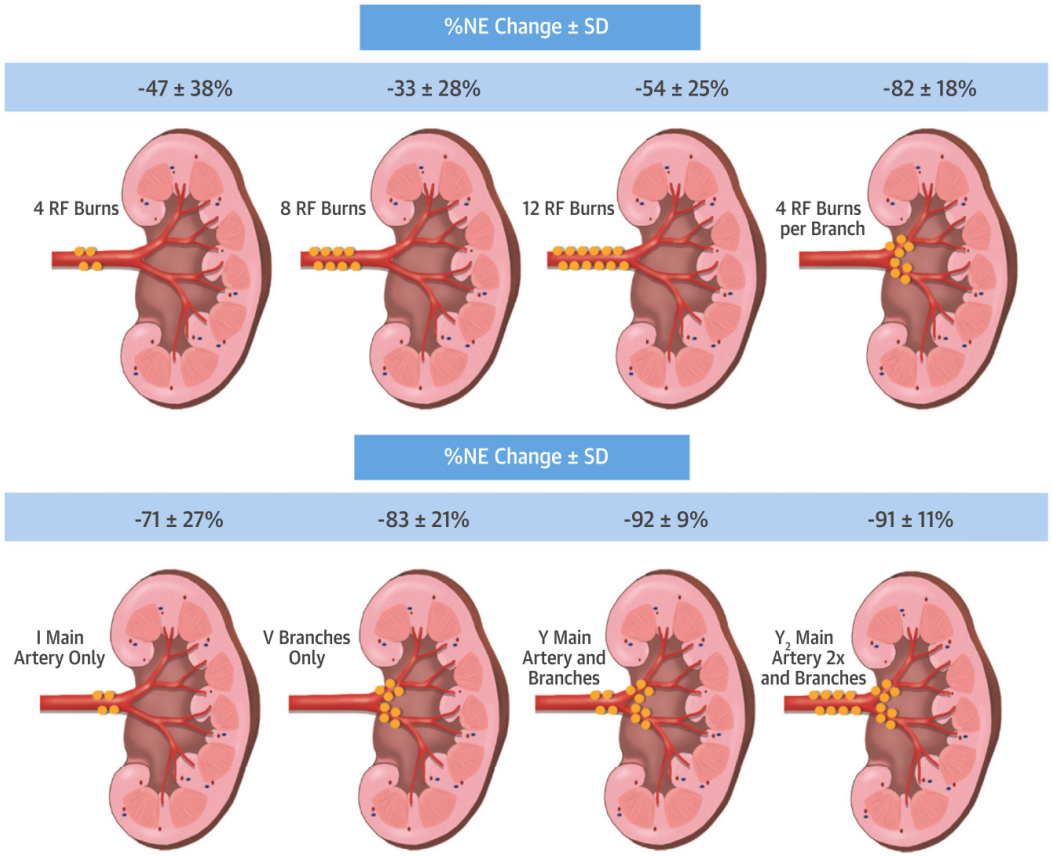
Vink et al[267] go some way to explain why RDN is only partially effective. Radiofrequency-induced damage to and around the vessel wall has a dome-shaped distribution field with limited penetration, leaving unaffected a large part of the nerves in (peri-) adventitial areas remote from the vascular lumen. This makes it unlikely that radiofrequency ablation would result in complete interruption of the continuity of all adventitial nerve bundles around the renal arteries:

Figure 14: *Histology of a Human Renal Artery After Renal Denervation*[267]
(A, adventitia; M, media)



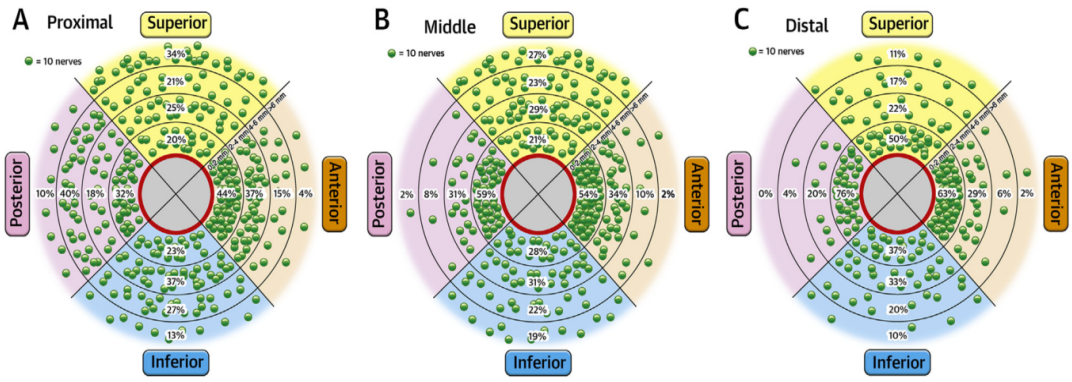
Simply increasing the number of radiofrequency lesions (4, 8, and 12) in the main renal artery is not sufficient to yield a clear dose-response relationship on norepinephrine content and axon density[268]. In contrast, targeted treatment of the renal artery branches or distal segment of the main renal artery results in markedly less variability of response and significantly greater reduction of both norepinephrine and axon density than conventional treatment of only the main renal artery. Combination treatment (main artery plus branches) produced the greatest change in renal norepinephrine and axon density with the least heterogeneity:

Figure 15: *Optimized Renal Denervation Techniques: Efficacy of Catheter-Based Radiofrequency Renal Denervation*[268]



As well as distal/branch therapy being recognised as increasingly important, the merits of four quadrant denervation have also been extolled[269]:

Figure 16: *Distribution and Density of Renal Sympathetic Nerves*[269]



Given these considerations, experience with renal denervation technique is now recognised as being particularly important to achieve successful therapy. Without such experience, denervation results can be affected[270].

Interestingly, post-hoc analysis of Symplicity HTN-3 revealed that more than half of operators performed at most two RDN procedures, and 31% performed only one RDN procedure during the trial. In the analysis[199], the lack of ablations in all four quadrants (~75%) and the number of ablation attempts were highly correlated with blood pressure reductions. These two variables may of course be inter-related but the directional changes in blood pressure were consistent across all measures of blood pressure assessment as well as heart rate. Comparison of propensity score-matched sham cohorts showed that this pattern of increasing response was less apparent in control patients and the RDN group receiving ≥14 ablations had significantly greater reductions in SBP.

Our cohort benefitted from the use of a second-generation device and an experienced operator, who was concurrently denervating patients in the EnligHTN II (NCT01705080) and EnligHTN III (NCT01836146) trials. The EnligHTN™ catheter has pre-specified electrode alignment, allowing a more predictable pattern of denervation. Despite notionally improving efficacy through more predictable four quadrant ablation, it yields comparably smaller and shallower lesions[271]. This may have important safety implications, suggests appropriate lesion targeting is even more important and, if Mauriello et al[272] are to be believed, implies meaningful denervation is still possible in a dialysis population with this catheter.

Mauriello et al[272] suggest dialysis patients may not necessarily need the extensive lesion size and depth imparted with the Symplicity catheter as their nerve distribution is more superficial. The authors demonstrated a significant increase in nerve density in the internal area of the peri-adventitial tissue (within the first 0.5 mm of the beginning of the adventitia) compared with controls. This may partially explain why our dialysis patients have demonstrated a positive sympatholytic result whereas other cohorts failed to do so.

4.8 Limitations

The open-label and uncontrolled nature of our small feasibility study is inherently prone to both placebo and Hawthorne effects. Despite these restrictions, we detected a significant reduction in MSNA at 12 months that was clinically relevant, and in keeping with previous results. The observed progressive and evolving pattern is also indicative of a true treatment effect of RDN, as Hawthorne and placebo effects are thought to wane over time[273].

Volume status is an important confounding factor as discussed in section 4.5. We didn't attempt to control volume during follow-up; each patient could have their target weight adjusted should the clinical need arise. We were not trying to prove volume-independency of RDN (indeed that would be counter-intuitive[274]), merely document the physiological changes that ensue from denervation in this population. Overall, despite individual weight fluctuations, there was no significant change in target weight at any point during the study period. This reflects the effective equipoise in ultrafiltration prescription that occurred post denervation. Despite the absence of change in ultrafiltration prescription, volume status itself was not static during the follow-up period (Figure 13; see section 4.5), a significant finding. That said, LV mass had fallen prior to changes in total body water, suggesting a volume- as well as blood pressure-independent effect.

Medication concordance has been a persistent problem with analysis of the effects of RDN, indeed Elmula et al suggest the technique has an uncertain BP-lowering effect once concordance has been ensured[275]. We documented medications from patient interviews at each follow-up visit, although didn't witness intake. This is a potential shortcoming in our study, although is commonplace amongst RDN and indeed wider resistant hypertension trials. Should the study be repeated in a larger cohort, witnessed intake of medications would be an appropriate methodological enhancement.

If concordance could be assumed to be non-dynamic following RDN, the documented changes in therapy should serve, if anything, to strengthen conclusions about the sympatholytic effect of RDN in ESKD, our primary outcome measure. At 12M (when the significant reduction in MSNA had been demonstrated), of the three patients who had data available, one patient had stopped cilazapril and another had started felodipine. Stopping cilazapril should conceivably *increase* not decrease MSNA, as should starting felodipine[276].

4.9 Future Study

This study was designed as a proof-of-principle trial that could inform the scientific community of the potential utility of RDN in ESKD. Inherently, a larger study should be undertaken against best medical care, potentially carvedilol add-on therapy[219], with long-term follow-up of hard cardiovascular endpoints mandated. The need to study just those with resistant hypertension is not essential, given the nature of the elevated sympathetic activity in ESKD regardless of blood pressure. Indeed, potentially widening the inclusion criteria to less severe forms of CKD is appropriate, with a view to ameliorating the progression of disease, as well as improving cardiovascular outcomes[277,278].

During the trial, several more specific areas for further investigation have been identified. Mechanistic clues surrounding the potential role of the baroreflex, as well as fluctuating plasma volume have been identified. Considering the literature review (and recent trial data[279]) I would also contend that endothelial function need be assessed post denervation in this population, a cohort that are particularly prone to dysfunction[280].

Alongside mechanistic considerations, novel denervation techniques may also offer the answer to sub-optimal reduction in sympathetic activity and require further research. Several techniques have been developed, including bipolar radiofrequency ablation, focused ultrasound, brachytherapy and chemical denervation. Although the field is prone to hyperbole as manufacturers strive to gain a footing in the marketplace, there is certainly potential merit to these new technologies.

Chemical renal denervation for instance seems able to get predictable circumferential nerve kill at substantial depth, to achieve efficient, complete and predictable denervation with minimal anatomical limitations, and without a need for capital equipment. This approach also allows one to target the nerves where they are located (in the adventitia), and thus minimise injury to the intima and media. Finally, in contrast to radiofrequency or ultrasound ablation this approach appears to be essentially painless. Key to the technique's purported efficacy is that alcohol can be spread diffusely, circumferentially, deeply, and distally and therefore has the potential to affect more sympathetic nerves. The downside is the lack of control and selectivity of the alcohol to the nerves and the risk that alcohol may potentially damage adjacent tissue. It is comforting to learn about the safety profile of the Peregrine System from the preclinical trials and from the feasibility study[281], although limited to 6 months angiographic and clinical follow-up.

Clearly more research is needed. In the words of Murray Esler, denervation's founding father, himself paraphrasing Winston Churchill, we are at "the end of the beginning" of RDN[282]. Quite what follows is open to much debate, and it is paramount the right direction is taken. Considering what I have learned during this project, I believe ESKD represents a meaningful direction for research, and feel an oft-neglected population can benefit from this evolving technology.

5 CONCLUSION

In this proof-of-concept study, RDN in ESKD patients demonstrated a statistically significant sympatholytic effect, led to marked regression of LVH and clearly reduced ambulatory BP. Such changes have substantial clinical implication in this high cardiovascular risk population. These preliminary findings need to be validated in a larger randomised, sham-controlled trial but are promising in their own right.

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