UiT The Arctic University of Norway

Cardiovascular Diseases Research Group UiT.

Department of Cardiology University Hospital of North Norway

RENAL SYMPATHETIC DENERVATION, POTENTIAL EFFECTS ON BLOOD PRESSURE AND GLUCOSE METABOLISM IN PATIENTS WITH SEVERE TREATMENT RESISTANT HYPERTENSION

THE RE-SHAPE CV-RISK STUDY

Atena Miroslawska

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1 ACKNOWLEDGMENTS

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2 SELECTED ABBREVIATIONS

AASI	Ambulatory arterial stiffness index
ABPM	Ambulatory blood pressure monitoring
ACEI	Angiotensin converting enzyme inhibitor
ARB	Angiotensin receptor blocker
ARV	Average real variability
BMI	Body mass index
BP	Blood pressure
BPV	Blood pressure variability
DBP	Diastolic blood pressure
EGR	Endogenous glucose release
ESH	European Society of Hypertension
FFA	Free fatty acids
GIR	Glucose infusion rate
HEC	Hyperinsulinemic-euglycemic clamp
HOMA-IR	Homeostasis model assessment, insulin resistance
IR	Insulin resistance
IRS	Insulin receptor substrate
IS	Insulin sensitivity
MAP	Mean arterial pressure
MSNA	Muscle sympathetic nerve activity
OGTT	Oral glucose tolerance test
PP	Pulse pressure
PWV	Pulse wave velocity
RDN	Renal denervation
RVLM	Rostral ventrolateral medulla
RAAS	Renin angiotensin aldosterone system
SBP	Systolic blood pressure
SCT	Sham-controlled trial
SD	Standard deviation
SNA	Sympathetic nerve activity
SNS	Sympathetic nervous system
TRH	Treatment resistant hypertension
WGD	Whole-body glucose disposal

3 LIST OF PAPERS

This thesis is based on the following papers.

3.1 Paper I

Miroslawska A, Solbu MD, Skjølsvik E, Toft I, Steigen TK.

Renal sympathetic denervation: effect on ambulatory blood pressure and blood pressure variability in patients with treatment-resistant hypertension. **The Re-Shape CV-risk study.** J Hum Hypertens 2016 Mar;30(3):153-7.

3.2 Paper II

Miroslawska A, Gjessing PF, Solbu MD, Fuskevåg OM, Jenssen TG, Steigen TK.

Renal Denervation for Resistant Hypertension Fails to Improve Insulin Resistance as Assessed by Hyperinsulinemic-Euglycemic Step Clamp. Diabetes 2016 Aug;65(8):2164-8.

3.3 Paper III

Miroslawska AK, Gjessing PF, Solbu MD, Norvik JV, Fuskevåg OM, Hanssen TA, Steigen TK

Metabolic effects two years after renal denervation in insulin resistant hypertensive patients. The Re-Shape CV-risk study. Clin Nutr. 2021 Mar 2;40(4):1503-1509.

4 ABSTRACT

Background: Denervation of renal sympathetic nerves (RDN) is a mini-invasive endovascular procedure introduced as an antihypertensive treatment with a potentially beneficial effect on insulin resistance. In this open-label non-randomized study we investigated non-diabetic patients with true treatment-resistant hypertension defined as office systolic blood pressure (BP)>140 mmHg and (mean) daytime (ambulatory) BP >135 mmHg, despite four or more antihypertensive drugs. **Methods***:* Bilateral RDN was performed with the Symplicity Catheter System (N=23), and patients were followed- for six and 24 months. BP measurements were performed after witnessed intake of antihypertensive drugs. BP variability and arterial stiffness indices were calculated from 24-hour BP recordings. Insulin sensitivity was assessed using a two-step hyperinsulinemic- euglycemic clamp with glucose tracer before and six months after RDN. Oral glucose tolerance test, levels of insulin, C-peptide, adiponectin and leptin were measured and surrogate insulin resistance indices were calculated before RDN and during follow-up. **Results**: Most of the patients were obese, had metabolic syndrome and severe insulin resistance at baseline. We found a statistically significant reduction of both office and ambulatory 24-hour BP as well as BP variability six months after RDN. Despite decline in BP, neither peripheral nor hepatic insulin sensitivity improved six months after RDN. Twenty patients continued to the two-year follow up. Some rebound in BP was found in most of patients. Arterial stiffness did not change during follow-up. All measured metabolic variables and insulin resistance surrogate indices remained essentially unaltered two years after RDN. **Conclusion**: Neither peripheral nor hepatic insulin sensitivity improved after RDN. Our study does not support the notion of a beneficial metabolic effect of RDN in patients with treatment resistant hypertension.

5 INTRODUCTION

Arterial hypertension is the most prevalent modifiable risk factor associated with cardiovascular events (1). Life style changes and use of several antihypertensive drugs do not always result in adequate decrease in blood pressure (BP), a condition defined as treatment resistant hypertension (TRH) (2, 3). In addition, high BP may be associated with impaired response of tissues to insulin that may lead to type 2 diabetes (4). Both hypertension and insulin resistance (IR) are associated with increased activity of the sympathetic nervous system (SNS) (5).

Renal denervation (RDN) is a procedure that aims to destroy sympathetic nerves along renal arteries by a radiofrequency catheter inserted through a minimally invasive technique. The Re-Shape CV-Risk Study was initiated to test the hypothesis that renal denervation could decrease BP and also improve insulin sensitivity (IS) in patients with TRH.

6 BACKGROUND

6.1 Hypertension

Hypertension has become a significant global health burden, and the prevalence is expected to increase with aging of the population (6). This is the most important modifiable, preventable risk factor for premature death as associated with cardiovascular disease, stroke, type 2 diabetes and atrial fibrillation as well (7).

Hypertension is classified as primary (90% of patients) or secondary if due to other diseases as renal failure, endocrine conditions, heart failure, drugs or sleep apnea (8). Primary hypertension results from a complex interaction of genes, older age and environmental factors including obesity, excessive salt ingestion, smoking and immobility.

Applying the European Society Hypertension (ESH) guidelines from 2018 hypertension is defined as office BP ≥140/90 mmHg (9). Many patients do not have sufficient effect of life style changes and medical treatment on their hypertension. This group is defined as patients with TRH. Knowing that TRH is associated with increased sympathetic nerve activity (SNA) (10), these patients were perceived as a potential target group for therapies able of modulating the activity of the autonomous nervous system, such as RDN. According to aforementioned guidelines, hypertension is defined as resistant when the recommended treatment (more than three different antihypertensive drugs, including a diuretic) strategy fails to lower office systolic BP <140 mmHg and/or diastolic <90 mmHg. In addition, TRH is diagnosed by ambulatory BP monitoring (ABPM) or home BP measurements, in patients whose adherence to therapy has been confirmed. TRH is not synonymous with uncontrolled hypertension, which includes patients who lack BP control secondary to poor adherence or inadequate treatment. Consequently, true TRH refers to a diagnosis of primary hypertension with exclusion of all other potential causes of uncontrolled BP. Although TRH was considered to be a common clinical problem, recent research show that exclusion of secondary and pseudo-resistance hypertension decreases the proportion of patients with true TRH from 40 % (3) to 10 % (11) of hypertensive patients. Pseudo-resistant hypertension can be caused by different factors, such as poor BP measuring technique, white-coat hypertension (12) and poor adherence to medication. There are many methods, reported in guidelines, on how to assess medical adherence. One of them is witnessed intake of medication.

This directly observed therapy followed by ABPM is an easy, available, reliable method to control the compliance of patients (13). Providing that they take antihypertensive drugs daily this method is safe, though in case of non-adherence, patients need to be observed due to potential hypotension complication after taking antihypertensive medications.

6.1.1 Blood pressure

A careful diagnosis of patients is crucial in selecting true TRH. Whereas classification of hypertension and guided treatment is based on office BP, hypertension diagnosed by ABPM appears to be a better predictor of organ damage and has been shown to have be associated with morbidity and even fatal events (14). In addition, the analysis of 24-hour BP profiles provides important insights into the physiological BP regulation and can give us more prognostic information than office BP alone. ABPM readings give the possibility to look at the BP changes during both day and night. In healthy individuals BP follows a circadian pattern, BP starts to decline in the evening, reaches a nadir around midnight and arise fast just after awaking in the morning (15). Thus, lowering or “dipping” of the BP during nights is a normal physiological variation which can be blunted by the severity of hypertension. A worsening of the dipping pattern and high night BP is associated with increased cardiovascular risk (16). Furthermore, ABPM give the possibility to assess BP changes during the transition from sleep to wakefulness as this period is associated with an increased risk of stroke and sudden cardiac arrest (17). Hence, potential effects of interventions that ameliorate the sympathetic nervous system should be assessed separately on day-and nighttime BP changes using ABPM.

6.1.2 Blood pressure variability

BP is not a constant variable, but it shows spontaneous oscillations over short-term (minutes to hours) and long-term (day and month) periods. Short-term BP variability (BPV) is usually defined as the oscillation of BP within 24 hours. Fluctuation of BP from minutes to hours mainly reflects the influence of central nervous system and autonomic modulation and the elastic properties of arteries (18). BPV is the result of complex interactions between extrinsic environmental and behavioral factors with intrinsic cardiovascular regulatory mechanisms (19). Thus, the reduction of the ability of the arterial and cardiopulmonary reflexes to buffer changes in BP can augment short-term BPV.

BPV increases proportionally to mean BP and seems to contribute independently to the presence and severity of target organ damage and cardiovascular events in hypertensive patients (20). BPV can be estimated by direct BP measurements or by using mathematical calculations, as in the present study.

6.1.3 Arterial stiffness

One of the main cardiovascular pathophysiologic changes associated with hypertension and aging is decrease in large artery compliance, especially in the aorta. The loss of elastic fibers in the vessel wall, a concomitant increased collagen deposition and calcification, together with an autonomously regulated abnormal arterial smooth muscle tone increases the stiffness of the wall. This process is often called “hardening of the arteries” (21). The expression of angiotensin type two receptors in vascular tissue leads to vascular wall hypertrophy and fibrosis (22). If the elasticity of conductance vessels decreases, diastolic BP (DBP) goes down. Then the ejection force cannot be offset by arterial distension, the pulse wave velocity (PWV) increases and reflex waves to the heart arrive earlier.

That augments systolic BP (SBP), which together with decreasing DBP results in an augmentation of the pulse pressure (PP). Then, when excessive PP is transmitted through the microcirculation of vital organs such as the brain and kidneys, extensive tissue injury tends to occur, leading to increased cardiovascular risk (23). Noninvasive carotid femoral PWV is considered the gold standard method for assessing vascular stiffness. This method is recommended by ESH as a tool for assessment of subclinical target organ damage (9). However, Staessen et al. have proposed ambulatory arterial stiffness index (AASI) as a surrogate index for arterial stiffness (24). This index is based on the rationale that the relationship between SBP and DBP is dynamic, and depends on the functional and structural characteristics of large arteries. DBP varies less for a given amount of change in SBP, resulting in a lower regression slope and, consequently, in a higher AASI. The stiffer the arterial tree, the closer the regression slope and AASI get to 1.

6.2 Insulin resistance

IR is a condition in which cells are no longer responding appropriately to circulating insulin. Since the discovery of insulin over 90 years ago, a wide range of IR definitions and explanations of pathogenesis have been applied. First, IR was linked to the observation that some of the diabetic patients needed large insulin doses to decrease glucose, secondary to antibodies directed against the therapeutic non-human insulin. Second, IR had been associated with vascular changes, the hypothesis was that structural and functional changes in the vasculature might limit the supply of hormones and substrates to target tissue (25). The reduction in the number of open capillaries could increase the distance that insulin must travel to reach the muscle cells (26). This might decrease glucose utilization, thereby leading to a pre-cellular form of IR.

Growing evidence indicates that IR develops mainly because of disturbances within cells, in insulin signaling pathways (27). Although the molecular mechanisms are not fully understood, one suspects that the strength of the insulin signal from its receptor to its final action is attenuated. Insulin affects cells through binding to their receptors on the surface of insulin-responsive tissues. The stimulated receptor phosphorylates itself and several substrates, including members of the insulin receptor substrate (IRS). Thus, insulin initiating down-stream signaling events that leads to control of glucose uptake (28). Insulin, via IRS, stimulates the translocation of the glucose transporter (GLUT-4) to the cell membrane, in order to bring glucose into the muscle and adipose tissue (29). The inhibition of these down-stream pathways dysregulates insulin signal transduction within cells causing IR.

Liver, kidney, skeletal muscle and adipose tissue are the major insulin-sensitive organs involved in glucose homeostasis.

6.2.1 Hepatic insulin resistance

The liver is the first organ to pick up nutrients that enter the body from the intestines and plays the role of a 'glucose-buffering system'. It takes up glucose and stores it in the form of glycogen, and releases it back into the blood when blood glucose concentration falls. When glucose concentration increases after a meal, insulin is released from β cells in the pancreas to the portal system. Glucose is taken into hepatocytes via receptors GLUT-2 independent of insulin (30). However, insulin binds to the insulin receptors on the hepatic cells and initiate a cascade of enzyme-phosphorylation, leading to activation of glycogen synthesis and reduction of gluconeogenesis (31). In the postabsorptive state, the liver is responsible for at least 75% of the total endogenous glucose production. In healthy humans, the hepatic glucose production rate is around two mg/kg body weight/min (32). The condition where the liver does not respond adequately to insulin, is classified as hepatic IR.

6.2.2 Peripheral insulin resistance

6.2.2.1 Adipose tissue

Adipose tissue is an active, endocrine tissue that produces adipokines: adiponectin, leptin and many proinflammatory cytokines such as TNF- alfa and IL-6 (33, 34). Exposure of cells to proinflammatory cytokines stimulates inhibitors of IRS phosphorylation. This is followed by attenuation of insulin signaling in insulin sensitive tissue, resulting in IR. Additionally, recent studies have revealed that expansion of white adipose tissue in an obese state leads to decreased secretion of adiponectin, for which the target organ is the liver. Adiponectin receptors in the liver (Adipo R2) reduce gluconeogenesis and free fatty acids (FFA) oxidation. Adiponectin directly increases hepatic IS, promotes fuel oxidation in skeletal muscle and decreases vascular inflammation. Hypoadiponectinaemia is also known to be consistently related to IR, obesity, type 2 diabetes, coronary heart disease, hypertension and atherosclerosis, based on both experimental and clinical studies (35).

Leptin, another important adipokine, was discovered in 1994, and named after the Greek word “leptos” meaning thin. Leptin, in opposite to insulin, is a catabolic hormone that increases lipolysis in adipocytes and decreases lipogenesis in the liver. However, obese individuals, for unknown reasons, become resistant to the satiety and weight-reducing effect of the hormone even though they preserve leptin-mediated sympathetic activation to non-thermogenic tissue, such as kidney, heart, and adrenal glands. Leptin has also been shown to influence nitric oxide production, natriuresis and chronic sympathetic activation, especially in the kidneys (36). Thus, hyperleptinemia appears to cause sodium retention, systemic vasoconstriction and BP elevation.

In the case of overactive SNS or large adipose tissue mass, as in obesity, excess secretion of FFA becomes part of an adverse process called lipotoxicity. Overflow of FFA leads to cell stress that dysregulates the insulin signaling pathway, not only inside adipose cells, but also in other cells (37). FFA that leave the fat cells, enter the circulation and are taken up by other organs, such as the liver and skeletal muscle that are unable to safely store large amounts of fat. Chronically increased plasma FFA stimulates gluconeogenesis, dysregulate cell pathways, and induces hepatic and muscle IR (38).

6.2.2.2 Skeletal muscle

Skeletal muscle utilizes both FFA and glucose as a fuel to produce energy and these processes are regulated by insulin, but also by the SNS. In lean healthy individuals insulin stimulates glucose uptake in skeletal muscle and suppress lipolysis. Defects in glucose metabolism in skeletal muscle are due to impaired insulin signaling, glucose transport by GLUT4, decreased glucose oxidation and impaired glycogen synthesis. One of the suggested explanations is an increase in intramyocellular fat content that dysregulates mitochondrial function, what could explain that weight reduction correlates with decreased peripheral IR (39, 40). The other, is related to inflammatory diseases, where cytokines are supposed to impair insulin signaling in skeletal muscle as may be observed e.g. in patients suffering from psoriasis, without being obese (41). Thus, skeletal muscle is a key tissue in whole-body energy metabolism and is responsible for IR associated with or without obesity and type 2 diabetes.

6.2.3 Assessment of insulin sensitivity

While, in many individuals the IR develops simultaneously in multiple organs, the severity of IR may differ among the various tissues. Since interventions that may improve IS are organ specific (e.g., physical activity for muscle IR, metformin for hepatic IR, and weight reduction and thiazolidinediones for both), it is important to quantitate the magnitude of IR separately (42, 43). There are many methods and surrogate indices used to asses hepatic and peripheral IS, based on fasting glucose and insulin, adipokines or oral glucose tolerance test (OGTT), but the gold standard is hyperinsulinemic euglycemic clamping (HEC). It is based on an infusion of insulin at a constant rate, while simultaneous infusion of glucose is titrated to euglycemia. Although HEC gives a picture of whole body IS, combined with tracer dilution method it can separately assess changes in hepatic and peripheral IS (30). A tracer is a labelled form of a substance, in this case labelled D-[6,6-$^{2}H_{2}$] glucose, that makes it detectable by liquid chromatography mass spectroscopy.

6.3 Metabolic syndrome

Hypertension is frequently accompanied by hyperinsulinemia, obesity and IR (4). The central hormone involved in this metabolic-BP cross-talk is insulin. As IR develops, more insulin is produced by β cells in the pancreas leading to hyperinsulinemia and gradually to type 2 diabetes. It is well known that fully functional adipose tissue is required for the maintenance of normal IS (44). IR is strongly associated with obesity (defined as excess of body fat accumulation). That in itself is not necessarily an adverse condition, as long as the fat is safely stored in healthy fat cells that respond to insulin. However, fat cells do not have an unlimited capacity to expand and dysregulation of fat metabolism plays a pivotal role in the development of IR.

Metabolic syndrome is a clustering of the aforementioned conditions such as hypertension, obesity and IR, all associated with increased cardiovascular risk. According to International Diabetes Federation (IDF) definition from 2005, diagnosis of the metabolic syndrome is based on central obesity and two other criteria as raised BP, fasting glucose or dyslipidemia (45). However, it is still unresolved whether overactivity of the sympathetic nervous system accompanied by inflammation, IR and obesity are the cause or an effect of the metabolic syndrome.

6.4 The sympathetic nervous system

The sympathetic nerves are a part of the autonomic nervous system that innervate many organs in the human body. Central sympathetic neurons are located in the rostral ventrolateral medulla (RVLM), which is a key area for regulation of arterial BP and metabolism (46, 47). The RVLM neurons conduct signals directly to the sympathetic preganglionic neurons located in the spinal cord that innervate several target organs and thus controlling cardiac output and blood flow to skeletal muscles and visceral organs. Feedback information is conveyed by a number of afferent inputs from carotid and organ receptors (e.g., mechanoreceptors, chemoreceptors) as well as hormonal mediators (48). Postganglionic neurons release the primary sympathetic neurotransmitter, noradrenaline. The endogenous natural receptors for the catecholamines adrenaline and noradrenaline are adrenergic receptors α and β, their activations have different effects depending on the target organ.

Moreover, signals from the brainstem and hypothalamus can also modulate the RVLM activation and alter SNA, e.g imidazoline I_1 receptor agonist acts centrally at the level of the RVLM to inhibit sympathetic drive (49).

6.4.1 Regulation of blood pressure

The cross-talk between RVLM and baroreceptors plays a central role in the regulation of BP. Sympathetic adrenoreceptors, α-receptors, act upon vascular smooth muscle in arterioles, leading to vasoconstriction, but β1 receptors in the heart act by increasing cardiac output. Thus, low BP sensed by baroreceptors reduces the output of the solitary nucleus, in this case stimulating RVLM. This leads to an increase in sympathetic stimulation of the heart and the vessels, in order to restore BP. In addition, increased renal sympathetic efferent outflow acts by an activation of adrenergic β1receptors in the kidney that releases renin from the juxtaglomerular apparatus and stimulates α1 receptors in the nephron tubule to increase sodium reabsorption and decrease renal blood flow (50, 51). This sympatho-renal axis including both efferent and afferent renal nerves define the dual contribution of the kidney in causing hypertension (52).

BP may be considered as a physiological marker of the autonomic nerve function. Both short and long BP fluctuation represents interactions among behavior, environment and neural central and peripheral reflexes within the sympathetic and the parasympathetic nervous system. In a well-regulated autonomic system, sympathetic withdrawal occurs during sleep, leading to a fall in night BP (53). The transition from sleep to awaking is linked to sympathetic activation and gain in plasma catecholamines which results in increased BP and heart rate. The mechanisms responsible for day-night BP changes are still not clear. Exaggerated BP responses to standing might be associated with increased basal SNA (54). In hypertension and obesity sympathetic outflow to skeletal muscle and kidney is increased contributing to continuous dysregulation in circadian BP pattern (55). Notably, an intervention modulating SNA, such as RDN, might decrease BPV, in addition to BP reduction.

6.4.2 Regulation of metabolism

The SNS plays an important role in regulation of daily energy expenditure by controlling of metabolic rate, food intake and temperature. It has been generally recognized that increased SNA produces catabolic effects on glucose and lipid metabolism whereas increased parasympathetic neural activity produces anabolic effects.

A number of afferent nerves from peripheral organs convey metabolic information that modulate activation of RVLM. Circulating factors such as insulin and angiotensin, which are able to cross the blood-brain barrier and have receptors distributed throughout the brain, can influence central sympathetic outflow and thereby modulate peripheral lipid and glucose metabolism (56). Leptin receptors are not only presented in the hypothalamus, but also in the solitary nucleus, contributing to SNA (57). Norepinephrine from sympathetic nerves and epinephrine released from the adrenal medulla affect glucose transport and metabolism in liver, pancreas, adipose tissue, and skeletal muscle. The liver, which plays a key role in glucose metabolism, is richly innervated by the autonomic components from the splanchnic sympathetic nerves and vagal parasympathetic nerves. Additionally, the part of sympathetic nerve fibers which innervate the liver arise directly from the hypothalamus - a center for the food intake and appetite regulation (58). SNA and catecholamines increase glucose by activation of $\alpha 1$ and $\beta 2$ receptors in the liver that leads to glycogenolysis and gluconeogenesis.

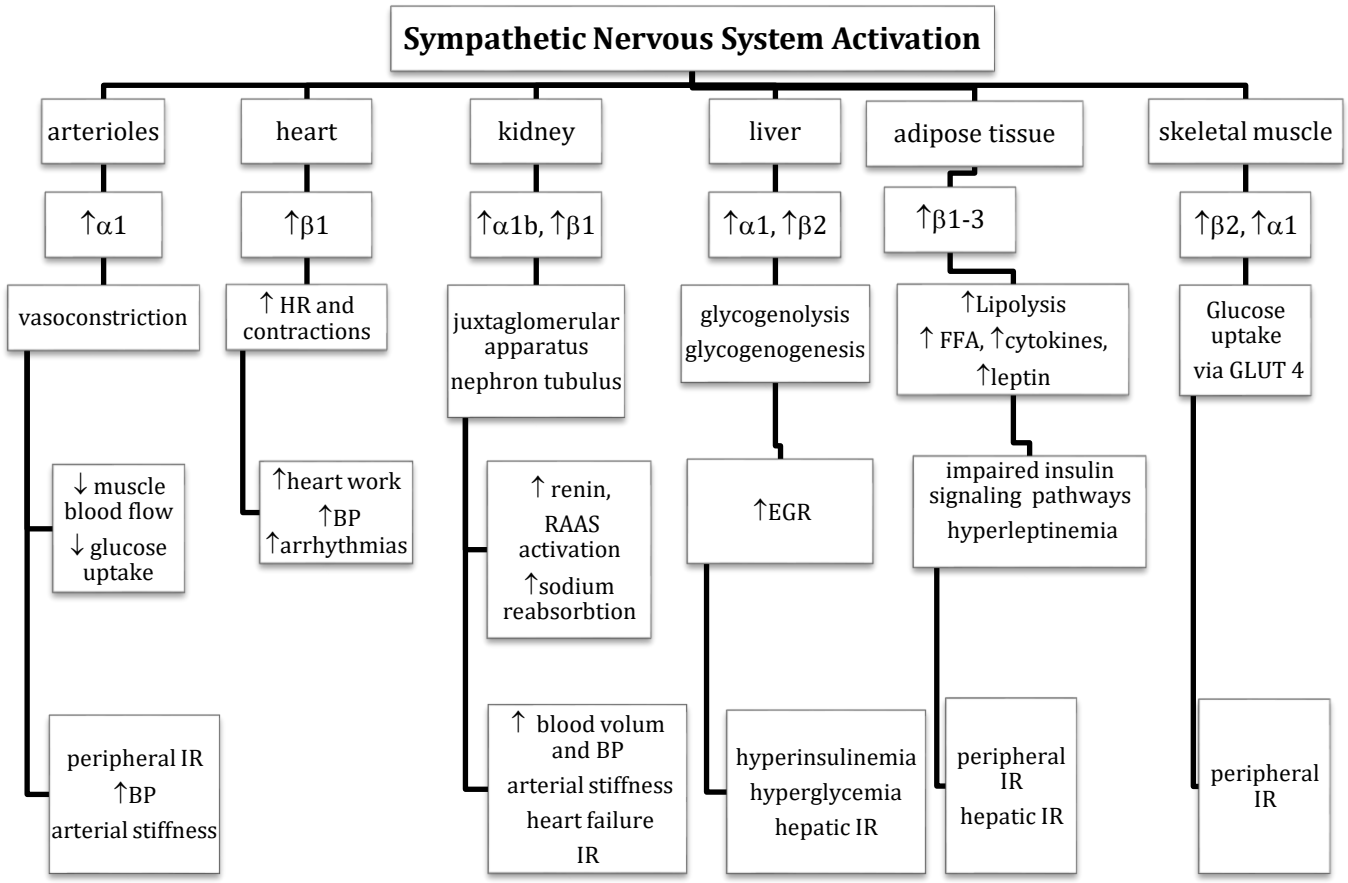
The sympathetic nerves innervating skeletal muscle can modulate glucose uptake and glycogenolysis independent of concomitant increase in plasma insulin levels, via activation of $\beta 2$ adrenergic receptors (59, 60). Of note, administering a medical β_2 agonist appears to improve glucose tolerance due to increased glucose uptake in skeletal muscle (61).

Conversely, neuronal stimulation of α-adrenergic receptors in arterioles, elicits vasoconstriction. Thus, reduction in the number of open capillaries may decrease glucose utilization leading to peripheral IR.

Compared to the liver, pancreas, and skeletal muscle (which are also under parasympathetic control), adipose tissue is only innervated by sympathetic nerves making the SNS an important regulator of lipid mobilization. The SNS stimulates adipocytes by β1-3 receptors, to increase lipolysis and to produce FFA (62, 63).

All these processes increase BP and glucose concentration in the blood, actions that are expected after activation of the SNS to protect vital organs and activate the body in case of danger. However, overactivity of the SNS, due to chronic increase of stimulating factors or decreased activation of the parasympathetic system, contributes to the development of many diseases such as TRH, obesity, metabolic syndrome and IR.

Figure 1. Role of central sympathetic nerve activation in BP control, glucose and lipid metabolism



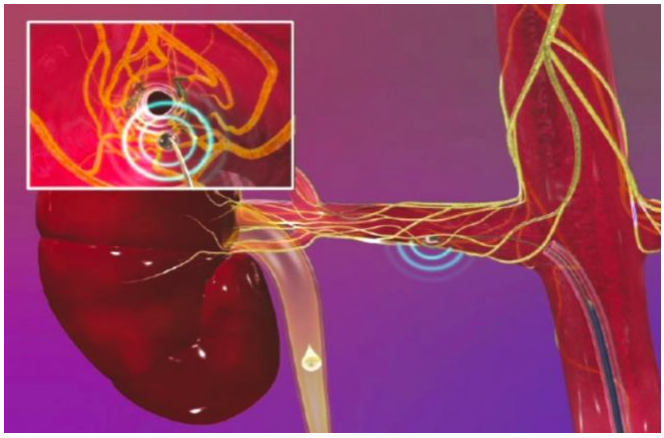
IR-insulin resistance, BP-blood pressure, HR-heart rate, RAAS-renin-angiotensin-aldosterone system, EGR-endogenous glucose release, FFA- free fatty acids, GLUT-4-glucose receptor

6.5 The rationale for renal sympathetic denervation

The renal sympathetic nerves run along the renal arteries in the adventitia. Afferent fibers from the kidneys convey signals to the brain, to regulate whole body sympathetic tone that contributes to the neurogenic mechanism of hypertension (48, 64). Renal efferent nerves innervate the kidneys from the para-vertebral ganglia at T11-L3, mediate in renal sodium retention (65) and stimulate the neuro-humoral renin-angiotensin-aldosterone axis. Knowing that BP depends primarily on neural control and volume regulation, sympathetic nerves plays an important role in BP regulation.

Thus, surgical thoracolumbar sympathectomy, resulting in renal denervation, was performed during the first half of the 20th century to treat malignant hypertension. The technique resulted in effective BP reduction. It was, however, accompanied by postural hypotension, erectile dysfunction and syncope (66, 67). Therefore, later availability of effective antihypertensive drugs put surgical antihypertension treatment aside. Despite a variety of antihypertensive drugs and combination of those, as well as resources to assist patients' adherence and lifestyle changes, BP and cardiovascular risk did not decrease in all patients, as expected. In the light of the development of invasive radiology and cardiology, the idea of a non-surgical sympathetic denervation emerged. Based on the anatomical availability of the renal nerves from a femoral access (via a 6F introducer through common femoral artery to the renal arteries) and given that efferent and afferent renal sympathetic nerve overactivity is thought to contribute to hypertension development, RDN has been developed to target these pathways to reduce BP. There are several types of RDN devices using different types of energy or chemical substances to damage nerves. Radiofrequency energy transform electrical current to high temperature resulting in localized tissue destruction and has been used in cardiology for many years to treat arrhythmias. RDN is a mini-invasive procedure using specialized radiofrequency ablation catheter with access to the renal arteries from one of the femoral arteries. The first-generation radiofrequency ablation catheters as Ardian system (Figure 2) applied usually, four to six two-minute treatments per renal artery to damage renal nerve fibers. The later evolution of RDN multielectrode devices led to an increased number of ablations including not only the main renal artery, but also its branches.

Figure 2. Nerve fibers along the renal artery and the Symplicity radiofrequency flex catheter



Picture of the Ardian system, with permission from Medtronic

The first RDN studies including SYMPLICITY HTN-1 and 2, demonstrated a significant reduction in BP after RDN (68, 69). Catheter-RDN has emerged as a new approach for TRH. Several studies have shown that RDN lowered SNA (as assessed by renal noradrenaline spillover) (68, 70), and muscle sympathetic nerve activity (MSNA) (71). Other RDN trials focused on an additional potential RDN effect on heart arrhythmias (72) and glucose metabolism (73). However, the publication of the SYMPLICITY HTN-3 changed the view of RDN research (74). This blinded trial did not show significant reduction in BP in patients with TRH six months after RDN, as compared with a sham control group. In the RDN group mean SBP measured in the office was reduced by an average of 14.1 ± 24 mmHg, whereas the corresponding SBP decline in the sham-controlled group was 11.7 ± 26 mmHg. Moreover, mean 24-hour SBP showed no significant between-group differences six months after the procedure. However, no drug adherence control and operator inexperience were major limitations of this study, suggesting the need for more carefully prepared and performed RDN trials.

In the later years RDN studies have focused on renal artery microanatomy, and identification of response based on renal nerve stimulation (75). Of note, proof of principle for the BP lowering effect of RDN have been demonstrated in some new, sham-controlled trials (SCT), even though the BT reduction was modest. These studies differed from previous RDN trials regarding patient selection, procedural and operator related aspects (76-78).

Due to the complex interactions between hypertension and IR it is difficult to indicate the primary insult that leads to overactivation of the SNS. This sympathetic cross-talk between the kidneys, peripheral tissue and the brain appears to play an important role in TRH and IR. Advances in technology and the availability of mini-invasive procedures as catheter-based devices enabled using of radiofrequency energy to ablate renal nerves in the aim to modulate SNA.

7 AIMS OF THE THESIS

The general aim of this thesis was to study the intraindividual changes in glucose metabolism after RDN, a new invasive method, applied in patients with TRH. The first step was to study BP and BPV change six months after RDN. Further, we wanted to test the hypothesis that IS, measured using the gold standard method, HEC, may be improved six months after RDN. Finally, we wanted to evaluate whether progression in IS might be delayed or even reversed at two-year follow-up after RDN, and whether changes in IS may be related to BP and arterial stiffness alterations during a two-year follow-up.

8 METHODS

8.1 Ethical approval

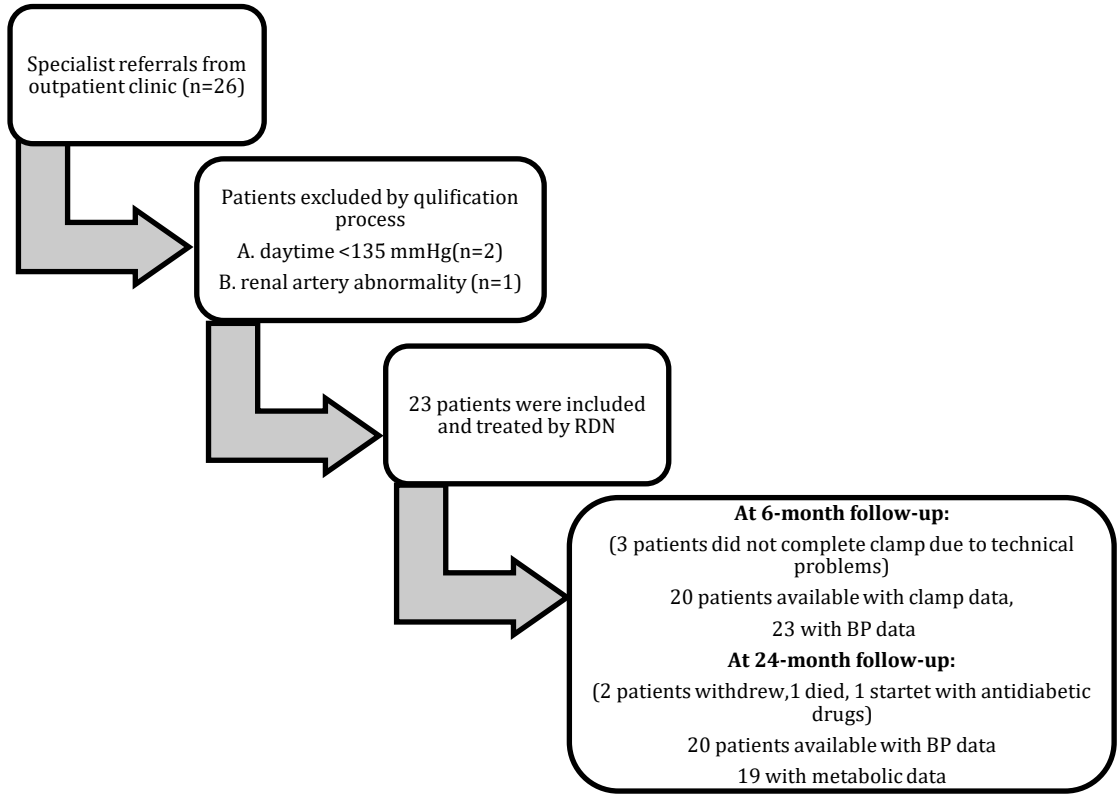
The study was conducted in accordance with the protocol, applicable regulatory requirements and the ethical principles of the Declaration of Helsinki and Title 45, U.S. Code of Federal Regulations, Part 46, Protection of Human Subjects, Revised 13 November 2001, effective 13 December 2001. The Regional Committee for Medical and Health Research Ethics as well as the Data Protection Officer at University Hospital of North Norway gave their approval. The included patients gave their written, informed consent. ClinicalTrials.gov Identifier: NCT01630928). All authors were funded by governmental nonprofit organizations in Norway. The study was supported by The Norwegian Diabetes Association, The North Norwegian Health authorities and UiT The Arctic University of Norway. The study also received an unrestricted grant from Medtronic. The funders had no access to the study data, and had no role in the design, conduct or reporting of the study.

8.2 Study population

Patients with TRH, classified according to a modified definition from the 2007 ESH guidelines (79) and confirmed by ABPM followed by hypertension specialists were eligible for inclusion in this study. The study was performed from 2013 to 2015. The patients had office BP >140/90 mmHg and were treated with four or more antihypertensive drugs, including a diuretic, in maximally tolerated doses. In addition, subjects had to have an average daytime SBP >135 mmHg, as measured by ABPM after an investigator witnessed the intake of their antihypertensive drugs. The antihypertensive medications were kept unchanged at least 14 days before starting the study.

Before being accepted as candidates for inclusion, secondary hypertension was excluded by standard clinical evaluation, and blood tests including measurements of serum aldosterone, thyroidal hormones, renin activity, normetanephrine and metanephrine. Exclusions criteria were as follow: <18 age > 68 years old, estimated Glomerular Filtration Rate (GFR)< 45 mL/min/1.73m² after MDRD formula (Modification of Diet in Renal Disease) (80), a previous diagnosis of diabetes, hemoglobin A1c (HBA1c) ≥ 6,5%, hemodynamically significant heart valve diseases, implanted pacemaker, contrast allergy, cancer last five years, pregnancy, previous renal transplantation, renal artery anatomy factors like diameter less than four mm, length <20mm (measured from ostium to first major side branch), renal artery stenosis or significant atherosclerosis and previous renal stenting. To achieve the power needed for the primary endpoint (20% change in basal EGR), 20-25 patients were needed. Twenty-three patients with TRH were included in the study and underwent RDN.

Figure 3. Flow chart of the present study



8.3 Measurements of blood pressure indices

8.3.1 Blood pressure

Patients were asked to bring their prescribed medication in original package to the clinical visit with one of the study nurses. Medication was documented, administered by the nurse and swallowed by the patient under continuous observation, to secure intake of the medication in prescribed doses. Patients were then continuously under observation by the nurse until 24-hour ABPM device had been mounted and tested. Information about lifestyle was assessed by a self-administered questionnaire. Medical history was taken by one of the study physicians. Patients were asked about obstructive sleep apnea symptoms, physical activity, diet and smoking. Changes of the antihypertensive medication was not allowed during the study, unless judged medically necessary.

ABPM readings were taken every 20 minutes during daytime (7:0 AM to 10:0 PM), and every 30 minutes during nighttime (10:0 PM to 7:0 AM).

Nocturnal hypertension was defined as (mean SBP ≥ 120 mmHg) (9). Mean arterial pressure (MAP) was calculated as [(2 × DBP) + SBP]/3 from ABPM. The degree of nocturnal BP fall (dip %) was calculated as 100 x (1- (average of nighttime BP/average of daytime BP)). Patients with nocturnal BP reduction less than 10 percent was defined as non-dippers.

Participants were classified as BP responders if they achieved a reduction in mean 24-h SBP ≥ 5 mm Hg from baseline to two-year follow-up (81).

Only ABPM with qualified recordings covering more than 70% of the 24 hours were regarded as technically sufficient for inclusion in the analyses. From the ABPM recordings, mean 24-hour SBP and DBP were computed. Office BP readings were taken in a seated position with an automatic oscillometric device after five minutes of rest. BP was measured on each arm, and the arm with the higher BP was used for all subsequent readings.

Averages of the two last measures were calculated and used for analysis. The same experienced nurses handled all BP measurements using the same calibrated devices in all patients at baseline and at follow-up. Office BP readings were taken by Casmed 740, (Infiniti Medical AS, USA) and ABPM was assessed using Schiller BR-102 plus (Diacor AS, Switzerland).

8.3.2 Blood pressure variability

Ambulatory BP recordings were analyzed and the standard deviation (SD) of 24-hour BP as well as SD of day and nighttime was calculated. Knowing that nocturnal BP fall is significantly and positively related to 24-hour BP SD, we assessed the weighted standard deviation (wSD) for each period of the day, to remove the mathematical interference from nighttime BP fall. We calculated wSD of SBP and DBP as the average of daytime and night time SD, divided by the duration of the day and night periods, respectively (82).

Current evidence suggests that average real variability index (ARV) adds significant prognostic information to ABPM monitoring, thus we calculated ARV as previously reported (83).

Morning BP surge was calculated as the difference between the average of SBP during two hours immediately after awakening and the average of the three SBP readings centered around the lowest night SBP value (after crosschecking the patients diary) (84).

The two-hour awake BP was defined as the average of four-five BP readings during the first two hours after morning arousal. The coefficient of variation of SBP and DBP was assessed by dividing SD by mean SBP and DBP, to examine whether the effect of BPV was independent of the BP level (85).

8.3.3 Arterial stiffness

PP was calculated as differences between mean systolic and diastolic ABPM. AASI, a surrogate measure of arterial stiffness, was calculated from ABPM readings as one minus the regression slope of DBP on SBP (24).

8.4 Assessment of metabolic variables and insulin sensitivity

8.4.1 Metabolic variables

From creatinine measurements eGFR was calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation (86).

Metabolic syndrome was diagnosed according to the IDF criteria from 2006 (45).

According to the American Diabetes Association criteria individuals were classified as having normal fasting glucose and tolerance, impaired fasting glucose or impaired glucose tolerance (87). Venous blood samples were drawn after an overnight fast (12 hours).

A standard (82.5 g of glucose monohydrate) OGTT was performed, with plasma samples obtained at 0, 30, 60, 90, and 120 minutes after the glucose load. Postload glucose and insulin responses were calculated as incremental area units during the two-hour sampling time, and were expressed as the area under the curve (AUC) for glucose and insulin.

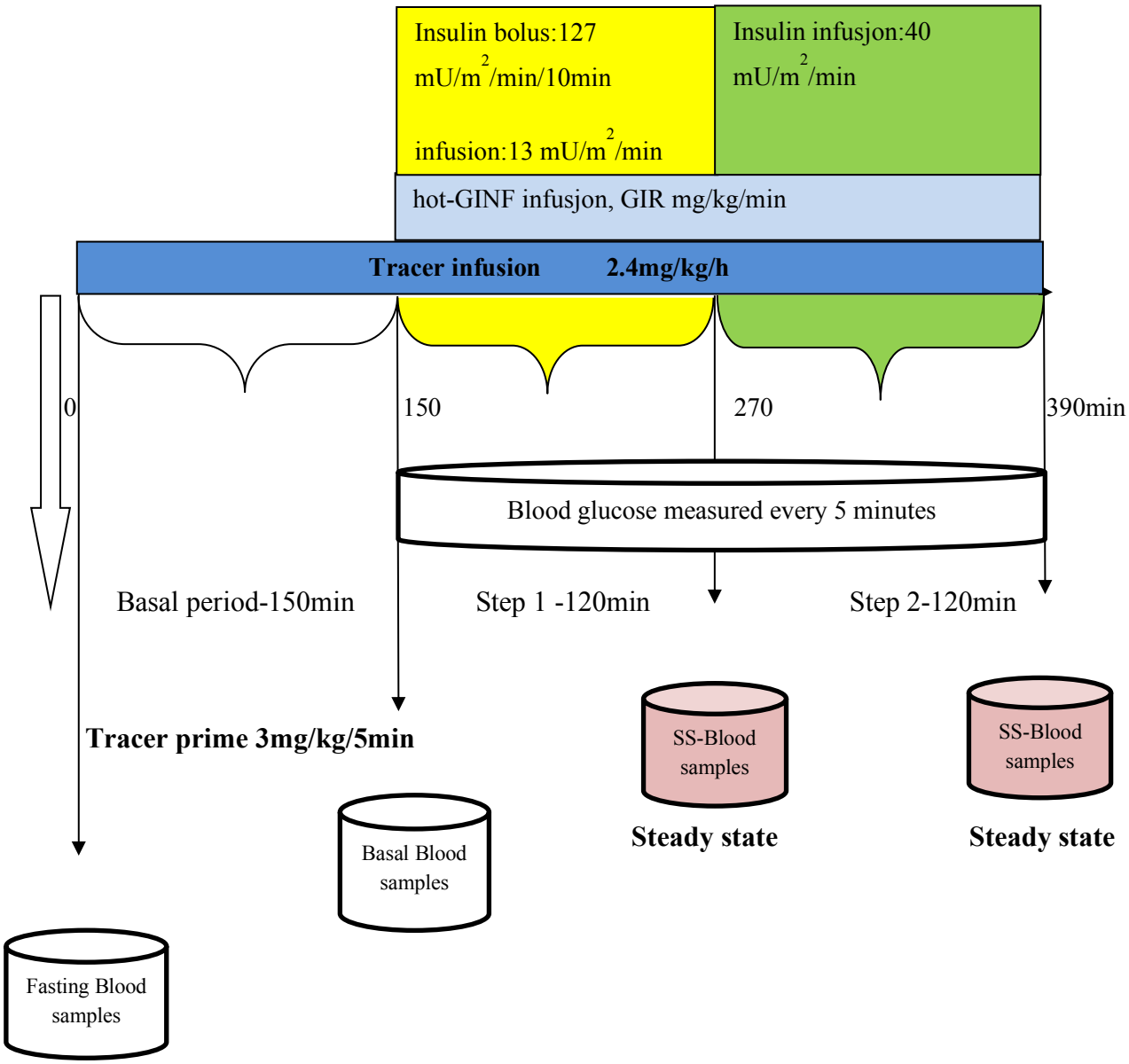
Insulin (endogenous and lispro) during HEC was measured with radioimmunoassay. Levels of insulin during the OGTT and C-peptide were measured by ELISA (EIA-1293 and EIA-2935 respectively; AH Diagnostics, Aarhus, Denmark). ELISA was used also to analyze leptin (EIA-2395), adiponectin (Acrp30).

8.4.2 Clamp procedure

Two step-HEC was performed after a 12-hour fast, as previously described (88). After the drawing of fasting blood samples, a primed (3 mg/kg/5 min), continuous (2.4 mg/kg/h) infusion of D-[6,6-$^2H_2$] glucose was performed for 150 min. to assess basal non-insulin stimulated endogenous glucose release (EGR) and whole-body glucose disposal (WGD). Tracer infusion was then continued, and a primed (127 mU/m^2/min for 10 min) infusion of human insulin (insulin lispro) was commenced at low (13 mU/m^2/min) and then high dose (40 mU/m^2/min), each lasting 120min. Glucose (200 mg/mL) enriched with D-[6,6-$^2H_2$] glucose at a 1.25 atom percent enrichment to improve the sensitivity of the method, so-called hot-GINF, was variably infused during the step-clamp to maintain normoglycemia (5 mmol/L).

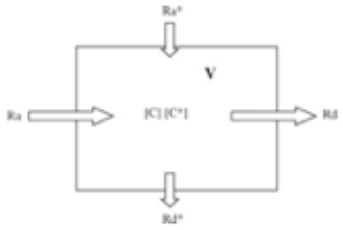
Glucose in arterialized blood was measured every five minutes during the step-clamp. From fasting blood samples glucose, insulin and C-peptide were measured. From basal sample and at every steady-state (last 40 minutes of step) new blood samples were obtained for insulin, C-peptide and tracer measurements. Sampling, chemical analysis, and the determination of tracer enrichment were performed as previously described, using liquid chromatography mass spectrometry for determination of tracer enrichment (89).

Figure 4. Schematic illustration of HEC



Whole-body IS was expressed as the glucose infusion rate (GIR) (mg/kg/min) during the last 40 min of each step of the clamp (steady state). The IS index (ISI) was calculated as the mean GIR divided by the mean insulin concentration at each step. Total glucose rate of appearance (*Ra*) and WGD as rate of disappearance (*Rd*) were calculated using modified versions of Steele's equations for non-steady state before step 1 (90, 91).

Figure 5. Ra and WGD calculations



$$Ra = \frac{F}{E(t)} - \frac{pV \times \left[C(t)/\left(1+E(t)\right)\right] \times \left(dE(t)/dt\right)}{E(t)} \quad \text{and} \quad WGD = Ra - pV \times \frac{dC}{dt}$$

F: the tracer infusion rate; E: the tracer enrichment; p: the pool fraction (0.65);

V: volume of compartment, the distribution volume of glucose taken as 230 ml/kg;

C: glucose concentration; C*: plasma tracer concentration;

Rd: rate of glucose disappearance; Rd*: rate of tracer disappearance;

Ra: rate of appearance; Ra*: tracer infusion rate;

F -consisted of the continuous tracer infusion alone prior to clamping, as opposed to during clamping where F was the sum of the continuous tracer infusion and tracer infused with the labelled glucose infusate during the last 40 min of each clamp. A linear curve was fit to the glucose concentration and tracer enrichment raw data by linear regression in order to minimize analytical variation and improve accuracy of the calculations.

EGR was calculated by subtracting the rate of exogenous GIR from the Ra of labeled glucose (EGR=Ra-GIR).

The following calibrated infusion pumps were applied: care fusion Alaris Guardrails (BD, San Diego, CA) syringe pumps were used for insulin, and infusions of D-[6,6-$^{2}H_{2}$] glucose and a tracer-enriched glucose solution were performed using Alaris Medsystem III (BD).

8.4.3 Surrogate insulin resistance indices

1. Homeostasis model assessment (HOMA-IR) = (fasting glucose [mmol/L] x fasting insulin [μIU/mL]/22.5) (92).

2. Quantitative IS Check Index (QUICKI) =1/(log [fasting glucose (mg/dL)]) + log(fasting insulin [μIU/mL] (93).

3.Simple Index Assessing IS OGTT (SIisOGTT) 1/(log [Σ glucose t 0–30–90–120] [mmol/L] + log [Σ insulin t 0–30–90–120] [μIU/mL]) (94).

4. The triglyceride and glucose (TyG)= Ln [fasting triglyceride (mg/dL) x fasting glucose (mg/dL) /2] (95).

5. The leptin-to-adiponectin (LAR) index-was calculated by dividing serum concentrations of fasting leptin (ng/ml) by fasting adiponectin (μg/ml)] (96).

6.The HOMA-adiponectin model assessment (HOMA-AD) was calculated with the formula: [fasting glucose (mmol/L) x fasting insulin (μIU /mL)] / [22.5 x fasting adiponectin (μg/ml)] (97).

8.5 The renal denervation procedure

RDN was performed with transfemoral access using the Symplicity Catheter System (Medtronic, Mountain View, CA), which was the first commercially available system used for RDN. Immediately before the procedure, renal anatomy was clarified using renal angiography, if not done before with computed tomography. The main trunk diameter should be more than four mm and length more than 20 mm. RDN procedure was performed via femoral artery catheterization with 6F catheter. After the cannulation of the artery, 5000 units of heparin were administered for anticoagulation. Then the flexible radiofrequency catheter was advanced into each renal artery under fluoroscopic guidance with the tip of the catheter placed in the ostium of the renal artery. The Symplicity catheter consists of a unipolar ablation catheter and a proprietary low-energy radiofrequency generator. Radiofrequency works by an alternating electrical current system (five to eight Watts) via a single electrode catheter with electrode tip size of two mm, that heats the tissue in contact with the catheter tip (up to four mm depth) and by thermal conduction (50-70 °C) to deeper tissue (98). Radiofrequency energy is applied to the endothelial lining, delivered energy causes local thermal destruction in the perivascular adventitia expecting to damage sympathetic nerves lying there. Native renal blood flow cools the intima and reduce endothelial injury. Between the ablations the catheter was drawn back one to two mm, rotated a little before another ablation was applied, to get a helical pattern of ablations to cover the circumference. Accessory branches were not denervated. This procedure was repeated four to five times before the same procedure was performed in the other renal artery. On average, each patient had 12 ablations of two minutes duration and the minimum number of complete ablations per side was more than four, as performed in other SYMPLICITY studies (69). The procedure duration was 50 to 90 minutes.

In case of a sudden rise in impedance, which could suggest overheating of radiofrequency catheters, the auto-feedback mechanisms prevented excessive temperature elevations. RDN was performed by one, experienced interventional cardiologist trained for the procedure (TKS). A product manager from the manufacturer (Medtronic) were present following all steps in the procedure for all patients. The perivascular neural bundle also contains sensory C fibers and thus neural destruction is accompanied by significant pain. Intraprocedural pain was managed with intravenous anxiolytics and narcotics (midazolam and morphine). Patients were hospitalized overnight and followed with self-administered BP measurements at home weekly the first month, later monthly, after written and practical instruction. After the procedure, all patients received Aspirin or Clopidogrel for at least one month. Six months and two years after the procedure all patients came for a follow-up visit with office BP and ABPM measurements after witnessed intake of medicines, as described above.

8.6 Statistical analysis and power calculation

Data were presented as mean ± SD if normally distributed or as median (min, max) if skewed. For continuous variables, we used paired Student's t-tests to compare differences between pre-RDN and six-month follow-up measurements and between pre-RDN and two-year follow-up. For variables with a skewed distribution we applied Wilcoxon signed-rank tests. Correlations were assessed using Pearson`s test. Statistical analyses were performed using SPSS software (IBM Corp. Released 2017. IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp.). Differences were considered statistically significant at $p<0.05$.

A 20% change in basal EGR (0.4 mg/kg/min) was considered to be clinically relevant. With an a level of 0.05 and a power of 80%, 20–25 patients were needed to demonstrate a 20% difference in basal EGR before and after intervention (99).

9 SUMMARY OF RESULTS

9.1 Paper I

In paper I we assessed the change in BP and short-term BPV from baseline to six months after RDN. Bilateral RDN was performed in 23 patients (mean age was 53 ± 8 years) without any periprocedural or late complications up to six months. The number of ablations for each patient were 12.6 ± 2. At baseline all patients used a diuretic and an angiotensin converting enzyme inhibitor (ACEI) or an angiotensin receptor blocker (ARB), 87% of patients used a calcium channel blocker, 48 % of patients used an aldosterone antagonist, minimum 25mg daily. The office SBP and ABPM fell significantly from 162 ± 20 mmHg to 139 ± 19 mmHg ($p=0.001$) and from 154 ± 20 mmHg to 144 ± 16 mmHg ($p= 0.038$) respectively. Reductions in office and ambulatory DBP were also significant. There was a statistically significant reduction in both systolic and diastolic mean and daytime BP, but not in nighttime SBP. We observed a reduction in office SBP ≥ 10 mmHg in 13 out of 23 patients and, accordingly, a decrease of mean 24-hour SBP more than five mmHg was seen in 12 of 23 patients (52%). There was a significant reduction in the number of prescribed drugs from a mean 4.8 to 4.2 ($p=0.02$). Heart rate did not change significantly from baseline (72 ± 12 beats per minute) to six months control (72 ± 12 beats per minute). Body mass index (BMI) and eGFR remained stable during the study.

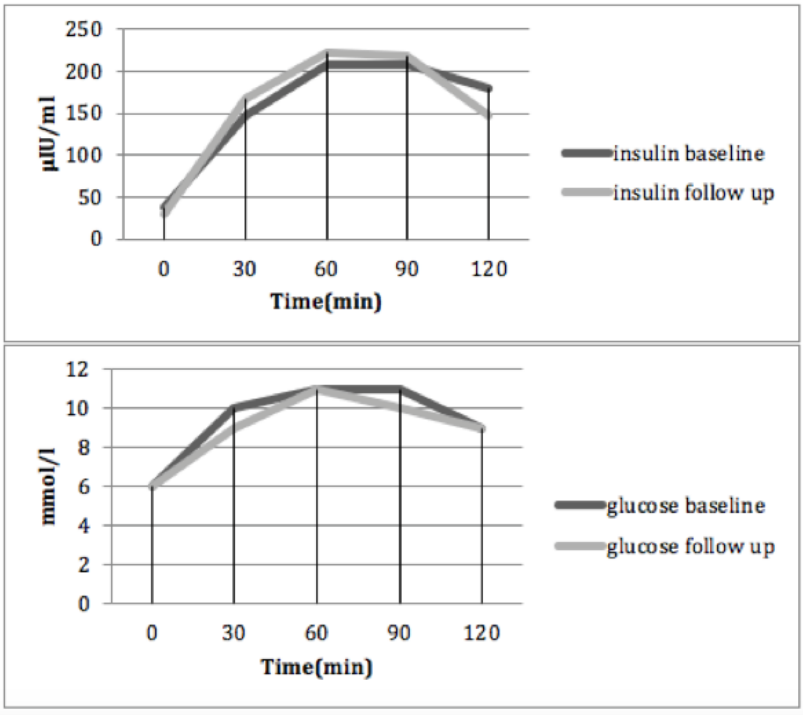
BPV measured as SD of 24-hour BP as well as SD of daytime BP fell significantly from baseline to six months, whereas no significant change in SD during nighttime was found. Significant decrease of both systolic and diastolic wSD and ARV was found after six months.

The morning BP surge and the two-hour awake BP decreased significantly from 29 ± 13 mmHg to 20 ± 14 mmHg (p=0.011) and from 157 ± 19 mmHg to 147 ± 16 mmHg (p=0.024) after six months. Systolic and diastolic dipping did not change significantly.

9.2 Paper II

In paper II, we assessed whether IS improved six months after RDN. Twenty-one of 23 patients had central obesity, and 18 patients had metabolic syndrome at baseline. Fifteen patients had normal fasting glycemia, eight patients had impaired fasting glycemia, and 17 patients had impaired glucose tolerance. Fasting plasma glucose and the OGTT-derived AUC for glucose and insulin remained unchanged at six-month follow-up-Figure 6.

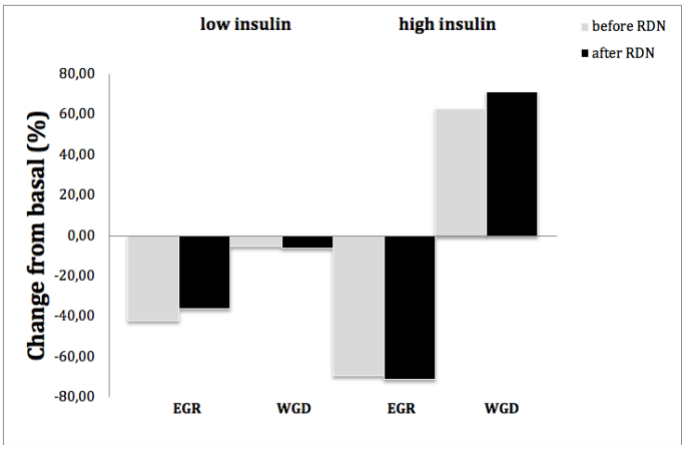
Figure 6. Plasma glucose and insulin response during 120min OGTT



High insulin and C-peptide concentrations were seen at baseline and remained unchanged after six months. Accordingly, the indirect indices of IR, QUICKI, SIisOGTT, and HOMA-IR were high at baseline and did not improve after RDN. Twenty-three patients were scheduled for a two-step HEC with glucose tracer and labeled glucose infusion before and six months after RDN. Three patients were excluded from the clamp measurements because of technical problems encountered during the clamp procedure. Basal EGR and WGD measured by glucose tracer infusion did not change significantly after RDN (2.12 ± 0.36 mg/kg/min vs. 2.15 ± 0.41 mg/kg/min (p=0.34), and 2.20 ± 0.36 mg/kg/min vs. 2.14 ± 0.40 mg/kg/min (p=0.35), respectively. During the two-step HEC, no significant changes in GIR and ISI were seen, indicating unaltered whole-body IS. Fasting and steady-state plasma C-peptide and insulin levels during the clamp remained unaltered after RDN.

The suppression of EGR decreased significant during low-dose insulin infusion, but remained unchanged during high-dose insulin infusion. The increase in WGD during high-dose insulin infusion was modest and remained unaltered at follow-up as presented below in Figure 7.

Figure 7. Change of EGR and WGD during two-step clamp before and six months after RDN



No improvement in IS was observed in a subanalysis of nine patients with extensive systolic mean ambulatory BP reduction (>10 mmHg) after RDN.

9.3 Paper III

We wanted to evaluate IR, adipokine profiles, BP and arterial stiffness changes two years after RDN. We also studied the correlation between gold standard measurements of IS and surrogate indices in this cohort of patients with TRH. Twenty patients continued to two-year follow-up (18/20 were men). There was a small, no significant reduction in the number of antihypertensive drugs from a median of 4.5 (4-8) different drug classes before RDN to 4.0 (0-7) (p=0.08) two years after RDN. Nocturnal hypertension was observed in 16 patients at baseline and 18 at two-year follow-up. Sustained reduction in DBP and MAP, but not SBP, was found two years after RDN. PP and AASI did not change significantly during follow-up.

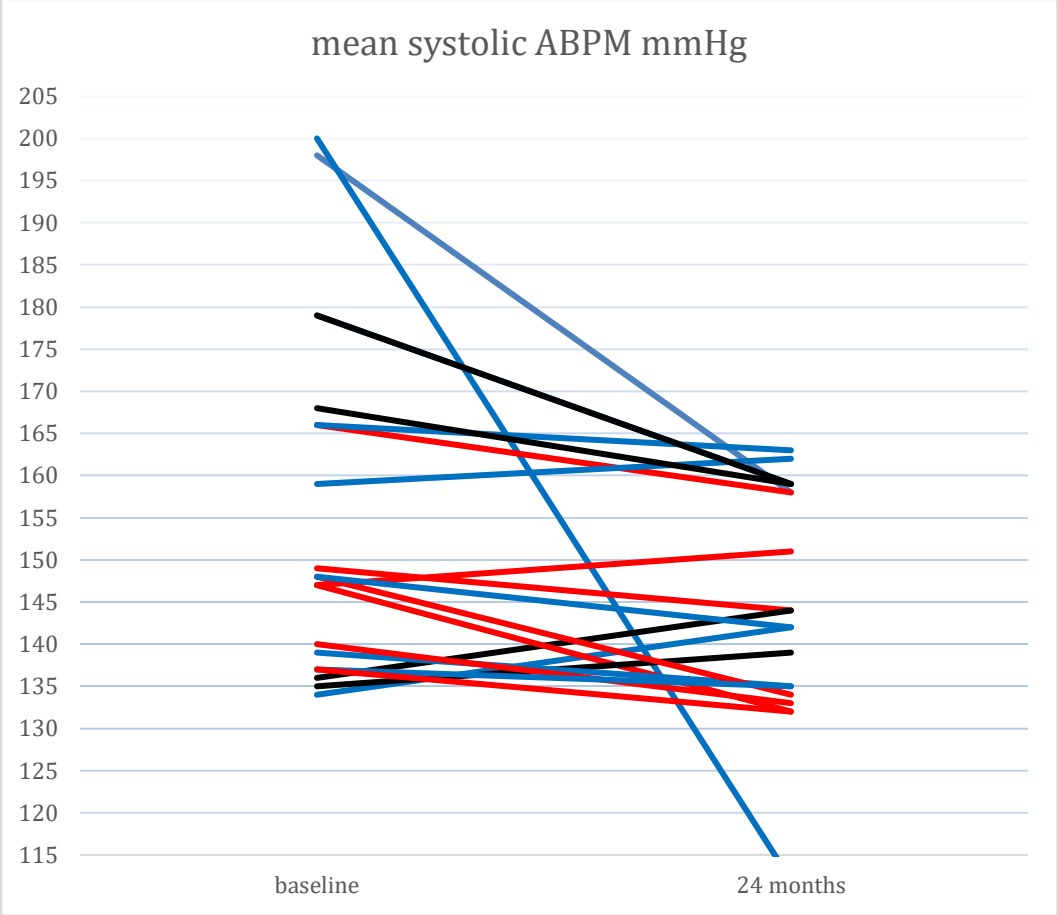
After two years two patients had developed type 2 diabetes. One of them received antidiabetic treatment at two years and was excluded from the two-year metabolic calculations. We found a borderline increase in BMI from 31.6 to 32.6 kg/m^2, p=0.05. Most of the metabolic parameters or IR surrogate indices were essentially unchanged two years after RDN, apart from a statistically significant increase in HOMA-AD and QUICKI indices, however, with vague clinical relevance.

There were no significant changes in the adipokines during the two-year study period.

There was modest correlation of the different indices of IR and HEC measurements prior to RDN. Peak of glucose concentration at 30 minutes during OGTT (OGTT 30 min.) correlated best with EGR reduction during low-dose insulin infusion. HOMA-IR correlated best with GIR and WGD increase during high-dose insulin infusion. Other indices, including TyG, LAR, C-peptide, adiponectin and leptin, correlated neither with hepatic nor peripheral clamp derived IS measurements. BP responders and non-responders did not show statistically different hepatic or peripheral IR prior to RDN.

There were no significant correlations between IR, adipokines, BP or AASI throughout two years of follow-up. As reported in Paper II, we found a statistically significant deterioration of hepatic IR six months after RDN. Nocturnal systolic BP and arterial stiffness before RDN correlated positively with a progression in hepatic IR at six-months follow-up.

Figure 8. Change in mean systolic ABPM from baseline to two-year follow-up relative to drug adjustment.



The colors depict changes in the number of antihypertensive drugs from baseline to two-year follow - up. blue- decreased, red- increased, black- unchanged number of drugs

10 METHODOLOGICAL CONSIDERATIONS

Participation in the study did not expose the patients to unacceptable risk. The benefits of a potential BP reduction outweighed the possible harm of the RDN and clamp procedures. The patients were carefully followed-up and received information about individual results at the end of the study.

10.1 Renal denervation

Efficacy

After the initial studies on RDN subsequent studies in the field suggested that the distribution pattern, density and distance to the lumen of the renal sympathetic nerves vary in animals and humans, hence this may have impact on the success of RDN (100, 101).

Starting the Re-Shape study in 2013, we applied the equipment that was available, the Symplicity Flex catheter. RDN was recommended to commence distally and to be performed by pulling and rotating the catheter tip to obtain a helical pattern of ablations. The procedure was performed empirically, as there is no intraprocedural test available to assess denervation effect. Given the spiral course of the nerve bundles, a nerve might cross to another quadrant between the ablation points and escape denervation. The manipulation of the Symplicity Flex catheter to achieve adequate contact and a circumferential ablation pattern is technically challenging, and thus requires rigorous training and great operator experience. Even though in our study the procedure was performed by one experienced interventional cardiologist (who participated in more than 20 RDN procedures in a specialized center in Germany, before treatment of the study patients) we cannot guarantee sufficient denervation in every patient.

Further research has brought new information, demonstrating that sympathetic nerves may not form a true renal plexus surrounding the proximal or ostial vessel segments which were focus in the first generation RDN trials (102). Many human studies have revealed that in a large percentage of kidneys, the main renal artery divide into many branches from the hilum and some nerves commonly bypasses from the preaortic ganglia to the branches (jumping over the main renal artery) (103). Other findings suggest that additional denervation of the distal artery and associated branches may contribute to better RDN effect (70)**.** This is crucial, because in Re-Shape study, only the main artery was treated. Thus, we cannot exclude that in some patients, we could have missed some renal nerves.

For a successful RDN, an accurate depth of the lesion is essential to sufficiently damage the periarterial nerves (104, 105). The Symplicity Flex catheter applies low energy and deliver about eight watts, which heats the tissue up to about four millimeters depth (98). Evolving evidence indicate that in human renal arteries, a substantial number of renal sympathetic nerves are located out of reach of the standard lesion depth delivered by radiofrequency catheters (100). Moreover, radiofrequency energy can be deflected by tendons and lymph nodes, adjacent to the renal arteries.

It also seems reasonable that a larger number of ablation points may increase the probability of adequately denervation of the kidney. In the newer RDN studies, spiral multi-electrode catheters were used. These new catheters may help to achieve complete circumferential nerve ablation, as the catheter does not need to be re-positioned between energy applications. The total number of ablations per patient, performed in our study, was 12, compared to SYMPLICITY HTN-1,2,3 with 7-8, 8-12, 13 ablations, respectively (68, 69, 74). However, applying multi-electrode catheter in SPYRAL HTN-OFF/ON MED trials the number of (main vessel and branches) ablations per patient was 44/46, respectively (77, 78).

Albeit, Lauder et al. showed, that ambulatory BP lowering was not related to renal artery length nor increasing number of ablations (106).

Indeed, it is still a matter of debate, what quantity of renal sympathetic nerves need to be ablated to achieve significant clinical results. It has been suggested from animal experiments that more than 50% of the periarterial renal nerves should be damaged to expect alterations in norepinephrine contents in the kidney (104). Given that the SNS induces different responses in many organs, the outcome of RDN cannot be limited to the evaluation of the one parameter. One of the major limitations of the available techniques is that there is no intraprocedural tests to assess proper ablation and effective destruction of the renal sympathetic nerves. In compliance with most RDN studies we did not measure SNA changes before and after RDN as there is still no good method to assess the procedural endpoint or the completeness of RDN. Summing up, the effectiveness of RDN procedure is unknown and new data make us question whether the Symplicity Flex catheter might be able to adequately ablate renal nerves. Thus, performance bias cannot be eliminated.

Durability

The durability of sympathetic modulation by RDN is an unanswered question, where nerve regeneration and cross-talk between denervated kidneys each may play a role (107, 108). Most of the patients that underwent surgical sympathectomy exhibited no rebound in BP over ten years (67). Albeit, only reports from animal research are available concerning the destruction and regrowth of renal nerves after RDN (109-112). The assertion about sustained BP reduction is mainly based on data from RDN registries which have shown a consistent BP reduction, both systolic and diastolic, even after three years (113, 114). However, a potential impact on BP results by improved adherence to antihypertensive treatment and patient's change of lifestyle was not controlled.

Safety

In our study, RDN was performed without any early complications. One patient experienced short-term orthostatic hypotension, but none of the patients experienced a hypertensive crisis or acute kidney injury. During the long-term follow-up, one patient died for unknown reasons two years after RDN. Due to the study design, we did not perform imaging of renal arteries after the RDN procedure, even in the patients who had experienced BP gain. However, a recent meta-analysis of 50 RDN trials including over 5700 patients with median follow-up of 11 months, estimated the incidence of new renal artery stenosis leading to revascularization to be 0.2% per year (115). In the longest follow-up study so far, presented by the Oslo RDN study, no renal stenosis has been revealed up to seven years after RDN (116). We limited safety control to the assessment of the kidney function, which was stable during two years of follow-up (117).

10.2 Clamp

The preceding studies that reported improvements in IS after RDN calculated changes in IS using surrogate indices with variable accuracy compared to the gold standard method. The two-step HEC with infusion of glucose tracer remains the only reliable noninvasive method to separately assess hepatic and peripheral IS. However, although considered gold standard method, some limitations of our clamp results must be revealed.

We did not measure glucagon, the major hormone that opposes the effects of insulin on hepatic glucose metabolism, functioning as a positive regulator of gluconeogenesis and glycogenolysis. SNA plays an important role in metabolic alterations, but via different pathways. Moreover, we did not assess FFA change during insulin infusion, which could contribute to whole body IS.

An evaluation of adipose tissue activity should not be limited only to adipokines, especially in case of an unequal gender distribution, as in our study (118). This is commented on in Paper III.

EGR was calculated as described above, and it should be taken into consideration that the liver is not the only glucose-producing organ during fasting conditions. The kidney cortex produces glucose by gluconeogenesis, and its relative contribution to EGR in the postabsorptive state is estimated to range from 5 % to 28 % (119, 120). Since the RDN intervention is performed directly on the kidneys, local changes in renal glucose metabolism could occur. Given that catecholamines and insulin play a role in the regulation of renal glucose release, as well as glucose reabsorption, this could have affected the results.

10.3 Blood pressure

The publication of the first RDN results in 2009 was met with a huge enthusiasm and thereafter many studies presented strong BP reductions after RDN procedures. Most of these trials lacked a sham-controlled group, and strict patient selection and control of adherence to medical treatment was not an issue. We used strict criteria related to both the number of prescribed drugs and daytime ABPM. Although the 2007 ESH guidelines did not require ABPM to diagnose TRH, we chose to exclude subjects having an average daytime SBP of <135 mmHg.

This was important, since ABPM appears to be associated with cardiovascular events (121). Another strength of our work was that secondary hypertension was carefully ruled out before acceptance into the study.

The patients' adherence to medical treatment was also an issue of utmost importance. To exclude nonadherent patients, we chose one of the methods recommended by guidelines, i.e. investigator witnessed intake of medications, proposed by the researches from the Oslo RDN study (13). In our study, none of the patients experienced hypotension after witnessed intake of their prescribed antihypertensive drugs. Only two patients had to be excluded from the trial after this carefully qualifying process, reflecting good patient selection. Unlike many other studies, we repeated the same adherence control at every step of follow-up.

However, the reliability of witnessed drug intake at a single instance to reflect general drug adherence is limited. After a single dose, one does not expect target levels of drug concentrations to be reached. Patients who take their medication irregularly, may not achieve stable levels of the drugs necessary to treat hypertension, to protect organ damage and vasculature changes. Therapeutic drug monitoring, which involves measurements of plasma or urine drug or drug metabolite concentrations, is considered a better approach that can assess adherence continually. In addition, this may help to personalize the treatment according to the patient 's individual pharmacokinetic properties. This method may also identify patients using drugs other than those prescribed (122, 123). The results from the SPYRAL HTN-ON MED trial showed that in 15% of the patients, nonprescribed medication was detected, which could not be demonstrated by applying witnessed intake. In the SYMPATHY study, almost 80% of patients with diagnosed TRH were either poorly adherent or completely nonadherent when participants and attending physicians were unaware of the drug measurement (124). In addition, about one third of the participants either increased or decreased adherence during follow-up, with a trend towards more pills being taken during follow-up. This finding was more pronounced in the control group than in the RDN treatment group, which may be explained by the more intensive follow-up during the study, but also by the absence of a sham intervention.

In the SPYRAL HTN-ON MED study, liquid chromatography mass spectrometry of urine and plasma was used in addition to witnessed intake to ensure drug adherence. Even then, 40% of patients were nonadherent, despite the awareness of compliance control (78). However, the ethical aspects in regard to involuntary drug control may be an issue of debate. Knowing that a witnessed intake before ABPM could not totally exclude fully or partly nonadherent patients, we acknowledge that this was a limitation in our selection of patients and that better adherence due to intensive follow-up could contribute to a BP drop after RDN. Adequate evaluation of adherence should be an obligatory part of TRH assessment.

10.4 Study design

The main weaknesses of our study were the nonrandomized design and the lack of control group, most preferably a sham procedure treated control group. When a study lacks a control group, it is impossible to determine whether the outcomes are attributable to the treatment or to other patients' characteristics and the natural history of the disease (e.g. decrease in DBP by age). The effects seen may be wholly or partly due to the intervention (RDN) or the placebo effect. Inclusion in a study may increase awareness of the disease, adherence to medications and other changes in patient behavior, known as the Hawthorn effect (125).

Another limitation was the sample size. Patients with TRH, but without type 2 diabetes are not abundant, so we struggled to find patients that matched our inclusion criteria. However, a different study design by reducing a number of drugs from four or more antihypertensive drugs to three or more (as recommended in the guidelines) would have enabled us to enroll more patients to the group. Although we had sufficient power to detect a significant change in IS; the changes in BP measurements and BP indices reduction could be underpowered.

The low number of patients limits statistical power and increases the probability of a type II error. The low sample size also prevented us from subgroup analyses. In addition, due to the small number of patients, we did not adjust the results for confounders.

11 DISCUSSION OF THE MAIN RESULTS

11.1 Blood pressure data

The Re-Shape study was addressed to patients with true TRH, where all possible antihypertensive drug classes, as well as lifestyle BP improving factors, had been attempted. In 2013, when our patients were included, TRH was defined as office BP> 140/90 mmHg despite the use of three or more BP lowering drug classes including a diuretic. Baseline characteristics, as reported in paper I, suggested that our patients were optimally treated. The mean number of antihypertensive drugs at baseline was 4.8 and all patients used diuretics, ARB/ACEI and most of them also spironolactone.

In paper I, we demonstrated a statistically significant reduction in both office and ambulatory SBP and DBP at six-month follow-up despite a decrease in the mean number of antihypertensive drugs from 4.8 to 4.2. The results, however, must be interpreted with caution. The BP changes from baseline to follow-up may be explained by regression to the mean, placebo effect or related to an improved adherence, as mentioned above. The mean difference between baseline and six-month follow-up in mean 24-hour SBP was -9 mmHg, and 12 out of 23 patients had an SBP drop ≥5 mmHg, which has been suggested to represent treatment response in previous studies (81). However, in SCTs presented by Bhatt et al., Desch et al. the mean 24-hour SBP fall in a sham group was -4, -3.5 mmHg, respectively (74, 126). In the work presented by Weber et al., mean 24-hour SBP reduction in the sham arm was -8.5 mmHg two months after RDN (in OFF MED period) (127).

This is also illustrated in the ReSET study when mean daytime SBP drop in the sham arm was zero after one month but then increased to six mmHg at three-month follow-up (128). This demonstrates how the placebo and the Hawthorn effects may operate on the results when adherence control is not meticulously executed.

Actually, in our study, we can also observe these non-RDN effects on BP reduction by looking at the discrepancy between mean office SBP reduction (-23 mmHg) and mean 24-hour SBP fall (-9 mmHg). This difference was even higher in the first generation studies (69), but quite small in the SCTs as in SPYRAL HTN-OFF/ ON MED and RADIANCE-HTN SOLO studies where the mean office SBP reduction compared to mean 24-hour SBP drop was 4.5, 0.4,3.8 mmHg, respectively (76-78).

In paper III we depicted an increase in BP two years after RDN despite an insignificant reduction in the total number of antihypertensive medications during follow-up. However, the mean number of drugs fell from 4.5 to 4.0 during the two years, which could contribute to a BP gain. Rebound in BP may be related to regeneration of renal nerves but also to an increase in a nonadherence and an attrition of the Hawthorn effect due to less attention given during long follow-up. Additionally, a raise in BP might be associated with later renal vasculature complications which were not assessed during follow-up. Finally, a disproportionate effect from a few patients on the overall result may always be a possibility in small studies.

Taking a close look at Figure 8, with individual BP recordings, we can observe wide dispersion of the BP fall. Some of the patients experienced huge BP drops, referred before as super-responders. Heterogenous response to RDN and its unpredictable effect is a key weak point of RDN. Many articles have been published about prediction of responders, however, mostly based on studies without sham-controlled group or global registries without adherence control of included patients (129).

An anatomic phenotype pattern of renal sympathetic nerves, denervation range, depth of lesion and finally a contribution of sympathetic nerve overactivity to hypertension, might make a difference in the RDN response. Indeed, it appears that RDN lowers BP in selected, but not all, patients, possibly due to the different mechanism leading to hypertension. However, knowledge in this field is still incomplete, and thus clinically useful recommendations for patient selection still have not been made.

An improvement in peripheral vascular resistance in addition to BP reduction is a potential treatment goal to reduce cardiovascular events. Many hypertensive drugs have vasodilator effect, and since vasoconstriction is a huge part of SNS activation, RDN may potentially also have an effect on vascular resistance. Previous RDN trials reported a drop in BP associated with an improved arterial stiffness and a decrease in total peripheral resistance independently of change in cardiac output (130, 131). Other RDN studies demonstrated a decrease in PWV (132) or improvement of aortic distensibility (133), unrelated to BP reduction. However, none of these studies were sham-controlled. The only RDN SCT revealed no significant changes in central aortic BP and PWV six months after the procedure in comparison with sham group despite significant within-group changes in the RDN group (134). In our study, we sought to assess potential changes in arterial stiffness by calculation of PP and AASI – an index presented as a negative predictor of BP response after RDN (135). We chose relatively young patients to avoid age dependent arterial stiffness. The results from paper III showed unaltered PP and AASI during the entire follow up. However, AASI and PP are surrogate indices that give limited information compared with PWV. In addition, in paper III we demonstrated a significant reduction in MAP during the entire study, however the sham-controlled study by Engholm et al. revealed an insignificant MAP reduction between RDN and sham group in addition to no improvement in microvascular impairment (136).

Thus, not applying a gold standard method for measuring vascular stiffness and not preforming SCT with adequate assessment of adherence, one still cannot conclude that RDN may improve vascular stiffness.

Looking at BP results in paper I and III it appears that the BP reduction after RDN was significantly more prominent during daytime than night time. In our study, insignificant changes were demonstrated for all nocturnal BP variables. Many of patients had nocturnal hypertension at baseline and even more at two-year follow-up. Assuming that our patients were adequately treated with diuretics, persisting nocturnal hypertension may suggest an unbalanced relationship between sympathetic and parasympathetic nervous system, which is typical for TRH patients. A possible explanation for the more pronounced effect during daytime may be that stress and activity increase SNA, thereby facilitating the RDN treatment effect being more pronounced during active hours. Further, SNA reduction occurs physiologically during sleep, causing BP reduction during night time to be less available for RDN (137). The SCTs have demonstrated conflicting results from a lack of nighttime BP drop (126) to a significant fall by -9.8 mmHg in RDN group compared to -2.1 mmHg in the sham arm (78). This inconsistency has no clear explanation; it is not unlikely that different patient selection criteria, differences in salt ingestion or presence of sleep disturbances may affect results. Additionally, the reduced frequency of BP readings during night- or fixed-time intervals for ABPM readings, might have affected the calculation of nocturnal BP and dipping. A successful reduction in nocturnal BP might have implications for cardiovascular endpoints. Growing evidence suggests that nighttime SBP is a stronger predictor for cardiovascular events than daytime SBP in hypertensive and diabetic patients (138). Of note, in paper III we presented that only nocturnal BP correlated with IR progression in our study.

In paper I, we looked at other BP parameters that are associated with cardiovascular events, such as BPV and morning surge. The transition from sleep to wakefulness is associated with a sympathetic activation and a rise in plasma catecholamines, which generally results in an increase in BP and heart rate. Another important aspect is that cardiovascular events have their greatest prevalence in the early morning period (17). In the current study, both the two-hour awake and morning BP surge fell significantly six months after RDN. In addition, we demonstrated a significant reduction in all indices of BPV (SD, wSD and ARV) both systolic and diastolic BP as well as SD of daytime BP, though not SD of nighttime BP.

The actual clinical value of morning BP surge is still a matter of debate, and conflicting results have been obtained (139). The value of BPV variables depends on the sensitivity of measurements, dependency on the BP level and high variability among individual patients. Thus, our study was too small to conclude about BPV variables, but our results may be considered as hypothesis generating. Even though another study with a larger sample size revealed improvement in BPV indices six months after RDN (140), none of the SCTs were studying the BPV changes as an additional potential surrogate outcome after SNS modulation.

11.2 Metabolic data

Because IR, hypertension and overactivated SNS are closely related, the previously reported amelioration of glucose metabolism after RDN has been followed with great interest. IR is also associated with polycystic ovary syndrome, nonalcoholic fatty liver disease and psoriasis (41), diseases linked to increased SNA, even without hypertension. Therefore, potential improvement of IS by RDN may be hypothesized to decrease cardiovascular risk, even in these group of patients.

When including patients to our study, we focused on patients with TRH that had not yet developed type 2 diabetes, a disease state with particularly high cardiovascular risk. As it turned out, included subjects were modestly obese and had high prediabetes risk. A greater part of them had already, before RDN, impaired fasting glucose and hyperinsulinemia, and 78% of them had metabolic syndrome. When obesity and hypertension are both present in the same patient, the degree of SNA is much greater than in those with either condition separately (5). Thus, our patients appeared to be a well selected target group for RDN, to improve IS.

In paper II we presented two steps clamp data to get a closer insight in IS changes. The method is considered gold standard method for assessment of IS. Tracer dilution during HEC gives the possibility to assess the ability of insulin to suppress EGR. During glucose tracer infusion basal EGR and WGD levels were where within normal range and did not change after RDN. Calculated EGR reduction from baseline was low and represented high hepatic IR before RDN. Six months after RDN we repeated the two-step clamp demonstrating even less EGR reduction, which suggested a deterioration of hepatic IR.

During step two of the clamp, insulin was infused at a rate that leads to the physiological hyperinsulinemia seen after a meal and that effectively increases WGD in individuals with normal IS values. The modest increase in WGD with increments in insulin infusion, both at baseline and at follow-up, supports the notion that our group of participants also experienced severe peripheral IR, which persisted after RDN. A trend toward increased GIR during clamp step two after RDN was probably related to the slightly higher serum insulin concentrations during clamping at follow-up, as the ISI, which corrects for the prevailing insulin concentrations, did not change after RDN. Taken together, our glucose tracer/clamp data did not show any change in basal glucose turnover nor improved hepatic or peripheral IS six months after RDN. Actually, they were in line with another small uncontrolled clamp study of eight nondiabetic patients with severe TRH, published in 2017 by Kampmann et al. (141).

The effect of the RDN procedure may be hypothesized to appear over a longer time period, which is thought to be related to gradually decreasing SNA (113, 142). Although the efferent fiber ablation may reduce a sympathetic input to the kidneys fast, the timing of the effect of renal sympathetic afferent fiber ablation with consequently remodulation of the central SNA may be delayed. Since the long-term effects of central SNA modulation on metabolic changes are unknown, we conducted the two-year follow-up.

Some RDN studies assessed the short effect of RDN on adipokines. It was hypothesized that RDN might affect adipokines, given that adipocytes have adrenergic receptors and leptin plays a role in the cross-talk between adipose tissue and the central nervous system. Hence, in paper III we assessed IR indices two years after RDN and adipokines profile during the entire follow-up. Although the included patients were slightly obese, they did not have hyperleptinemia, and their mean level of adiponectin was borderline low at baseline. During two-year follow-up no changes in their adipokine profiles were registered.

As opposed to our findings, other studies exhibited a rise in adiponectin, yet none of them demonstrated any change in the leptin level after RDN (143, 144). Differences in the use of renin angiotensin aldosterone system (RAAS) inhibitors may be one of the factors explaining the opposing study results. In previous studies, an increase in circulating adiponectin after a two-month treatment with losartan in hypertensive patients has been reported (145, 146). There are other experimental data showing an enhanced adiponectin expression in response to long-term use of ARB, and indeed, in our study, all of patients used RAAS inhibitors that could diminish the effect of RDN on adipokines (147, 148).

In paper III we also presented correlations between IS assessed by the gold standard clamp method and commonly used IR surrogate indices. In previous non-RDN metabolic trials, HOMA-IR was thought to represent hepatic IR and could possibly underestimate peripheral IR (149, 150). In our research HOMA-IR correlated best with total IS, whereas OGTT 30min correlated best with hepatic IR. These variations in results may have been caused by assessing different groups of patients representing different kinds of IR. This emphasize the need for the gold standard method when evaluating IS in selected groups of patients.

Collectively, in paper II and III, both clamp measurements and surrogate indices confirmed that our patients had severe IR before RDN, with no improvements in IS after short- and long-term follow-up. Rather, a smaller reduction in EGR during the low-dose insulin clamp at follow-up was seen, suggesting deterioration of hepatic IR.

After two years, most of the surrogate indices tended to be higher, although only HOMA-AD demonstrated a significant worsening in IS. Knowing that our results showed only modest correlations between clamp data and surrogate indices, we cannot exclude that performing a clamp two years after RDN could have resulted in findings showing IR progression at the end of follow-up.

To our knowledge, our study is by far the largest assessing IS using the time consuming and cumbersome gold standard method in TRH patients undergoing RDN. Therefore, comparison of our results with other studies is currently limited. Some small uncontrolled metabolic RDN human trials showed contradictory results. Even though the inconsistency cannot be easily explained, the use of different IR indices, RDN devices and inclusion criteria might be the reasons. Additionally, an absence of adherence control of both antihypertensive and antidiabetic drugs makes these studies difficult to interpret. Even more, no RDN SCT focusing on metabolic changes has been published until now; thus, the hypothesis that RDN may improve IS has not yet been elucidated.

12 MAIN CONCLUSIONS

The main hypothesis of this research was, that a decrease in SNA may improve IS in patients with TRH. Additionally, one expected an amelioration of BP indices. The study assumed that RDN is a method able to modulate SNA.

The results of our study do not support the hypothesis that RDN leads to beneficial metabolic effects or can alleviate IR in patients with long-term TRH, providing that the RDN procedure was successful. Knowing the unpredictable degree of denervation, negative metabolic results may represent an ineffective procedure or the resistance of IS improvement by SNA modulation in patients with TRH.

Thus, the hypothesis has not been resolved by this study. Whether autonomic nerve system modulation may ameliorate peripheral IR by decreasing vasoconstriction in advanced stage hypertensive patients with vascular changes, has not yet been settled. To what extent devices may decrease SNA to improve metabolism in overworked adipose tissue, is another conundrum. Finally, there is vagueness concerning whether this perturbed autonomic nervous system function in severely hypertensive patients may be reversible at all, of any device modulating SNA.

12.1 Paper I

The results of this study demonstrated a statistically significant reduction in office and ABPM six months after RDN. In addition, we observed decreases in all BPV indices apart from nocturnal BPV. The RDN procedure was done without any major adverse effects. However, due to the absence of a sham-controlled group and lack of meticulously adherence assessment, we cannot conclude whether the observed BP changes were related to denervation or other factors.

12.2 Paper II

Neither peripheral nor hepatic IS improved six months after the RDN treatment in this group of insulin resistant patients without diabetes and with TRH, as measured with gold standard methods, “two steps HEC”. However, due to the study design, we could not justify whether a lack of IS improvement was related to resistance towards SNA alterations or reflect only that RDN was not enough effective to modulate SNA.

12.3 Paper III

Two years after RDN, IS and parameters of glucose metabolism had not improved. The limited accuracy of several IR surrogate indices, compared to HEC, was demonstrated in this well-defined group of patients. There were no correlations between the magnitude of IR prior to RDN and BP changes. Patients with higher nocturnal SBP and arterial stiffness presented a further deterioration of hepatic IR during follow-up.

13 FURTHER RESEARCH

RDN research has gone through major enthusiasm and flames during the last ten years. Much has been learnt from RDN studies about the importance of adherence, BP measurements and careful selection of patient with uncontrolled hypertension. Negative results forced scientists to broaden their knowledge of renal artery microanatomy and to improve RDN devices. Learning from older RDN research, new proof-of-concept studies were performed, showing BP reduction, however still quite small and not superior to well-adjusted antihypertensive drug treatment. Is it the end of the road for RDN? What about the patients who struggle with adherence? What about the patient's preference? Is the idea of moving RDN from TRH to younger patients with mild hypertension the right way to go? The aspects of adherence, quality of life due to polypharmacy and potential impact of RDN on heart failure, kidney function, arrhythmias, IR and other consequences of increased SNA may be issues bringing RDN further in the clinical world. Nowadays, RDN is not recommended in clinical practice guidelines. Concerns, such as the target group for RDN treatment, device type, control of denervation effectiveness, and diagnostic systems for renal nerves mapping are each and all issues yet to be resolved. In spite of this, the setbacks of RDN should not decrease the interest for research in a device technology aiming to modulate SNA. These are all complex issues, and high-quality, investigator-initiated research is needed to understand the potential of new device-based technologies to modulate SNA, especially in conditions as IR, where effects of medical treatment and lifestyle changes is difficult to achieve. Improvement in IS may be a key factor to protect against type 2 diabetes development, and thus decreases cardiovascular risk. None of the RDN trials was designed to look at hard clinical end points as primary outcomes. Such results are warranted before getting SNA modulating devices into routine clinical practice.

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15 PAPERS I-III

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ORIGINAL ARTICLE

Renal sympathetic denervation: effect on ambulatory blood pressure and blood pressure variability in patients with treatment-resistant hypertension. The ReShape CV-risk study

A Mirosławska[1], M Solbu[2], E Skjølsvik[1], I Toft[2,†] and TK Steigen[1,3]

Renal sympathetic denervation (RDN) represents a potential treatment option for highly selected patients with resistant arterial hypertension. In this open label study, we aimed to investigate the response of blood pressure (BP) and short-term BP variability (BPV) to RDN 6 months after procedure. We defined treatment-resistant hypertension as office systolic BP > 140 mm Hg, despite maximum tolerated doses of ⩾4 antihypertensive drugs, including a diuretic. In addition, daytime systolic ambulatory blood pressure (ABPM) > 135 mm Hg was required after witnessed intake of antihypertensive drugs. Bilateral RDN was performed with the Symplicity Catheter System ($n = 23$). The mean systolic office BP and ABPM fell from 162 ±20 mm Hg to 139 ±19 mm Hg ($P < 0.001$) and from 154 ±20 mm Hg to 144 ±16 mm Hg ($P < 0.038$), respectively. In addition, we observed a significant reduction in diastolic office BP and ABPM. The current study also demonstrated a significant decrease of both systolic and diastolic average real variability, weighted standard deviation (s.d.) as well as conventional s.d. of mean and daytime BP, but not of s.d. of nighttime BP. RDN after witnessed intake of ⩾4 antihypertensive drugs reduced both office BP and ABPM at 6 months in patients with truly resistant hypertension. Also BPV improved, possibly reflecting an additional effect from intervening on the sympathetic nerve system.

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INTRODUCTION

Hypertension (HT) is a significant global health burden, as it affects > 25% of the adult population.[1–3] Many patients do not have sufficient effect of lifestyle changes and medical treatment on blood pressure (BP) and ~ 10% of patients treated for HT remain with elevated BP despite prescription of > 3 antihypertensive drugs.[4] However, the identification of truly treatment-resistant hypertensive patients is controversial and some authors suggest that poor adherence to prescribed treatment explains a large part of the reported treatment resistance.[5] The analysis of 24-h BP profiles provides important insights into physiological regulation and can give us more prognostic information than office BP alone. BP variability (BPV) is the result of complex interactions between extrinsic environmental and behavioral factors with intrinsic cardiovascular regulatory mechanism both humoral and neural where central sympathetic drive has an important role.[6]

Renal sympathetic denervation (RDN) is an invasive treatment option for patients with resistant HT if optimal medical treatment fails.[7–10] Recently, data from the randomized, blinded Symplicity HTN-3 trial have cast doubt on the BP-lowering effects of RDN.[11] This trial did not show a significant reduction of systolic BP (SBP) in patients with resistant HT 6 months after RDN compared with a sham-treated control group. The objective of the present study was to investigate the BP-lowering effect of RDN in a cohort of highly selected patients with true resistant arterial HT, defined by stricter criteria than those applied in previous studies. We also aimed at assessing the BPV as an additional factor that may be modulated by RDN.

METHODS

Patient selection

Patients with treatment-resistant HT followed by HT specialists were eligible for inclusion in this study performed from March 2013 to February 2014. The study is a part of a larger study focused on insulin resistance in patients undergoing renal denervation (ClinicalTrials.gov Identifier: NCT01630928). Before being accepted as candidates for inclusion, secondary HT was excluded by standard clinical evaluation and blood tests including measurements of serum aldosterone, renin activity, plasma normetaphrine and metanephrine. Treatment-resistant HT was defined as office SBP > 140 mm Hg despite regular intake of maximally tolerated doses of ⩾4 antihypertensive drugs, including a diuretic. The medication was kept unchanged at least 14 days before enrollment. In addition, subjects had to have an average daytime systolic BP > 135 mm Hg as measured by ambulatory BP monitoring (ABPM) after investigator witnessed intake of their antihypertensive drugs. Patients could be 18–68 years old and the estimated glomerular filtration rate should be > 45 ml min^{-1} per 1.73 m^2. The exclusion criteria included: diabetes mellitus, HBA1c > 6.5%, aortic valve stenosis with valve area < 1.0 cm^2, implanted pacemaker or implantable cardioverter defibrillator, contrast allergy, history of cancer within 5 years, pregnancy and renal transplantation. Qualification to renal denervation required anatomical criteria: renal artery diameter > 4 mm and artery length > 2 cm, no significant renal artery stenosis and no previous renal artery stenting.

[1]Department of Cardiology, University Hospital of North Norway, Tromsø, Norway; [2]Department of Nephrology, University Hospital of North Norway, Tromsø, Norway and [3]Institute of Clinical Medicine, University of Tromsø, Tromsø, Norway. Correspondence: Dr TK Steigen, Department of Cardiology, University Hospital of North Norway, Sykehusveien 38, Tromsø N-9038, Norway.
E-mail: Terje.Steigen@unn.no
[†]Deceased.
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The included patients gave their written, informed consent. The study was conducted in accordance with the protocol, applicable regulatory requirements and the ethical principles of the Declaration of Helsinki. The Regional Committee for Medical and Health Research Ethics as well as the Data Protection Officer at University Hospital of North Norway gave their approval.

Procedures

Patients were asked to bring their prescribed medication in original package to the clinical visit with one of the study nurses. Medication was documented, administered by the nurse and swallowed by the patient under continuous observation, to secure intake of the medication in prescribed doses. Patients were then continuously under observation by the nurse until 24-h ABPM device had been mounted and tested. Ambulatory blood pressure readings were taken every 20 min during daytime (0700 hours to 2200 hours), and every 30 min during nighttime (2200 hours to 0700 hours). Only ABPM with recordings over 70% were regarded as technically sufficient for inclusion in the analyses. From the ABPM recordings, mean 24-h systolic and diastolic BP were computed. From the diary, based on the times of getting up and going to bed, mean systolic and diastolic daytime BP and mean systolic and diastolic BP at rest were calculated.[12]

Office BP readings were taken in a seated position with an automatic oscillometric device after 5 min of rest. At baseline, BP was measured on each arm, and the arm with the higher BP was used for all subsequent readings. Averages of the two last measures were calculated and used for analysis.

The same experienced nurses handled all BP measurements using the same calibrated devices in all patients. Office BP readings were taken by Casmed 740, (Infiniti Medical, Menlo Park, CA, USA) and ABPM was assessed using Schiller BR-102 plus (SCHILLER-Reomed AG, Dietikon, Switzerland).

RDN was performed using the Symplicity Catheter System (Medtronic, Mountain View, CA, USA) by one experienced interventional cardiologist trained for the procedure (TKS) as described by others,[8] with a product manager from the manufacturer (Medtronic) present following all steps in the procedure for all patients. Pain was treated with appropriate analgesics. On average, each patient had 12 ablations of 2-min duration (complete ablation) carried out, and the minimum number of complete ablations per side was $\geqslant 4$, as recommended. Patients were hospitalized overnight and followed with self-administered BP measurements at home weekly the first month, later monthly, after written and practical instruction.

Six months after the procedure all patients came for a follow-up visit with office BP and ABPM measurements after witnessed intake of medicines, as described above.

Variables

Ambulatory BP recordings were analyzed and the standard deviation (s.d.) of 24-h BP as well as s.d. of day- and nighttime, directly from all individual readings were computed. All subjects had recordings of good technical quality (mean number of BP readings was 59 and minimum 55). The 'weighted' s.d. (wSD) of systolic and diastolic BP was calculated as previously published (for example, the average of daytime and nighttime s.d., divided by the duration of the day and night periods, respectively).[13] Another parameter, average real variability (ARV), recently proposed by other investigators,[14] was also calculated by the following formula:

$$\mathrm{ARV} = \frac{1}{n-1}\sum_{k=1}^{n-1}|\mathrm{BP}_{k+1} - \mathrm{BP}_k|$$

where n is the number of BP readings during 24 h.

Morning BP surge was calculated as the difference between the average of SBP during 2 h immediately after awakening and the average of the three SBP readings centered around the lowest night SBP value (after crosschecking the patients diary).[6,15,16] The 2-h-awake BP was defined as the average of 4–5 BP readings during the first 2 h after morning arousal (taken from diary report). The coefficient of variation (CV) of SBP and DBP was assessed by dividing s.d. by mean SBP and DBP, to examine whether the effect of BPV was independent of the BP level.[6] The degree of nocturnal BP fall (dip%) was calculated as$100 \times (1 -$ average of nighttime BP/average of daytime BP ratio).

The study nurses measured height and weight and body mass index was calculated. Information about lifestyle was assessed by a self-administered questionnaire. Medical history was taken by one of the study physicians. Fasting blood samples were drawn at baseline and at follow-up. From creatinine measurements, estimated glomerular filtration rate was calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation.[17]

Statistical analysis

SPSS software version 22 for Windows (IBM, Armonk, NY, USA) was used for all statistical analyses. We used paired two-sided *t*-test to calculate differences between baseline and 6 months after RDN for normally distributed data, Wilcoxon non-parametric test was used for non-normally distributed data (s.d. of daytime SBP).

RESULTS

Two patients were excluded after the qualifying witnessed drug intake, due to an average systolic daytime ABPM below 135 mm Hg.

RDN was performed in 23 patients without any periprocedural or late complications up to 6 months. No vasovagal syncope was reported. One patient developed orthostatic hypotension and one was treated ambulatory for high BP. Both recovered after adjustment of medication. The majority of patients were middle-aged, slightly obese men. Baseline characteristics are given in Table 1.

The mean systolic office BP and ambulatory BP fell significantly from 162 mm Hg ± 20 mm Hg to 139 mm Hg ± 19 mm Hg ($P < 0.001$/95% confidence interval 10–35) and from 154 mm Hg ± 20 mm Hg to 144 mm Hg ± 16 mm Hg ($P < 0.038$/95% confidence interval 0.6–18), respectively (Figure 1). Reductions in office and ambulatory diastolic BP were also significant (Table 2).

There was a significant reduction in both systolic and diastolic mean and daytime BP, but not in nighttime SBP (Table 2). Heart rate did not change significantly from baseline (72 ± 12 beats per minute) to 6 months control (72 ± 12 beats per minute).

We observed a reduction in office SBP $\geqslant 10$ mm Hg in 13 out of 23 patients and, accordingly, a decrease of mean 24-h SBP > 5 mm Hg was seen in 12 of 23 patients (52 %).

The number of ablations on both sides was 12.6 ± 2.

Antihypertensive medications before and 6 months after RDN are given in Table 3. At baseline, all patients used diuretics and angiotensin converting enzyme inhibitors or angiotensin receptor

Table 1. Baseline characteristics of patients ($n = 23$)

Variable	*All (n = 23)*
Age, years	53 ± 8.4
Women, *n*	5
BMI (kg m^{-2})	32 ± 5
Smoker, *n*	3
Previous smoker, *n*	10
OSA treated, *n*	5
Serum total cholesterol, mmol l^{-1}	5 ± 1
Serum sodium, mmol l^{-1}	142 ± 3
Serum potassium, mmol l^{-1}	4 ± 0.5
Serum creatinine, μmol l^{-1}	88 ± 23
eGFR (ml min^{-1} per 1.73 m^2)	83.9
History of stroke, *n*	3
Atrial fibrillation, *n*	1
Peripheral artery disease, *n*	1
Coronary artery disease, *n*	2

Abbreviations: BMI, body mass index; eGFR, estimated glomerular filtration rate; OSA, obstructive sleep apnea. Values are given as mean ± (s.d.) or number of patients.

blockers, 87% of patients used calcium channel blockers, 48% of patients used an aldosterone antagonist, minimum 25 mg daily.

Eleven patients had reduced the number of prescribed antihypertensive drugs after RDN from 4.8 ± 1.1 to 4.2 ± 1.2 ($P < 0.02$)

Body mass index and estimated glomerular filtration rate remained stable during the study (P = NS for both).

BPV measured as s.d. of 24-h BP as well as s.d. of daytime BP fell significantly from baseline to 6 months, whereas no significant change in s.d. during nighttime was found. Significant decrease of both systolic and diastolic wSD and ARV was revealed after 6 months (Table 2).

The morning BP surge and the 2-h awake BP decreased significantly from 29 to 20 mm Hg ($P < 0.011$) and from 157 to 147 mm Hg ($P < 0.024$) after 6 months. Systolic and diastolic dipping did not change significantly.

There was no correlation between the s.d. and office and ambulatory BP reduction.

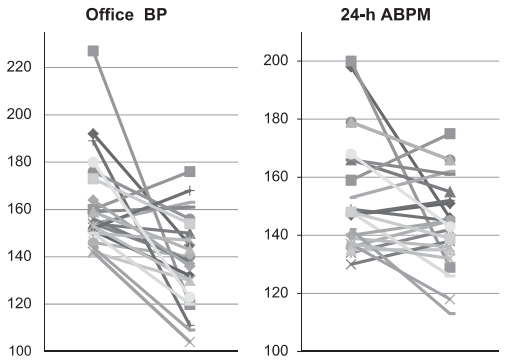

Figure 1. Individual changes in systolic OBP and systolic 24-h ABPM from baseline to 6 months control after RDN.

DISCUSSION

In this group of carefully selected patients with resistant HT using ⩾4 antihypertensive drugs, we found that both office and ambulatory SBP was significantly decreased 6 months after treatment. BPV measured as s.d., wSD and ARV fell significantly. We also found a significant reduction in diastolic office BP and ABPM. This has been reported in the Symplicity trials[8] and also observed in the recently published Prague-15 study.[18]

To our knowledge this is the second study published that used witnessed intake of drugs to ascertain adherence to medication.[5,19] Contrary to the one experience in the Oslo RDN studies, none of our patients experienced hypotension after witnessed intake of their prescribed antihypertensive drugs. Only

Table 3. Antihypertensive medications before and 6 months after renal denervation

	Before renal denervation	*6 months follow-up*
No. of antihypertensive drug classes	4.8 ± 1.2	4.2 ± 1.2
Patients on five or more medications	11	10
Patients receiving drug class		
ACE	8	8
ARB	14	13
Direct renin inhibitors	1	0
β-Blockers	18	16
Calcium channel blockers	20	19
Loop diuretics	16	13
Thiazides	9	8
Aldosterone antagonists	11	7
Selective alpha 1 receptor blockers	3	4
Nitroglycerin	1	1
Central alpha 2 sympatholytic	7	5
Amiloride	2	1

Abbreviations: ACE, angiotensin converting enzyme inhibitor; ARB, angiotensin II receptor antagonist. Data are shown as means ± (s.d.) or number of patients.

Table 2. BP levels and indices of BPV at baseline and 6 months after RDN

	Systolic BP			*Diastolic BP*		
	Before RDN	*6 months after RDN*	P-*value*	*Before RDN*	*6 months after RDN*	P-*value*
Mean levels of BP mm Hg						
Office BP	162 ± 20	139 ± 19	< 0.001	108 ± 18	91 ± 14	< 0.001
24-h ABPM	154 ± 20	144 ± 16	< 0.038	97 ± 13	89 ± 11	< 0.003
Daytime	157 ± 20	147 ± 16	< 0.032	99 ± 13	91 ± 12	< 0.003
Nighttime	140 ± 22	136 ± 18	< 0.297	87 ± 13	81 ± 13	< 0.025
Dip %	11 ± 9	8 ± 8	< 0.064	13 ± 8	11 ± 9	< 0.239
BP variability						
Standard deviation, mm Hg						
24 h	19 ± 4	16 ± 3	< 0.017	18 ± 4	14 ± 3	< 0.000
Daytime	23 ± 21	15 ± 3	< 0.020	17 ± 5	12 ± 3	< 0.000
Nighttime	14 ± 6	12 ± 4	< 0.117	14 ± 5	13 ± 3	< 0.542
wSD	16 ± 4	14 ± 2	< 0.006	15 ± 4	12 ± 2	< 0.000
ARV	14 ± 3	12 ± 2	< 0.002	13 ± 4	10 ± 3	< 0.001
Coefficient of variation	13 ± 3	11 ± 2	< 0.060	18 ± 3	15 ± 3	< 0.001
Average of BP 2 first hours after wake up	157 ± 19	147 ± 16	< 0.024			
Morning BP surge	29 ± 13	20 ± 14	< 0.011			

Abbreviations: ABPM, ambulatory BP monitoring; ARV, average real variability; BP, blood pressure; BPV, BP variability; RSD, renal sympathetic denervation; wSD, weighted s.d.

two patients had to be excluded from the trial after this qualifying step, reflecting good patient selection by the referring HT specialists. We used witnessed drug intake both at baseline and at control after 6 months. One could speculate that the reason we got more BP reduction in our study than in the Oslo studies was that drug adherence decreased at 6 months in the two Oslo studies, thus 'hiding' part of the RDN-related blood pressure decrease, as they used witnessed drug intake as a qualifying measurement only at baseline.[5,19] Moreover, the RDN groups in the Oslo studies were small ($n = 6$ and $n = 9$, respectively), and firm conclusions should be avoided.

Patients and physicians were instructed not to change antihypertensive medication during the study period. However, antihypertensive drug regimen was reduced during follow-up in some patients as a result of confirmed low office BP, or the development of symptoms of hypotension.

In this study, some patients with a clear reduction in office BP had unchanged or increased 24-h BP after RDN. Office BP measurements have several limitations including the inability to track the dynamic behavior of BP, the inherent inaccuracy of the technique, and especially the white coat effect. Twenty-four hours ABPM is considered superior to office BP measurements for predicting cardiovascular events and mortality.[20] Thus, office BP alone may no longer be considered as sufficient to assess the effect of antihypertensive treatment, especially in patients with resistant HT.[21,22]

Growing evidence suggest that nighttime SBP is a stronger predictor than daytime SBP in hypertensive patients.[23,24] We observed a significant change in both systolic and diastolic mean and daytime ABPM but only in nighttime DBP. High nighttime BP and non-dipping patterns have been associated with increased sympathetic activity in hypertensive patients. In line with the Symplicity trials, we found no significant nocturnal BP fall after RDN in our study. This has no clear explanation, but it is likely that a more pronounced reduction of daytime BP than nighttime BP can be observed after RDN. Also, the fixed time intervals for ABPM readings during day and night periods might affect calculation of nocturnal dipping. Moreover, 6 months is a relatively short observation time, and it cannot be excluded that further improvements may be observed after longer observation. We are planning a per protocol 2 years follow-up.

Increased BPV is associated with diabetes mellitus, advanced age, female gender and also, most likely, sympathetic activity.[25] s.d. can only be precisely estimated from analysis of beat-to-beat BP tracing. In this study, we used the simplest and probably the most established measure of short-term BPV, which is s.d. of mean BP. We also analyzed day and night periods s.d. separately and wSD for each period of day to remove the mathematical interference from nighttime BP fall. The weighted 24-h s.d. of BP seems to be superior to conventional 24-h s.d. to correlate with end-organ damage.[13] ARV has also been considered as a more specific measure of BPV than s.d. and stronger associated with cardiovascular risk.[26] Our results showed a significant decrease of 24-h s.d. and wSD both systolic and diastolic BP as well as s.d. of daytime BP but not s.d. of nighttime BP. Decrease of 24-h s.d. and s.d. of daytime without effect on nighttime has been previously reported in one small study.[27] A possible explanation of the more pronounced effect during daytime may be that stress and activity increase sympathetic activity and thereby facilitates the RDN treatment effect. It is also likely that reduced frequency of BP readings during night might affect calculations.

Unlike another study,[28] analysis of our data showed significant reduction of all indexes of BPV (s.d., wSD and ARV) after RDN. This has never been shown before. BPV indexes at baseline in our study were higher than in the previous studies,[27,28] which might be related to the fact that we used more strict inclusion criteria to select patients with truly resistant HT than others.

We did not observe a significant change in the systolic coefficient of variation, which may indicate that the systolic BPV decrease was dependent on the BP lowering *per se*. In one recently published paper, high diastolic 24-h s.d. was considered to correspond with a 48% increased risk of cardiovascular events.[16] However, the IDACO database showed that indices of 24-h BPV were independent predictors of outcomes, but they improved the prediction of fatal plus nonfatal events by only 0.1%, and did not contribute much to risk stratification over and beyond 24-h BP.[29]

Cardiovascular events have their greatest prevalence in the early morning period. Whether this is attributable to an arousal dependent BP increase is not clear. The transition from sleep to wakefulness is associated with a sympathetic activation and an increase in plasma catecholamines, which results in increase of BP and heart rate. In the current study, both the 2-h awake and morning BP surge decreased significantly. The actual prognostic value of morning BP surge is still a matter of debate and conflicting results have been obtained.[16] In a recently published retrospective study from Italy where 2051 subjects were assessed with ABPM, morning BP surge was not found to be an independent predictor of CV death or high cardiovascular risk.[15]

Of note, in the present study, a pronounced decrease in BPV was observed despite the fact that a majority of patients used calcium channel blockers at baseline, the antihypertensive agent considered to improve BPV most efficiently.[30]

The present study has several limitations. The main weaknesses of our study are the non-randomized design, the small sample size and the lack of a control group. The low number of patients limits the possibilities for subgroup analyses. Because of the lack of control group, no strict conclusions can be made about a causal effect of RDN on the lowering of BP and BPV. Finally, automatic BP readings spaced by 20 min may not satisfactorily capture a highly dynamic phenomenon such as the increase in BP from sleep to wakefulness, which take place in a matter of seconds.

In conclusion, the results of the current study indicate that well selected patients with truly resistant HT may benefit from RDN. In addition to significant BP lowering after 6 months, we observed decreases in BPV indexes that were probably dependent on the BP lowering. Further randomized studies are needed to evaluate whether the effect of RDN on 24-h ABPM profiles translates into reduced numbers of cardiovascular events.

What is known about this topic?

- Renal denervation may reduce blood pressure in some groups of patients with resistant hypertension.
- Improving blood pressure variability may be an additional effect of RDN.

What this study adds?

- We have used stricter criteria for resistant hypertension than other studies, in addition to observed drug intake. This increases the possibility to select truly treatment-resistant patients.
- Our study is one of the few to focus on short time blood pressure variability in patients treated by RDN and the first which shows reduction on both conventional s.d. and also weighted s.d. as well as average real variability

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Atena K. Miroslawska,[1,2] Petter F. Gjessing,[3,4] Marit D. Solbu,[5,6] Ole M. Fuskevåg,[7] Trond G. Jenssen,[6,8] and Terje K. Steigen[1,2]

Renal Denervation for Resistant Hypertension Fails to Improve Insulin Resistance as Assessed by Hyperinsulinemic-Euglycemic Step Clamp

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METABOLISM

We assessed whether insulin sensitivity improved after renal denervation (RDN) for resistant hypertension. Twenty-three patients underwent a two-step hyperinsulinemic-euglycemic clamp (HEC) with glucose tracer and labeled glucose infusion and oral glucose tolerance test (OGTT) before and 6 months after RDN. Eighteen patients had metabolic syndrome at baseline. Blood pressure declined significantly after RDN, whereas mean (SD) fasting plasma glucose concentration (5.9 ± 0.7 mmol/L), median (minimum–maximum) insulin concentration (254 pmol/L [88–797 pmol/L]), and median C-peptide concentration (2.4 nmol/L [0.9–5.7 nmol/L]) remained unchanged. Endogenous glucose release during HEC was less suppressed after RDN, suggesting a slight decrease in hepatic insulin sensitivity. During high-dose insulin infusion, whole-body glucose disposal was low and remained unchanged after RDN, indicating persistent peripheral insulin resistance (IR). Area under the curve for 0–120 min for glucose and insulin during OGTT, Quantitative Insulin Sensitivity Check Index, Simple Index Assessing Insulin Sensitivity Oral Glucose Tolerance, and HOMA-IR were high, and did not improve after RDN. Despite a significant decrease in blood pressure, neither peripheral nor hepatic insulin sensitivity improved 6 months after RDN treatment in this group of insulin-resistant patients without diabetes and with resistant hypertension, as measured with gold standard methods.

More than 50% of patients with essential hypertension are considered to have insulin resistance (IR) (1). The link between these two disorders is partly unknown, but it has been hypothesized that increased sympathetic nerve activity (SNA) plays an important role (2). Sympathetic activation results in peripheral vasoconstriction through the release of norepinephrine, which acts upon vascular muscle adrenoreceptors. A subsequent reduction in skeletal muscle blood flow leads to reduced whole-body glucose utilization (3). Consequently, increased SNA may reduce insulin sensitivity (IS) through direct effects on the regulation of glucose uptake by skeletal muscle (4,5).

Targeting both sympathetic tone and IR might be of relevance to reduce the cardiovascular risk associated with hypertension. Renal denervation (RDN) has been shown to lower SNA by the ablation of both afferent and efferent nerves (6). The first study showing improved IS after RDN used only surrogate indexes of whole-body IR (7). To investigate the possible effects of RDN on IR in more detail, we applied a two-step hyperinsulinemic-euglycemic clamp (HEC) with glucose tracer infusion and labeled glucose infusate to separately assess glucose turnover, as well as hepatic and peripheral IS before and after RDN. Patients were also subjected to an oral glucose tolerance test (OGTT).

[1]Department of Cardiology, University Hospital of North Norway, Tromsø, Norway
[2]Cardiovascular Diseases Research Group, UiT The Arctic University of Norway, Tromsø, Norway
[3]Gastrosurgery Research Group, UiT The Arctic University of Norway, Tromsø, Norway
[4]Department of Digestive Surgery, University Hospital of North Norway, Tromsø, Norway
[5]Section of Nephrology, University Hospital of North Norway, Tromsø, Norway
[6]Metabolic and Renal Research Group, UiT The Arctic University of Norway, Tromsø, Norway
[7]Department of Laboratory Medicine, University Hospital of North Norway, Tromsø, Norway
[8]Department of Organ Transplantation, University Hospital of Oslo Rikshospitalet, Oslo, Norway

Corresponding author: Terje K. Steigen, terje.steigen@unn.no.

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A.K.M. and P.F.G. are co–first authors.

RESEARCH DESIGN AND METHODS

Twenty-three patients with treatment-resistant hypertension were included in the study and underwent RDN. Resistant hypertension was defined as office systolic blood pressure (BP) of >140 mmHg despite regular intake of maximally tolerated doses of four or more antihypertensive drugs, including a diuretic. In addition, subjects had to have an average daytime systolic BP of >135 mmHg, as measured by ambulatory BP monitoring after an investigator witnessed the intake of their antihypertensive drugs. Patients with known diabetes or an HbA_{1c} level of ≥6.5% were excluded. Details about the selection criteria have been published previously (8) (clinical trial reg. no. NCT01630928, clinicaltrials.gov).

The included patients gave their written, informed consent. The study was conducted in accordance with the protocol; applicable regulatory requirements; and the ethical principles of the Declaration of Helsinki and Title 45, U.S. Code of Federal Regulations, Part 46, Protection of Human Subjects, Revised 13 November 2001, effective 13 December 2001. The Regional Committee for Medical and Health Research Ethics as well as the Data Protection Officer at University Hospital of North Norway gave their approval.

Measurements and Calculations

All measurements were obtained within the last 4 weeks before and 6 months after RDN. Metabolic syndrome was diagnosed according to the International Diabetes Federation criteria from 2006 (9). Venous blood samples were drawn after an overnight fast (12 h). Levels of insulin during the OGTT and C-peptide were measured by ELISA (C-peptide, EIA-1293; insulin, EIA-2935; AH Diagnostics, Aarhus, Denmark). Insulin (endogenous and lispro) during HEC was measured with radioimmunoassay methods (HI-14K; Millipore, Billerica, MA) at the Hormone Laboratory, Oslo University Hospital (Aker, Norway). HEC was performed after a 12-h fast, as previously described (10). After the drawing of fasting blood samples, a primed (3 mg/kg/5 min), continuous (2.4 mg/kg/h) infusion of D-[6,6-$^2H_2$] glucose was given for the measurement of basal glucose turnover. Tracer infusion was continued, and a primed (127 mU/m^2/min for 10 min), continuous (13 mU/m^2/min) infusion of human insulin (insulin lispro) was commenced after 150 min. Glucose (200 mg/mL) with D-[6,6-$^2H_2$] glucose added at a 1.25 atom percent enrichment was variably infused to maintain normoglycemia (5 mmol/L). The second step clamp began after 270 min, when insulin infusion was increased to 40 mU/m^2/min, and continued for 120 min. Sampling, chemical analysis, and the determination of tracer enrichment were performed as previously described (11) using liquid chromatography mass spectroscopy.

Whole-body IS was expressed as the glucose infusion rate (GIR) (in milligrams per kilogram per minute) during the last 40 min of each step of the clamp (steady state). The IS index (ISI) was calculated as the mean GIR divided by the mean insulin concentration at each step. Endogenous glucose release (EGR) and whole-body glucose disposal (WGD) were calculated using modified versions of Steele's equations (12,13). The following calibrated infusion pumps were applied: care fusion Alaris Guardrails (BD, San Diego, CA) syringe pumps were used for insulin, and infusions of D-[6,6-$^2H_2$] glucose and a tracer-enriched glucose solution were performed using Alaris Medsystem III (BD).

A standard (82.5 g of glucose monohydrate) OGTT was performed, with plasma samples obtained at 0, 30, 60, 90, and 120 min after the glucose load. Postload glucose and insulin responses were calculated as incremental area units during the 2-h sampling time, and were expressed as the area under the curve (AUC) for glucose and insulin (14).

HOMA-IR was calculated as follows: HOMA-IR = (glucose $t0$ [mmol/L] × insulin $t0$[μIU/mL]/22.5) (15). Simple Index Assessing IS OGTT (SIisOGTT) and Quantitative IS Check Index (QUICKI) were calculated using the following formulas: 1/(log [Σ glucose t 0–30–90–120] [mmol/L] + log [Σ insulin t 0–30–90–120] [μIU/mL]) and 1/(log [fasting glucose (mg/dL)]) + log (fasting insulin [μIU/mL]), respectively (16,17).

Statistical Analysis

A 20% change in basal EGR (~0.4 mg/kg/min) was considered to be clinically relevant. With an α-level of 0.05 and a power of 80%, 20–25 patients were needed to demonstrate a 20% difference in basal EGR before and after intervention (18).

Frequency distribution was checked for all variables. Data were reported as the mean ± SD if normally distributed, and median (minimum–maximum) if distribution was skewed. Accordingly, paired t tests and Wilcoxon signed rank tests were used for the comparison of variables between baseline and follow-up. Significance was accepted at $P < 0.05$. All analyses were performed with the SPSS statistical package version 22 (IBM, Armonk, NY).

RESULTS

Baseline characteristics are displayed in Table 1. The mean age was 53 ± 8 years. Twenty-one of 23 patients had central obesity, and 18 patients had metabolic syndrome (9) at baseline. Three patients were excluded from the clamp because of technical problems encountered during the procedure. The mean systolic and diastolic office BP and ambulatory BP fell significantly after RDN treatment despite a significant reduction in the number of prescribed drugs, as previously reported (8). Fifteen patients had normal fasting glycemia, 8 patients had impaired fasting glycemia, and 17 patients had impaired glucose tolerance. The OGTT-derived AUC for glucose and insulin remained unchanged after RDN (Table 1). High insulin and C-peptide concentrations were seen at baseline and remained unchanged after 6 months (Table 1). Accordingly, the indirect indices of IR, QUICKI, SIisOGTT, and HOMA-IR were high at baseline and did not improve after RDN (Table 1).

Fasting and steady-state plasma C-peptide and insulin levels during the clamp remained unaltered after RDN (Table 2).

Table 1—Characteristics of body composition, cholesterol and glucose concentrations, and results of OGTTs and ISIs (n = 23)

	Before RDN	After RDN	P value
BMI, kg/m^2	32 ± 5	32 ± 5	0.66
Waist-to-hip ratio	1.0 ± 0.1	1.0 ± 0.1	0.75
HbA_{1c}, % (mmol/mol)	5.7 ± 0.3 (39)	5.6 ± 0.3 (38)	0.02
Cholesterol, mmol/L	5.1 ± 1	4.9 ± 0.9	0.47
Fasting glucose, mmol/L	5.9 ± 0.7	6.0 ± 0.6	0.20
Glucose after 120 min, mmol/L	8.9 (3.9–16.8)	8.4 (5.2–18.6)	0.70
AUC glucose for 0–120 min, mmol/L	1,191 ± 215	1,153 ± 184	0.70
AUC insulin for 0–120 min, pmol/L	19,609 ± 8,172	20,607 ± 11,745	0.70
Fasting insulin, pmol/L	254 (88–797)	201 (90–791)	0.34
Insulin after 120 min, pmol/L	1,312 ± 854	1,125 ± 521	0.40
Fasting C-peptide, nmol/L	2.4 (0.9–5.7)	2.7 (1.5–5.6)	0.12
HOMA-IR	7.7 (2.9–20.2)	7.4 (1.9–25.5)	0.45
SIisOGTT	0.2 (0.2–0.3)	0.2 (0.2–0.3)	0.93
QUICKI	0.3 ± 0.2	0.3 ± 0.2	0.38

Values are reported as the mean ± SD, or as the median (minimum–maximum) if distribution is skewed.

Basal EGR and WGD measured by glucose tracer infusion did not change significantly after RDN (2.12 ± 0.36 vs. 2.15 ± 0.41 mg/kg/min [P = 0.34], and 2.20 ± 0.36 vs. 2.14 ± 0.40 mg/kg/min [P = 0.35], respectively). During the two-step HEC, no significant changes in GIR and ISI were seen, indicating unaltered whole-body IS (Table 2). The suppression of EGR decreased from 0.9 ± 0.4 to 0.8 ± 0.4 mg/kg/min (P = 0.02) during low-dose insulin infusion, but remained unchanged during high-dose insulin infusion. The increase in WGD during high-dose insulin infusion was modest and remained unaltered at follow-up (Table 2). No improvement in IS was observed in a subanalysis of nine patients with extensive systolic ambulatory BP reduction (>10 mmHg) after RDN.

DISCUSSION

To our knowledge, this is the first study in which peripheral and hepatic IS have been separately assessed by gold standard methods before and 6 months after RDN in a group of patients with IR and treatment-resistant hypertension. Despite a significant reduction in BP, we found no improvement in IS, as measured by two-step HEC with infusion of a glucose tracer and a labeled glucose infusate. OGTT-based ISIs gave the same findings.

Because IS and SNA are closely associated, the previously reported amelioration of glucose metabolism after RDN in this group of patients has been followed with great interest (7). Most authors have calculated changes in IR according to HOMA-IR, a simple surrogate index that is most sensitive for changes in hepatic IR (15) and does not actually measure IS accurately on an individual basis.

The currently used two-step HEC with infusion of glucose tracer and a labeled glucose infusate remains the only reliable noninvasive method to separately assess hepatic and peripheral IS. During step clamp 2, insulin was infused at a rate that leads to the physiological hyperinsulinemia seen after a meal and that effectively increases WGD in individuals with normal IS values. The modest increase in WGD with increments in insulin infusion, both at baseline and at follow-up, supports the notion that our group of treatment-resistant hypertensive patients also experienced severe peripheral IR, which persisted after

Table 2—Glucose and insulin during two-step HEC (n = 20)

	Before RDN Step 1	After RDN Step 1	P value	Before RDN Step 2	After RDN Step 2	P value
GIR, mg/kg/min	0.69 (0.11–2.69)	0.50 (0.00–1.53)	0.08	2.57 (1.21–4.97)	3.04 (1.26–5.59)	0.13
ISI, μmol/kg/min/pmol	0.02 (0–0.08)	0.02 (0–0.06)	0.15	0.03 (0.01–0.06)	0.03 (0.01–0.06)	0.50
WGD increase from baseline (mg/kg/min)	−0.12 ± 0.36	−0.14 ± 0.42	0.86	1.38 ± 1.18	1.60 ± 1.11	0.27
EGR reduction from baseline, mg/kg/min	0.95 ± 0.37	0.77 ± 0.38	0.02	1.51 ± 0.51	1.53 ± 0.61	0.89
S-insulin, pmol/L	271 ± 67	243 ± 104	0.62	569 ± 104	604 ± 118	0.10
C-peptide, nmol/L	1.83 (0.76–4.95)	2.13 (1.01–4.48)	0.53	1.62 (0.50–4.46)	1.68 (0.73–3.45)	0.39

Values are reported as the mean ± SD, or as the median (minimum–maximum) if distribution is skewed. Step 1, low insulin infusion; step 2, high insulin infusion.

RDN. A substantial residual endogenous insulin release was present even during the highest insulin infusion rate in our study population, as indicated by the lack of decline in C-peptide levels during clamping. Interestingly, it has previously been shown that insulin inhibition of insulin secretion during HEC, as measured by the decrease in C-peptide levels, is correlated with IS (19).

Tracer dilution during HEC further makes it possible to assess the ability of insulin to suppress EGR. In order to optimize the detection of changes in hepatic IS, the insulin infusion rate during step clamp 1 was chosen to achieve a level of circulating insulin near to the median effective dose at the steep part of the sigmoidal-shaped hepatic insulin dose-response curve. Most of our patients had impaired fasting glucose levels and hyperinsulinemia, as well as high HOMA-IR index, indicating reduced hepatic IS. A smaller reduction in EGR during the low-dose insulin clamp at follow-up was statistically significant, but its clinical relevance is doubtful. A trend toward increased GIR during clamp 2 after RDN was probably related to the slightly higher serum insulin concentrations during clamping at follow-up. Accordingly, the ISI, which corrects for the prevailing insulin concentrations, did not change after RDN.

Importantly, and similar to the findings of Mahfoud et al. (7), patients in the current study experienced a significant reduction in office BP after RDN. However, in contrast to our study, 15 of 16 patients with type 2 diabetes mellitus in the study of Mahfoud et al. (7) used metformin, and adherence to the prescribed drugs was not rigorously assessed. Thus, one cannot exclude that improved patient compliance and a more rigorous metformin intake after RDN could have explained the findings in that study. In our study, HbA_{1c} level was significantly reduced after RDN, but the magnitude of this decrease was small and probably without clinical relevance.

Although the first RDN studies were promising, the sham-controlled HTN-3 Trial revealed no significant treatment effect of RDN on BP. Furthermore, in the DREAMS Study (20), which included only patients with metabolic syndrome, no effect on glucose metabolism was found 6 and 12 months after RDN, and neither HOMA-IR nor SIisOGTT values were changed after 6 months. Almost all patients in our study had metabolic syndrome with central obesity at baseline, and, although we did not investigate sympathetic drive directly, there are reasons to believe that increased SNA was prevalent at baseline in this group. In contrast to the DREAMS Study, in which almost drug-naive participants were included and measurements were performed during drug-free intervals, all our patients used four or more antihypertensive drug classes and were examined after witnessed drug intake. Despite these obvious differences in study populations and examination methods, no effect of RDN on IR was revealed in either study. However, even with witnessed intake and a significant reduction in the number of antihypertensive drugs used (8), we cannot exclude the possibility that improved compliance could have contributed to the fall in BP.

Although the recommended number of bilateral ablations was performed (8), we cannot exclude the possibility that the failure of RDN treatment to improve IR in our study might also be due to either insufficient ablation or true recurrence, possibly due to nerve regeneration or the sprouting of nerve fibers from the injured sympathetic chain. Indeed, previously reported improvements in IS measured by HOMA, OGTT (7), or HEC (21) were registered 3 months after RDN, whereas follow-up performed 6 months after RDN in the DREAMS Study and the current study had negative results with regard to these metabolic parameters.

The lack of a control group and the inclusion of only Caucasian patients are both important limitations of our data.

The current study shows that IS did not improve 6 months after successful RDN treatment in a highly selected group of treatment-resistant hypertensive patients with IR.

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Author Contributions. A.K.M. and P.F.G. conceived and conducted the study, researched and analyzed the data, and reviewed and edited the manuscript. M.D.S., T.G.J., and T.K.S. conceived and conducted the study, analyzed the data, and reviewed and edited the manuscript. O.M.F. analyzed clamp samples and reviewed the manuscript. T.K.S. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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Original article

Metabolic effects two years after renal denervation in insulin resistant hypertensive patients. The Re-Shape CV-risk study

A.K. Miroslawska [a, b], P.F. Gjessing [c], M.D. Solbu [d, e], J.V. Norvik [d, e], O.M. Fuskevåg [f], T.A. Hanssen [g], T.K. Steigen [a, b, *]

[a] Department of Cardiology, University Hospital of North Norway, Tromsø, Norway
[b] Cardiovascular Research Group, Institute of Clinical Medicine, Faculty of Health Sciences, UiT-The Arctic University of Norway, Tromsø, Norway
[c] Gastrosurgery Research Group, UiT, The Arctic University of Norway, Norway
[d] Section of Nephrology, University Hospital of North Norway, Tromsø, Norway
[e] Metabolic and Renal Research Group, UiT The Arctic University of Norway, Tromsø, Norway
[f] Department of Laboratory Medicine, University Hospital of North Norway, Tromsø, Norway
[g] Department of Health and Care Sciences, Faculty of Health Sciences, UiT The Arctic University of Norway, 9037 Tromsø, Norway

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SUMMARY

Background & aims: Denervation of renal sympathetic nerves (RDN) is an invasive endovascular procedure introduced as an antihypertensive treatment with a potential beneficial effect on insulin resistance (IR). We have previously demonstrated a reduction in blood pressure (BP) six months after RDN, but severe hepatic and peripheral IR, assessed by glucose tracer and two step hyperinsulinemic-euglycemic clamp (HEC), did not improve. The aim of the current study was to evaluate IR and adipokines profiles in relation to BP and arterial stiffness changes two years after RDN.

Methods: In 20 non-diabetic patients with true treatment-resistant hypertension, ambulatory and office BP were measured after witnessed intake of medications prior to, six and 24 months after RDN. Arterial stiffness index (AASI) was calculated from ambulatory BP.

Insulin sensitivity (IS) was assessed using an oral glucose tolerance test (OGTT), the Homeostasis Model Assessment (HOMA-IR), HOMA-Adiponectin Model Assessment (HOMA-AD), the Quantitative Insulin Sensitivity Check Index (QUICKI), the Triglyceride and Glucose Index (TyG) and the Leptin-to-Adiponectin Ratio (LAR). These surrogate indices of IS were compared with tracer/HEC measurements to identify which best correlated in this group of patients.

Results: All measured metabolic variables and IS surrogate indices remained essentially unchanged two years after RDN apart from a significant increase in HOMA-AD. OGTT peak at 30 min correlated best with reduction in endogenous glucose release (EGR) during low insulin HEC ($r = -0.6$, $p = 0.01$), whereas HOMA-IR correlated best with whole-body glucose disposal (WGD) ($r = -0.6$, $p = 0.01$) and glucose infusion rate ($r = -0.6$, $p = 0.01$) during high insulin HEC. BP response was unrelated to IS prior to RDN. Nocturnal systolic BP and arterial stiffness before RDN correlated positively with a progression in hepatic IR at six-month follow-up.

Conclusion: IR, adiponectin and leptin did not improve two years after RDN. There was no correlation between baseline IS and BP response. Our study does not support the notion of a beneficial metabolic effect of RDN in patients with treatment resistant hypertension.

1. Introduction

A majority of patients with essential hypertension also suffer from insulin resistance (IR) [1]. Sympathetic overactivity may induce IR and hyperinsulinemia, which again contributes to maintaining a high sympathetic activity, resulting in a vicious cycle involving hypertension, obesity and diabetes mellitus [2].

* Corresponding author. University Hospital of North Norway, Dept. Of Cardiology, Sykehusveien 38, N-9019, Tromsø, Norway.
E-mail address: Terje.Steigen@UNN.no (T.K. Steigen).

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Abbreviations

AASI	ambulatory arterial stiffness index
ABPM	ambulatory blood pressure
AUC_{GLC}	area under the curve for glucose
BMI	body mass index
BP	blood pressure
CV risk	cardiovascular risk
DBP	diastolic blood pressure
EGR	endogenous glucose release
GIR	glucose infusion rate
HEC	hyperinsulinemic-euglycemic clamp
HOMA-AD-	HOMA adiponectin model assessment
HOMA-IR	homeostasis model assessment, insulin resistance
IR	insulin resistance
IS	insulin sensitivity
LAR	leptin to adiponectin ratio
MAP	mean arterial pressure
OGTT	oral glucose tolerance test
PP	pulse pressure
RDN	renal sympathetic nerve denervation
SBP	systolic blood pressure
SNA	sympathetic nerve activity
TyG	triglyceride and glucose index
WGD	whole-body glucose disposal
QUICKI	quantitative insulin sensitivity check index

Hyperinsulinemia may play an important role in the pathogenesis of primary hypertension, not only by activation of sodium reabsorption and water retention [3], but also by changes in the arterial wall leading to increased arterial stiffness [4]. Research has shown that adipose tissue, which is innervated by sympathetic nerves, is involved in whole-body insulin sensitivity (IS). Excess sympathetic nerve activity (SNA) and adipose tissue mass in obesity leads to increased circulating levels of free fatty acids, leptin, and pro-inflammatory cytokines [5]. These cytokines have been suggested to impair intracellular insulin signaling, and thereby glucose uptake in skeletal muscle [6,7]. In addition, chronic hyperleptinemia appears to contribute to development of hypertension via activation of the sympathetic nervous system and decreased natriuresis [8,9].

Minimally-invasive catheter based endovascular renal sympathetic nerve denervation (RDN), as a non-surgical approach to modulate SNA and blood pressure (BP), was first reported in 2009 [10]. The procedure and devices by which it is applied has undergone continuous development [11], but the results of the first sham controlled randomized clinical trial was disappointing [12]. However, later three separate randomized proof of principle trials have shown superiority over sham treatment [13–15]. Animal studies have shown that surgical denervation of renal arteries improves hepatic IS [16,17]. Human studies on the effect of RDN on IS, inflammation and adipokine profile [18,19] have shown contradictory results, probably due to differences in patient selection, the use of different surrogate indices of IS at different time points of follow-up as well as variable concomitant antidiabetic treatment [20,21].

The previous Re Shape study results, at six-month follow-up, demonstrated that RDN did not improve hepatic and peripheral IR found in non-diabetic patients with TRH [22]. However, the long-term effects of RDN on metabolic changes are unknown. RDN registries reported a gradual increase in BP fall over time, as potential result of the remodeled activation of sympathetic nervous system [23]. Additionally, both human and animal studies showed an extended effect of RDN on intracellular processes, including genetic modifications [24,25]. However, it is still uncertain how long these changes last and how an increased BP fall could affect IS over time. Thus, we sought to assess whether RDN could ameliorate IS and adipokines profile in relation to BP and arterial stiffness alterations after long term observation. The IS was examined using several surrogate indices. We also described the degree of correlation of these indices with our previously published pre-RDN and 6 months follow-up measurements using HEC with glucose tracer technique [22].

2. Material and methods

2.1. Selection of participants

Twenty-three patients with treatment resistant hypertension were included in the study.

Treatment resistant hypertension was defined as office systolic BP (SBP) > 140 mmHg despite regular intake of maximally tolerated doses of ≥4 antihypertensive drugs, including a diuretic, in addition to daytime systolic ambulatory BP (ABPM) > 135 mmHg after witnessed intake of the prescribed BP lowering drugs. Patients with known diabetes or an HbA_{1c} level ≥6.5% were excluded. Details about the selection criteria and detailed methods description have been published previously [26]. (Clinical trial reg. no. NCT01630928, clinicaltrials.gov).

Twenty out of the 23 patients attended the two-year follow-up. The included patients gave their written, informed consent. The study was conducted in accordance with the protocol, applicable regulatory requirements, and the ethical principles of the Declaration of Helsinki. The Regional Committee for Medical and Health Research Ethics as well as the Data Protection Officer at University Hospital of North Norway gave their approval.

3. Methods

Bilateral RDN was performed using the Symplicity Catheter System (Medtronic, Mountain View, CA, USA) by one experienced interventional cardiologist trained for the procedure (n = 23). BP measurements were performed in the same way during the entire study, as previously described [26]. To ensure compliance to the antihypertensive drug treatment, patients brought their prescribed BP medication in original package to the study nurse. Medications were documented and swallowed while the patients were under continuous observation until the ABPM device was mounted. Pulse pressure (PP) was calculated as differences between mean systolic and diastolic ABPM.

The ambulatory arterial stiffness index (AASI), a surrogate measure of arterial stiffness, was calculated from ABPM readings as one minus the regression slope of diastolic BP (DBP) on SBP [27].

Mean arterial pressure (MAP) was calculated as [(2 × DBP) + SBP]/3 from ABPM.

Participants were classified as BP responders if they achieved a reduction in mean 24-h SBP ≥5 mm Hg from baseline to two-year follow-up [28].

Prior to RDN and after six months and two years, IS was assessed using surrogate indices based on fasting blood samples and an oral

glucose tolerance test (OGTT). Area under the curve for glucose (AUC_{GLC}), homeostasis model assessment for insulin resistance (HOMA-IR), quantitative insulin sensitivity check index (QUICKI) were calculated as described before [22]. The triglyceride and glucose (TyG) index was calculated with established formulas according to previous studies TyG = Ln [triglyceride (mg/dL) x glucose (mg/dL)/2] [29]. The leptin-to-adiponectin (LAR) index-was calculated by dividing serum concentrations of leptin (ng/ml) by adiponectin (μg/ml) [30]. The HOMA-adiponectin model assessment (HOMA-AD) was calculated with the formula: [fasting glucose (mmol/L) x fasting insulin (mU/L)]/[22.5 × fasting adiponectin (μg/ml)] [31]. As we did not perform HEC at the two-year follow-up, pre-RDN data were used to identify the surrogate IS indices that correlated best with step-HEC/glucose tracer measurement of hepatic IS (expressed as endogenous glucose release (EGR) during low-dose insulin clamp) and peripheral IS and whole-body IS (expressed as whole-body glucose disposal (WGD) and glucose infusion rate (GIR) during the high-dose insulin clamp). ELISA methods were utilized to measure C-peptide and insulin (EIA-1293 and EIA-2935 respectively; AH Diagnostics, Aarhus, Denmark) and to analyze leptin (EIA-2395), adiponectin (Acrp30).

3.1. Statistical analysis

Data are presented as mean ± SD if normally distributed or as median (min, max) if skewed. For continuous variables, we used paired Student's t-tests to compare differences between pre-RDN and six months follow-up measurements and between pre-RDN and two-year follow-up. For variables with a skewed distribution we applied Wilcoxon signed-rank tests. Correlations were assessed using Pearson's test. Statistical analyses were performed using SPSS software (IBM Corp. Released 2017. IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp.). Differences were considered statistically significant at $p < 0.05$.

4. Results

4.1. Blood pressure

There was a small, non-significant reduction in the number of antihypertensive drugs from a median of 4.5 [4–8] different drug classes before RDN to 4.0 (0–7) ($p = 0.08$) two years after RDN. Mean age was 53 ± 8 years and 18/20 were men. BP measurements and arterial stiffness calculations are presented in Table 1.

Nocturnal hypertension (mean SBP ≥ 120 mmHg between 10 pm and 7 am) was observed in 16/20 patients at baseline and 18/20 at two-year follow-up. There were 12 BP responders after two years. MAP was significantly lower after two years compared to baseline. PP and AASI did not change significantly during follow-up (Table 1).

4.2. Metabolic data

4.2.1. Relationships between surrogate indices of IS and HEC measurements

Correlation coefficients of the different indices of IS and HEC measurements prior to RDN are displayed in Table 2. Peak of glucose concentration at 30 min during OGTT (OGTT 30 min) correlated best with EGR reduction during low-dose insulin infusion. HOMA-IR correlated best with GIR and WGD increase during high-dose insulin infusion.

Other indices, including TyG, LAR, c-peptide, adiponectin and leptin, correlated neither with hepatic nor peripheral clamp derived IS measurements.

4.2.2. Metabolic changes two years after RDN

We found a borderline increase in body mass index (BMI) from 31.6 to 32.6 kg/m^2, $p = 0.05$, shown in Table 3.

After two years two patients had developed diabetes mellitus. One of them received antidiabetic treatment at two years and was excluded from the two-year metabolic calculations. Most of the metabolic parameters or IS surrogate indices were essentially unchanged two years after RDN, apart from a significant increase in HOMA-AD and QUICKI indices, as shown in Table 3. However, the clinical relevance of the QUICKI index change of this magnitude is doubtful.

There were no significant changes in adipokines (adiponectin and leptin) during the two-year study period, as illustrated in Fig. 1.

Table 2
Pearson's correlation analysis between clamp measurements and surrogate IS index calculations prior to RDN (n = 23).

IS index	Hepatic IS		Peripheral IS		Total IS	
	r	p	r	p	r	p
HOMA-IR	−0.5	0.03	−0.6	0.01	−0.6	0.01
LogHOMA-AD	−0.5	0.04	−0.4	0.05	−0.5	0.01
QUICKI	0.5	0.02	0.5	0.03	0.5	0.01
AUCGLC	−0.4	0.05	−0.4	0.06	−0.5	0.04
OGTT 30min	−0.6	0.01	−0.4	0.07	−0.5	0.04

HOMA-IR-homeostasis model assessment insulin resistance; HOMA-AD: HOMA-adiponectin model assessment; QUICKI-quantitative insulin sensitivity check index; AUC_{GLC}- area under the curve for glucose; OGTT 30min.- glucose concertation at 30 min during oral glucose tolerance test.

Table 1
Blood pressure and arterial stiffness indices prior to RDN and during follow-up (n = 20).

	Pre-RDN	Six-month follow-up	Two-year follow-up	p -value Pre-RDN vs two-year follow-up
Office SBP, mmHg	164 ± 21	139 ± 19	150 ± 18	0.06
Office DBP, mmHg	108 ± 20	89 ± 13	97 ± 13	0.03
Mean ambulatory SBP, mmHg	156 ± 21	145 ± 14	147 ± 14	0.06
Mean ambulatory DBP, mmHg	97 ± 14	89 ± 11	90 ± 11	0.02
Daytime ambulatory SBP, mmHg	159 ± 21	148 ± 15	147 ± 14	0.04
Daytime ambulatory DBP, mmHg	99 ± 14	91 ± 12	92 ± 11	0.02
Nighttime ambulatory SBP, mmHg	141 ± 22	137 ± 17	142 ± 17	0.96
Nighttime ambulatory DBP, mmHg	87 ± 14	82 ± 12	84 ± 12	0.27
Mean arterial pressure, mmHg	117 ± 15	108 ± 11	108 ± 11	0.03
Pulse pressure, mmHg	59 ± 12	57 ± 10	56 ± 10	0.36
AASI, units	0.46 ± 0.2	0.47 ± 0.1	0.49 ± 0.2	0.41

Values are reported as the mean ± SD.
SBP-systolic blood pressure; DBP-diastolic blood pressure; AASI-ambulatory arterial stiffness index.

Table 3
Metabolic variables (n = 19).

	Baseline	Two-year follow-up	p-value
BMI, kg/m^2	31.6 ± 4.9	32.6 ± 5.1	0.05
HbA_{1c}, %	5.6 ± 0.3	5.6 ± 0.5	0.93
Cholesterol, mmol/L	5.0 ± 1.2	5.0 ± 1.0	0.97
LDL, mmol/L	3.6 ± 1.0	3.4 ± 0.9	0.21
HDL, mmol/L	1.0 ± 0.2	1.1 ± 0.3	0.33
TG, mmol/L	1.7 ± 0.6	2.0 ± 0.9	0.10
Fasting glucose, mmol/L	5.9 ± 0.8	5.7 ± 0.9	0.08
Fasting insulin, pmol/L	134 ± 85	159 ± 54	0.29
Fasting c-peptide, pmol/L	1242 (890–2509)	1477 (1002–2295)	0.13
OGTT peak at 30min, mmol/L	9.8 ± 1.5	9.7 ± 1.7	0.75
OGTT peak at 120 min, mmol/L	9.5 ± 3.1	9.2 ± 2.9	0.35
$AUC_{GLC\ 0-120min}$ mmol/L	1211 ± 216	1196 ± 270	0.71
LAR	1.9 (0.5–9)	2.3 (0.4–10)	0.21
HOMA-IR	6.3 ± 3.9	7,0 ± 2.9	0.45
HOMA-AD	0.9 (0.3–5)	1.5 (0.3–4)	0.04
QUICKI	0.3 ± 0.3	0.3 ± 0.1	0.02
TyG	8.9 ± 0.4	9.0 ± 0.5	0.34

Values are reported as the mean ± SD or median (minimum–maximum) if skewed distributed.
BMI-body mass index; HbA_{1c}-glycosylated haemoglobin; OGTT- oral glucose tolerance test.
AUC_{GLC}- area under the curve for glucose.
LAR-leptin-to-adiponectin ratio; HOMA-IR-homeostasis model assessment insulin resistance.
HOMA-AD: HOMA-adiponectin model assessment; QUICKI-quantitative insulin sensitivity check index.
TyG-triglyceride and glucose index.

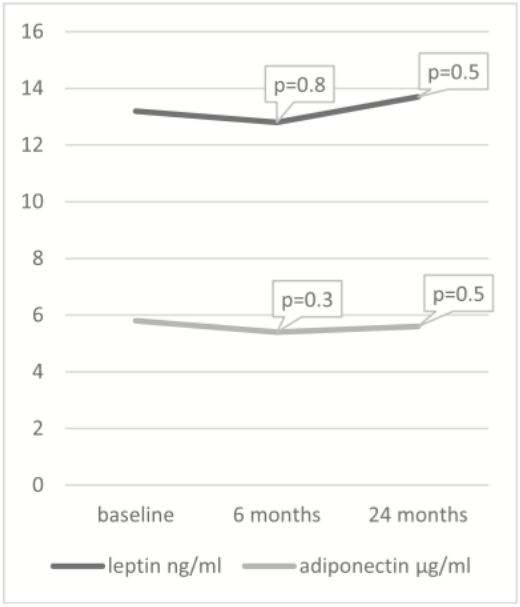

Fig. 1. Adiponectin and leptin profiles during two-year follow-up (n=20). Leptin and adiponectin changes between baseline vs six-month and baseline vs 24-month follow-up.

4.2.3. *Correlations between IS, BP and AASI*

BP responders and non-responders did not show statistically different hepatic or peripheral IS prior to RDN (Fig. 2).

There were no significant correlations between IR measurement, adipokines, BP or AASI throughout two years of follow-up.

Nocturnal systolic BP and arterial stiffness before RDN correlated positively with a progression in hepatic IR at six-month follow-up as shown in Fig. 3. However, the effect of RDN on BP did not correlate with IR two years after RDN (Table 4).

5. Discussion and conclusion

In this study we investigated whether RDN could improve the substantial IR seen in a cohort of true treatment resistant hypertensive patients on the long term. We analyzed IS and other parameters of glucose metabolism, as well as adipokines, from baseline to two years after RDN. All metabolic variables remained essentially unchanged apart from an increase in HOMA-AD, suggestive of a deterioration of IS. However, this surrogate index showed modest correlations with IS measured by HEC. Moreover, we found no associations to BP change and indices of arterial stiffness.

Provided that IR is partly associated with impaired microcirculation in the skeletal muscle and adipose tissue, a reduction in vasoconstriction and renin-angiotensin system activation induced by RDN may in theory be a mechanism by which IS could be improved [32,33]. As a result of decreased SNA followed by a decline in BP and reduction of hyperinsulinemia, one would expect an improvement of both hepatic and peripheral IR in patients treated by RDN.

IS assessed by glucose tracer and HEC methods did not show improvement after six months of observation. Thus, we aimed to assess IS and adipokines changes after longer follow-up. At two-year follow-up we did not perform HEC but we used surrogate IS indices which correlated best with our previous clamp data in this specially selected group of patients.

Our results show that hepatic IS correlated best with the first stage of OGTT (OGTT 30min.) as expected, since EGR is closely related to fasting hyperglycemia and postprandial glycemia. In contrast to a previous study by Abdul-Ghani et al., we found that the first step of OGTT correlated better with EGR than AUC_{GLC} [34]. Peripheral IS (i.e. WGD increase during high insulin infusion) correlated best with HOMA-IR. GIR during high-dose insulin infusion was best associated with HOMA-IR, but also moderately with HOMA-AD, QUICKI, AUC_{GLC} and OGTT 30min.

Reassessment after two years of these indices as well as fasting glucose, insulin, c-peptide, and $HbA1_c$ showed no change, apart from HOMA-AD.

We therefore conclude that our patients suffered from severe hepatic and peripheral IR prior to and six months after RDN, and that this had not improved during the two-year follow-up.

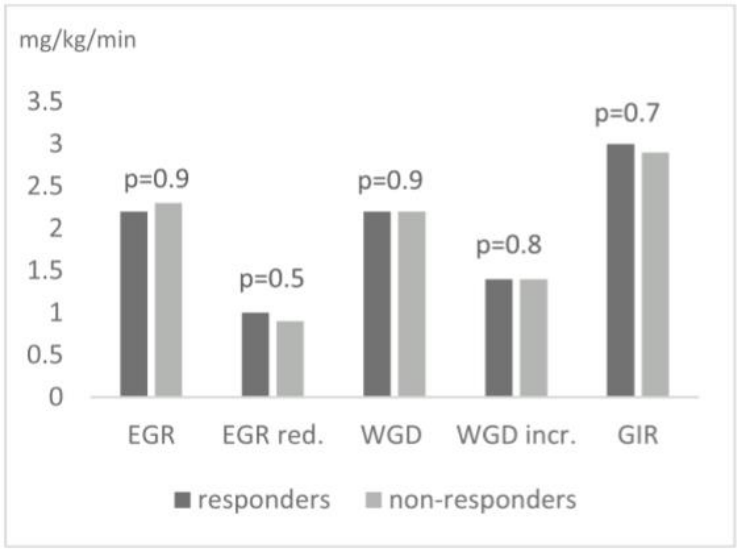

Fig. 2. Insulin sensitivity characteristics prior to RDN for blood pressure responders and non-responders at two-year follow-up. EGR; Basal endogenous glucose release during glucose tracer infusion (before insulin infusion). WGD; Basal whole-body glucose disposal (before insulin infusion). EGR red.; Endogenous glucose release reduction during low-dose insulin infusion. WGD incr.; Whole-body glucose disposal increase during high-dose insulin infusion. GIR; Glucose infusion rate during high-dose insulin infusion. 12 Responders and 8 non-responders defined as mean systolic ambulatory BP≥5mmHg between baseline and two-year follow-up. Significant changes if P<0.05

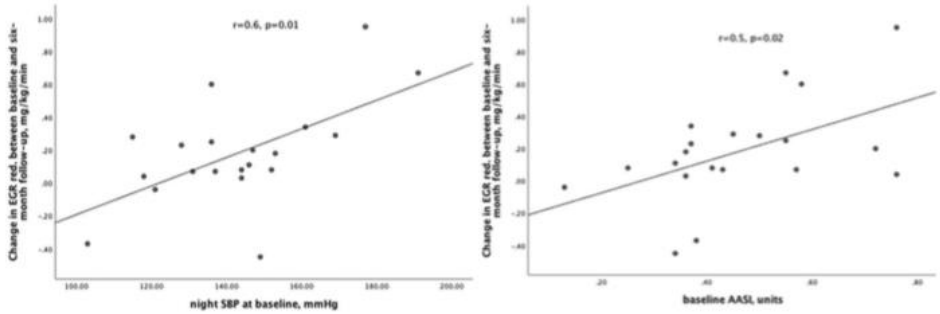

Fig. 3. Development of hepatic insulin resistance six months after RDN in relation to nocturnal hypertension and arterial stiffness prior to RDN. EGR red.- endogenous glucose release reduction during low-dose HEC; night SBP-night systolic BP prior to RDN; AASI- ambulatory arterial stiffness index prior to RDN

Table 4
Correlations between BP changes after RDN and IR indices at two-year follow up (n = 19).

ΔBP 0–24 months	OGTT-30min 24 months		HOMA-IR 24 months	
Systolic	r	p	r	p
Mean BP	−0.14	0.57	−0.03	0.92
Daytime BP	−0.25	0.33	−0.05	0.86
Nighttime BP	0.01	0.97	−0.32	0.21
Diastolic	r	p	r	p
Mean BP	−0.12	0.66	−0.21	0.41
Daytime BP	−0.23	0.38	−0.26	0.32
Nighttime BP	0.18	0.47	−0.01	0.99

HOMA-IR-homeostasis model assessment insulin resistance; OGTT 30min.- glucose concertation at 30 min during oral glucose tolerance test.

Parallel to the lack of improvement in IS we observed no significant change in adipokines after RDN. Adiponectin enhances IS by several intracellular pathways [35]. Increase in adiponectin with unchanged leptin three months after RDN has been reported by others [19]. In our study, the increase in BMI was borderline significant, but adiponectin and leptin remained unchanged. Despite a plausible pathophysiological connection between leptin and hypertension in obese patients [36] we did not find correlations between BP and adipokines in our study. Additionally, adipokines did not correlate with IS or arterial stiffness assessed by PP and AASI. However, one need to take into consideration that 18/20 patients were men. That could potentially affect changes in adipokines, knowing the differences in lipid metabolism and fat distribution between men and women [37].

In line with unaltered IS and adipokines, PP and AASI were also not affected by RDN during follow-up. PP is known to be higher in diseases involving the vascular system such as type 2 diabetes, and

PP > 60 mmHg may result from increased arterial stiffness [38]. In our study, mean PP was 60 mmHg and mean AASI 0.46 units, which are borderline results, suggesting increased arterial stiffness. In a previous RDN study it was demonstrated that RDN improved arterial stiffness, and that patients with the lowest pulse wave velocity had better BP response [39]. Moreover, Sata et al. found that AASI >0.51 predicted lack of BP response after RDN [40]. Thus, the patients in our cohort may have developed irreversible vascular changes due to long-term hypertension and metabolic disturbances, no longer responsive to RDN. However, the gold standard method for assessing arterial stiffness is pulse wave velocity. AASI is a surrogate index that mostly reflects blood pressure variability as it is calculated from ABPM measurements.

In spite of unchanged metabolic data during follow-up we observed a significant reduction of BP, with a concomitant non-significant decrease in antihypertensive drugs and borderline BMI gain. It is unknown how basal peripheral or hepatic IR may affect BP changes over time. In a previous study BP was mainly related to peripheral IR [41]. In our study we observed a modest, non-significantly higher total whole-body IS in BP responders, but BP reduction did not correlate with IR after this long observation time, however a small sample size and non-randomized design do not allow us to draw conclusions. Incomplete denervation cannot be excluded as a cause of discrepancy between BP and metabolic changes. Previous studies showed that the anatomical region of RDN and type of device might be reasons for partial denervation, leading to fragmentary effect of denervation [42–44].

During follow-up, discrepancy between daytime and nocturnal BP changes was observed as in previous RDN studies [13,45]. Most of the patients in our study had nocturnal hypertension which can refer to an imbalance between the sympathetic and the parasympathetic nervous system and seems to be an important target factor for reducing cardiovascular risk in patients with diabetes mellitus [46]. While in some previous studies a relation between nocturnal BP and IR were found, hepatic and peripheral IS was not assessed separately [47]. However, we revealed a progression of hepatic IR six months after RDN, which correlated with increased nocturnal SBP as well as arterial stiffness measured before RDN. These changes were not detected by surrogate IS indices at this point of follow-up. However, two-year metabolic data might suggest deterioration of total whole-body IS.

There are some important limitations to the design of our study and the generalizability of its findings. The main limitations are the small sample size, the non-randomized design and the lack of a control group. The low number of patients limits the possibilities for subgroup analyses. Another limitation is that IS was assessed by calculated surrogate indices at two years follow-up, not by the gold standard method.

6. Conclusions

Two years after RDN in true treatment resistant hypertensive patients, IR and parameters of glucose metabolism had not improved. Limited accuracy of several IS surrogate indices, compared to HEC, was demonstrated in this well-defined group of patients. There were no correlations between the magnitude of IR prior to RDN and adipokines and BP change. Patients with higher nocturnal SBP and arterial stiffness presented a further deterioration of hepatic IR during follow-up. This study does not support the hypothesis that RDN leads to beneficial metabolic effects or can alleviate IR in patients with long-term, treatment resistant hypertension.

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Author contributions

A.K.M., M.D.S., T.K.S., J.V.N and P.F.G. conceived and conducted the study, collected and analyzed the data, drafted, reviewed and edited the manuscript. O.M.F. analyzed the metabolic samples and reviewed and edited the manuscript. T.A.H. contributed to conception, data analysis, data interpretation and critical revision of the manuscript. All authors approved the final manuscript for submission.

T.K.S. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Conflict of interest

No potential conflict of interest was reported by the authors.

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