**Diploma Thesis**

# Metabolic Effects of Renal Sympathetic Denervation in Resistant Hypertension


Submitted by

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Graz, 28th of April 2014 David Philipp Lang

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*Vitae humanae tempus, punctum; materia fluens; sensus obscurus; totius corporis compages ad putredinem vergens; animus, turbo; fortuna, res perplexa; fama, judicii expers: ut paucis dicam, omnia corporis, fluvius; omnia animi, somnium et fumus; vita, bellum et peregrini commoratio; fama posthuma, oblivio. Quid igitur est, quod deducere potest? Unicum et solum, philosophia.*

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Marcus Aurelius Antoninus Augustus

“ta eis heautòn”, II.17

171/172 A.D.

# I. Acknowledgement

The end of my studies at the Medical University of Graz is drawing nearer, so it seems the right moment now, to review the both instructive and joyful years I have had in this great city. Still, my time here would not have been the same without the great people I had the honour to share it with.

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# II. Contents

# III. Abbreviations

**11β-HSD1**… 11-Beta Hydroxysteroid Dehydrogenase

**ABP**… (24-hour) Ambulatory Blood Pressure

**ACE-I**… Angiotensin-Converting-Enzyme Inhibitor

**ARB**… Angiotensin Receptor Blocker

**ATP III**… Adult Treatment Panel III

**BMI**… Body Mass Index

**BP**… Blood Pressure

**CCL2**… Chemokine (C-C motif) Ligand 2

**CKD** Chronic Kidney Disease

**CNS**… Central Nervous System

**CRP**… C-Reactive Protein

**CT**… Computed Tomography

**CV(D)**… Cardiovascular (Disease)

**DAG**… Diacylglycerol

**ESRD**… End Stage Renal Disease

**ET-1**… Endothelin 1

**FFA**… Free Fatty Acids

**FPG**… Fasting Plasma Glucose (Concentration)

**$fT_4$**… free Thyroxine (Concentration)

**(e)GFR**… (estimated) Glomerular Filtration Rate

**GLUT-4**… Glucose Transporter Type 4

**HbA1c**… glycated haemoglobin (A1c)

**HDL**… High Density Lipoprotein

**HOMA**… Homeostasis Model Assessment

**HPA-axis**… Hypothalamic–Pituitary–Adrenal axis

**HPT-axis**… Hypothalamic–Pituitary–Thyroid axis

**HT**… Hypertension

**IBD**… Inflammatory Bowel Disease

**IDF**… International Diabetes Federation

**IKK**… IκB Kinase

**Il-6**… Interleukin-6

**IR**… Insulin Resistance

**IRS-1**… Insulin Receptor Substrate 1

**$IS_{QUICKI}$**... Quantitative Insulin Sensitivity Check Index

**JIA**… Juvenile Idiopathic Arthritis

**JNK1**… c-Jun N-terminal Kinase 1

**LDL**… Low Density Lipoprotein

**LPL**… Lipoprotein Lipase

**MC4R**… Melanocortin 4 Receptor

**MCP-1**… Monocyte Chemotactic Protein-1

**MDRD**… Modification of Diet in Renal Disease (formula)

**MetS**… Metabolic Syndrome

**MRT**… Magnetic Resonance Tomography

**MSNA**… Muscle Sympathetic Nerve Activity

**NA (NE)**… Noradrenaline (Norepinephrine)

**NADPH**… Nicotinamide Adenine Dinucleotide Phosphate

**NCEP**… National Cholesterol Education Program

**NEFA**... Non-Esterified Fatty Acid

**NHANES**… National Health and Nutrition Examination Survey

**NK (-cell)**… Natural Killer (Cell)

**NO**… Nitric Oxide

**NSAID**… Non-Steroidal Anti-Inflammatory Drug

**OBP**… Office Blood Pressure

**OSAS**… Obstructive Sleep Apnoea Syndrome

**PKC**… Protein Kinase C

**POMC**… Proopiomelanocortin

**RA**… Rheumatoid Arthritis

**RAAS**… Renin Angiotensin Aldosterone System

**ROS**… Reactive Oxygen Species

**RSD**… Renal Sympathetic Denervation

**SAT**… Subcutaneous Adipose Tissue

**SNA**… Sympathetic Nerve Activity

**SNS**… Sympathetic Nervous System

**TG**… Triglyceride

**TIA**… Transient Ischemic Attack

**TNF-α**… Tumor Necrosis Factor α

**TRH**… Thyrotropin-Releasing Hormone

**TSH**… Thyroid-Stimulating Hormone

**U.S.(A)**... United States (of America)

**UPR**… Unfolded protein response

**VLDL**… Very Low-Density Lipoprotein

**WC**… Waist Circumferenc

**MSH**… Melanocyte-Stimulating Hormone

# IV. Abstract

Metabolic Effects of Renal Sympathetic Denervation in Resistant Hypertension

**Objective:**

Renal sympathetic denervation (RSD) with radiofrequency ablation substantially reduces blood pressure (BP) in patients with treatment-resistant hypertension. We investigated the effects of RSD on BP, body mass index (BMI), glucose- and lipid metabolism, as well as on inflammatory-, renal function-, and endocrine parameters.

**Design and Method:**

We conducted a retrospective analysis of those mentioned parameters among n=78 patients treated with RSD. BMI, lipid metabolism- and endocrine parameters were assessed before and six months after RSD, all other parameters before, six and twelve months after RSD.

**Results:**

We found, that 24-h ambulatory, but not office measured BP was significantly reduced one year after RSD. BMI, thyroxine- and Interleukin-6 concentration and leukocyte count had significantly decreased during follow up, while HDL-cholesterol, triglyceride concentration, Cystatin C and free plasma cortisol had significantly increased. No sustained significant alterations could be detected concerning glucose metabolism and renal function parameters. We found, that ambulatory rather than office BP values were correlated with the investigated parameters and their changes during follow up. Also, ambulatory BP reduction upon RSD may be predicted by baseline Interleukin-6 and TSH concentrations.

**Conclusions:**

RSD may be associated with a reduction in BP and BMI, with changes in lipid metabolism as well as endocrine parameters and may furthermore exhibit anti-inflammatory properties. This analysis did neither reveal meaningful effects on glucose metabolism, nor on renal function. Our results also suggest, that 24-h ambulatory BP may be a superior parameter in the evaluation of RSD results than office measured BP.

# Zusammenfassung


Stoffwechseleffekte der Perkutanen Renalen Denervierung bei Therapieresistenter Hypertonie

**Einleitung:**

Die perkutane renale Denervierung (PRD) durch Radiofrequenzablation bei Patienten mit therapieresistenter Hypertonie führt zu Senkung des Blutdruckes (BD). Wir untersuchten die Auswirkungen der PRD auf BD, body mass index (BMI), Zucker- und Fettstoffwechsel, Entzündungs- und Hormonparameter, sowie Nierenfunktion.

**Methoden:**

Wir führten eine retrospektive Analyse der besagten Parameter unter n=78 Patienten, die mit PRD behandelt wurden, durch. BMI, Fettstoffwechsel und endokrine Parameter wurden vor der Denervierung und sechs Monate danach erhoben, alle anderen Parameter davor, sowie sechs und zwölf Monate danach.

**Ergebnisse:**

Die Analyse ergab, dass die Werte der 24-h Langzeitblutdruckmessung, nicht jedoch die in Praxisblutdruckwerte nach einem Jahr signifikant reduziert waren. BMI, Leukozytenzahl, Thyroxin- und Interleukin-6 Konzentration zeigten sich im Verlauf signifikant vermindert, während signifikante Anstiege bei HDL-Cholesterin, Triglycerid-, Cystatin C- und Plasmacortisolkonzentration zu verzeichnen waren. Indes waren die Parameter bezüglich Glucosestoffwechsel und Nierenfunktion unverändert. Des Weiteren zeigte sich, dass die 24-h Langzeit- eher als die Praxisblutdruckwerte Korrelationen mit den untersuchten Parametern vor dem Eingriff oder deren Änderungen im Verlauf aufwiesen. Außerdem fanden wir eine Korrelation zwischen dem Ausmaß der Änderung der Langzeitblutdruckwerte und der Interleukin-6, sowie der TSH-Konzentration vor der PRD.

**Diskussion:**

Unsere Ergebnisse deuten darauf hin, dass die PRD zu Reduktion von BD und BMI, sowie Änderungen in Fettstoffwechsel und bei endokrinen Parametern führen kann, weiters scheint sie auch entzündungshemmende Effekte hervorzurufen. Wir fanden keine eindeutigen Auswirkungen auf Zuckerstoffwechsel oder Nierenfunktion. Des Weiteren scheint die 24-h Langzeitblutdruckmessung geeigneter als die Praxisblutdruckmessung, um die Resultate der PRD zu beurteilen.

# 1. Background

## 1.1. Hypertension in Obesity and Metabolic Disorders

### 1.1.1. Epidemiology and Definition

High blood pressure represents one of the major causes of morbidity and mortality worldwide, with about 15% of total deaths attributable to hypertension.[1] According to recent data collected by the National Health and Nutrition Examination Survey (NHANES), overall age-adjusted prevalence of hypertension among adults in the United States is estimated 29,1%. Of those, 75,7% were taking antihypertensive medication, but only 51,9% had their BP controlled, defined as values being below a threshold of 140/90mmHg. The highest prevalence in the U.S. is found among non-Hispanic black adults.[2]

Blood pressure itself and the prevalence of hypertension vary with gender, race and region. The chance to develop hypertension increases with age and is related to obesity, dietary salt intake and urine sodium-to-potassium ratio.[1] Genetic components are likely to contribute to the development of hypertension in a polygenic manner, possibly affecting genes involved in function and control of the RAAS, β-receptors or renal tubular sodium reabsorption.[1,3,4] However, according to current knowledge, genetic effects only seem to play a minor role in the pathogenesis of hypertension, compared to other "traditional" risk factors like obesity.[5]

There is evidence, that blood pressure is closely associated with BMI and waist-to-hip ratio, probably slightly differing by age and gender.[6,7] A large part of hypertension cases may be attributable to obesity,[8] and it is a common finding in animal and human studies, that blood pressure increases with weight gain and decreases with weight loss.[9] Elevated blood pressure in obesity and the metabolic syndrome could therefore be regarded as a kind of "secondary hypertension".[10]

The risk of cardiovascular disease, heart failure, stroke and renal disease increases continuously with systolic and diastolic blood pressure levels.[1,11] Among individuals aged 40-69 years, each 20mmHg increase in systolic BP or 10mmHg in diastolic BP entails a twofold augmentation of CVD-risk, beginning with 115/75mmHg.[12] Patients with prehypertension (130/80-139/89mmHg) do not (yet) require pharmacological treatment, still, they are at double risk to develop hypertension.[13] The diagnosis of hypertension should be based upon mean blood pressure values of at least two office visits. Measurement should be conducted, after the patient has been seated quietly for a minimum of five minutes, with

a proper sized cuff.[11] According to the Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure (JNC 7), the following threshold values should be used in diagnosis and therapy of hypertension:[11]

*Table 1. Classification and management of blood pressure for adults, modified after the Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure (11)*

| **BP Classification** | **Systolic/Diastolic BP** (mmHg) | **Initial Therapy** (without compelling indication*) |
|---|---|---|
| Normal | <120/<80 | - |
| Prehypertension | 120-139/80-89 | Lifestyle modification |
| Stage 1 Hypertension | 140-159/90-99 | Lifestyle modification + single-drug therapy (thiazide, ACE-I/ARB, β-blocker, calcium-channel-blocker etc.) |
| Stage 2 Hypertension | ≥160/≥100 | Lifestyle Modification + 2-drug combination (thiazide + ACE-I/ARB or β-blocker or calcium-channel-blocker) |

** Heart failure, post-myocardial infarction, high coronary risk, diabetes, chronic kidney disease, recurrent stroke prevention*

The recently published JNC 8 guidelines aimed to take a critical approach to the current classification of hypertension with its thresholds and therapeutic options, by applying evidence from randomized controlled studies to the previous guidelines. Major new findings include, that the goal for patients >60 years should be a systolic BP of <150/90mmHg, while patients <60 years should reach a target diastolic BP <90mmHg. Currently, there is no evidence for a systolic threshold in this group, so it is suggested, that BP should be below 140/90mmHg, as it is also recommended for hypertensive patients with diabetes or CKD. Evidence also suggests, that in white hypertensives an ACE-I or ARB should be the primary choice for treatment, given their additional renoprotective and metabolic features.[14]

24-h ambulatory BP monitoring offers insights into BP regulation throughout the day, including BP in physical activity and also in sleep.[1,11] ABP monitoring is considered a more powerful diagnostic tool than office BP measurement, as ABP levels have been shown to be correlated with target-organ injury and cardiovascular risk to a higher extent.[1,15] 24-h ABP levels are usually lower than office measured values, therefore ABP should be below a threshold of 135/85mmHg in awake patients and <120/75mmHg when asleep.[1,11] Night ABP

levels should be decreasing by 10-20%, attenuation of this phenomenon referred to as "dipping", is accompanied by an increased risk of cardiovascular events.[11,16]

### 1.1.2. Resistant and Refractory Hypertension

Resistant hypertension is a condition defined as the inability to reach target blood pressure despite the administration of a triple or higher combination of antihypertensive drugs at proper doses, including one diuretic.[17,18]

The prevalence of resistant hypertension has been reported 12,8% in a large US cohort, with considerably higher odds ratios for black race and obesity and -less distinct- for male sex and older age.[18] Similarly, in a Spanish cohort, prevalence of resistant hypertension was 12,2% using office BP measurement. Consecutive ABP monitoring among these patients revealed 37,5% as being "white coat hypertensive".[19]

Other possible reasons for so called "pseudo-resistant hypertension" include inaccurate measurement of blood pressure due to wrong cuff size or other technical problems, inadequate drug prescription by the physician or lack of compliance by the patient.[20]

When pseudo-resistance has been ruled out, the Austrian Society of Hypertension suggests the following diagnostic algorithm to detect and treat factors possibly causing resistant hypertension:[20]

*Table 2. Diagnostic algorithm in resistant hypertension, modified after Weber et al., 2012 (20)*

| | |
|---|---|
| • Life style factors | Obesity, high salt intake, inordinate alcohol intake |
| • Unfavourable comedications | NSAID, corticosteroids, drug abuse, sympathomimetics, contraceptive pill, immunosuppressants, etc. |
| • Secondary hypertension | OSAS, renal-parenchymal/renovascular hypertension, hyperaldosteronism, pheochromocytoma, Mb. Cushing, etc. |

Patients displaying resistant hypertension, which cannot be controlled with maximal pharmaceutical therapy effort at a specialized hypertension unit are considered suffering from "refractory hypertension".[21] In a recent study, patients with refractory hypertension displayed a significantly higher heart rate and a decreased antihypertensive response to spironolactone therapy than patients with "ordinary" resistant hypertension, suggesting the influence of neural mechanisms like the sympathetic nervous system.[21]

## 1.2. Pathophysiological Concepts of Hypertension

### 1.2.1. The Renin-Angiotensin-Aldosterone-System

Increased systemic activity of the RAAS in obesity and the metabolic syndrome has been reported in older studies and was therefore thought to contribute to hypertension.[22,23,24] However, in contrast to that assumption, a recent population study among third generation Framingham Heart Study participants reported no correlation of systemic RAAS activity parameters with subcutaneous or visceral fat mass.[25] Still, the lack of evidence concerning systemic RAAS activity does not ostracise the important role the RAAS plays in the metabolic syndrome, but it probably rather acts at a local, tissue specific level than at a systemic one.[26,27] Angiotensinogen gene expression has been reported to be especially increased in adipose tissue, when visceral obesity is present,[28] and may be crucial in the development of obesity-induced hypertension.[29]

Vasoconstriction by angiotensin II, arising from an activated local or systemic RAAS, is suspected to cause direct glomerular injury to the kidney.[30] Consistently, ACE-I and ARB have been shown to reduce proteinuria in CKD,[31] and also the incidence of ESRD in patients with proteinuria, especially in obese subjects.[32] Furthermore, older animal studies suggest, that pharmacological RAAS inhibition may cause stronger antihypertensive effects in obese subjects than in lean ones.[23]

Plasma aldosterone concentrations are reported to be elevated in obese hypertensive subjects,[33,34] with BMI being closely correlated with plasma aldosterone concentrations.[35] Also, mineralocorticoid-receptor antagonists have recently been demonstrated to improve endothelial function by a degree dependent on measures of adiposity in obese subjects.[36] This finding may partly explain the increased prevalence of resistant hypertension in obese patients and consistently, aldosterone antagonists are being successfully used in drug-resistant hypertension.[37] Furthermore, mineralocorticoid receptor antagonists may exhibit renoprotective features,[38] also when added to an ACE-I therapy.[39] However, this advantage may be achieved at the price of an increased risk of complications like hyperkalaemia, so that the actual benefits of dual RAAS blockade are still discussed controversially.[40]

### 1.2.2. Microcirculation and Endothelial Function in Obesity and Hypertension

Microcirculation is an important regulator of overall peripheral vascular resistance and thus of blood pressure.[41] Microvascular abnormities can either be cause or consequence of hypertension.[42] Defects in microvascular structure and control can lead to increased vasoconstriction, reduced vasodilatation and vascular rarefaction within muscle and skin vascular beds, which has been reported in hypertension,[43] as well as in obesity and in insulin resistance.[44] This goes in line with the finding, that obese, hypertensive humans display vasoconstriction as a response to insulin rather than the normally expected vasodilatation.[45] Microvascular control is sustained by the balance of several intracellular signalling pathways and is probably -at least in part- insulin-dependent.[42] Research results suggest, that insulin resistance in hypertension is associated with endothelial dysfunction, which may be caused by local NO and ET-1 imbalance in small blood vessels.[46] The molecular basis for this mechanism may be found in the insulin-dependent activation of intracellular signalling pathways, leading to synthesis of vasodilatatory NO on the one hand and vasoconstrictive Endothelin-1 on the other hand.[42] The balance between the two systems may be crucial in microvascular control and has been shown to be disturbed in hypertension and obesity.[46,47] Adipose tissue factors like FFA, adiponectin, angiotensinogen and TNF-α may play a causative role those processes:[42,48] FFA activate PKC-θ, leading to inactivation of IRS-1, which ultimately affects NO-synthesis, while TNF-α may have similar effects by activating JNK. So ultimately, both substances may impair insulin mediated vasodilatation, while the insulin-mediated Endothelin-1 pathway is thought to remain active, shifting the vascular balance towards vasoconstriction.[49] In line with these hypotheses, measures of microvascular dysfunction were found to be positively correlated with measures of visceral and subcutaneous adiposity as well as with the levels of inflammatory parameters like CRP, Il-6 and TNF-α.[48] Summing up, adipose tissue factors like inflammatory markers and FFA, but also adiponectin, leptin, and angiotensinogen seem to be participating in microvascular processes, possibly linking hypertension to obesity and insulin resistance.[42]

Importantly, similar microvascular alterations may not only be found in skin or muscle blood vessels, but also in the kidneys, perhaps triggering subtle renal injury and salt sensitivity, essential factors in the development of hypertension.[50,42] Consistently, de Jongh et al. reported, that salt sensitivity was inversely correlated with measures of skin microvascular function.[51]

## 1.2.3. The Kidney in Hypertension

According to most pathophysiological models, alterations in structure and function of microcirculation cannot not be the solitary cause of hypertension, as temporarily elevated blood pressure by increased peripheral resistance should normally be balanced by increased pressure-natriuresis in the kidneys.[1,42] Essential hypertension therefore may also require an impairment in renal pressure-induced sodium excretion to develop, as integrated in the "Borst-Guyton concept" of salt sensitivity in hypertension. There may be no form of hypertension in which the kidneys are not involved in initiation or perpetuation of elevated blood pressure levels.[4] Johnson et al. suggested three major mechanisms of renal involvement in hypertension including 1) response to decreases in glomerular filtration rate, 2) impairment in tubular sodium transport and 3) renal ischemia by vasoconstriction, oxidative stress and inflammation.[4]

Decreases in GFR are known to be closely associated with hypertension,[52] probably caused by sodium retention and volume expansion, additionally linked with disturbed tubular sodium excretion.[4] Several genetic polymorphisms, for example in the epithelial sodium channel of the collecting duct, are known to predispose to hypertension,[4] still, although hypertension incidence is known to be increased within families,[3] only a minor part of blood pressure variance could be attributed to genetic models, compared to the traditional risk factors.[5] The incredible increase in the prevalence of hypertension, especially in last century, suggests an environmental rather than a genetic influence,[4] which is likely to be found in the epidemic of obesity and the metabolic syndrome,[53,54,55,56] which itself is associated with numerous factors that may contribute to hypertension, as reviewed in this thesis.

## 1.2.4. Renal Sodium Handling and Renal Injury

Renal ischemia, like originally suggested by Goldblatt already in the 1940s, may actually be a major factor in the development of hypertension. However, as we know today, this process is not caused by constriction of the renal arteries, but happens at a much more microvascular level.[50] Johnson et al. suggested, that kidney injury, ultimately responsible for the perpetuation of hypertension would proceeded in two steps.

Episodes of renal vasoconstriction, especially affecting the afferent arteriole, are thought to initiate the process leading to hypertension.[4] This temporary vasoconstriction can -for example- result from increased SNS activity,[57] as found in borderline hypertension especially in younger subjects,[58] activation of the RAAS,[59] or endothelial dysfunction with

consecutive depletion of NO,[60] as mentioned earlier in this thesis. At this early stage, hypertension is thought to be salt-resistant, as the ability of the kidneys to excrete sodium is not impaired yet and the elevation in blood pressure is caused by those extrarenal factors. However, as microvascular and tubulointerstitial injury develop with the progression of hypertension, sodium excretion may become impaired and the salt resistant may turn to salt sensitive hypertension.[50]

This hypothesis, especially with a focus on angiotensin II as major contributor to renal injury, has been successfully tested in animal models,[59] and led to another important finding: Vascular damage in the kidneys is associated with T-cell and macrophage infiltration into the interstitium of the kidneys, a process which may be source of further production of angiotensin II and oxidants reducing local NO production.[4,61,62] Consistent with this finding, salt-sensitive hypertension could be shown to be prevented by the systemic application of mycophenolate mofetil, a substance suppressing immune cell proliferation.[60,63]

### 1.2.5. Low Nephron Numbers and Hypertension

Another major renal-specific risk factor in the development of hypertension may already be determined in the fetal period, as there is evidence, that congenitally low nephron numbers are associated with future elevations of blood pressure.[64,65] Animal and human studies suggest, that infants with low birth weight or intrauterine growth retardation have lower nephron numbers and are more likely to develop hypertension and salt sensitivity.[66,67,68] However, in humans, this association could only be proven in Caucasians up to now,[69] but not in African-Americans,[68] suggesting additional effects of other, race specific mechanisms. Altogether, the pathophysiological mechanism underlying this association may be rather be an increased vulnerability to renal microvascular disease and to tubulointerstitial inflammation, than the low nephron number itself.[4]

### 1.2.6. Renal Compression by Visceral Adipose Tissue

Weight gain increases renal sodium reabsorption and attenuates pressure natriuresis, leading to a higher GFR, probably in order to restore renal sodium excretion to back normal at the price of an elevated blood pressure.[70,71] A possible cause for this mechanism may be physical compression of the kidneys by surrounding adipose tissue and penetration of fat into the renal sinuses.[71] Increased amounts of renal sinus fat have been reported to be associated with increased risk of hypertension and chronic kidney disease.[72] Animal models of obesity caused by high fat feeding demonstrated increased renal sinus fat and hypertension,[73] as well

as augmented sodium reabsorption and volume retention.[70] Hypothetically, this may be caused by increased interstitial pressure within the kidneys via compression of renal veins and lymphatic vessels.[72,73] Artificial constriction of the renal vein in animals has been reported to cause an augmented sodium reabsorption in the loop of Henle,[74] possibly caused by increased pressure and consecutively reduced flow rate.[71] Low sodium concentrations at the macula densa could then cause further sodium reabsorption by activation of the RAAS via the renal feedback system.[71]

Furthermore, not only fat around the kidneys, also lipid content in the renal parenchyma is increased in obesity,[73,75] suggesting an additional involvement of lipotoxicity, oxidative stress and inflammation in the kidney,[72] similar to the processes in muscle or other tissues, underlying insulin resistance, as discussed later in this thesis.

# 1.3. The Sympathetic Nervous System in Hypertension and Metabolic Disorders

## 1.3.1. The SNS in Cardiovascular and Metabolic Control

It is commonly agreed on the fact, that acute sympathetic activation causes an elevation in cardiac output, next to sodium retention and renin release in the kidneys increasing circulating volume and peripheral resistance.[1,10] General metabolic effects of sympathetic activation include lipolysis with an elevation in free fatty acids, gluconeogenesis in the liver and inhibition of insulin release by the pancreas.[76] These short term cardiovascular and metabolic effects suggest, that chronic sympathetic over-activity may be an important factor in diseases like hypertension, obesity, chronic kidney disease, insulin resistance and lipid metabolism disorders, as integrated in the concept of the “metabolic syndrome”.

## 1.3.2. Alterations of SNS-Activity in the Metabolic Syndrome

Recently, increased sympathetic nerve activity has emerged as a new major factor not only in the pathogenesis of hypertension, but also in various metabolic disorders integrated the “metabolic syndrome”. Mancia et al. even referred to the sympathetic nervous system as the “primum movens” of the cardiovascular and metabolic alterations characterizing the metabolic syndrome,[77] as there is evidence, that most of its components, like obesity or insulin resistance, are associated with an elevation in adrenergic activity.[76,77]

Heart rate, used as a marker of sympathetic activity,[78] has been reported to be significantly increased in patients meeting the criteria for the metabolic syndrome.[55] Noradrenaline

spillover rate to plasma from total body,[79] kidney,[80] or brain,[81] is increased in hypertension. Notably, renal NA spillover is elevated in obesity with or without hypertension.[79,80] This finding is may indeed be a possible explanation, of how obesity can cause hypertension by impairing renal function via the sympathetic nervous system.

Measuring of efferent muscle sympathetic nerve activity (MSNA), assessed by microneurography indicated elevated sympathetic nerve activity in hypertension,[82,83] diabetes,[83] obesity,[79,84] and in the metabolic syndrome in general.[84] Increased MSNA in the MetS has been reported even in normotensive subjects, but hypertension further increases the levels of muscle sympathetic nerve activity.[85] Gender may also play a role in sympathetic activation in obesity, as it has been recently reported, that muscle sympathetic nerve activity was related to blood pressure in women, but to measures of obesity in men and weight loss only reduced MSNA in men, but not in women.[86] Furthermore, the arterial baroreflex has been shown to be impaired in the metabolic syndrome.[84]

Obstructive sleep apnoea, widely known to be highly prevalent in obesity the MetS, has been reported to be accompanied by increased sympathetic nerve activity in lean and even more in obese subjects,[87] suggesting that the presence of OSAS may rather add an additional activation to, than being the exclusive cause for sympathetic over-activity in obesity.[76]

### 1.3.3. The SNS and Insulin Resistance

Insulin action and sympathetic nerve activity seem to be closely related to each other, although results are varying between different studies. Evidence suggests, that increased sympathetic activity reduces insulin sensibility by modifying local haemodynamics.[77] One the one hand, sympathetically mediated vasoconstriction of small muscle vessels decreases glucose uptake,[88] on the other hand, insulin locally prevents sympathetically mediated vasoconstriction in healthy subjects.[89]

In lean subjects, elevation of plasma insulin levels increases sympathetic nerve activity to skeletal muscle and also local blood flow, but this vasodilatatory response seems to be blunted in obese persons,[45,90] who also had higher MSNA at baseline.[90] These findings may establish a connection between SNS activity and microvascular function, especially concerning the response to insulin, that has already been discussed earlier in this thesis.

The question, whether sympathetic activation or other metabolic alterations are the primary cause of insulin resistance is still not fully understood. Noradrenaline spillover from the kidneys to plasma is not increased by hyperinsulinaemia[79] and weight gain in non-obese subjects has been shown to increase MSNA, systolic blood pressure, plasma leptin

concentrations and plasma renin activity, but not insulin concentration.[91] Mauso et al. reported, that in young, non-hypertensive Japanese, elevations in markers of sympathetic activation were present ten years before they developed insulin resistance and hypertension.[92] Similarly, another study demonstrated, that future insulin resistance was independently predicted by elevated parameters of sympathetic reactivity.[93] Together, these findings strongly suggest, that hyperinsulinaemia and insulin resistance do not cause increased SNS activity in the metabolic syndrome.[76]

### 1.3.4. The SNS and Adipose Tissue Factors

Factors released by adipose tissue may play an important role in sympathetic activation in obesity and the metabolic syndrome, as especially visceral adiposity is associated with an increased adrenergic drive.[94]

Levels of Non-esterified fatty acids (NEFA), also known as free fatty acids (FFA), are associated with a deterioration of glucose tolerance,[95] and systemic NEFA infusion increases muscle sympathetic nerve activity as well as blood pressure,[96] which potentially leads to insulin resistance in muscle. However, the putative role of systemic FFA/NEFA levels in insulin resistance and the metabolic syndrome remains to be questioned,[97] as it has also been reported, that whole body and renal NA spillover is rather decreased than increased upon NEFA infusion.[98]

Concentrations of the adipokine leptin are known to be increased in obesity and the metabolic syndrome.[99,100] Furthermore leptin is believed to increase sympathetic nerve activity and reduce appetite in order to promote weight loss[101,102]. The appetite-suppressing effect is thought to be lost in obesity due to selective central nervous system resistance to its action, while the sympathoexcitatory properties may persist.[77,103] Fasting plasma leptin levels have been shown to be correlated with the extent of noradrenaline spillover to plasma, but not with MSNA.[104] Still, evidence concerning a causative role of leptin in sympathetic nerve activity is no fully conclusive,[76] as Masuo et al. demonstrated, that elevated baseline markers of adrenergic activity could predict weight gain and blood pressure elevations in future, but leptin could not.[105] A new possible mechanism, which may link leptin with sympathetic nerve activity and hypertension at the level of the central nervous system, referred to as the leptin melanocortin pathway,[30] will be discussed later in this thesis.

Adiponectin has been reported to act anti-inflammatory and anti-atherogenic and its concentration is decreased in obesity and insulin resistance.[106,107,108] Low levels of adiponectin are independently associated with increased risk of hypertension,[109] and

antihypertensive therapy with rilmenidine significantly increased plasma adiponectin concentration without significant changes in BMI or insulin sensitivity.[110] In mouse studies, a decrease of renal SNS activity and BP was observed after application of adiponectin.[110]

### 1.3.5. The Leptin-Melanocortin System

Leptin may be a key factor linking obesity with hypertension and SNS activity. As mentioned before, leptin is an important regulator of the energy household, acting by reducing appetite and increasing energy consumption, probably via the SNS.[30] Leptin concentrations are increased in obesity,[99] and associated with markers of sympathetic nerve activity.[101,104] Furthermore, administration of leptin increases blood pressure and heart rate,[112] which may represent a marker of SNA.[78]

Genetically leptin deficient mice and humans are extremely prone to develop obesity and other features of the metabolic syndrome, but exceptionally they do not display hypertension and have rather reduced SNS activity.[113,114] The molecular basis for the action of leptin to increase blood pressure is assumed to be found the CNS: Leptin receptors on POMC (proopiomelanocortin) neurons in the hypothalamus may cause activation of MC4R (melanocortin)-receptors in the paraventricular nucleus and the brainstem, via the release of α-MSH.[30] Consistent with this hypothesis, deletion of the leptin receptor on POMC neurons in mouse models led to a decrease in blood pressure as a response to leptin application instead of the normally expected increase.[115] Similar to those findings, humans and animals with POMC or MC4R mutations are obese and insulin resistant, but not hypertensive and have no increased or even lower SNS activity compared to controls.[116,117,118,119]

### 1.3.6. Connections between SNS and RAAS

Augmented efferent sympathetic outflow to the kidneys causes activation of the RAAS by an increase in renin release upon stimulation of β-1 receptors in juxtaglomerular granular cells.[120] Thus, it is likely, that RAAS and SNS activity are closely connected to each other.

Some studies report, that the RAAS is activated in obesity with significantly elevated plasma renin activity,[22] either due to local production in adipose tissue,[28,29] or due to a feedback mechanism between the SNS and the RAAS at a peripheral or central nervous system level.[121] Adipocytes in obese hypertensive subjects have been reported to display an up-regulation of the renin, ACE and angiotensin II type 1 receptor genes.[122] Although specific pathways have not yet been found, these findings underpin, that local RAAS activity in adipose tissue may be a possible link between obesity, increased SNA and hypertension.

### 1.3.7. The Kidney and the Sympathetic Nervous System

It is likely, that renal afferent sympathetic signalling to the CNS is increased as a response to the process of renal injury, which is linked with the pathogenesis of hypertension.[4] This hypothesis is supported by the fact, that measures of sympathetic nerve activity have been repeatedly shown to be increased in hypertension,[82,85,123] and also in renal failure.[124,125,126] Furthermore, sympathetic nerve traffic from the CNS to the kidneys is stimulated in hypertension, whereupon efferent renal specific sympathetic over-activation of the kidneys has been reported in several studies.[123,127]

As mentioned before, augmented efferent SNA to the kidney can cause arteriolar vasoconstriction, increased renal sodium reabsorption and impaired pressure natriuresis.[30] All of those renal processes may contribute to the vicious circle ultimately leading to the onset and perpetuation of hypertension. Consistently, in a recent study, Foss et al. reported, that in a genetic salt-sensitive hypertension rat model, targeted sympathetic nerve ablation resulted in distinct decrease in salt-sensitivity compared to controls.[128]

Obesity related hypertension has been reported to be associated with increased sympathetic outflow to the kidney and to skeletal muscle,[76] however, increased sympathetic nerve activity is also present in obesity without hypertension.[22,85,86] This underlines the hypothesis, that increased sympathetic nerve activity may not only be a consequence of renal injury in chronic hypertension, but also be an important factor in the initiation of the whole process ultimately leading to hypertension.[4] Therefore, this finding may help us understand, why and how obesity can initiate hypertension by affecting the kidney.

## 1.4. The Metabolic Syndrome

### 1.4.1. Epidemiology and Definition

The metabolic syndrome represents a constellation of certain co-occurring metabolic abnormities increasing the risk of cardiovascular disease and diabetes mellitus. Those metabolic dysfunctions include central obesity, lipid metabolism disorders, hypertension and hyperglycaemia.[108,129,130]

The most commonly used diagnostic criteria for the metabolic syndrome were edited by the International Diabetes Foundation (IDF) in 2006,[129] based on the guidelines by the National Cholesterol Education Program, Adult Treatment Panel III (NCEP: ATPIII) from 2001.[130] The IDF-Criteria emphasise the importance of abdominal obesity as a main criterion in the

diagnosis of MetS, as waist circumference is easier to assess than insulin resistance and has been proven to be independently related with insulin resistance and visceral obesity.[129,131]

*Table 3. NCEP ATP III Criteria for the Metabolic Syndrome 2001 (130)*

| **Three or more of the following:** |
|---|
| • Abdominal obesity: Waist circumference >102cm (m), >88cm (f) |
| • Hypertriglyceridemia: Triglycerides ≥150mg/dl or specific medication |
| • Low HDL cholesterol: <40mg/dl (m) and <50mg/dl (f) or specific medication |
| • Hypertension: blood pressure ≥130/85mmHg or specific medication |
| • Fasting plasma glucose ≥110mg/dl or specific medication or previously diagnosed type 2 diabetes |

*Table 4. IDF Criteria for the Metabolic Syndrome 2006 (129)*

| **Waist circumference:** |
|---|
| Ethnicity specific; ≥94cm (m), ≥80cm (f) for Europeans |
| **Plus any two or more of the following:** |
| • Fasting triglycerides >150mg/dl or specific medication |
| • HDL cholesterol <40mg/dl (m) and 50mg/dl (f) or specific medication |
| • Blood Pressure >130mmHg systolic or >85mmHg diastolic or treatment of previously diagnosed hypertension |
| • Fasting plasma glucose ≥100mg/dl or previously diagnosed type 2 diabetes |

In the U.S. population, age-adjusted prevalence of the metabolic syndrome according to the NCEP ATP III guidelines has been reported 23,7% in 2002.[53] Overall prevalence in Germany, assessed in 2005, accounted for 19,8%, with a higher rate among men than among women (22,7% and 18,0% respectively).[54] In an Italian population (PAMELA-study), the metabolic syndrome was present in 16,2%, in which 95,4% were hypertensive, 77,1% had high levels of plasma triglycerides and 72,2% had low plasma HDL concentrations. Central obesity was present in 58,5% and impaired fasting glucose in 31.5%.[55]

Other metabolic abnormities, such as elevations in atherogenic small dense low-density lipoprotein or apolipoprotein B and indicators of proinflammatory and prothrombotic state, associated with visceral obesity, are discussed to be included into future diagnostic criteria.[77,129]

## 1.4.2. Pathophysiological Concepts

### 1.4.2.1. Visceral Obesity and Lipid Metabolism Disorders

#### 1.4.2.1.1. Visceral Obesity in the MetS Definition

In a study published in 2004, Carr et al. demonstrated, that intra-abdominal fat measured by CT imaging was significantly increased in patients meeting the NCEP ATP III criteria and independently associated with all metabolic syndrome criteria. Insulin sensitivity however, was only independently associated with HDL cholesterol, TG and fasting plasma glucose concentration[131]. The amount of intra-abdominal fat may therefore be the most important measure to determine the presence of the metabolic syndrome.

Another interesting finding was, that waist circumference (WC) had a stronger relation to the amount of intra-abdominal adipose tissue than to subcutaneous fat and WC was independently associated with visceral adiposity as well as with insulin resistance.[131] Waist circumference is easy to assess and therefore poses the most effective measure to identify visceral adiposity in clinical practice, as the true body fat distribution can only be judged by imaging techniques like computed tomography scanning.[129,131]

#### 1.4.2.1.2. The Hypertriglyceridaemic Waist

A co-occurrence of increased waist circumference and fasting hypertriglyceridemia, referred to as the "hypertriglyceridaemic waist" may be an even better marker of visceral adiposity.[132] This constellation may also be a simple, but powerful tool for screening vascular risk factors like hyperinsulinaemia and the "atherogenic triad" of hypertriglyceridemia, elevated apolipoprotein B and small, dense LDL,[133] identifying persons at high risk for concomitant diseases like coronary artery disease.[131,133,134]

#### 1.4.2.1.3. Causes and Consequences of Dyslipidaemia

Dyslipidaemia in obesity and the metabolic syndrome is characterized by elevated triglyceride and small dense LDL-cholesterol, as well as low HDL-C levels. Pathophysiological models for explanation suggest, that lipid metabolism in obesity is shifted towards high levels of fasting and postprandial triglycerides. This causes an increased exchange of TG from VLDL and LDL to HDL and of cholesterol-esters from HDL to VLDL and LDL. Thus, HDL-C is decreased and small dense LDL is formed, as the hepatic lipase removes TG and phospholipids from LDL.[135] Concurrently, high TG and low HDL-C levels have been found to be correlated with the occurrence of small dense LDL, while

hyperinsulinaemia and increased visceral adipose tissue mass, despite being significantly correlated to, could not independently predict the small dense LDL phenotype.[136]

However, it is still not fully resolved what ultimately causes these lipid metabolism alterations in the MetS. Lipoprotein-Lipase (LPL) plays a crucial role in postprandial lipolysis of TG-rich chylomicrons and is normally activated by the postprandial rise of insulin concentration in order to shift lipid components to fat storages.[135,137] Deregulation of LPL in obesity may therefore be associated with impairments in fat metabolism, leading to postprandial hypertriglyceridemia,[138] ectopic fat deposition, for example in liver and skeletal muscle,[139] and lipotoxicity, all ultimately increasing insulin resistance.[140,141]

#### 1.4.2.1.4. The RAAS in Lipid Metabolism Disorders

The RAAS may also play a fundamental role in the control of blood lipid metabolism.[27] On the one hand, hypercholesterinaemia has been shown to stimulate the expression of RAAS components, especially in vascular cells, which may play an important role in the development of atherosclerosis .[27] On the other hand, angiotensin II increases the oxidation of LDL in macrophages,[142] and promotes foam cell formation[143], both hallmarks of atherosclerosis. In line with the potential involvement of the RAAS in lipid metabolism and atherosclerosis, several large scale studies have shown properties of ACE-I and ARB to improve the blood lipid profile, especially TG, HDL and LDL and also glucose metabolism.[144] Remarkably, the lowering of TG and increasing of HDL-C was reported to be considerably stronger in patients with the metabolic syndrome.[145]

#### 1.4.2.1.5. Free Fatty Acids in the Metabolic Syndrome

In the last decades, the main driving force behind the pathophysiological processes resulting in insulin resistance and lipid metabolism alterations was thought to be an elevated level of plasma FFA/NEFA, derived from increased visceral adipose tissue lipolysis.[135] Systemic application of FFA was shown to increase both insulin resistance in muscle and triglyceride production in the liver.[108,136] However, the “portal theory”, stating, that visceral adipose tissue lipolysis selectively caused accumulation of FFA in liver cells, has been abandoned,[77] as there is evidence, that only a minor part of FFA in systemic and portal circulation originates from visceral adipose tissue.[146] In recent review, Karpe et al. reported, that also the compelling theory behind systemic FFA levels being increased in obesity and causing insulin resistance probably was to be condemned, as they could not prove an association of FFA release into systemic circulation with increased adipose tissue mass. Also, FFA concentrations were not necessarily increased in states of insulin resistance. Thus, the

authors suggested, that impaired fat storage or adipokines and inflammatory cytokines from increased adipose tissue mass were more likely to cause lipid and glucose metabolism alterations in the metabolic syndrome than FFA.[97]

## 1.4.2.2. Adipose Tissue Inflammation and Adipokines

### 1.4.2.2.1. Inflammation in Obesity and the MetS

There is extensive evidence, that markers of inflammation, like CRP, Il-6, TNF-α and white blood cell count are increased in obesity, type 2 diabetes mellitus and the metabolic syndrome itself.[107,108,147-149] Numerous studies in the last decades have produced evidence, that increased inflammatory marker concentrations are associated with an increased risk for cardiovascular disease, diabetes or sudden cardiac death.[150,151,152]

The amount of visceral adipose tissue has been shown to be independently associated with high concentrations of CRP, Il-6, urinary isoprostanes and MCP-1,[147] and there is evidence, that weight loss significantly decreases proinflammatory cytokine concentrations.[148]

Importantly, due to portal circulation and hepatic metabolism of biomarkers excreted by visceral adipose tissue, their concentration in peripheral blood may not always reflect their true degree of production.[147] This was underlined by findings, that Il-6 concentration was 50% higher in the portal vein blood than in peripheral artery blood.[153] Furthermore, Il-6 levels in portal vein blood were directly correlated with peripheral artery CRP levels, suggesting a mechanistic link between Il-6 and hepatic CRP production,[153] which has already been proposed in earlier studies.[154]

### 1.4.2.2.2. Adipose Tissue Macrophages in Obesity

Proinflammatory state in obesity is likely to be associated with infiltration of macrophages into adipose tissue. Macrophage numbers are increased in obese adipose tissue, where they account for almost all of TNF-α expression and a great part of Il-6 expression.[155]

The underlying molecular mechanisms are still unclear, however MCP-1 (macrophage chemoattractant protein 1), also known as CCL2 (C-C motif chemokine ligand 2), plays an important role in macrophage recruitment. Its secretion is increased in visceral obesity,[147,156] and decreases with weight loss.[157]

### 1.4.2.2.3. Adipokines in the Metabolic Syndrome

In the past decade, adipokines -protein messenger substances produced by adipocytes- apart from those known as inflammatory markers, have been subject to intensive research. Many

of them may exert substantial influence on energy balance, immunity and vascular homeostasis either in a positive or in a negative way.[107,158]

Leptin for instance, mainly produced by adipocytes, is known to be an important factor in energy balance, acting in the central nervous system as well as in the pancreas, liver and immune system.[158] Its concentration is associated with fat mass and BMI,[99,100] as well as with markers of inflammation. Especially its relationship to soluble TNF-α receptor level suggests, that leptin may influence inflammatory activity in adipose tissue.[100] At the hypothalamic level, leptin is thought to suppress appetite,[102] which however, does not fit with the increased leptin levels in obesity, suggesting a mechanism of some sort of resistance to the action of leptin.[158] Leptin may also be involved the CNS regulation of sympathetic nerve activity and blood pressure through the leptin-melanocortin-pathway.[30]

Adiponectin has been reported to be decreased in obesity and diabetes and is inversely correlated with insulin sensitivity and obesity.[106,107] It may be involved in the regulation of liver gluconeogenesis and play a protective role against atherosclerosis,[159,160] possibly by interacting with the TNF-α induced inflammatory response in endothelial cells.[161]

TNF-α levels are increased in obesity and decrease with weight loss.[148] The major part of TNF-α is not produced by adipocytes, but by non-fat cells in adipose tissue,[162] mainly macrophages.[153,155] It may have substantial effects on glucose metabolism and be a major link between inflammation and insulin resistance,[163] possibly by interfering with the microvascular response to insulin.[42,44,48] Furthermore, it has been demonstrated in several small studies, that pharmacological TNF-α blockade, for example used in rheumatoid arthritis, may improve insulin sensitivity.[164,165,166]

Interleukin-6 is increased in obesity[147,148,156] and may have variable, tissue-dependent effects on glucose metabolism.[167] Especially high concentrations of Il-6 were measured in portal vein blood,[153,156] which may have direct adverse effects on hepatic insulin metabolism and hepatic production of acute-phase-proteins like CRP.[156,167]

As mentioned before, monocyte chemoattractant protein-1 (MCP-1) has also been reported to be increased in obesity and plays an important role in macrophage infiltration of adipose tissue.[147,155] MCP-1 may also be involved in the inflammatory processes in the arterial wall, marking atherosclerosis,[158] as its levels have been reported to be elevated in coronary and periphery artery disease.[168,169]

#### 1.4.2.2.4. Adipose Tissue RAAS

Angiotensinogen, precursor molecule of angiotensin-II in the renin-angiotensin-aldosterone system, is widely known to be produced in the liver. Obesity has been repeatedly reported to be associated with activated systemic RAAS measures in animal and humans,[22,23,24] however, other studies failed to provide evidence for a connection between circulating RAAS activity and measures of obesity.[25]

Still, angiotensinogen, renin, angiotensin-converting enzyme and angiotensin II type 1 receptor gene expression was shown to be considerably increased in obese adipose tissue.[28,122] In accordance with that, a recent study reported, that adipocyte angiotensinogen-deficient mice developed no obesity-induced hypertension,[29] which underlines the important role of local adipose tissue RAAS in obesity hypertension. An activated RAAS may also counteract with glucose metabolism, as meta-analyses have demonstrated, that RAAS inhibition by ACE-I or ARB improves insulin sensitivity and decreases the incidence of type 2 diabetes.[170] These findings will be reviewed in a more detailed way in another, subsequent chapter of this thesis.

### 1.4.2.3. Insulin Resistance and Diabetes

#### 1.4.2.3.1. Insulin Resistance in the Metabolic Syndrome

Insulin resistance represents a hallmark of the MetS and its associated diseases like type 2 diabetes, atherosclerosis or non-alcoholic fatty liver disease.[108,171] Still, the role of insulin resistance in either causing the MetS or being caused by other metabolic alterations has not been fully explained yet, as extensively reviewed in previous chapters. There are several possible mechanisms like microvascular dysfunction, ectopic lipid accumulation, deficient mitochondrial oxidative phosphorylation, and systemic inflammation, that may -acting alone or together- ultimately lead to insulin resistance.[107,108,171]

#### 1.4.2.3.2. Insulin and Microcirculation

Next to its effects on blood pressure by regulating peripheral resistance, microcirculation, including arterioles, capillaries and venules, may also be closely linked to glucose metabolism due to its influences on insulin-dependent muscle perfusion.[42] The physiologic ability of insulin to increase skeletal muscle blood flow and blood volume has been shown to be decreased in insulin resistance, probably due to impairments in capillary recruitment in insulin sensitive tissues like muscle.[172] Microvascular recruitment as a response to insulin has been shown to be impaired in obese and insulin resistant subjects.[44,173] Altogether, impaired microvascular response to food intake or insulin application may pose an important

link between obesity and insulin resistance as well as with hypertension.[41,174] The underlying mechanism for the blunted microvascular response to insulin is likely to be endothelial dysfunction, resulting in imbalance in intracellular vasodilatatory and vasoconstrictory signalling,[42] as described in prior chapters.

#### 1.4.2.3.3. Insulin Resistance and Ectopic Lipid Accumulation

Lipid metabolism is closely linked with insulin sensitivity. Older studies focussed on the glucose metabolism effects of circulating lipids, like free fatty acids. Conversely, lipid content in muscle cells has been reported to predict insulin resistance better than free fatty acid concentration in circulation.[175] This and other findings in the last decade suggested a major role for ectopic accumulation of lipids in insulin responsive tissues as a new approach to the explanation of the pathogenesis of insulin resistance.[176]

According to this hypothesis, insulin resistance may -for instance- be caused by increased intramyocellular diacylglycerol (DAG) concentrations,[176] exerting influence on intracellular signalling pathways by activating different protein kinase C (PKC) isoforms.[177] Protein kinase C isoenzymes, especially the subclass of novel PKCs (nPKC δ, ε, η and θ), that are activated by DAG, pose an important connection between lipid accumulation and insulin resistance. Novel PKCs may play a substantial role in muscle and hepatic insulin resistance and its connections to intracellular inflammatory pathways.[176]

Ectopic lipid accumulation in the liver, probably involved in causing non-alcoholic hepatic steatosis, is accompanied by hepatic insulin resistance.[176] Dietary weight loss in poorly controlled type 2 diabetic subjects has been reported to remarkably reduce intrahepatic lipid amount, which was associated with a decrease of hepatic glucose production and hepatic insulin resistance, but not with intramyocellular lipid amount or peripheral glucose uptake.[178] In addition, contrary to earlier assumptions, hepatic insulin resistance may not be that closely related to visceral adipose tissue mass, but more to intrahepatic lipid content.[179]

Lipoprotein lipase (LPL) represents a key enzyme in peripheral lipid uptake. In rodent models, tissue-specific overexpression of LPL has been reported to result in increased lipid uptake and insulin resistance in muscle because of impaired insulin-stimulated glucose uptake.[176] The same changes were seen in the liver, where insulin resistance was probably caused due to impaired suppression of endogenous glucose production.[180]

Also, not only the total lipid content in various tissues may be crucial in the development of insulin resistance, but also their exact localisation within the cells. It is reported, that

"adiposomes", intracellular lipid droplets, may play an important role as a major site of intracellular lipid metabolism.[181]

Intracellular lipid intermediates like diacylglycerol and ceramides may also participate in the development of insulin resistance, as they possibly exert effects on intracellular signalling pathways partly due to their physiological function as second messengers.[176]

Another recent field of research in insulin resistance is "endoplasmic reticulum stress", also referred to as the "unfolded protein response" (UPR). Activation of the UPR enzymes is thought to happen in order to reduce accumulation of unfolded proteins within the endoplasmic reticulum caused by increased protein production due to higher demands. The UPR may especially play an important role in linking hepatic insulin resistance to lipogenesis, lipid export and lipid accumulation in the liver.[176] In accordance with that, markers of UPR in adipose tissue were shown to be reduced after weight loss.[182] In a recent study of liver biopsies obtained from obese patients undergoing bariatric surgery, links between insulin resistance and hepatic steatosis were examined. Hepatic DAG content was demonstrated to be significantly associated with hepatic triglyceride content and hepatic PKCε activity. DAG content of lipid droplets in cytoplasm was identified to be the best predictor of insulin resistance (HOMA-IR). No significant association could be found between insulin resistance and JNK1 activity and only limited connections with other markers of endoplasmatic reticulum stress could be identified, questioning the role of UPR in insulin resistance. Furthermore, expression of TNFα, IL-1β, IL-6 and CRP in the liver were not related to HOMA-IR and neither was hepatic ceramide content.[183]

#### 1.4.2.3.4. Insulin Resistance and Inflammation

As mentioned before, proinflammatory cytokines levels have been repeatedly reported to be elevated in diabetes and obesity, possibly due increased number and activity of macrophages and other immune cells in adipose tissue.[184] Still, molecular mechanisms linking insulin resistance to inflammation are only incompletely understood. These mechanisms include the mitogen activated protein kinase pathways (JNK1) and the inhibitor of nuclear factor κ-B kinase pathway (IKK). However, up to now, the limited knowledge in this field is almost entirely based on rodent models, and its relevance for humans is still uncertain[176]. However, limited evidence exists, that TNFα and Il-1R1 blockade,[164,165,185] as well as the anti-inflammatory substance salsalate,[186] may have beneficial effects on glucose metabolism, supporting the role of inflammation in insulin resistance.[184]

#### 1.4.2.3.5. Insulin Resistance and the RAAS

RAAS blocking drugs like ACE-I or ARB were reported to be able to prevent new-onset-diabetes in patients with impaired glucose tolerance and CVD risk factors,[187] or in patients with hypertension.[188] It was also demonstrated, that glucose metabolism parameters improved under such medication,[189] suggesting an important role of the RAAS insulin resistance. Angiotensin II is known to cause insulin resistance,[190] an effect, that may partly be attributable to peripheral vasoconstriction in insulin sensitive tissues,[191] but recent research results suggest, that effects on intracellular signalling pathways are more likely.[190] The activation of the mitochondrial NADPH oxidase, also playing an important role in atherosclerosis,[142] and consecutive oxidative stress due to reactive oxygen species (ROS) have been reported to inhibit insulin dependent glucose transport via IRS-1 and GLUT-4 in muscle, thus possibly causing insulin resistance.[192]

High levels of glucose and insulin may in return promote RAAS activity,[27] and thereby exert influence on the inflammatory response in vascular cells, probably contributing to the typical diabetic vascular complications.[193]

The influence of the RAAS on insulin sensitivity may not only be confined to peripheral effects in various insulin-sensitive tissues, but also to the pancreas itself: Lupi et al. reported local expression of RAAS genes in isolated pancreatic islets to be increased in hyperglycaemia and markers of oxidative stress like NADPH oxidase to be concomitantly overexpressed. Application of ACE-I reduced insulin secretion and oxidative stress.[194] These effects of Angiotensin II on pancreatic insulin secretion may be due to local vasoconstriction on the one hand and oxidative stress on the other, which is likely to interfere with intracellular insulin signalling and glucose transport, like already mentioned concerning the skeletal muscle.[192] Furthermore, Angiotensin II induces the expression of MCP-1 in pancreatic islets,[195] supporting evidence for a causative role of local pancreatic islet inflammation in insulin resistant states.[196,197]

### 1.4.2.4. Endocrine Parameters in Obesity and Inflammation

#### 1.4.2.4.1. Hypothalamic-Pituitary-Adrenal Axis in Hypertension and the Metabolic Syndrome

Stress response in human organism is effected by two systems, acting together. These are, on the one side, the autonomic nervous system, and on the other side, the HPA-axis. Acute stress causes changes in behaviour and metabolism in order to regain the balance disturbed

by the stressor. While acute stress reaction is necessary in daily life, chronic stress, however, is associated with disease.[198]

Animal studies report, that subordinate animals in a social group are more likely to develop visceral obesity, atherosclerosis and ovarian dysfunction, probably caused by chronic social stress.[199] In humans, there is evidence, that depression and work related stress are associated with the metabolic syndrome and increased HPA-axis activity.[200-203]

#### 1.4.2.4.2. HPA-Axis in Obesity and the Effects of Leptin

Although the results are varying, depending on the measures used to assess HPA axis function, most studies suggest, that markers like 24-hour urinary-free cortisol or morning salivary cortisol are elevated in obesity and correlated to BMI and waist-to-hip ratio.[198,204]

Analogous to the increased expression of inflammatory cytokines in obese adipose tissue, also the gene expression and in vitro-activity of 11β-HSD1, the enzyme regenerating active cortisol from cortisone, has been found to be increased in subcutaneous adipose tissue (SAT) of obese individuals and to be correlated with BMI and waist circumference.[205,206] However, to which extent this local adipose tissue-specific cortisol metabolism causes systemic effects, or if it rather acts at a paracrine or autocrine level, remains unclear.[198]

The adipokine leptin plays a crucial role in energy household and its plasma concentration is known to be increased in proportion to fat mass.[99,100] A connection between leptin and the sympathetic nervous system, the so-called leptin-melanocortin-system has been described earlier in this thesis. Similar links may exist between leptin and the HPA-axis: Administration of dexamethasone has been reported to increase serum leptin concentrations,[207] but this effect may be confined to obese women, suggesting gender specific pathways.[208] Other studies discovered, that this effect probably can only be seen after glucose and insulin infusion or after food intake.[209,210] Leptin release follows a circadian rhythm, with the highest levels after midnight and the lowest before noon, probably reflecting the suppression and activation of appetite at the proper times.[198,211] The diurnal pattern of hormones like thyrotropin (TSH) and melatonin is similar to that of leptin,[212] while adrenocorticotropin and cortisol show an inverse regulation,[213] suggesting a connection between leptin and the HPA-axis at some level could be possible.

#### 1.4.2.4.3. HPA-Axis and Chronic Inflammation

Inflammatory cytokine levels are known to be increased in the metabolic syndrome,[107,108,147-149] and especially TNF-α, Il-1 and Il-6 are able to activate the HPA-axis, possibly affecting all its components.[214] Therefore, increased cytokine concentrations in the metabolic

syndrome may be a major cause of increased HPA-axis activity. Application of cortisol decreases concentrations of inflammatory markers,[198] but the anti-inflammatory response to cortisol has been demonstrated to be blunted in obesity.[215]

Impaired responsiveness of the HPA-axis, leading to glucocorticoid resistance has been reported in states of chronic inflammation or autoimmunity like in IBD, rheumatoid arthritis and asthma.[216-218] For example, in rheumatoid arthritis, the circadian cortisol curve is flattened, when the disease is active, while it appears to be normal when the disease activity is low. In rheumatoid arthritis patients, peaks of inflammatory cytokines in the early morning are higher and last longer than in controls, probably due to inadequate suppression by the smaller morning rise in endogenous glucocorticoids.[218,219] This phenomenon may partly be responsible for the typical joint stiffness, increased pain and decreased grip strength measured in early morning hours.[218,219]

Inflammation may, on the one hand, affect the HPA-axis either directly at the hypothalamic, at the pituitary or at the adrenal level. One the other hand, also glucocorticoid availability in the periphery may be disturbed, possibly via local factors like corticosteroid binding globulin, 11β-HSD-1/2 or the glucocorticoid receptor itself.[220] Alterations of the HPA-axis are possible, although systemic glucocorticoid levels may be normal or even elevated.[220] Inflammatory cytokines may directly cause an impairment in glucocorticoid receptor function, or increase the production of inactive receptor isoforms.[221] Glucocorticoid receptor dysfunction may also be a possible explanation to the often observed association of chronic stress with increased susceptibility to the new onset or exacerbations of inflammatory or autoimmune diseases,[220] but also with obesity and the metabolic syndrome.[222]

#### 1.4.2.4.4. HPA-Axis, Stress and Sympathetic Nervous System

Straub et al. reviewed the connection between short term and long term stress mainly in rheumatoid arthritis and juvenile idiopathic arthritis, as well as in animal models. They concluded, that acute stress rather stimulated immune response and inflammatory reactions, while strong prolonged stress supressed those, although the effects of sustained stress are controversial. The authors suggested a dual role of both HPA axis and SNS in responding to short or long term stress, as well as of their products cortisol and noradrenaline.[223]

Just like cortisol, noradrenaline has both immuno-stimulatory as well as immunosuppressive properties. One the one hand, it seems to stimulate the immune system by enhancing Il-8 production and thus chemotaxis,[224] as well as complement production by macrophages.[223]

On the other hand, it also inhibits functions of the innate immune system, like NK-cells and neutrophils.[223]

In patients with RA and JIA, β-receptors on immune cells have been shown to be diminished,[226] while $\alpha_1$-receptors on leukocytes seem to be increased, resulting in an augmented production of Il-6 as a response to chatecholamines.[223] Also, the density of sympathetic nerve fibres in synovial tissue in rheumatoid arthritis is significantly reduced,[227] suggesting a process of separation of the inflamed area from the rest of the body.[223] This, together with the "beta-to-alpha shift" on immune cells suggest, that states of chronic inflammation may go along with altered effects of the SNS on immune system control, as well as with increased SNS activity.[223] Consistent with this hypothesis, measures of sympathetic nerve activity, were repeatedly shown to be increased in RA patients.[228,229] Furthermore, a recent study reported a significant decrease in ABP levels especially in the morning hours upon TNF-α therapy,[230] consistent with previously mentioned hypothesis concerning the increase in morning cytokine levels due to a disturbed circadian cortisol production.[218,219] The mentioned study also reported significant decreases in plasma norepinephrine levels and plasma renin activity.[230]

These findings once more underline the connection between inflammation, the SNS and the RAAS. Angiotensin II is well known for its role in inflammatory processes, like those in the kidney, marking renal injury in the pathogenesis of hypertension,[69,61] or in atherosclerosis.[142] The underlying molecular mechanism is thought to be an activation of the NADPH-oxidase, causing oxidative stress and leading to alterations in intracellular signalling pathways, which may also be associated with a deterioration of glucose transport in muscle, causing insulin resistance.[192] Concordantly, as previously mentioned, TNF-α inhibitors were demonstrated to improve insulin sensitivity in patients with rheumatoid arthritis.[164-166]

Inflammatory cytokines are widely known to be elevated in hypertension, obesity and the metabolic syndrome.[107,108,147-149] Thus, it seems probable, that the HPA-axis deregulation, described in RA and other inflammatory diseases, may also be present in the metabolic syndrome. Indeed, alterations of the HPA-axis are reported in hypertension,[231] coronary artery disease,[232] and obesity, associated with BMI and waist circumference.[233] Obstructive sleep apnoea syndrome was recently reported to be associated with lower basal cortisol levels and with decreased HPA-axis responsiveness, compared to controls.[234] Similarly, a trend towards lower cortisol levels in hypertensive compared to normotensive subjects was also reported in a study among Caucasian and African women.[235]

Another interesting finding was, that administration of dexamethasone could prevent the sympathetic stimulation normally caused by application of insulin.[236] Furthermore, long-term dexamethasone administration significantly reduced MSNA in obese, but not in lean subjects,[237] once more underlining the involvement of the HPA-axis in obesity and its association with increased SNS activity.

#### 1.4.2.4.5. Thyroid Function in the Metabolic Syndrome

In a cross-sectional population study among patients without present or previous thyroid disease, Knudsen et al. found a positive association between BMI and TSH and a negative connection between BMI and fT4, suggesting, that differences in thyroid function, even within the normal range, are associated with variations in body weight. Furthermore, they found out, that serum TSH levels were significantly positively correlated with an increase in body weight within the next 5 years and significantly associated with obesity, defined as BMI>30kg/m$^2$.[238] Intuitively, it seems logical, that thyroid hormones play an important role in obesity and the MetS, given their properties to influence energy expenditure, thermogenesis and body weight, as especially seen in severe thyroid disorders.[212,238] However, up to now, evidence for the exact role of thyroid hormones in metabolism is rare. Thyroid hormone therapy as a treatment for obesity has been proposed,[239] but success was reported to be only limited.[238]

As already mentioned, studies among adults and children suggested, that obesity is associated with relatively higher levels of TSH, which decrease with weight loss,[238,240-242] while results concerning free thyroid hormones vary between the studies.[240-242] Thyroid volume has been observed to be correlated with BMI, waist circumference and measures of body fat. Furthermore, it has been reported to decrease, when weight is lost.[241] A recent study demonstrated, that especially $T_3$ was significantly lowered by weight loss and the $T_3$:$fT_4$ ratio decreased, suggesting that weight loss may have influences on the peripheral conversion of $T_4$ to $T_3$.[243]

#### 1.4.2.4.6. Thyroid Function and Leptin

Changes in thyroid function in obesity and upon weight loss suggest an association with adipose tissue mass. Leptin has been proposed to be a possible link, as both TSH and leptin levels are increased in obesity.[212,238] It has been suggested, that the leptin-melanocortin-system could modulate the HPT-axis, especially in states of fasting.[244] Leptin may exert influence on the feedback regulation of thyroid hormones, probably at the hypothalamic level.[212] Furthermore, leptin may stimulate the conversion of $T_4$ to $T_3$ under certain

circumstances, as demonstrated in rats.[245] Altogether, several studies imply connections between thyroid function and leptin at various levels, but those seem to be complex. The underlying mechanisms and links between the systems are only incompletely understood and evidence in this fields is up to now mostly limited to experimental studies in animals.

#### 1.4.2.4.7. Thyroid Function and Inflammation

Another possible link between obesity and thyroid dysfunction next to leptin may be inflammation, known to be present in obesity and hypertension[147-1449]. Analogously to the previously described alterations of the HPA-axis in states of inflammation, it seems obvious, that also the hypothalamic-pituitary-thyroid (HPT) axis may be disturbed by inflammatory cytokines.[246] However, such disturbances should rather result in lower TSH levels, than in the elevated levels previously described.[238] Findings in critically ill patients or patients after surgical stress, highlight another possible explanation: These exhibit a characteristic disturbance of the HPT axis, referred to as the "non-thyroidal illness-syndrome" (NTIS) or "low $T_3$ syndrome".[247,248] Patients display low $T_3$ levels and usually inadequately normal or decreased TSH concentration, which may be caused by effects on thyroid hormone metabolisms in peripheral tissues,[248] as well as by changes in the hypothalamus, where the expression of TRH has been shown to be reduced in such states.[247] Thyroid hormone concentrations in critically ill patients can independently predict survival and decrease of $fT_3$ together with $fT_4$ is associated with a further increase in mortality.[249]

Changes in peripheral thyroid hormone metabolism may occur more likely in acute illness, while central neuroendocrine effects like low TSH, or loss of response to TRH stimulation mark prolonged or chronic states of disease.[248-250] Importantly, HPT-axis response to TRH has been demonstrated to predict outcomes in critically ill patients and increases in TSH concentration may mark recovery.[248,250] The major part of $T_3$ is derived from peripheral conversion of $T_4$ by iodothyronine selenodeiodinase type 1 (D1) and type 2 (D2), while the type 3 deiodinase (D3) inactivates both kinds of peripheral thyroid hormones.[248] Consistent with the observed alterations in states of disease, liver D1 activity in intensive care patients has been shown to be decreased, while D3 activity was increased.[251]

A possible cause of these observations, may be the presence of inflammatory cytokines like Il-6 and TNF-α. Il-6 concentration in myocardial infarction is, for instance, inversely correlated with $T_3$ concentration, possibly due to putative inhibitory effects on deiodinase activity. However, direct inhibitory cytokine effects on deiodinase activity have been repeatedly challenged, as they could not be reproduced in laboratory settings.[253]

Another possible process underlying the pathophysiology of NTIS, is oxidative stress and again, Il-6 may play an important role.[248] Il-6, next to other cytokines, is believed to cause oxidative stress by activation of the NADPH-oxidase pathway and by overexpression of the angiotensin II type 1 receptor.[254,255] The resulting changes in intracellular redox state may disturb the function of deiodinase enzymes.[248] In line with this hypothesis, a recent study reported, that effects of Il-6, inhibiting $T_4$ to $T_3$ conversion by interfering with D1 and D2 could be prevented by the addition of antioxidants to the cell culture, which strongly suggests influences of ROS. Inactivation of $T_3$ was shown to be increased by Il-6, probably, because D3 may not be prone to oxidative stress.[256] Similarly, oxidative stress by $H_2O_2$ was demonstrated to activate D3 and decrease the reactivity of D2 to various stimuli, whereas both effects could be prevented by the application of anti-oxidative agents.[257]

# 1.5. Therapeutic Options in the Metabolic Syndrome

Although it remains controversial, whether the metabolic syndrome itself increases cardiovascular risk more than its single factors taken together,[258] there is no doubt that patients meeting those criteria exhibit a considerably increased risk for all-cause mortality and cardiovascular events, as well as an about threefold increased risk for developing diabetes.[55,77,259] Thus, diagnosis of the metabolic syndrome should entail maximum efforts in treatment, involving lifestyle modification, weight loss and drug therapy.[108,129,130]

## 1.5.1. Weight Reduction as the Primary Target

As obesity is the hallmark of the metabolic syndrome, treatment should primarily aim at weight reduction.[10,129,130] This should be achieved by increased general physical activity, caloric restriction and changes in diet, with reduced intake of saturated fat and salt and increased intake of fibre.[129] Weight loss, even to a small extent, has been repeatedly reported to significantly lower blood pressure and decrease the risk of developing hypertension.[260] Physical exercise and dietary intervention may even be able to improve BP control without any weight loss happening at all.[261,262]

Drugs supporting weight loss, like sibutramine or orlistat can be helpful,[10,263] although sibutramine is not recommended in patients with cardiovascular diseases, as it may increase CV risk.[76] Although several studies indicate effectivity concerning weight loss and also improvement of glycaemic control under the use of weight loss drugs, given their possible adverse effects and the low number of approved substances, there is only limited experience in clinical use up to now.[76] In patients with a BMI>40kg/m$^2$ bariatric surgery can also be

considered,[108] which has been reported to result in not only weight loss, but also improvements in glucose and lipid metabolism.[76]

### 1.5.2. Pharmacotherapy in the Metabolic Syndrome

According to the IDF and the NCEP ATP III guidelines, LDL cholesterol should be lowered to <100mg/dl in patients with the metabolic syndrome, just like in patients with diabetes,[108] TG and HDL-cholesterol levels should be in the range suggested by the to the ATP III goals.[129,130] These targets should be acquired by lifestyle modification and drug treatment, using statins and fibrates or the combination of both.[129]

Blood pressure should be treated according to the current international guidelines,[11,14] using an ACE-I or ARB as first line therapy if possible, as those exhibit positive effects on glucose and lipid metabolism, as well as the potential to prevent new-onset diabetes.[32,108,144,187-189] Furthermore, ARB therapy with the substance Telmisartan has recently been reported to decrease the concentrations of the proinflammatory cytokines Il-6 and TNF-α.[264]

β-Blockers may have metabolic disadvantages like weight gain, aggravation of insulin resistance and dyslipidaemia, but those changes tend to be minimal,[10] so these substances are not contraindicated in the MetS.[108,129] A recent study demonstrated, that among patients with impaired glucose tolerance, diuretics and statins were associated with an increased risk of new onset diabetes, while β-blockers and calcium-channel-blockers were not.[265] Furthermore, β-Blockers may lower blood pressure to a larger extent in obese individuals, than in lean ones, possibly due to presence of increased adrenergic activity in obesity.[266]

Insulin resistance should be primarily treated by biguanides (metformin) and thiazolidinediones (TZD, “glitazones”), that both improve general insulin sensitivity and insulin action in the liver, where they also suppress gluconeogenesis.[108] Glitazones also enhance glucose metabolism in muscle and adipose tissue. The diagnosis of diabetes in the MetS further increases CVD risk, so that antidiabetic therapy should be a primary target.[129]

### 1.5.3. Targeting the Sympathetic Nervous System

Given the previously discussed extensive contribution of the SNS to the metabolic syndrome, sympathetic inhibition seems to be a promising way of intervention.[77]

Weight loss has been reported to reduce MSNA as well as total body NA spillover rate and to increase baroreflex sensitivity.[104] Those changes were accompanied by an improvement in insulin sensitivity and additionally, the changes in noradrenaline spillover rate were independently correlated with the changes in leptin concentration,[104] once more indicating a

close relationship between the SNS and leptin.[77] Especially renal SNA, measured by NA spillover rate to plasma can be markedly reduced by aerobic exercise training, which also reduces blood pressure and increases total oxygen consumption.[267]

Pharmacological inhibition of the SNS can be achieved by β-blockers, as mentioned above and by blockers of the RAAS, that impact by inhibiting the sympathostimulatory effects of Angiotensin II.[268] ACE-I or ARB may be the most beneficial drugs in the treatment of the metabolic syndrome, given their substantial effects on not only blood pressure, but also glucose and lipid metabolism.[32,144,187-189]

Alpha1-receptor-blockers like Doxazosin may have favourable effects in the MetS next to lowering BP.[269] Doxazosin has been reported to reduce plasma LDL, triglycerides and FPG concentration and increase HDL and insulin sensitivity in patients with uncontrolled hypertension and insulin resistance.[270] Additionally, it seems to unfold better antihypertensive effects among obese hypertensive patients,[271] probably due to an increased SNS activity in those. Similarly, centrally acting substances like the imidazoline receptor binding agents rilmenidine or moxonidine have been reported to improve various metabolic parameters:[77] In a group of hypertensive women with the metabolic syndrome, treatment with rilmenidine significantly increased HDL and decreased FPG compared to patients on lisinopril.[272] Another study on hypertensive patients with hypertriglyceridaemia or impaired glucose tolerance demonstrated a positive effect of rilmenidine on glucose metabolism parameters, compared to amlodipine, while antihypertensive effects were similar.[273] Furthermore, rilmenidine has been reported to decrease noradrenaline spillover rate to plasma, as a marker of whole body SNA, in hypertensive patients at rest, while the physiologic responses to mental stress and tilting were preserved.[274] Rilmenidine therapy also led to a significant increase in adiponectin in a small group of hypertensive patients, while BMI, body fat content and measures of insulin sensitivity did not change significantly.[110] Of special interest, in a large study group of hypertensive patients with obesity or metabolic syndrome, treated with the substance moxonidine, not only marked blood pressure reductions were reported, but also a mean weight loss by -1,4kg within eight weeks.[275] Another study on uncontrolled hypertensive patients meeting the MetS criteria reported BP reductions by -24,5/-12,6mmHg and a mean decrease of body weight by -2,1kg, next to improvements in FPG and fasting triglyceride concentrations.[276]

These findings once more imply, that activation of the SNS represents a hallmark of the metabolic syndrome and that sympathetic inhibition by various means can not only improve blood pressure control, but also metabolic parameters and probably even body weight.

# 1.6. Renal Sympathetic Denervation

## 1.6.1. Theoretical Background

Sympathetic activation of the kidney is mediated by β-1 receptors in juxtaglomerular granular cells, containing renin, by α-1b receptors located at the basolateral membrane of the renal tubular cells and by α-1a receptors on vascular smooth muscle cells.[120] Increased sympathetic outflow to the kidney thus causes vasoconstriction, leading to a decrease in renal blood flow and GFR. Besides, tubular sodium reabsorption increases and renin is released, causing activation of the RAAS, ultimately leading to volume expansion.[120,277]

Importantly, efferent nerve fibres, sending signals from brain to kidney, as well as afferent nerves, signalling from the kidneys to the CNS are involved in the renal sympathetic innervation. In models of renal injury caused by phenol injection, afferent and efferent renal sympathetic nerve activity were increased, as well as blood pressure.[278]

Evidence for increased SNS activity and hypertension in chronic renal failure, which could be decreased by nephrectomy suggests, that renal injury might increase SNA and blood pressure via afferent signalling to the CNS.[124-126,278,279] In line with those findings, rats having undergone selective afferent renal denervation by dorsal rhizotomy were shown to display salt-sensitivity, which underlines the importance of afferent renal sympathetic nerves for adaptation of renal sodium excretion in order to avoid hypertension.[280] On the other hand, genetically salt-sensitive rats, displayed a decrease in salt-sensitivity after targeted renal and/or splanchnic sympathetic nerve ablation.[128]

As mentioned before in this thesis, research in the last decades has assembled a large body of evidence indicating a prominent role of the sympathetic nervous system in the onset and maintenance of essential hypertension.[281] Sympathetic over-activity has reported in obesity with and without hypertension and in the metabolic syndrome.[76,77,79,80,84]

## 1.6.2. Development and Method

Renal sympathetic nerves have been known to modulate renal function and blood pressure, both by afferent and efferent signalling, for a long time. Radical surgical pelvic, abdominal or thoracic sympathectomy in hypertensive patients performed in the 1940s, could

successfully lower blood pressure. However, due to the high rate of complications and particularly also the emerging new pharmacologic antihypertensive agents like β-receptor-blockers, this method was abandoned.[20,120]

The fact, that over 90% of the renal sympathetic nerves lie within 2mm of the renal artery lumen,[282] and the enormous progress in vascular intervention technology in the last decades suggested new ways for sympathectomy: Thus, a novel minimal invasive technique has been developed, using a percutaneous access for an endovascular radiofrequency ablation of the renal sympathetic nerves.[283] Accessing the femoral artery in the groin, a catheter is advanced into the renal arteries and a renal angiogram is performed. After placement of the catheter within the distal renal artery in contact with the vessel wall, radiofrequency energy is applied, increasing local temperature to up to 70°C for 2 minutes. For security reasons, temperature and impedance is continuously measured, while the ablation is automatically conducted. This procedure is repeated in a stepwise fashion for up to six times per side, as the catheter is moved both longitudinally and rotationally in the vessel from distal to proximal, resulting in a spiral pattern.[20,283] The procedure causes diffuse visceral pain while radiofrequency energy is delivered, which should be managed by intravenous analgetic and sedative drugs.[283] In most cases, ablation causes characteristic irregularities of the vessel wall, which are visible in renal angiography immediately after energy application and may be caused by local spasm and edema of the vessel wall. However, those “notches” were shown to have resolved within days in follow up angiograms and MRT examination of the renal arteries after six months did not show any irregularities at the ablation points any more.[283]

The observed reductions in blood pressure, as exhaustively described below may be due to blockade of efferent signalling to the kidney on the one hand, as renal noradrenaline spillover rate to plasma has been reported to be decreased by 47,5% after the procedure.[283] On the other hand, also whole body NA spillover rate, MSNA measured by microneurography and plasma renin activity were reported to be decreased in a single patient case report.[284] This argues for a substantial effect of RSD on central sympathetic outflow to the periphery, probably caused by changes in afferent sympathetic signalling from kidney to brain.[120] Nevertheless, a prospective case series published in 2012 failed to show significant reductions in resting heart rate, resting muscle sympathetic nerve activity and pharmacological baroreflex control of heart rate and MSNA, raising the question, whether the effects of RSD on central sympathetic outflow are predictable or not.[285]

One frequently mentioned possible limitation of this new method may be regrowth of the renal sympathetic nerves. Efferent nerves are known to be able to partly regrow to transplanted organs like heart or kidney,[286-288] but probably without ever reaching the original extent again. In contrast, regrowth of afferent fibres seems to be unlikely,[120] as its absence has been reported in human heart transplants.[289]

Intuitively, a potential side effect of RSD may be damage to the renal artery or the kidney itself. However, aside from few renal artery complications associated with the procedure itself, no significant worsening of renal function or occurrence of ESRD has been reported during follow up to twelve months in various studies.[290]

### 1.6.3. Resistant Hypertension and RSD Inclusion Criteria

Inclusion criteria for the first studies on the application of renal sympathetic denervation in resistant hypertension included the previously mentioned criteria for resistant hypertension with systolic office BP limits of 160mmHg for patients without and 150mmHg for patients with type 2 diabetes, respectively. Anatomic criteria concerning the renal artery to be met were the absence of a hemodynamically significant renal artery stenosis, previous intervention, renal artery diameter <4mm or length <20mm or more than one main renal artery. Furthermore, individuals with an eGFR (MDRD) of <45mL/min/1,73m$^2$, type 1 diabetes, hemodynamically significant valvular heart disease, pregnancy, cerebrovascular events within 6 months previously or history of CVD including myocardial infarction or unstable angina were excluded.[291]

### 1.6.4. Clinical Trials of RSD in Resistant Hypertension

The non-randomized first-in-man and proof-of-principle study SIMPLICITY HTN-1 published in 2009 included 45 patients undergoing RSD. A significant reduction of office-measured blood pressure by -14/-10, -21/-10, -22/-11, -24/-11 and -27/-17mmHg at 1, 3, 6, 9 and 12 months, respectively was reported, and systolic 24-hour ABP among n=9 OBP responders changed by -11mmHg. Office and ambulatory systolic BP changes were significantly correlated with each other. Renal NA spillover rate was reported to be considerably reduced by -47% in 10 patients studied, while there were no significant changes in heart rate. Only few adverse events, including one renal artery dissection and one femoral artery pseudoaneurysm were reported, renal function (GFR) remained without significant change.[283] These reported blood pressure reductions were shown to be of a similar extent in an extended group of 153 patients, including the initially treated individuals and were

sustained up to two years.[292] The final 3-year report of the SYMPLICITY HTN-1 study, published in 2013, reported OBP reductions at an average of -32/-14mmHg in the remaining 88 patients, who completed long-term follow up. The proportion of patients considered responders in terms of renal denervation, defined as having a decrease in office measured systolic BP by ≥10mmHg, rose from 69% at one month to 81% at six months, 83% at one year and further to 93% at three years. However, compared to baseline, there was a significant increase in serum creatinine concentration from baseline 83,8 to 92µmol/L at three years, associated with a decrease in eGFR from 83,6 at baseline to 74,3mL/min/1,73m$^2$ at the end of follow up.[293]

In 2010, the SMPLICITY HTN-2 Trial, a randomised, controlled, prospective trial could confirm the significant decrease in OBP six months after denervation in the intervention group of 52 patients, while there was no change from baseline in the control group of 54 patients. Home measured ambulatory BP data was available in 20 patients in the RSD group, who witnessed a significant reduction by -11/-7mmHg. Again, no serious procedure or device-associated adverse events could be reported and renal function, assessed by serum creatinine, eGFR and Cystatin C remained without statistically significant change.[291] In terms of a crossover after six months, 35 patients from the initial control group underwent renal denervation per protocol. Those patients witnessed a significant reduction of BP similar to the group, undergoing RSD in first place. Besides, the BP lowering effect was shown to be unchanged after 12 months in the initially treated cohort.[294]

Very recently, the SYMPICITY HTN-3 study was published, which was designed as a randomized controlled trial, including over 500 patients, receiving bilateral RSD or a sham procedure in a 2:1 ration in a single-blind manner. Furthermore, measurement of ABP was be assessed in all patients, in order to refine the inclusion criteria as well as the BP lowering effects of RSD.[295] Contrary to the previous studies, no significant difference between the denervation and the sham procedure group could be detected. Office BP was significantly reduced in both groups, however, average blood pressure difference between the two groups accounted for only -2,39mmHg, which was tested non-superior using a margin of 5mmHg. Similarly, average ABP difference between RSD and sham group, amounting to -1,96mmHg, deemed non-significant using a margin of 2mmHg for superiority. Safety profiles, heart rate, HbA1c levels and renal function did not differ between the groups.[296]

This study seems to be a major backlash for the method of RSD, as it clearly contradicts the previously published data, in which large BP reductions have been reported. However,

further clinical trials are needed to assess the true effects of RSD, also in applications beyond hypertension and with other devices than those used in the SYMPLICITY studies.[277]

### 1.6.5. Possible Limitations of RSD

Apart from the fact, that the now presented SYMPLICITY HTN-3 study may have caused a shift in the general notion of RSD from enthusiastic to sceptic, the yet published results concerning BP reduction and also the applications beyond seem to be promising. The scientific background of targeting the SNS in various diseases appears compelling, still, a large number of questions remain unanswered: As previously mentioned, regrowth of the sympathetic nerve fibres may be possible and injury to the kidney and its vessels by the procedure, although it has not yet been reported, constitutes a further uncertainty.[277]

Further questions are raised by the recent prospective case series by Brinkmann et al., who could neither re-enact the previously reported decreases in measures of SNA, nor in office BP,[285] which now seems to be confirmed by the HTN-3 study.[296]

Other recently criticized points in the application of renal sympathetic denervation in resistant hypertension include the lack of ABP measurement in patient selection for the intervention and reported BP results upon RSD. The fact, that only a minor part of patients in the SYMPLICITY HTN-1 and HTN-2 studies received ABP monitoring and that the reported blood pressure effects were much more attenuated in ambulatory than in office measuring, may indicate a significant white coat effect,[297] which is also supported by the recent studies.[296] Lately, Mahfoud et al. published a report of ABP data of 346 patients treated with RSD at ten different centres. ABP significantly decreased among all patients, but especially after the exclusion of 43 patients considered being “pseudo-resistant” hypertensive (baseline systolic ABP <130mmHg). The remaining 303 “true-resistant” hypertension patients had significant systolic ABP reductions by -10,2 and -11,7mmHg at six and twelve months respectively, while in the “pseudo-resistant HT” group no significant changes could be detected.[298] ABP measurement before RSD may therefore probably improve outcomes concerning BP reduction by selecting patients more likely to benefit from the intervention. Still, despite the implementation of these methodological improvements in the SYMPLICITY HTN-3 study, no superiority of ABP and OBP reduction by RSD versus sham procedure could be demonstrated.[296]

## 1.6.6. RSD in Other Fields of Application

Next to the primarily reported BP lowering effects of RSD, soon other possible fields of application for this novel method emerged. These include insulin resistance, congestive heart failure, chronic kidney disease and atrial fibrillation. However, all of those studies only included small patient numbers and were designed in a proof-of-concept fashion, so there is still no evidence of a therapeutic effect of RSD in the mentioned conditions.[277]

### 1.6.6.1. RSD in Chronic- and End Stage Renal Disease

Sympathetic nerve activity is increased in CKD and ESRD,[123-127] and may be involved in initiation as well as in the perpetuation of hypertension.[4,76,128] Hering et al. conducted a study among n=15 patients with CKD stages 3 and 4 receiving bilateral RSD. Results concerning BP and safety profile were similar to those reported in previous RSD studies among patients without CKD.[299] Similarly, Schlaich et al. reported, that RSD in n=9 patients with ESRD could reduce blood pressure. Measures of SNA (renal noradrenaline spillover rate and MSNA), assessed in a small group of patients, were shown to be increased before RSD and decreased after the procedure. However, three patients could not undergo denervation due to atrophic renal arteries, a condition which may limit the application of RSD in ESRD.[300]

### 1.6.6.2. RSD in Insulin Resistance

In a pilot study published in 2011, Mahfoud et al investigated glucose metabolism parameters in a group of n=37 patients, most of them included in the randomized controlled SYMPLICITY HTN-2 trial. N=20 of those patients were diagnosed with type 2 diabetes, n=16 received antidiabetic drugs. After three months FPG, insulin and C-peptide levels, as well as the 2-hour glucose levels in the oral glucose tolerance test were shown to be significantly reduced. Insulin sensitivity, measured by HOMA-IR and $IS_{QUICKI}$ improved significantly. $HbA_{1c}$ levels did not differ from baseline, as reported probably due to the short time of follow up. OBP was significantly decreased in the patients according to previous studies, however, systolic or diastolic BP reduction did not correlate with the changes in FPG or insulin levels.[301] In addition, the SYMPLICITY HTN-3 study reported no significant differences in changes in HbA1c fraction between denervation and sham group.[296] More evidence will possibly arise from the currently ongoing DREAMS study (Denervation of the Renal Artery in Metabolic Syndrome), in which glucose metabolism parameters and MSNA will be assessed in patients meeting the criteria for the metabolic syndrome.[277,302]

# 2. Methods

## 2.1. Study Setting

We performed a retrospective analysis of ambulatory and office BP values, body weight, as well as of metabolic-, renal function-, inflammatory- and endocrine parameters among n=78 patients treated with RSD for resistant hypertension. This study was conducted according to the Declaration of Helsinki and approved by the ethics committee of Upper-Austria.

## 2.2. Patient Characteristics

We analysed the previously mentioned parameters in a group of n=78 patients, who received renal sympathetic denervation in the years 2010 and 2011 at the General Hospital of Linz (Allgemeines Krankenhaus der Stadt Linz). Mean age among the study patients was 62,8 years, 42% were female, 58% male. Before RSD, 40% had a history of coronary artery disease, 13% of previous stroke or TIA, 31% were diabetic and hyperlipidaemia was present in 54%. Patients received an average of 3,6 antihypertensive drugs, 18% of the study patients were taking ACE-I, 31% ARB, 51% renin inhibitors, 76% β-blockers, 15% α-blockers, 50% calcium-channel blockers, 88% diuretics and 12% vasodilatators. Oral antidiabetic drugs were administered in 21% of cases, 6% were on insulin treatment. Patients received an average of 5,2 ablations in the left and 5,5 ablations in the right renal artery.

Patients were thoroughly screened for eligibility for the intervention according to the recommendations of the Austrian Society of Hypertension published in 2012. All patients gave written informed consent before RSD. After the intervention, all treated patients were appointed to follow up examinations within one month, three months and twelve months consecutively. The follow up examination at six months was conducted in an in-patient setting, as all individuals received MR-angiography of the renal arteries at this visit. Furthermore, an extended laboratory check-up, including blood lipid metabolism parameters was performed during the hospital stay.

## 2.3. Study Procedure

After the approval of the study by the ethics committee of Upper-Austria, patient data was collected at the Cardiology Department of the General Hospital of Linz (Abteilung Interne 1, Allgemeines Krankenhaus der Stadt Linz).

Laboratory data was extracted from the local SAP-based hospital information system, body weight was taken from the scan-documented patient charts and blood pressure data was collected using printed copies of admission notes and ABP report sheets in the patients' medical records. All data was collected in a Microsoft Excel table. Patient data was made anonymous by assigning consecutive numbers chronologically by treatment date.
The data used in statistical analysis, as well as the results published here does not contain any identifiable patient data.

## 2.4. Statistical Analysis

All statistical analyses were performed with IBM SPSS statistics 20 software. Baseline and follow up data are presented as mean ± standard deviation, differences between baseline values and follow up data, as mean and 95% confidence interval.
Means of descriptive statistics were used to characterize baseline, 6 months and 12 months data values and differences between baseline data and follow up visits. Comparison between baseline and follow up values was performed using a paired-samples t-test or the Wilcoxon signed-rank test for normally and non-normally-distributed data, respectively. Group differences were examined by applying an independent samples t-test, given normal distribution or the Mann-Whitney U-test in the other case. Normal distribution was determined using the Shapiro-Wilk-test. A two-tailed probability value of <0,05 was regarded statistically significant.
Separate analyses were conducted for pseudo- and true-resistant hypertension, defined as baseline systolic ABP of ≤/>130mmHg and for RSD responders and non-responders, defined as having a systolic ABP reduction of ≤/>5mmHg at six months.
Correlations of baseline values and differences from baseline values during follow up with baseline ABP/OBP and their changes at six months were primarily investigated by graphic analysis of point cloud diagrams and calculation of a univariate linear regression with the coefficient of determination ($R^2$). Pearson's correlation coefficients were then calculated, whereupon the reported probability values are for deviation from 0 and a correlation coefficient with a probability value of <0,05 was regarded statistically significant.

## 2.5. Role of the Funding Source and Conflicts of Interest

Authors received no funds from any source or sponsor and declare no conflicts of interest.

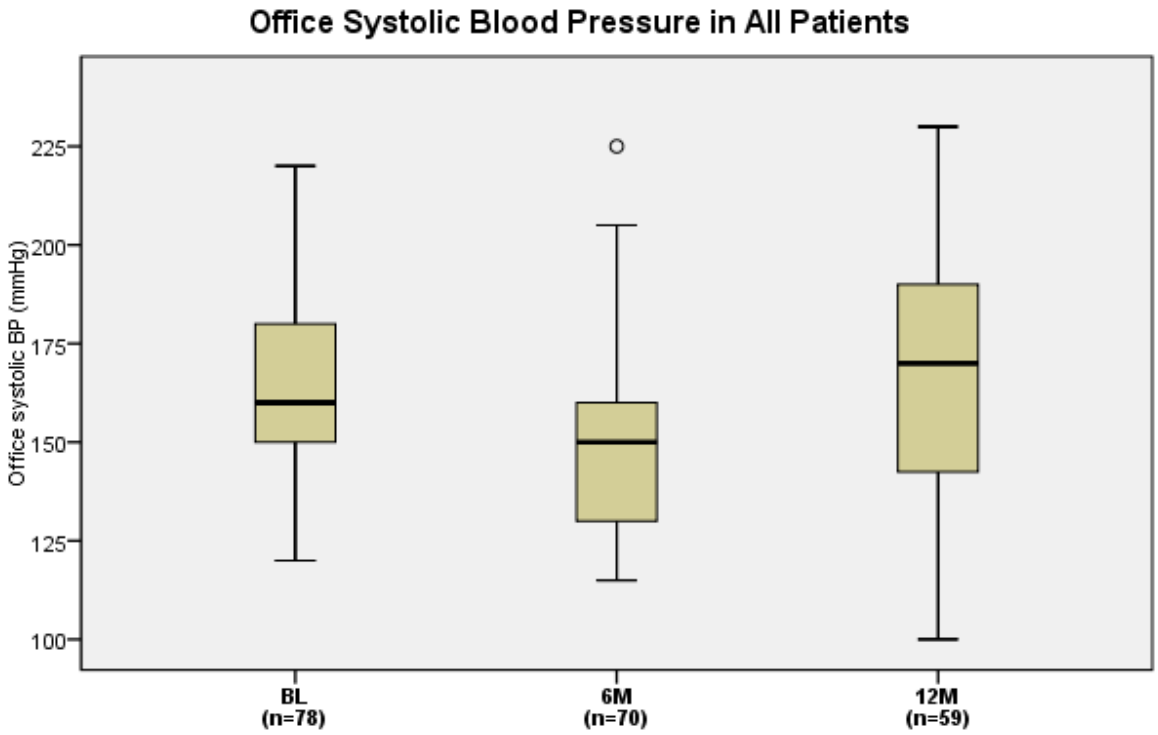
# 3. Results

## 3.1. Office and Ambulatory Blood Pressure

### 3.1.1. Office Blood Pressure (OBP)

#### 3.1.1.1. Office Blood Pressure in All Patients

Office blood pressure was available in all n=78 patients at baseline, n=70 at six months and n=59 patients at one year. Among those, average BP was 164/89mmHG (21,1/13,1) at baseline, 150/83mmHG (20,2/14,2) at six and 167/91mmHg (27,9/17,8) at twelve months.



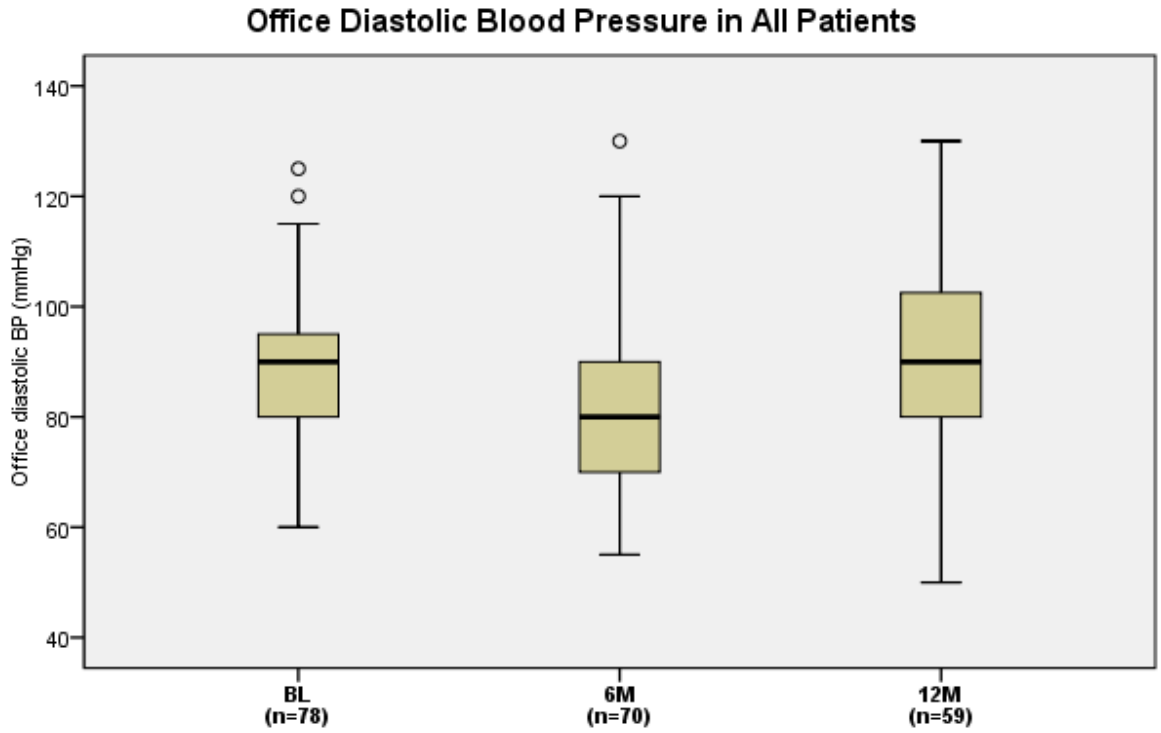
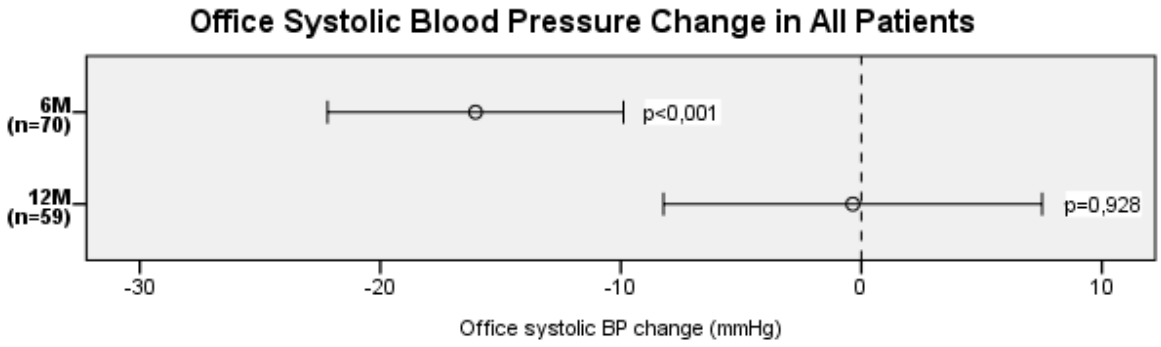



*Figure 1. Office systolic and diastolic blood pressure (OBP) in all patients at baseline, 6 and 12 months after renal sympathetic denervation. Whiskers indicate 5 and 95 percentile.*

Mean OBP reduction at the six months follow up visit (n=70) was -16,0/-6,4mmHg (-22,2;-9,9/-10,4;-2,4). In the group of patients, who completed one year follow up (n=59), OBP changed by -0,4/0,6mmHg (-8,2;7,5/0,6;-4,6). Systolic and diastolic office BP differences at six months were found to be statistically significant (p<0,001/p=0,002), while the changes at one year deemed non-significant (p=0,928/p=0,824).



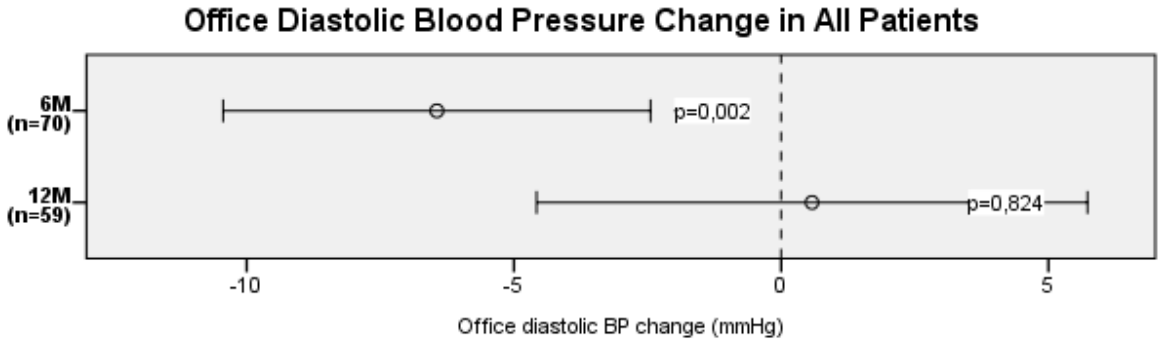



*Figure 2. Office systolic and diastolic blood pressure (OBP) changes in all patients at 6 and at 12 months after renal sympathetic denervation. Whiskers indicate 5 and 95 percentile, p-values are for comparison with baseline values.*

### 3.1.1.2. Office Blood Pressure in Pseudo-/True Resistant Hypertension

Mean office systolic and diastolic BP in the "pseudo-resistant HT" group was 157/84mmHg (15,1/13,6) at baseline (n=13), 148/84mmHg (20,6/12,9) at six months (n=11) and 166/93mmHg (33,3/21,5) at twelve months (n=8). In the "true resistant HT" group, average OBP amounted to 166/90mmHg (21,9/12,9) at baseline (n=65), 151/82mmHg (20,3/14,5) at six months (n=59) and 168/90mmHg (27,3/17,3) at twelve months (n=51).

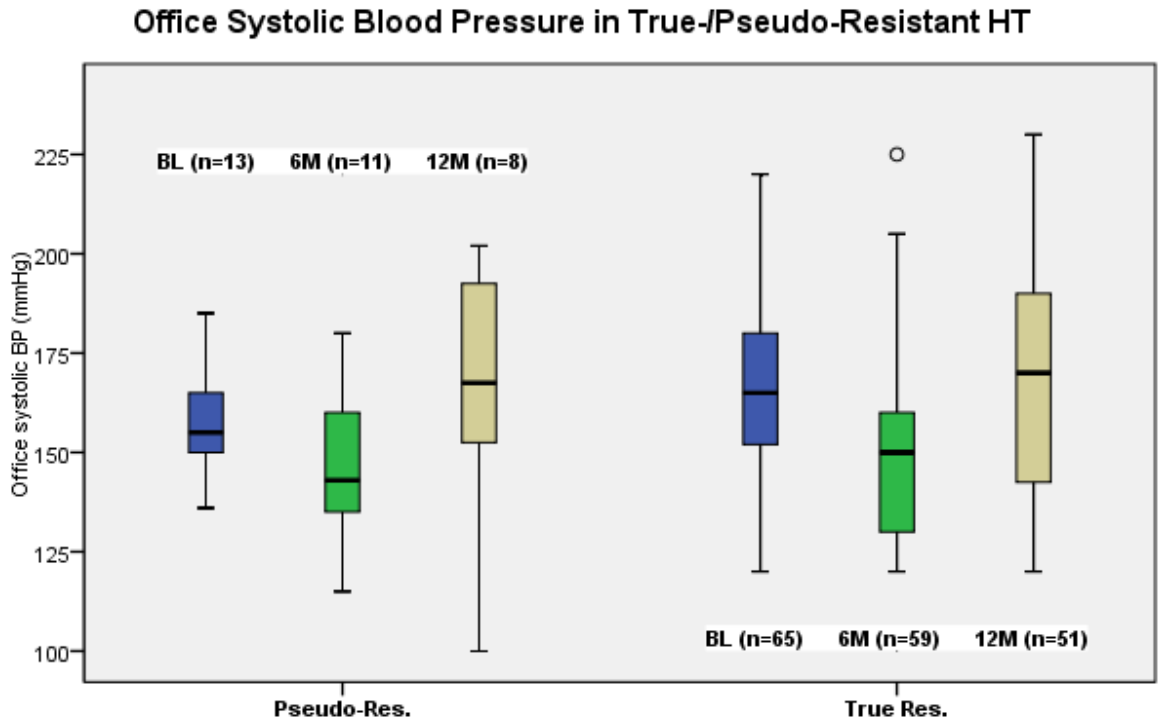



*Figure 3. Office systolic blood pressure (OBP) at baseline, 6 and 12 months after renal sympathetic denervation in the pseudo-resistant HT and the true resistant HT group, respectively. Whiskers indicate 5 and 95 percentile.*

Among the patients considered having “true resistant HT”, paired six months follow up data was available in n=59 patients, whose average OBP levels were reduced by -16,8/-7,4mmHg (-23,8;-9,9/-11,6;-3,2), which was considered statistically significant (p<0,001/p=0,001). One year follow up was completed by n=51 patients in this group, who witnessed a mean OBP change by -1,0/-0,4mmHg (-9,4;7,4/-5,7;4,9), which was considered statistically non-significant (p=0,808/p=0,877). Among the “pseudo-resistant HT” patients, in whom complete follow up of values was available, mean OBP change amounted to -11,8/-1,5mmHg (-25,7;2,1/-14,7;11,8) at six months (n=11) and to 3,9/6,9mmHg (-24,9;32,6/-14,5;28,3) at twelve months (n=8). Changes at six months (p=0,088/0,812) and at twelve months (p=0,759/0,472) were tested statistically non-significant.

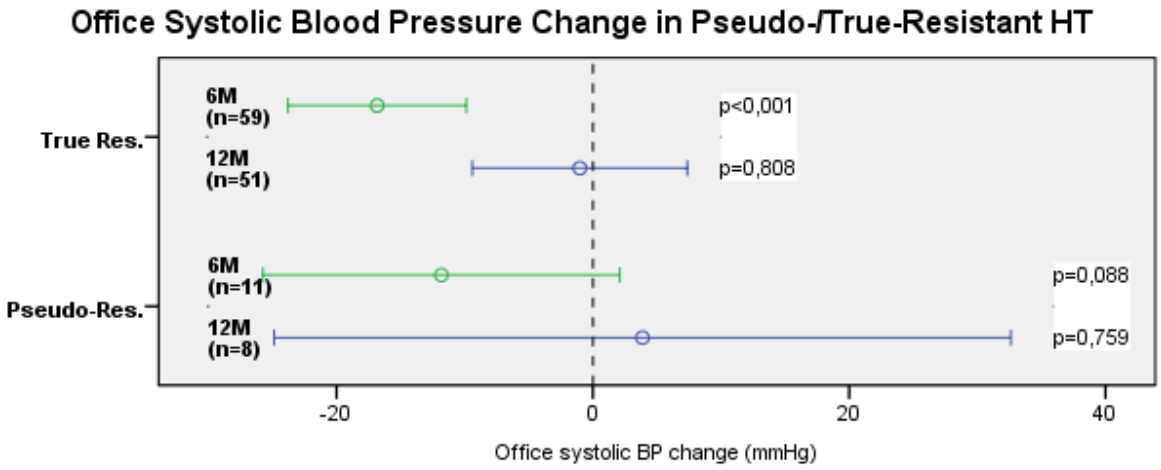



*Figure 4. Office systolic blood pressure (OBP) changes at 6 and 12 months after renal sympathetic denervation in the true resistant HT and the pseudo-resistant HT group, respectively. Whiskers indicate 5 and 95 percentile, p-values are for comparison with baseline values.*

No statistically significant difference between the “pseudo-“and the “true resistant HT” group could be detected concerning baseline OBP values (p=0,158/p=0,172), differences at six months (p=0,558/p=0,286) or the differences at one year (p=0,674/p=0,337).

### 3.1.1.3. Office Blood Pressure in RSD Non-/Responders

Patients, who witnessed a reduction in systolic ambulatory BP by ≥5mmHg at six months after the intervention were considered RSD responders. Among those, average office systolic and diastolic OBP was 165/90mmHg (17,6/12,9) at baseline (n=33), 148/81mmHg (20,0/14,6) at six months (n=33) and 164/88mmHg (30,5/19,4) at twelve months (n=29). In the RSD non-responders group, OBP levels were 167/90mmHg (20,3/12,8) before

denervation (n=32), 152/85mmHg (21,6/14,2) at six months (n=32) and 170/94mmHg (26,5/16,3) at one year (n=26).

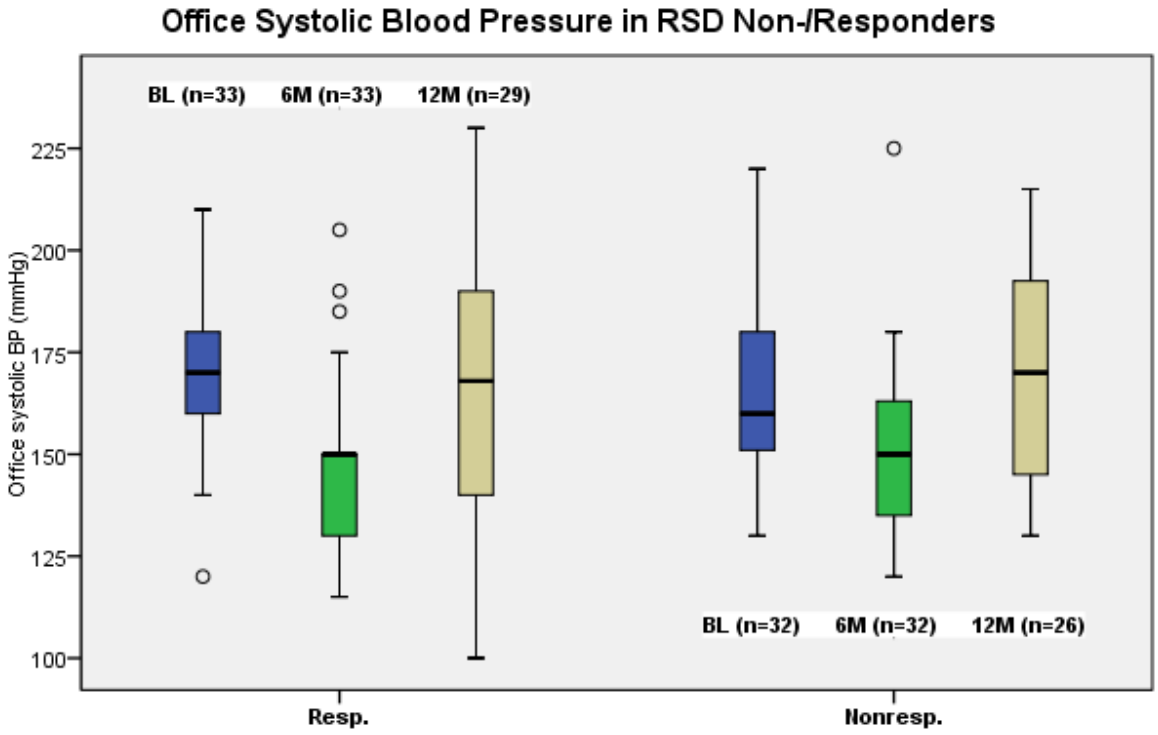



*Figure 5. Office systolic blood pressure (OBP) at baseline, 6 and 12 months after renal sympathetic denervation in the RSD responder and the non-responder group, respectively. Whiskers indicate 5 and 95 percentile.*

Among the RSD responders, who completed six months follow up of values (n=33), systolic and diastolic OBP decreased by -17,5/-9,1mmHg (-26,1;-8,9/-14,3;-3,9) on average. In the one year follow up group (n=29), mean difference averaged out at -1,6/-2,0mmHg (-14,4;11,2/-10,0;6,1). OBP changes in the RSD responders group were considered significant at six months (p<0,001/p=0,001) and non-significant at twelve months (p=0,797/p=0,622). In non-responders, who attended the six months follow up visit (n=32), OBP decreased by -14,6/-4,1mmHg (-23,9;-5,2/-10,9;2,7) on average, while patients with complete follow up to one year (n=26) had a mean BP change by 0,4/3,7mmHg (-10,5;11,3/-4,0;11,4). Systolic OBP changes at six months were considered statistically significant (p=0,003), mean diastolic change at six months (p=0,229) and the BP differences at twelve months (p=0,937/p=0,331) were tested non-significant.

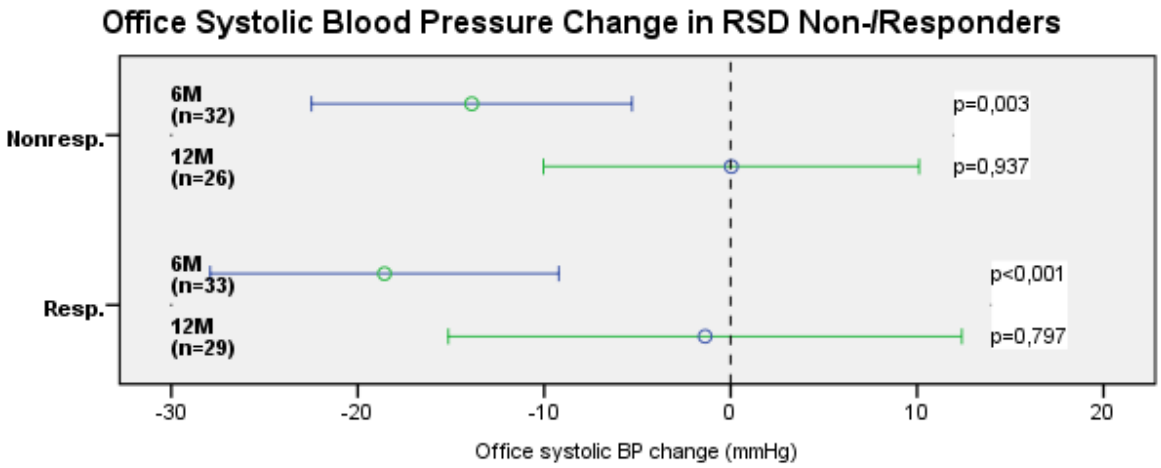



*Figure 6. Office systolic BP (OBP) changes at 6 and 12 months after renal sympathetic denervation in the RSD non-responder and responder group, respectively. Whiskers indicate 5 and 95 percentile, p-values are for comparison with baseline values.*

No statistically significant difference between the RSD responders and non-responders group could be detected, neither concerning baseline OBP values (p=0,729/p=0,928), nor in mean changes at six months (p=0,640/p=0,236) or at twelve months (p=0,806/p=0,304).

## 3.1.2. Ambulatory Blood Pressure (ABP)

### 3.1.2.1. Ambulatory Blood Pressure in All Patients

ABP values were available in n=78 patients at baseline, n=65 at six and n=51 at twelve months. Among those, mean ABP amounted to 145/86mmHg (18,4/12,5) before RSD, 142/84mmHg (16,2/12,7) at six months and 138/82mmHg (15,6/13,2) after one year.

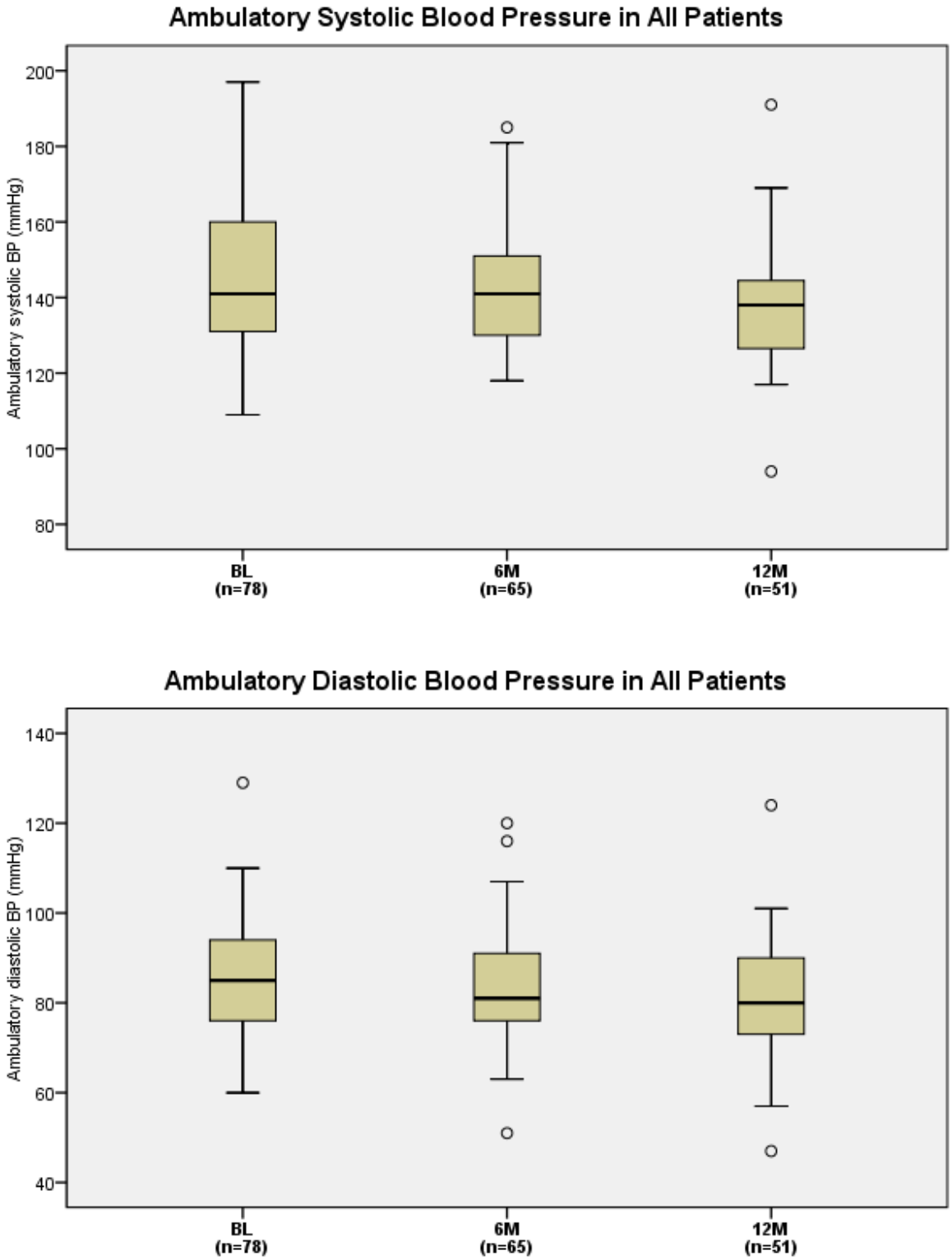



*Figure 7. Ambulatory systolic and diastolic blood pressure (ABP) at baseline, 6 and 12 months after renal sympathetic denervation in all patients. Whiskers indicate 5 and 95 percentile.*

In the group of patients, who attended the six months follow up examination (n=65), mean ABP changed by -2,6/-1,4mmHg (-7,1;1,9/-3,9;1,2). In the one year follow up group (n=51),

ABP levels decreased by a mean difference of -8,2/-3,8mmHg (-13,5;-2,9/-7,0;-0,5). ABP changes at six months, were tested non-significant (p=0,136/p=0,113), while one year results indicated significant reductions of both systolic and diastolic blood ABP (p=0,002/p=0,021).

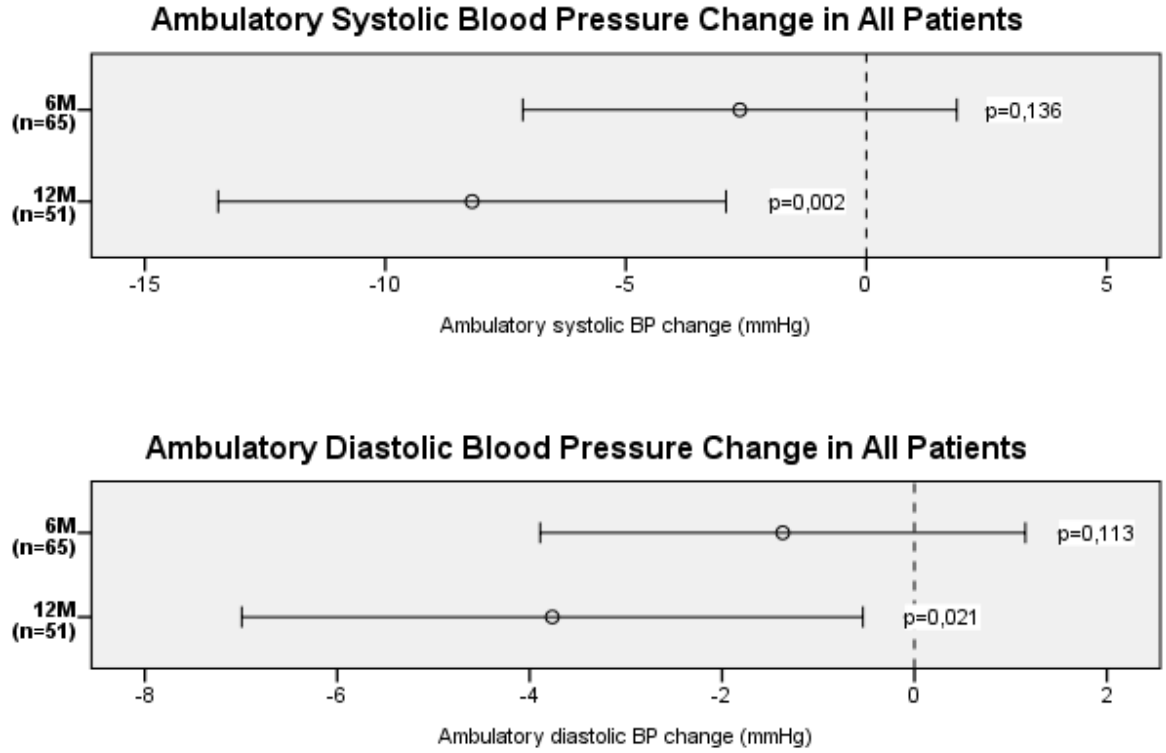



*Figure 8. Ambulatory systolic and diastolic blood pressure (ABP) changes at 6 and at 12 months after renal sympathetic denervation in all patients. Whiskers indicate 5 and 95 percentile, p-values are for comparison with baseline values.*

After excluding three patients with extraordinarily high ABP alterations (one with a decrease of more than 60mmHg at six months and two with an increase of more than 40mmHg at one year), the following results were seen: ABP decreased from 145/85mmHg (18,4/12,3) to 141/83mmHg (14,9/12,1) and to 138/81mmHg (12,4/10,9) in n=75, n=62 and n=49 patients during follow up. In the n=62 patients, in whom complete six months follow up data was available, ABP changed by -3,7/-1,9mmHg (-7,7;0,3/-4,1;0,3), the n=49 patients, who attended the one year follow up visit, had a mean decrease by -8,1/-3,3mmHg (-12,5;-3,6/-5,6;-1,0). ABP changes at six months were considered statistically non-significant (p=0,067/p=0,087), changes at one year deemed significant (p=0,001/p=0,005).

#### 3.1.2.2. Ambulatory Blood Pressure in Pseudo-/True-Resistant Hypertension

In the "pseudo-resistant HT" group, systolic and diastolic ABP values developed from 123/74mmHg (5,5/8,6) to 140/80mmHg (19,5/14,0) and 132/73mmHg (7,9/10,8) at baseline

(n=13), six months (n=11) and one year among (n=7), respectively. In the "true-resistant HT" group, baseline ABP was 150/88mmHg (16,6/11,9) in n=65 patients. Six months after RSD, values among the n=54 remaining patients had decreased to 142/84mmHg (15,6/12,5) and further to 139/83mmHg (16,3/13,1) among n=44 patients after one year.

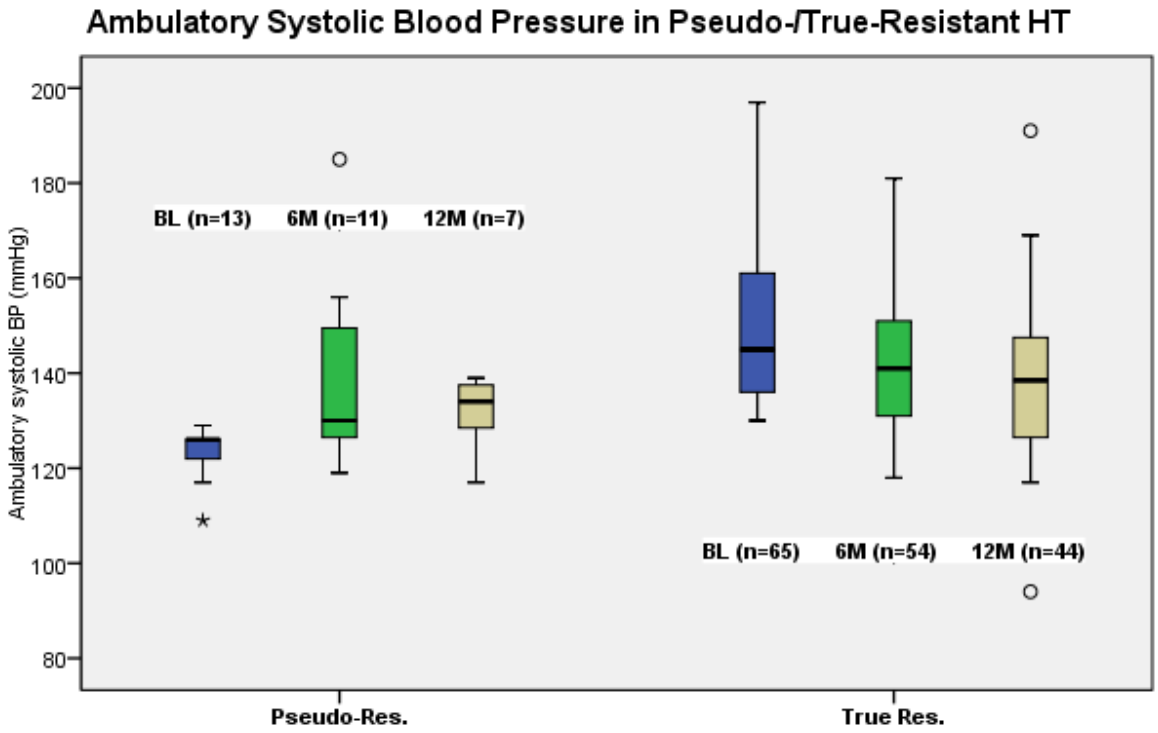



*Figure 9. Ambulatory systolic blood pressure (ABP) at baseline, 6 and 12 months after renal sympathetic denervation in the pseudo-resistant HT and the true resistant HT group, respectively. Whiskers indicate 5 and 95 percentile.*

Among "true-resistant HT" patients, who attended the six months follow up visit (n=54), ABP significantly fell by an average of -6,3/-2,9mmHg (-10,5;-2,1/-5,4;-0,5 p=0,006/p=0,015). One year follow up of ABP values was available in n=44 subjects in this group, who had a mean decrease by -10,5/-4,6mmHg (-16,3;-4,8/-8,3;-0,9 p<0,001/p=0,009). ABP levels in the "pseudo-resistant HT" group increased by 15,4/6,4mmHg (1,5;29,2/-1,9;14,6) at six months (n=11), which was considered statistically significant for the systolic (p=0,029), but not for diastolic values (p=0,139). At one year (n=7), ABP values had changed by 6,4/1,4mmHg (-2,4;15,3/-2,0;4,9 p=0,172/p=0,400).

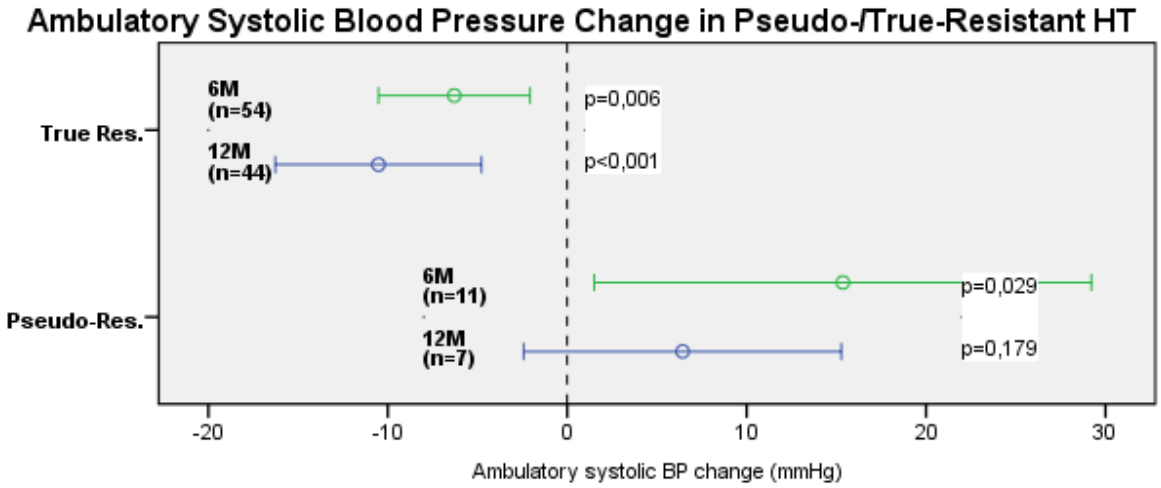



*Figure 10. Ambulatory systolic blood pressure (ABP) changes at 6 and 12 months after renal sympathetic denervation in the true resistant HT and the pseudo-resistant HT group, respectively. Whiskers indicate 5 and 95 percentile, p-values are for comparison with baseline values.*

Significant differences between the "pseudo-" and the "true-resistant HT" group could be demonstrated for systolic and diastolic ABP at baseline (both p<0,001), for ABP differences at six months (p=0,001/p=0,020) and for the systolic difference (p=0,013) at one year, but not for the diastolic ABP difference at one year (p=0,100).

#### 3.1.2.3. Ambulatory Blood Pressure in RSD Non-/Responders

Among RSD responders, ABP values were available in n=33 patients at baseline, n=33 at six months and n=29 at twelve months, systolic and diastolic ABP developed from 151/88mmHg (16,1/14,7) to 135/80mmHg (13,5/13,2) and to 135/79mmHg (14,1/12,7). RSD non-responders had ABP values averaging out at 137/82mmHg (13,9/10,3) at baseline (n=32), 149/88 (15,8/11,0) at six (n=32) and 141/84 (16,1/13,9) at twelve months (n=21).

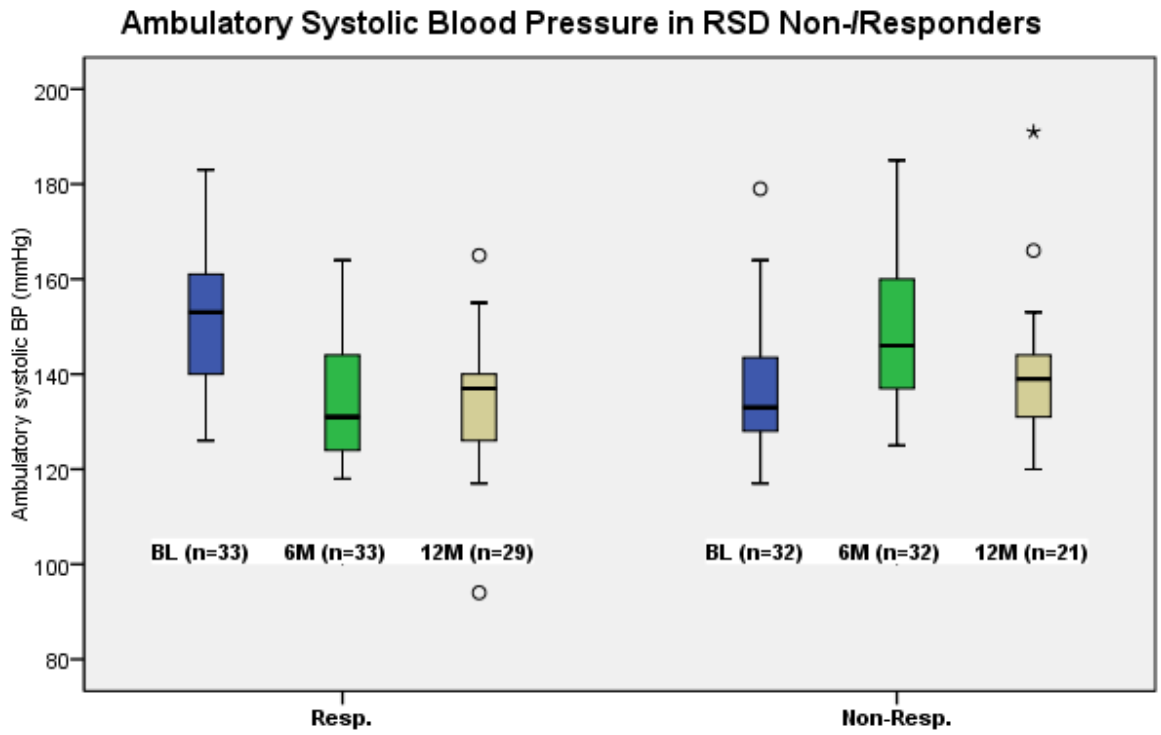



*Figure 11. Ambulatory systolic blood pressure (ABP) at baseline, 6 and 12 months after renal sympathetic denervation in the RSD responder and the non-responder group, respectively. Whiskers indicate 5 and 95 percentile.*

In the RSD responders group, paired six months ABP follow up data was available in n=33 patients, who had a significant mean decrease by -16,4/-8,1mmHg (-19,8;-13,1/-10,3;-5,8 p<0,001/p<0,001). Patients, who attended the one year follow up visit (n=29), had a significant mean decrease by -15,9/-7,6mmHg (-22,4;-9,4/-12,1;-3,1 p<0,001/p=0,001).
RSD non-responders, in whom complete six months follow up of values was available (n=32), witnessed an increase by 11,6/5,5mmHg (6,8;16,4/2,4;8,7 p<0,001/p=0,001). Twelve months follow up of values was completed by n=21 non-responders, who had a mean change adding up to 3,4/1,1mmHg (-3,4;10,2/-3,1;5,2 p=0,394/p=0,489).

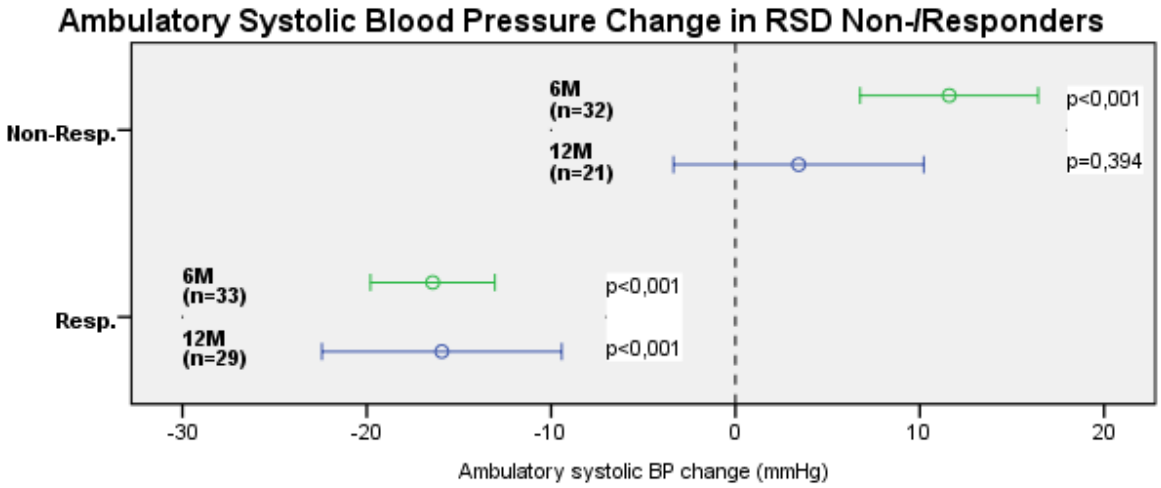



*Figure 12. Ambulatory systolic blood pressure changes (ABP) at 6 and 12 months after renal sympathetic denervation in the RSD responder and non-responder group, respectively. Whiskers indicate 5 and 95 percentile, p-values are for comparison with baseline values.*

Statistical testing revealed a significant difference between the RSD responders and non-responders group concerning baseline systolic ABP (p=0,001), systolic and diastolic ABP differences at six months (p<0,001 for both) and at twelve months (p<0,001/p=0,003).

### 3.1.3. Correlations of Office and Ambulatory Blood Pressure Data

Graphical and mathematical analysis of correlations between ABP and OBP values at baseline and the BP differences at six months was conducted using the modified dataset for ABP values.

Analysis revealed a significant positive correlation between baseline ABP and baseline OBP values (r=0,452; p<0,001). OBP differences at six months were significantly correlated with baseline OBP (r=-0,643; p<0,001) and ABP differences at six months were significantly correlated with baseline ABP values (r=-0,574; p<0,001).

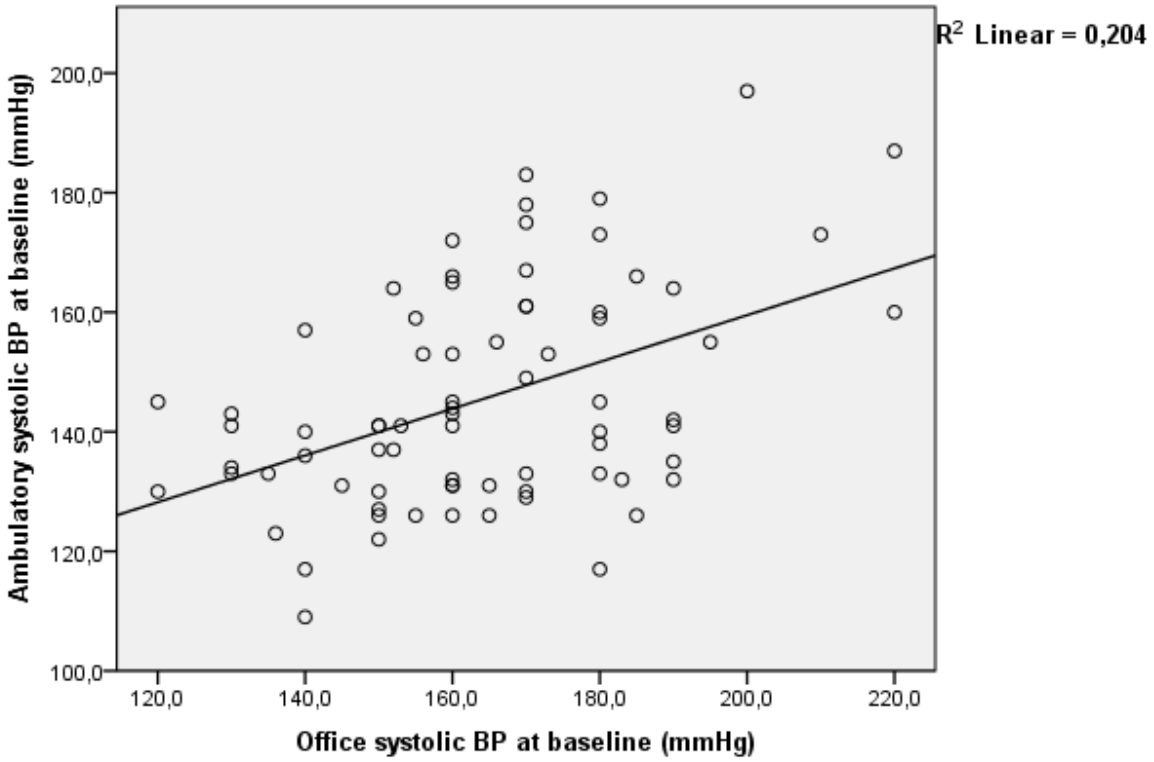



*Figure 13. Relationship between ABP and OBP values at baseline (n=75).*

# 3.2. Weight and Body Mass Index (BMI)

## 3.2.1. Body Weight and Body Mass Index in All Patients

Body weight was assessed among all n=78 patients at baseline and in n=63 at the six-month follow up visit. Among all patients, average BMI amounted to 30,4 kg/m$^2$ (4,7) at baseline and to 30,2kg/m$^2$ (5,2) after six months of follow up.

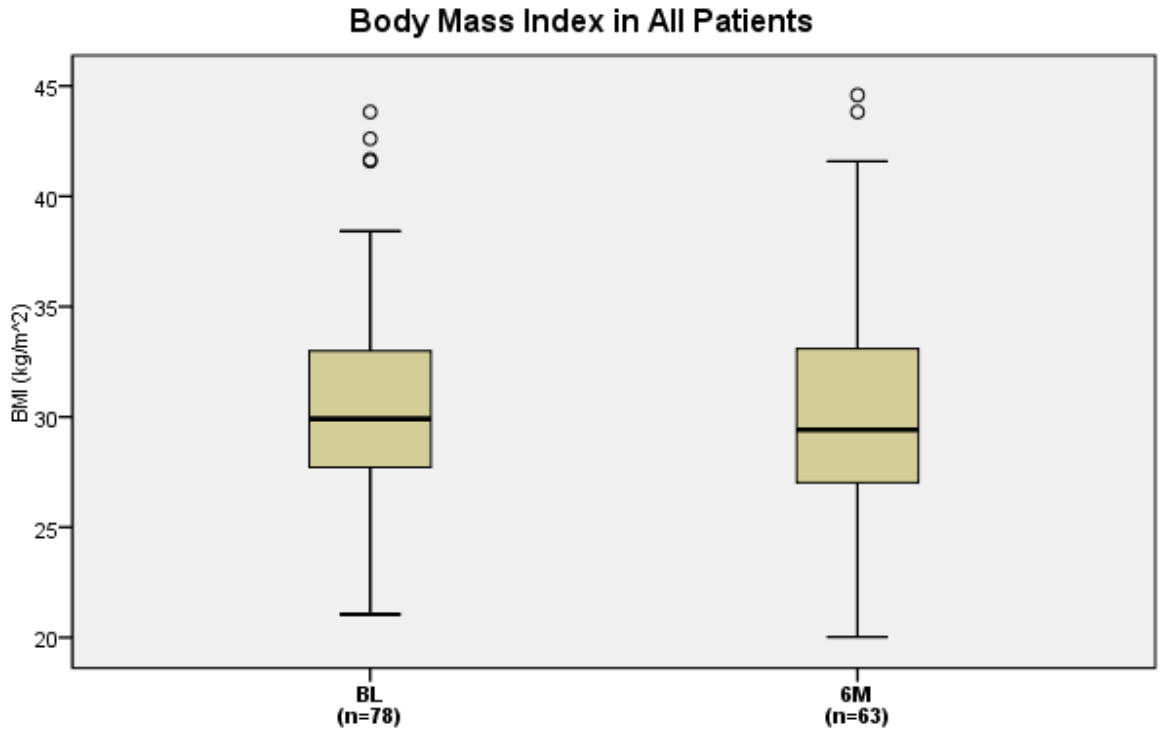



*Figure 14. Body mass index (BMI) in all patients at baseline and 6 months after renal sympathetic denervation. Whiskers indicate 5 and 95 percentile.*

In the group of n=63 patients, in whom paired six month follow up BMI data was available, average BMI significantly decreased by -0,5kg/m$^2$ (-0,8;-0,2 p=0,004), representing an average weight loss of -1,4kg (-2,4;-0,5 p=0,006).

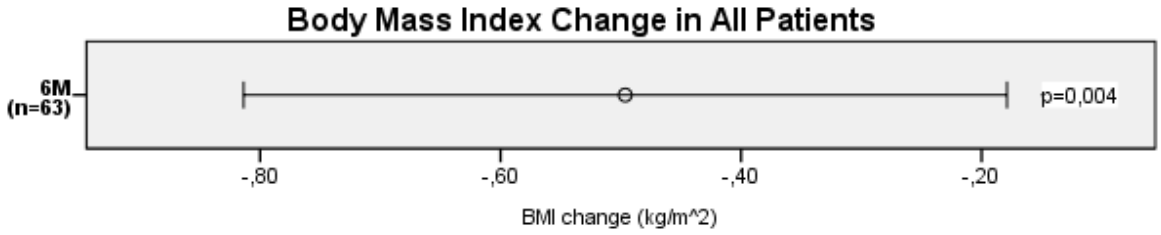



*Figure 15. Body mass index (BMI) change in all patients at 6 months after renal sympathetic denervation. Whiskers indicate 5 and 95 percentile, p-value is for comparison with baseline value.*

After excluding one patient with an extraordinarily great weight loss of 15kg, average baseline BMI among the remaining n=62 patients was 30,8kg/m$^2$ (4,9) and 30,3kg/m$^2$ (5,1) at the six month visit, resulting in a significant mean reduction by -0,4kg/m$^2$ (-0,7;-0,1 p=0,004) in BMI and by -1,2kg (-2,1;-0,4 p=0,005) in body weight.

### 3.2.2. Body Mass Index in RSD Non-/Responders

In the RSD responders group, average BMI was 30,2kg/m$^2$ (4,1) at baseline (n=33) and 29,4kg/m$^2$ (4,6) at six months (n=29). The RSD non-responder patients had a baseline BMI of 30,8kg/m$^2$ (5,1) before denervation (n=32) and 30,6kg/m$^2$ (5,2) six months after (n=32).


*Figure 16. Body mass index (BMI) at baseline and 6 months after renal sympathetic denervation in the RSD responder and the non-responder group, respectively. Whiskers indicate 5 and 95 percentile.*

Among RSD responders, in whom paired BMI follow up data was available (n=29), mean BMI significantly decreased by -0,6kg/m$^2$ (-1,2;-0,1 p=0,028) on average. Mean difference among RSD non-responders (n=30) was -0,4kg/m$^2$ (-0,8;0,0 p=0,058).

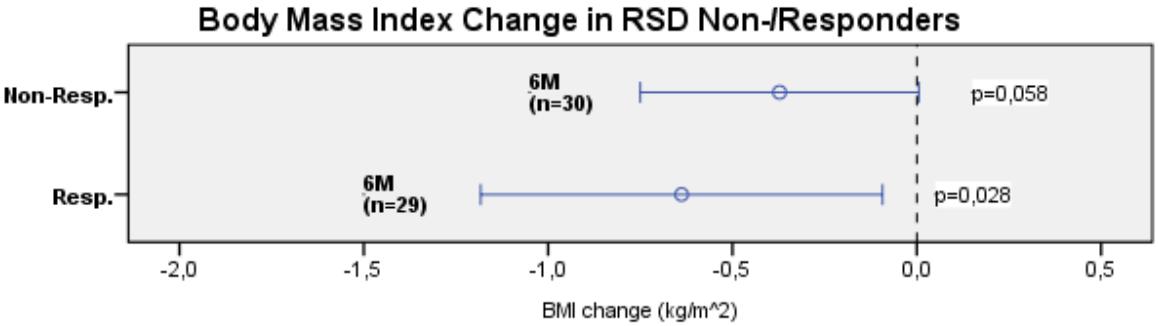



*Figure 17. Body mass index (BMI) change at 6 months after renal sympathetic denervation in the RSD responder and non-responder group, respectively. Whiskers indicate 5 and 95 percentile, p-values are for comparison with baseline values.*

Neither baseline BMI (p=0,823) nor the changes at six months (p=0,543) could be proven to differ significantly between the RSD responders and non-responders group.

### 3.2.3. Correlations of Body Mass Index and Blood Pressure Values

Graphical and mathematical analysis of correlations between BMI, ABP and OBP values at baseline and the BP differences at six months was conducted using the modified datasets both for BMI and ABP values.

Analysis revealed a significant positive correlation between ABP and BMI differences at six months (r=0,273; p=0,041).

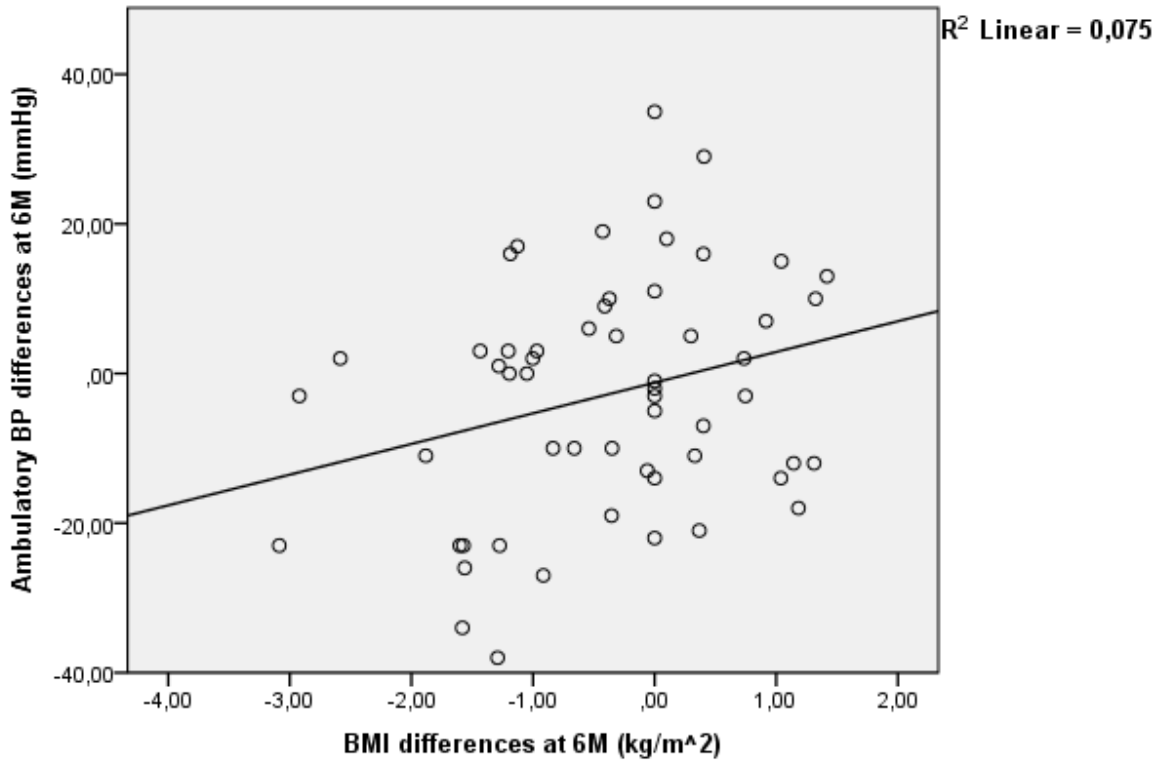



*Figure 18. Relationship between BMI differences and ABP differences at 6M (n=56). Modified datasets were used for ABP and BMI values.*

## 3.3. Glucose Metabolism Parameters

### 3.3.1. Fasting Plasma Glucose Concentration (FPG)

#### 3.3.1.1. Fasting Plasma Glucose Concentration in All Patients

Plasma fasting glucose concentration was measured in all n=78 patients at baseline, n=70 at six months and n=60 at twelve months. FPG levels among all patients averaged out at

112,9mg/dL (28,9) at baseline, 112,0mg/dL (36,5) at six months and 127,7mg/dL (50,1) at one year. In the group with complete six months follow up of values available (n=70), mean concentration difference amounted to -1,5mg/dL (-9,7;6,7 p=0,202). One year follow up of FPG data was completed by n=60 patients, mean concentration in those increased significantly by 13,8mg/dL (4,1;23,5 p=0,012).

After excluding all patients on insulin treatment (n=5), mean FPG concentration was 111,0mg/dL (26,9) at baseline (n=73), 111,1mg/dL (29,2) at six months (n=65) and 121,7mg/dL (41,7) at twelve months (n=57).

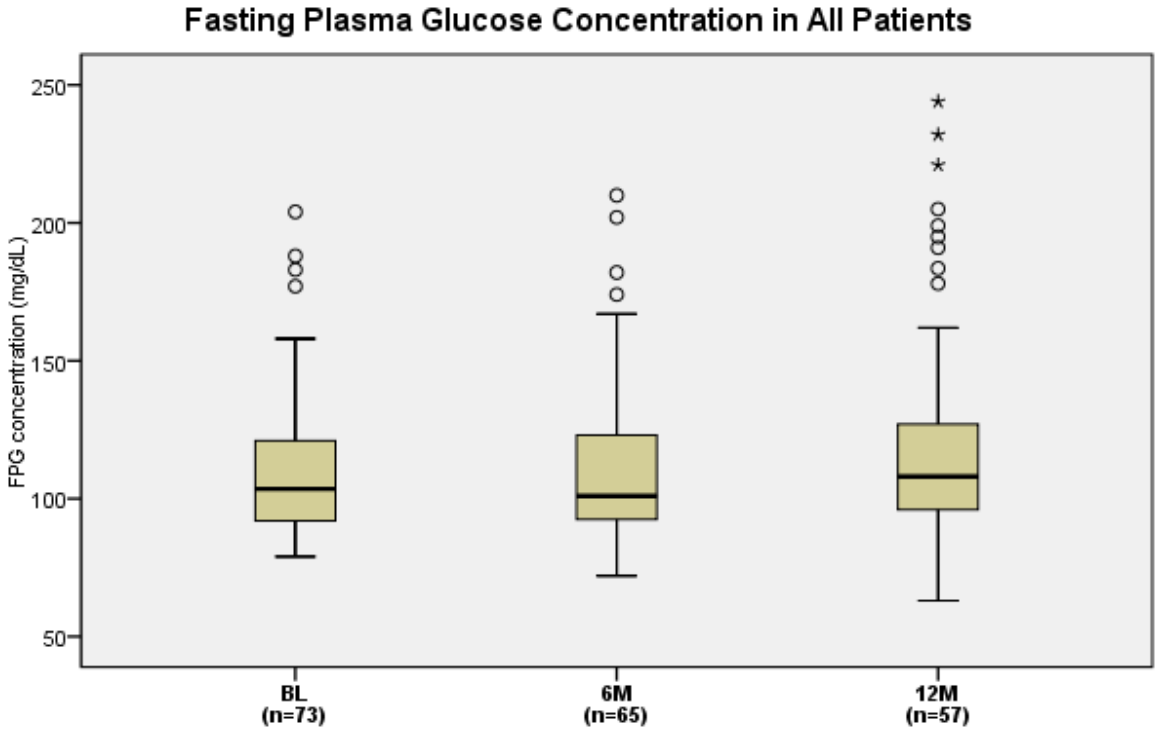



*Figure 19. Fasting plasma glucose (FPG) concentration in all patients at baseline, 6 and 12 months after renal sympathetic denervation. Whiskers indicate 5 and 95 percentile. Patients on insulin treatment (n=5) were excluded from this analysis.*

In the six months follow up group (n=65), mean FPG concentration changed by an average of -0,4mg/dL (-7,2;6,5 p=0,338), while patients, who attended the one-year follow up visit (n=57), witnessed a significant mean increase by 10,7mg/dL (1,8;19,6 p=0,036).

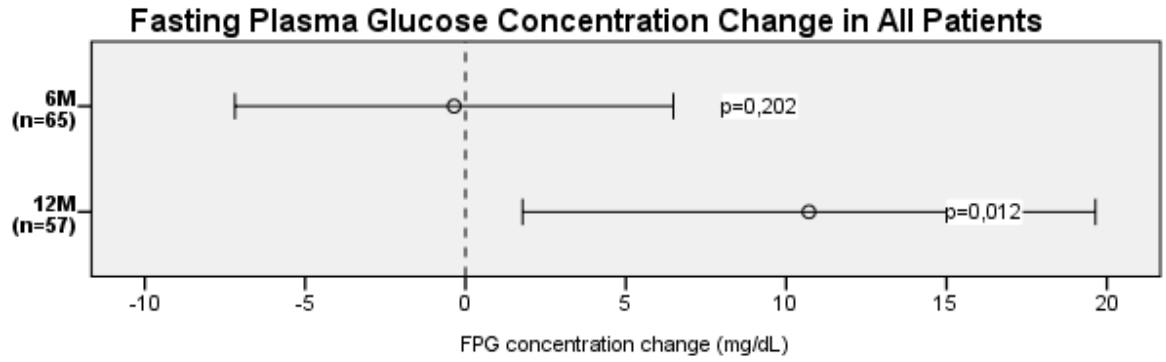



*Figure 20. Fasting plasma glucose concentration changes in all patients at 6 and at 12 months after renal sympathetic denervation. Whiskers indicate 5 and 95 percentile, p-values are for comparison with baseline values. Patients on insulin treatment (n=5) were excluded from this analysis.*

#### 3.3.1.2. Fasting Plasma Glucose Concentration in RSD Non-/Responders

The modified dataset excluding all patients on insulin treatment (n=5) was used in this analysis. In the RSD responders group, mean FPG concentration was measured in n=32 patients at baseline at a mean concentration of 117,8mg/dL (32,6), that developed to 111,3 (27,5) at six months (n=32) and 130,7mg/dL (48,1) at twelve months (n=30). FPG concentration among RSD non-responders averaged out at 105,7mg/dL (17,7) at baseline (n=29), 109,2mg/dL (26,7) at six (n=29) and 109,0mg/dL (29,1) at twelve months (n=25).

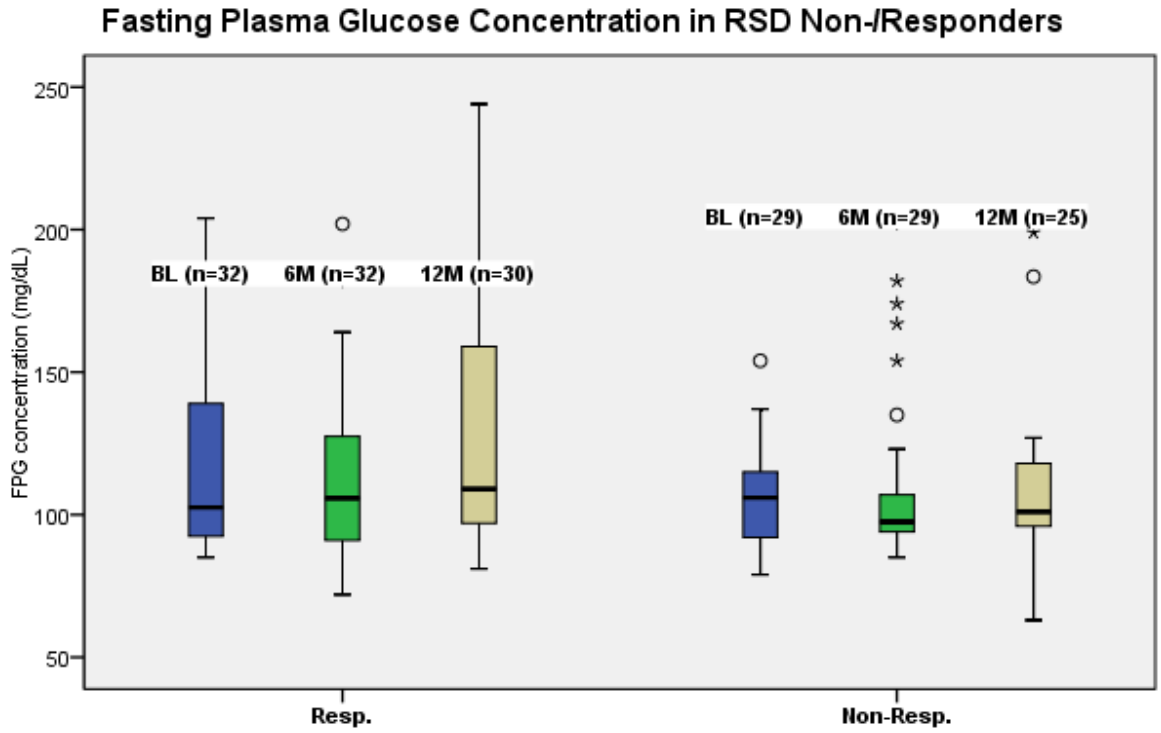



*Figure 21. Fasting plasma glucose concentration at baseline, 6 and 12 months after renal sympathetic denervation in the RSD responders and non-responders group, respectively. Whiskers indicate 5 and 95 percentile. Patients on insulin treatment (n=5) were excluded from this analysis.*

Among RSD responders, who completed six months follow up (n=32), mean FPG concentration changed by an average of -6,5mg/dL (-13,6;0,6 p=0,162). Twelve months follow up was available in n=30 responders, whose mean FPG concentration significantly increased by 14,2mg/dL (0,1;28,2 p=0,034). Patients considered RSD non-responders, who attended the follow up examinations, had a mean change by 3,5mg/dL (-8,2;15,1 p=0,866) at six months (n=29) and by 4,4mg/dL (-7,0;15,9 p=0,686) at twelve months (n=25).

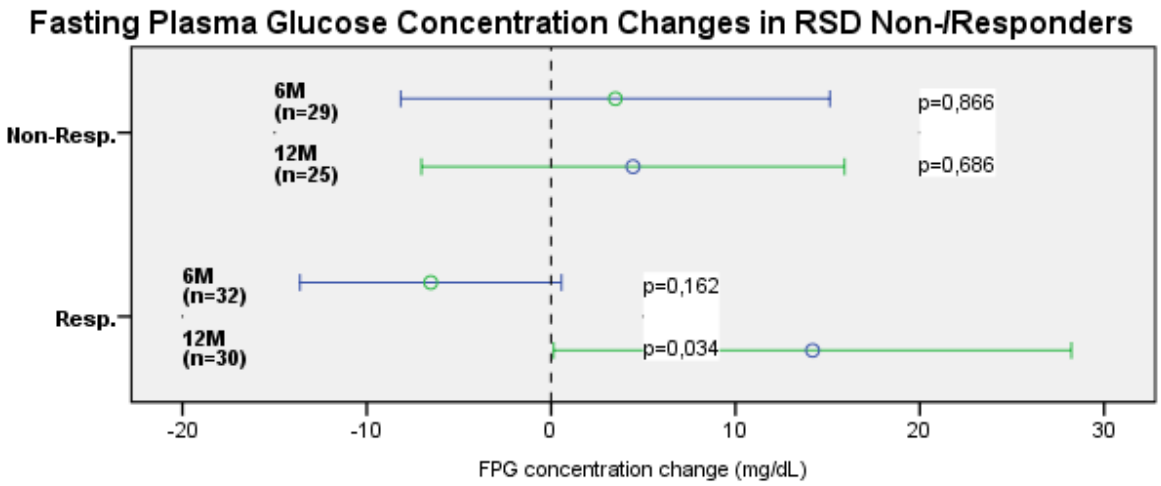



*Figure 22. Fasting plasma glucose (FGP) concentration changes at 6 and 12 months after renal sympathetic denervation in the RSD responder and non-responder group, respectively. Whiskers indicate 5 and 95 percentile, p-values are for comparison with baseline values. Patients on insulin treatment (n=5) were excluded from this analysis.*

Neither FPG concentration at baseline (p=0,319), nor the differences at six (p=0,461) or twelve months (p=0,272) differed statistically significant between the two groups.

#### 3.3.1.3. Correlations of Fasting Plasma Glucose Concentration and Blood Pressure Values

Graphical and mathematical analysis of correlations between FPG concentrations, ABP and OBP values at baseline and the differences at six months was conducted using the modified datasets both for FPG concentrations and ABP values.

Analysis revealed a significant positive correlation between baseline ABP and FPG values (r=0,235; p=0,049). FPG differences at six months were significantly correlated to baseline FPG concentrations in a negative way (r=-0,418; p=0,001).

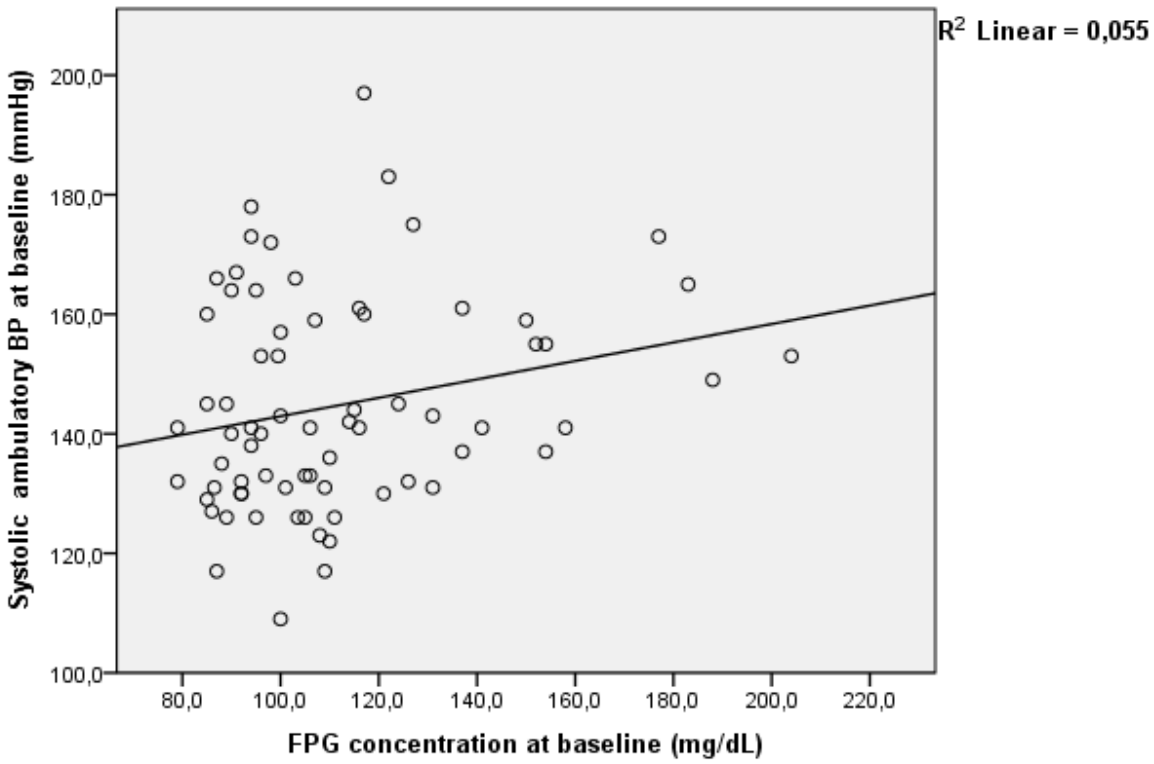



*Figure 23. Relationship between baseline FPG concentrations and baseline ABP values (n=71). Modified datasets were used for FPG and ABP values.*

## 3.3.2. Glycated Haemoglobin Fraction (HbA1c)

### 3.3.2.1. HbA1c Fraction in All Patients

HbA1c fraction data was collected among n=70 patients at baseline, n=65 at six months and n=50 at twelve months. Among those, values developed from 6,03% (0,89) to 6,12% (0,88) and 6,07% (0,96) during follow up. In the group with complete six months follow up of values available (n=63), mean HbA1c change accounted for -0,06% (-0,03;0,15 p=0,083). In the one year follow up group (n=49), average difference was -0,13% (-0,00;0,26 p=0,057).

After the exclusion of all patients on insulin treatment (n=5), average percentage of HbA1c was 5,91% (0,75) at baseline (n=65), 6,03% (0,79) at six months (n=60) and 5,98% (0,82) at twelve months (n=48).

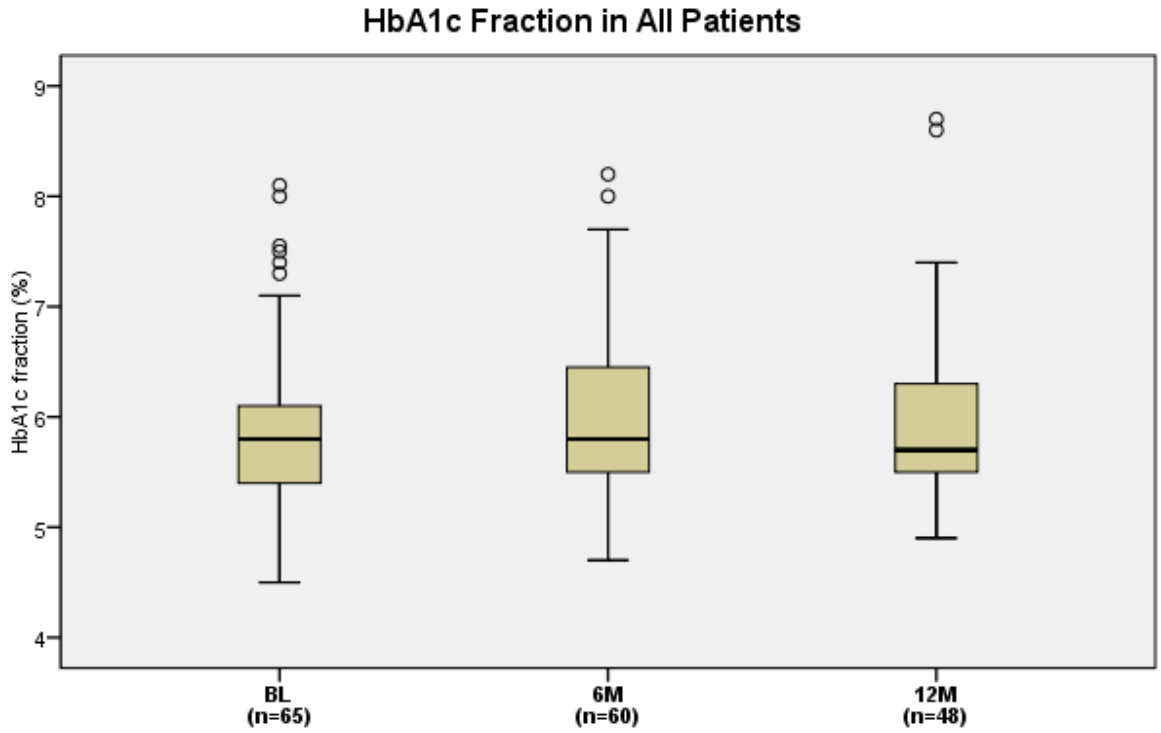



*Figure 24. HbA1c fraction in all patients at baseline, 6 and 12 months after renal sympathetic denervation. Whiskers indicate 5 and 95 percentile. Patients on insulin treatment (n=5) were excluded from this analysis.*

Among the patients, who attended the six months follow up visit (n=58), mean fraction of HbA1c significantly increased by 0,09% (0,01;0,17 p=0,015). After one year of follow up (n=47), analysis revealed a non-significant difference of 0,09% (-0,02;0,19 p=0,098).

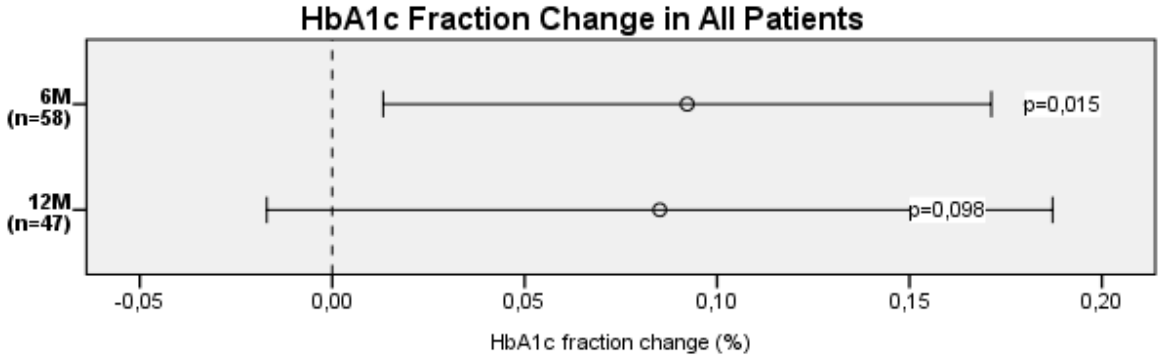



*Figure 25. HbA1c fraction changes in all patients at 6 and at 12 months after renal sympathetic denervation. Whiskers indicate 5 and 95 percentile, p-values are for comparison with baseline values. Patients on insulin treatment (n=5) were excluded from this analysis.*

### 3.3.2.2. HbA1c Fraction in RSD Non-/Responders

The modified dataset excluding all patients on insulin treatment (n=5) was used in this analysis. Patients considered RSD responders had a baseline (n=32) HbA1c fraction of 6,00% (0,84), that developed to 6,08% (0,88) at six months (n=32) and to 5,98% (0,91) at

twelve months (n=25). RSD non-responders had a mean HbA1c fraction of 5,79% (0,54) at baseline (n=27), 5,90% (0,60) at six months (n=24) and 5,87% (0,47) at one year (n=21).

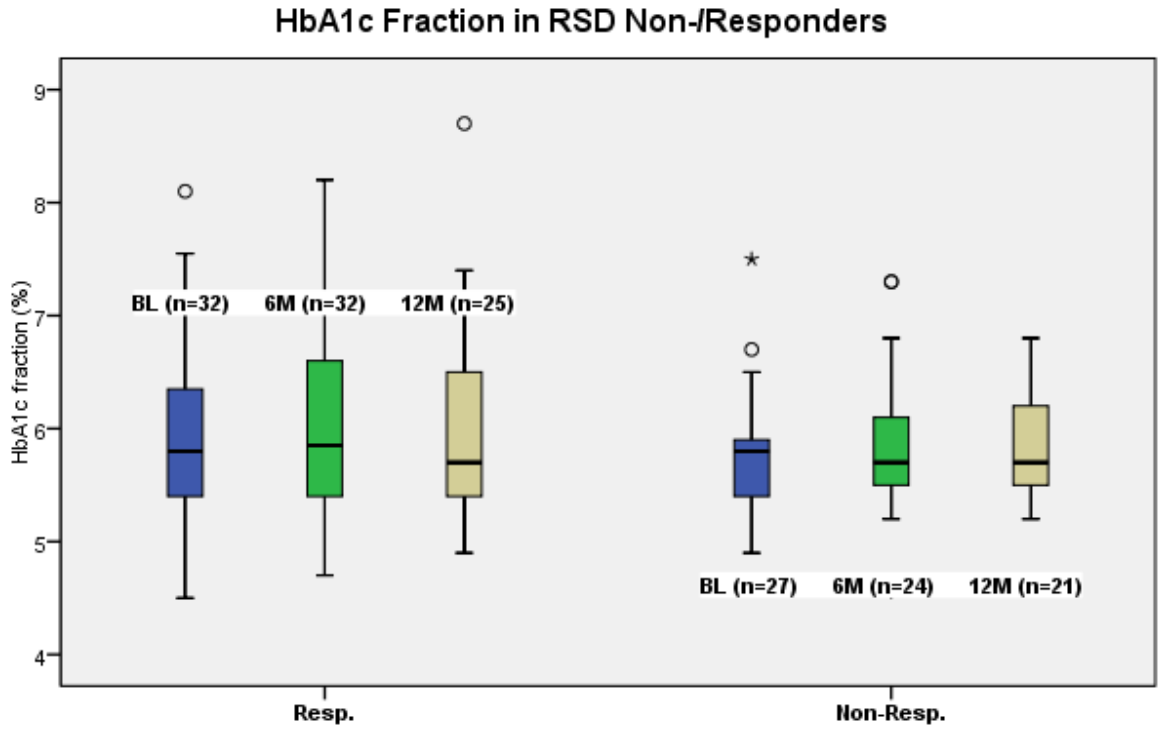



*Figure 26. HbA1c fraction at baseline, 6 and 12 months after renal sympathetic denervation in the RSD responders and non-responders group, respectively. Whiskers indicate 5 and 95 percentile. Patients on insulin treatment (n=5) were excluded from this analysis.*

Among RSD responders, who attended the six months follow up examination (n=32), HbA1c fraction changed by 0,08% (-0,03;0,18 p=0,081) on average. Mean difference in the one year follow up group (n=25) accounted for 0,08% (-0,10;0,25 p=0,410). RSD non-responders, who completed six months follow up of values (n=23) witnessed an average change in HbA1c fraction by 0,12% (-0,03;0,26 p=0,109). In the one year follow up group of non-responders (n=20), mean change amounted to 0,08% (-0,03;019 p=0,159).

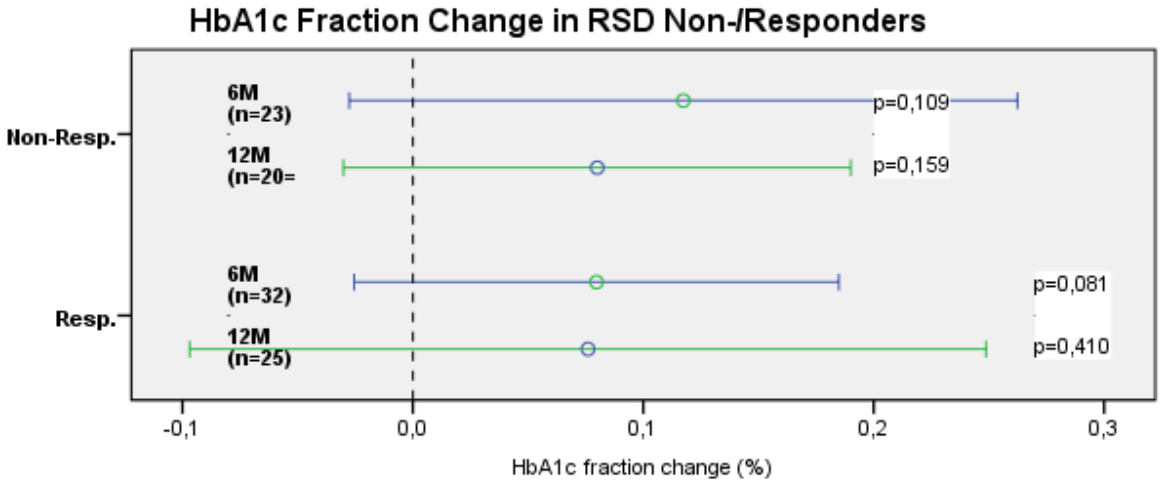



*Figure 27. HbA1c fraction changes at 6 and 12 months after renal sympathetic denervation in the RSD responder and non-responder group, respectively. Whiskers indicate 5 and 95 percentile, p-values are for comparison with baseline values. Patients on insulin treatment (n=5) were excluded from this analysis.*

No statistically significant difference between the groups, neither concerning baseline values (p=0,478), nor changes at six (p=0,829) or twelve months (p=0,747) could be found.

### 3.3.2.3. Correlations of HbA1c Fraction and Blood Pressure Values

Graphical and mathematical analysis of correlations between HbA1c fraction, ABP and OBP values at baseline and the differences at six months was conducted using the modified datasets both for HbA1c and ABP values.

Analysis revealed a significant positive correlation between baseline HbA1c fraction and baseline ABP values (r=0,253; p=0,045).

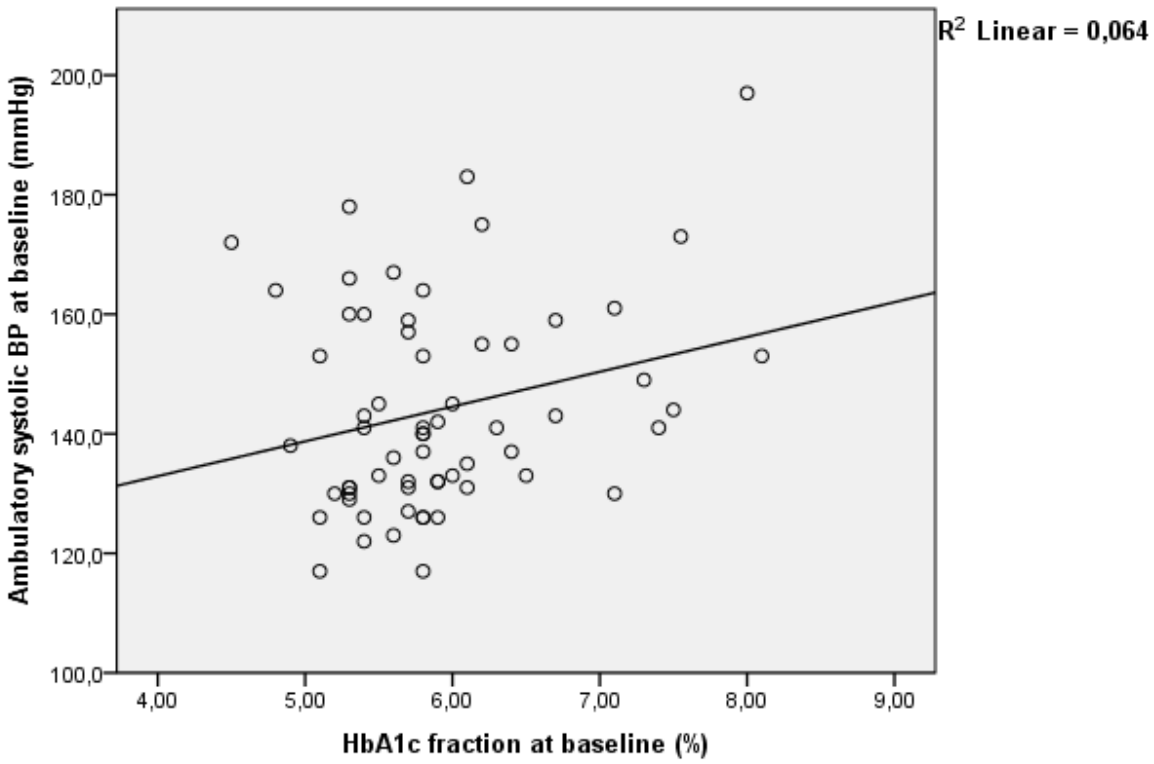



*Figure 28. Relationship between baseline HbA1c fraction and baseline ABP values (n=63). Modified datasets were used for ABP and HbA1c values.*

# 3.4. Lipid Metabolisms Parameters

## 3.4.1. Low-Density Lipoprotein Cholesterol (LDL-C)

### 3.4.1.1. LDL-Cholesterol in All Patients

LDL-C concentration was assessed in n=72 patients at the baseline examination and in n=50 patients at the six month follow up visit. In those, mean baseline LDL-C concentration was 112,2mg/dl (40,0) and increased to 116,2mg/dl (39,2) at six months.

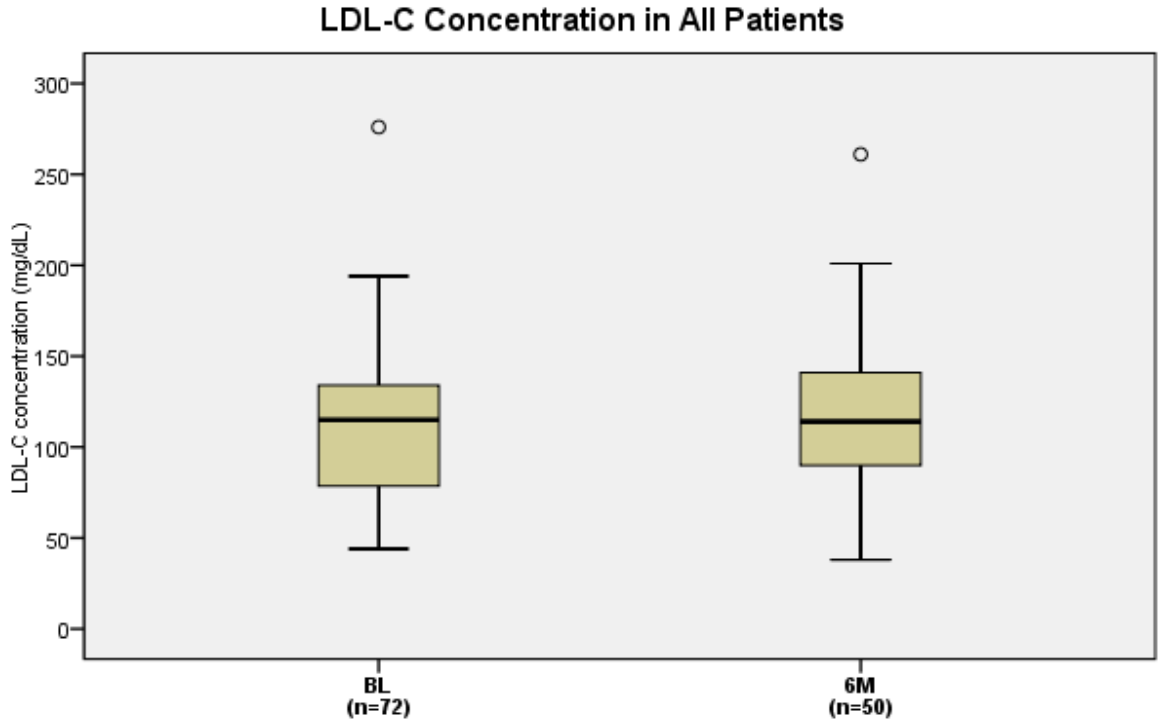



*Figure 29. LDL-cholesterol concentration in all patients at baseline and 6 months after renal sympathetic denervation. Whiskers indicate 5 and 95 percentile.*

Complete six months follow up of values was available in n=46 patients, whose mean LDL-C concentration changed by 1,6mg/dL (-5,9;9,1 p=0,676).

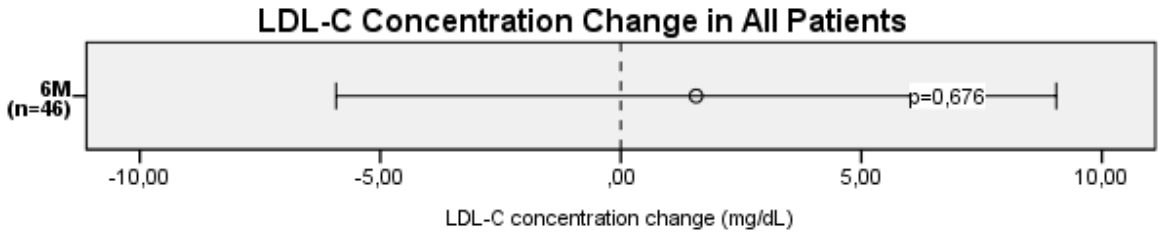



*Figure 30. LDL-cholesterol concentration change in all patients at 6 months after renal sympathetic denervation. Whiskers indicate 5 and 95 percentile, p-value is for comparison with baseline value.*

### 3.4.1.2. LDL-Cholesterol in RSD Non-/Responders

In the RSD responders group, LDL-C concentration was assessed in n=29 patients at baseline and n=24 patients at six months, average values were 103,1mg/dL (35,5) and 115,1mg/dL (34,0), respectively. Among non-responders, LDL-C at baseline (n=30) amounted to 119,2mg/dL (47,6) and to 119,9 (43,5) at six months (n=23).

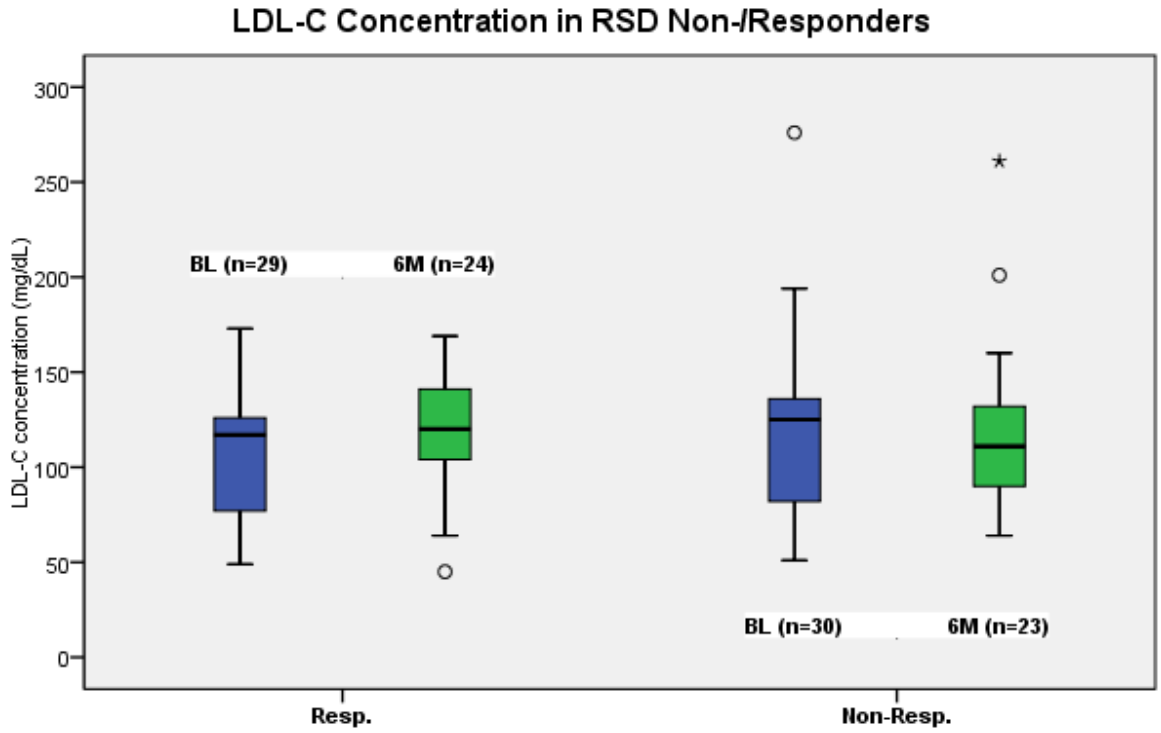



*Figure 31. LDL-cholesterol concentration at baseline and 6 months after renal sympathetic denervation in the RSD responder and the non-responder group, respectively. Whiskers indicate 5 and 95 percentile.*

In the group of patients considered RSD responders, paired follow up data was available in n=21 cases, mean change in those accounted for 9,5mg/dL (-1,6;20,7 p=0,266). Among non-responders (n=22), average LDL-concentration changed by -2,5mg/dL (-12,6;7,7 p=0,495).

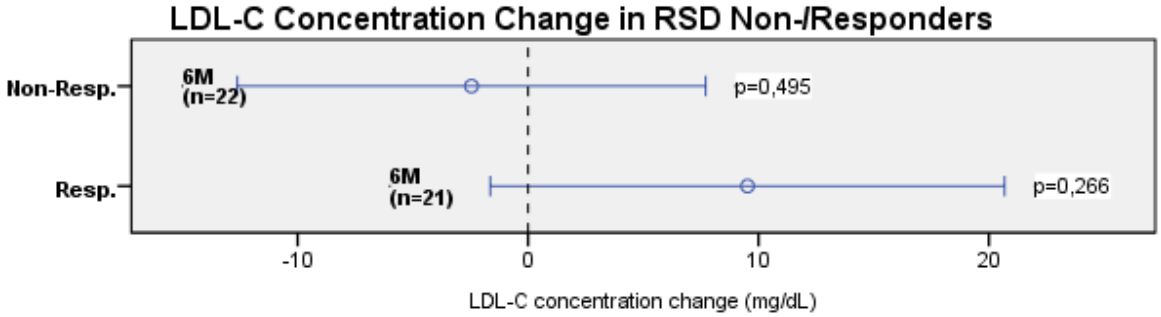



*Figure 32. LDL-C concentration changes at 6 months after renal sympathetic denervation in the RSD responder and non-responder group, respectively. Whiskers indicate 5 and 95 percentile, p-values are for comparison with baseline values.*

Neither baseline LDL-C concentrations (p=0,187), nor the mean difference at six months (p=0,101) could be proven to differ statistically significant between the two groups.

#### 3.4.1.3. Correlations of LDL-C and Blood Pressure Values

Graphical and mathematical analysis of correlations between LDL-C, ABP and OBP values at baseline and the differences at six months was conducted using the modified dataset for ABP values.

Analysis revealed no significant correlation between those values. LDL-C differences at six months were significantly correlated with baseline LDL-C (r=-0,413; p=0,004).

### 3.4.2. High-Density Lipoprotein Cholesterol (HDL-C)

#### 3.4.2.1. HDL-Cholesterol in All Patients

Baseline HDL-C concentrations were available in n=77 patients, averaging out at 52,4mg/dL (15,8) and in n=64 patients at six months with a mean concentration of 52,9mg/dL (15,2).

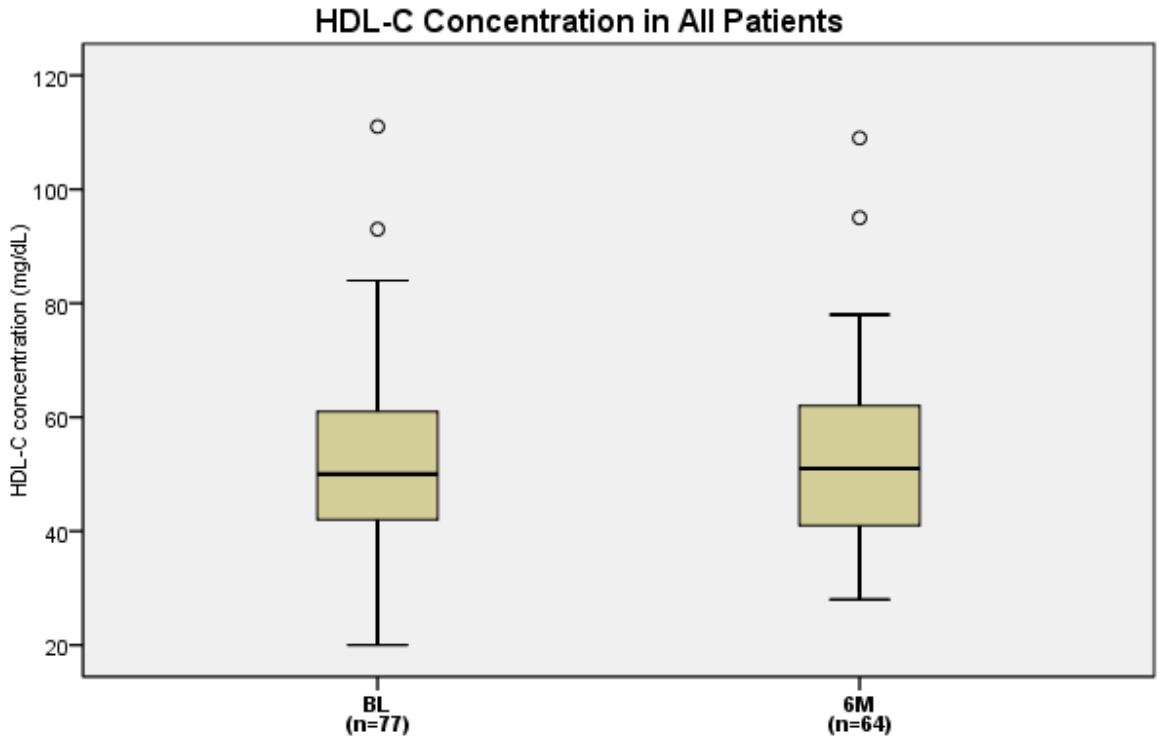



*Figure 33. HDL-cholesterol concentration in all patients at baseline and 6 months after renal sympathetic denervation. Whiskers indicate 5 and 95 percentile.*

During six months of follow up (n=63), mean HDL-C significantly increased by an average of 1,1mg/dL (-0,8;2,9 p=0,031).

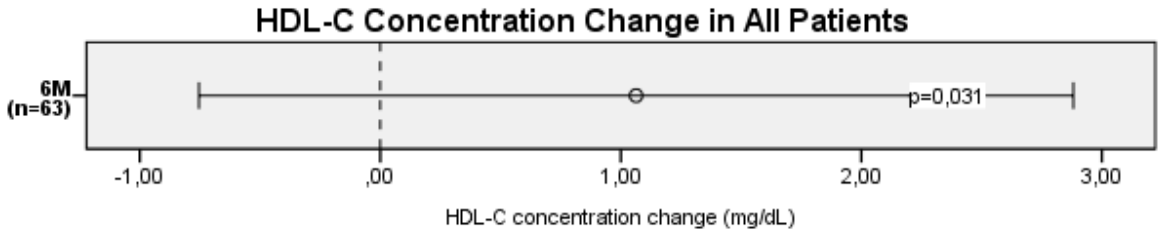



*Figure 34. HDL-cholesterol concentration change in all patients at 6 months after renal sympathetic denervation. Whiskers indicate 5 and 95 percentile, p-value is for comparison with baseline value.*

### 3.4.2.2. HDL-Cholesterol in RSD Non-/Responders

Among RSD responders, values developed from 50,9mg/dL (16,6) to 54,3mg/dL (15,5) during follow up (n=32 at baseline and n=30 at six months, respectively). Patients considered RSD non-responders had an average HDL-C of 51,5mg/dL (15,5) at baseline (n=32) and 51,6mg/dL (15,9) at six months (n=30).

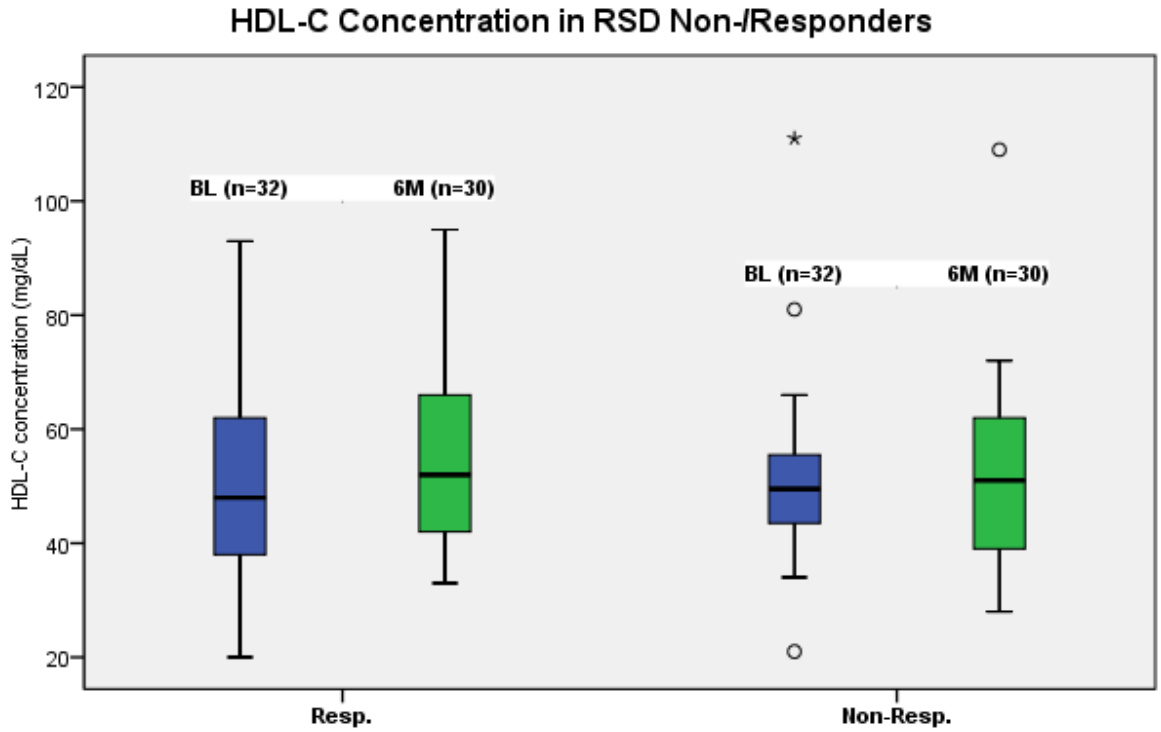



*Figure 35. HDL-cholesterol concentration at baseline and 6 months after renal sympathetic denervation in the RSD responder and the non-responder group, respectively. Whiskers indicate 5 and 95 percentile.*

Among RSD non-responders, who completed six months follow up of values (n=30), mean HDL-C changed by 0,6mg/dL (-2,0;3,2 p=0,367). Meanwhile, RSD responders (n=29) had a significant increase in HDL-C by 2,9mg/dL (0,6;5,2 p=0,002).

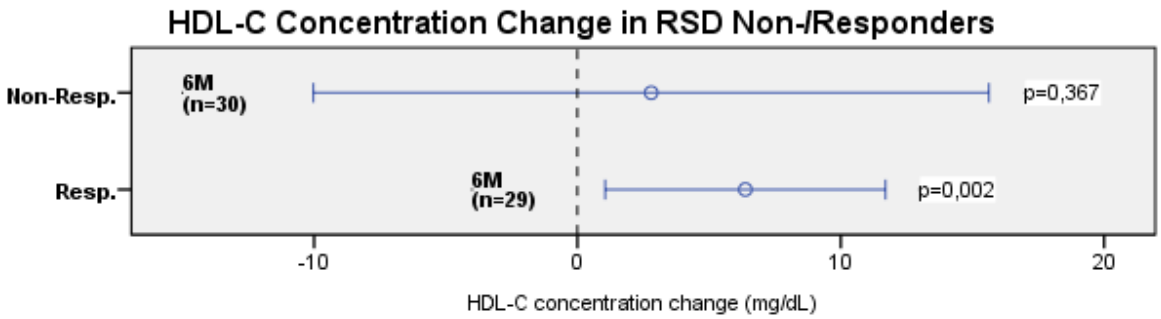



*Figure 36. HDL-cholesterol concentration changes at 6 months after renal sympathetic denervation in the RSD responder and non-responder group, respectively. Whiskers indicate 5 and 95 percentile, p-values are for comparison with baseline values.*

Both baseline values (p=0,783) and mean differences at six months (p=0,349) did not differ significantly between the two groups.

#### 3.4.2.3. Correlations of HDL-C and Blood Pressure Values

Graphical and mathematical analysis of correlations between HDL-C, ABP and OBP values at baseline and the differences at six months was conducted using the modified dataset for ABP values.

Analysis revealed no significant correlation between those values. HDL-C differences at six months were significantly correlated with baseline HDL-C values (r=-318; p=0,011).

### 3.4.3. Total Blood Cholesterol Concentration

#### 3.4.3.1. Total Cholesterol Concentration in All Patients

Total blood cholesterol concentration was measured in n=77 patients at baseline and n=64 at six months, average concentrations at those examination dates were 194,5mg/dL (42,2) and 197,0mg/dL (40,1).

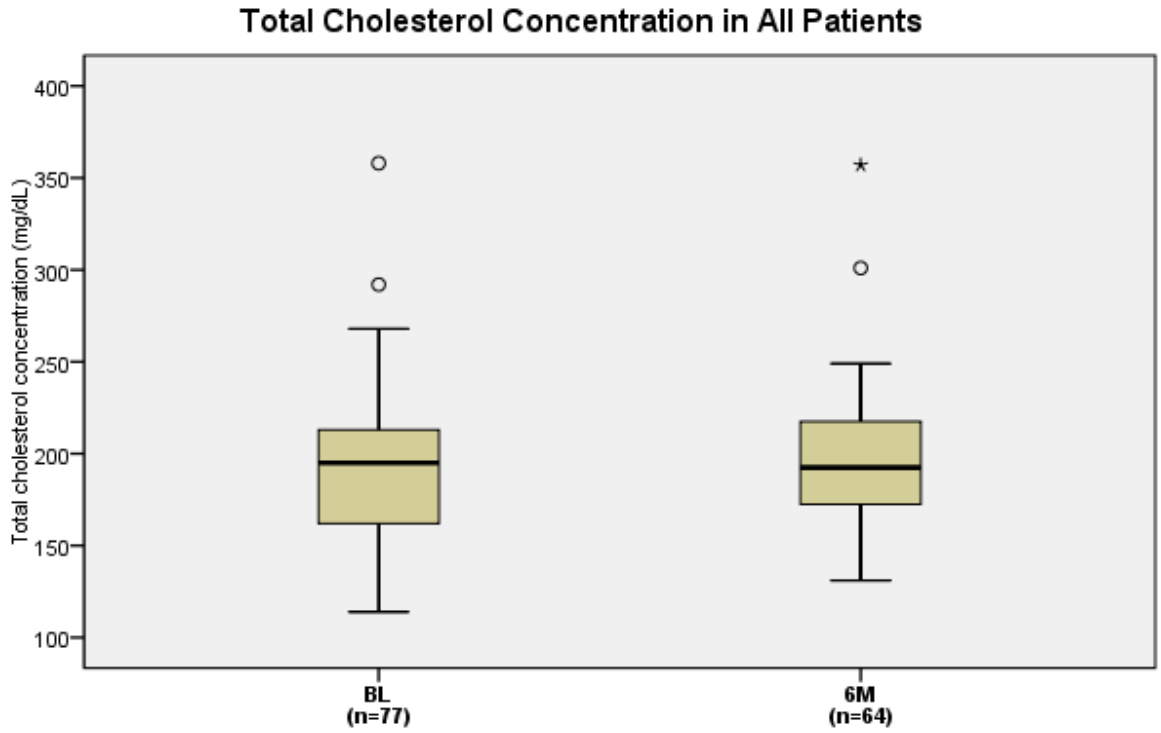



*Figure 37. Total cholesterol concentration in all patients at baseline and 6 months after renal sympathetic denervation. Whiskers indicate 5 and 95 percentile.*

In the group of patients, who attended the six months follow up visit (n=63), mean total cholesterol concentration change amounted to 4,5mg/dL (-1,7;10,7 p=0,153).

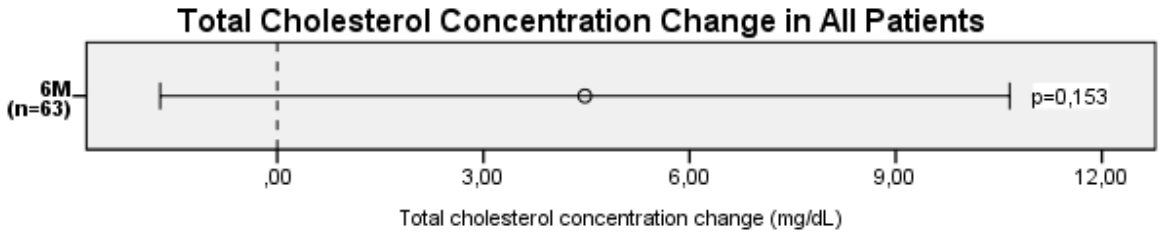



*Figure 38. Total cholesterol concentration change in all patients at 6 months after renal sympathetic denervation. Whiskers indicate 5 and 95 percentile, p-value is for comparison with baseline value.*

### 3.4.3.2. Total Cholesterol Concentration in RSD Non-/Responders

In the RSD responders group, baseline total cholesterol concentration levels were assessed in n=32 patients, whose mean concentration amounted to 184,2mg/dL (34,8) and to 194,4mg/dL (35,5) after six months of follow up (n=30). Among RSD non-responders, total cholesterol concentration averaged out at 200,6mg/dL (52,0) at baseline (n=32) and at 198,7mg/dL (46,2) at six months (n=30).

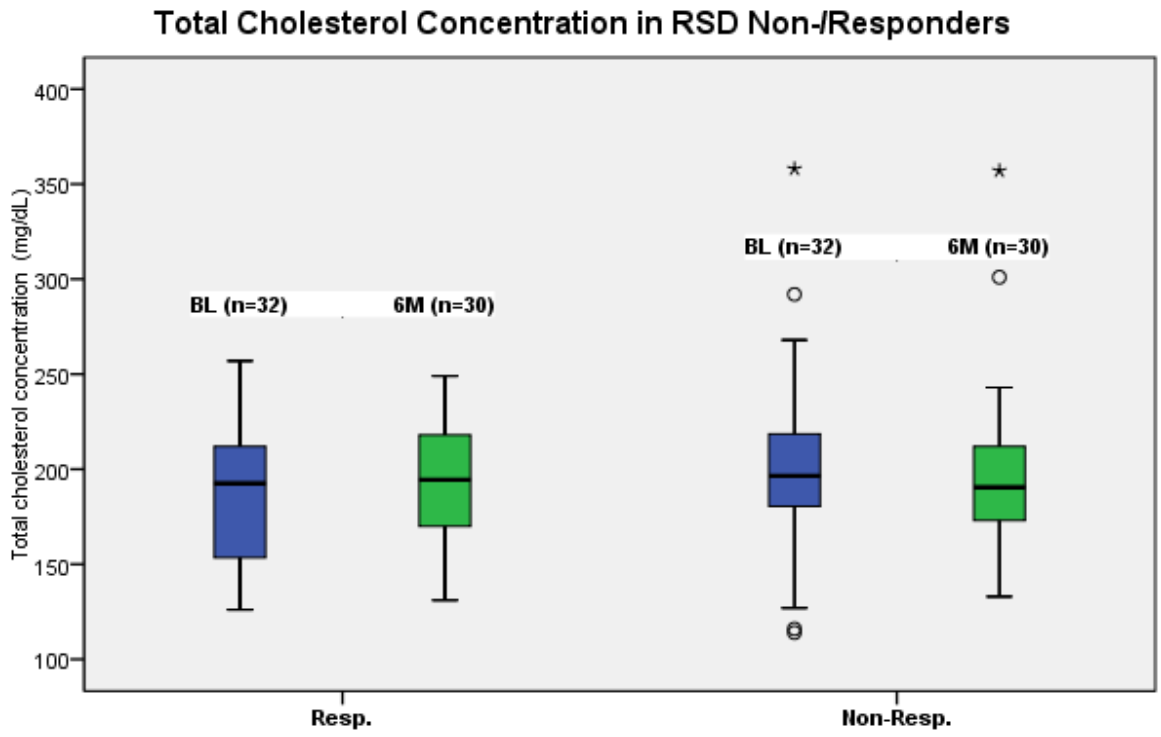



*Figure 39. Total cholesterol concentration at baseline and 6 months after renal sympathetic denervation in the RSD responder and the non-responder group, respectively. Whiskers indicate 5 and 95 percentile.*

In the patients considered RSD responders, who completed follow up of values (n=29), mean change in total cholesterol concentration was 11,3mg/dL (1,6;21,0 p=0,066). RSD non-responders (n=30) had a mean difference averaging out at -0,2mg/dL (-8,7;8,3 p=0,854).

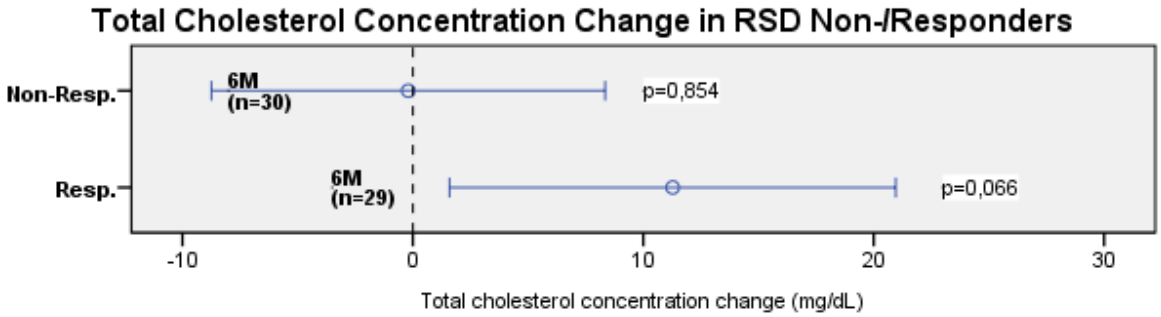



*Figure 40. Total cholesterol concentration changes at 6 months after renal sympathetic denervation in the RSD responder and non-responder group, respectively. Whiskers indicate 5 and 95 percentile, p-values are for comparison with baseline values.*

Between the two groups, no statistically significant differences concerning baseline concentrations (p=0,334) or differences at six months (p=0,203) could be detected.

#### 3.4.3.3. Correlations of Total Cholesterol and Blood Pressure Values

Graphical and mathematical analysis of correlations between total cholesterol concentration, ABP and OBP values at baseline and the differences at six months was conducted using the modified dataset for ABP values.

Analysis revealed no significant correlation between those values. However, total cholesterol concentration differences at six months were significantly correlated with baseline total cholesterol concentrations (r=-0,448; p<0,001).

### 3.4.4. Non-HDL-Cholesterol (non-HDL-C)

#### 3.4.4.1. Non-HDL-Cholesterol Concentration in All Patients

Non-HDL-C was assessed in n=77 patients at baseline and in n=64 at six months, average values at those examination dates were 146,0mg/dL (53,9) and 144,0mg/dL (39,4).

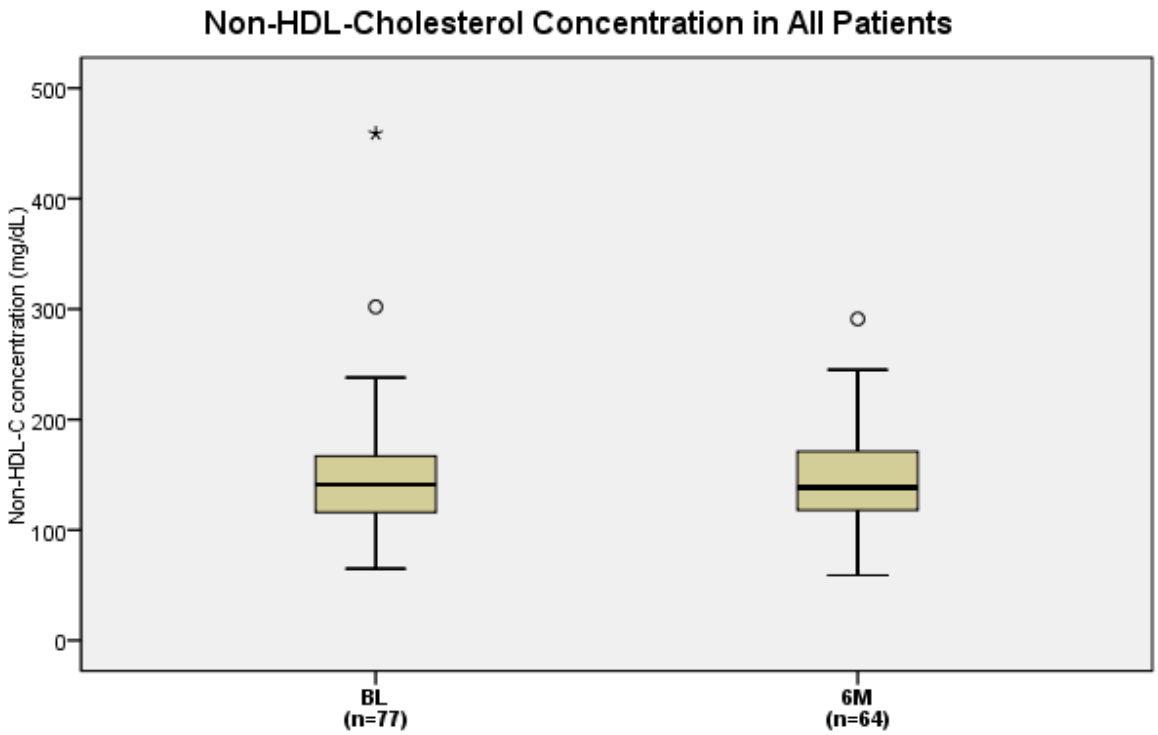



*Figure 41. Non-HDL-cholesterol concentration in all patients at baseline and 6 months after renal sympathetic denervation. Whiskers indicate 5 and 95 percentile.*

Among the patients, who attended the six month follow up visit (n=63), non-HDL-C levels changed by an average of 3,4mg/dL (-2,5;9,3 p=0,537).

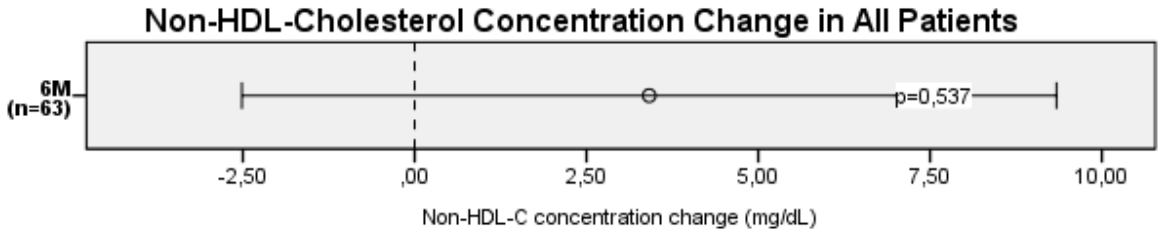



*Figure 42. Non-HDL-cholesterol concentration change in all patients at 6 months after renal sympathetic denervation. Whiskers indicate 5 and 95 percentile, p-value is for comparison with baseline value.*

### 3.4.4.2. Non-HDL-Cholesterol in RSD Non-/Responders

In the RSD responders and non-responders group, non-HDL-C concentration was assessed in n=32 patients at baseline and in n=30 at six months. Mean concentration among responders amounted to 133,3mg/dL (33,3) at baseline and to 140,1mg/dL (36,0) at six months. RSD non-responders had a mean baseline concentration of 149,1mg/dL (48,9), that changed to 147,1mg/dL (44,0) during follow up.

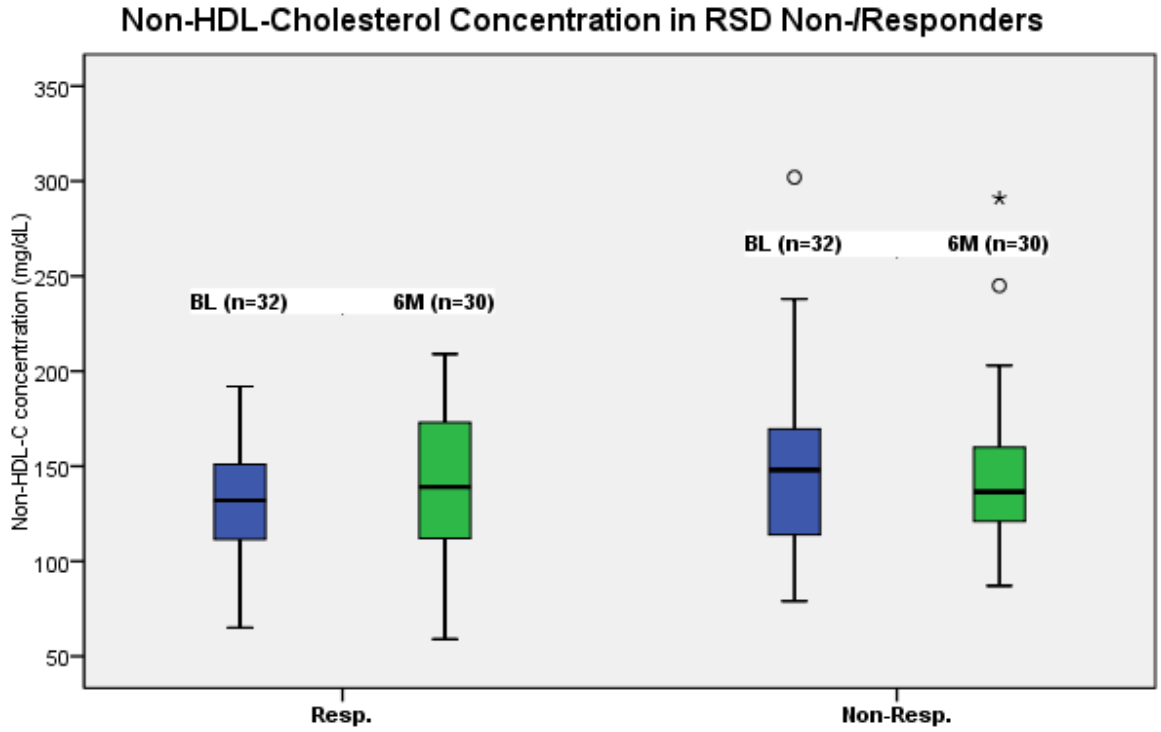



*Figure 43. Non-HDL-cholesterol concentration at baseline and 6 months after renal sympathetic denervation in the RSD responder and the non-responder group, respectively. Whiskers indicate 5 and 95 percentile.*

Among RSD responders, complete follow up of values was available in n=29 patients, whose non-HDL-C changed by 8,4mg/dL (-1,3;18,0 p=0,087) on average. RSD non-responders (n=30) witnessed a mean change of -0,8mg/dL (-9,00;7,5 p=0,850) within six months.

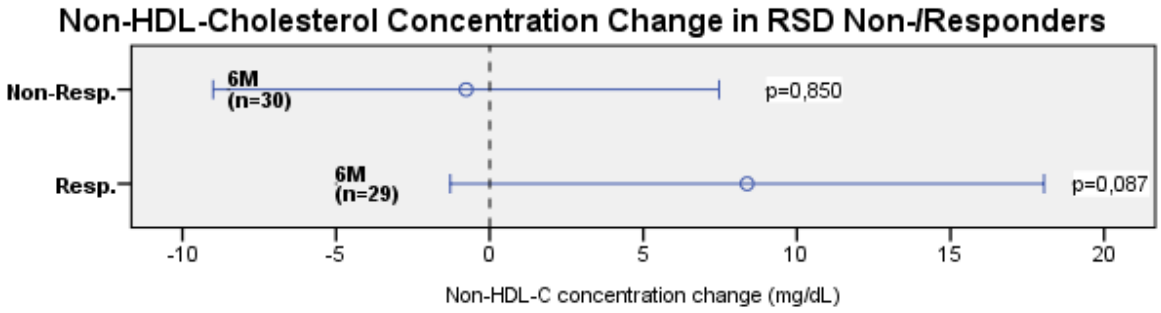



*Figure 44. Non-HDL-cholesterol concentration changes at 6 months after renal sympathetic denervation in the RSD responder and non-responder group, respectively. Whiskers indicate 5 and 95 percentile, p-values are for comparison with baseline values.*

Neither differences in baseline values (p=0,204), nor in changes at six months (p=0,146) were proven to differ statistically significant between the two groups.

#### 3.4.4.3. Correlations of Non-HDL-Cholesterol and Blood Pressure Values

Graphical and mathematical analysis of correlations between and non-HDL-C, ABP and OBP values at baseline and the differences at six months was conducted using the modified dataset for ABP values.

Analysis revealed no significant correlation between those values. However, non-HDL-C concentration differences at six months were significantly correlated with baseline non-HDL-C levels (r=-0,397; p=0,001).

### 3.4.5. Fasting Triglycerides

#### 3.4.5.1. Fasting Triglyceride Concentration in All Patients

Fasting blood triglyceride concentrations were available in n=77 patients at baseline, averaging out at 167,4mg/dL (123,7), and in n=64 at six months with a mean concentration amounting to 166,1mg/dL (92,2). Among the patients, in whom complete follow up of values was available (n=63), mean fasting triglyceride concentration significantly increased by 7,7mg/dL (-10,4;25,8 p=0,033).

After exclusion of all patients with triglyceride concentrations >400mg/dL (n=4), the remaining n=73 patients at baseline had an average concentration amounting to 144,0mg/dL (63,6), while mean concentration at six months (n=61) averaged out at 155,8mg/dL (68,2).

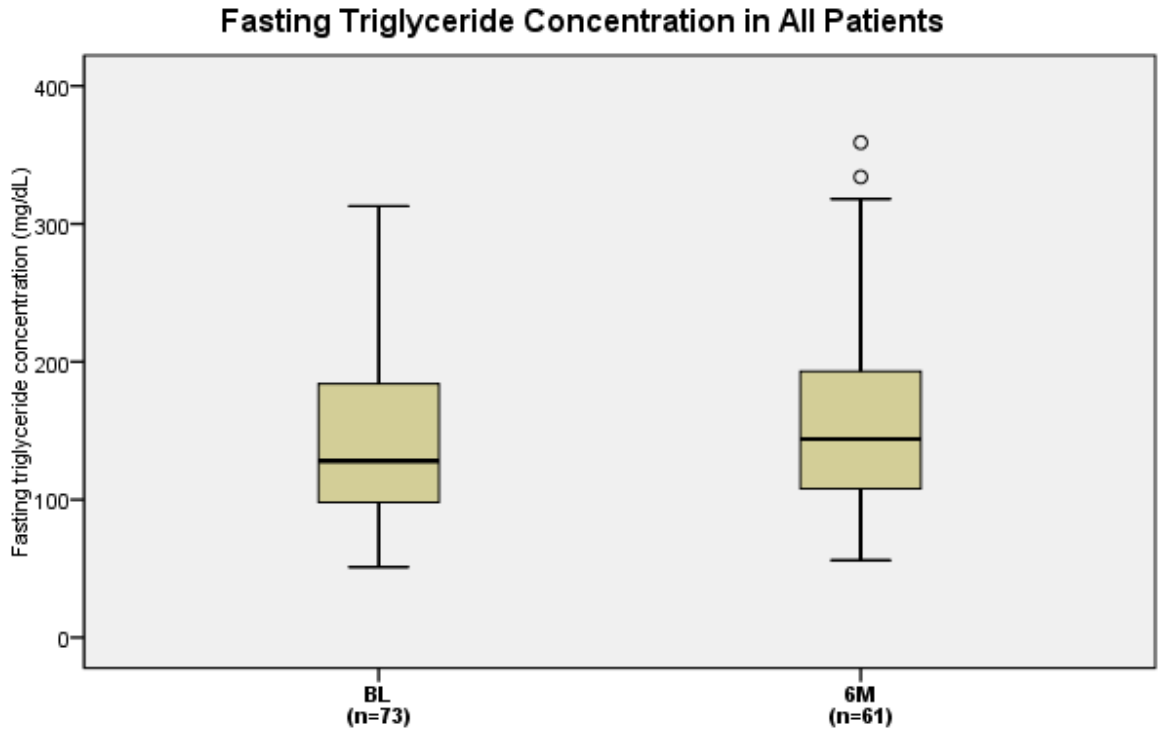



*Figure 45. Triglyceride concentration in all patients at baseline and 6 months after renal sympathetic denervation. Whiskers indicate 5 and 95 percentile. Patients with triglyceride concentrations >400mg/dL (n=4) were excluded from this analysis.*

Patients, in whom complete follow up of values was available (n=60), had a significant increase in fasting triglyceride concentration at by 14,4mg/dL (2,3;26,4 p=0,020).

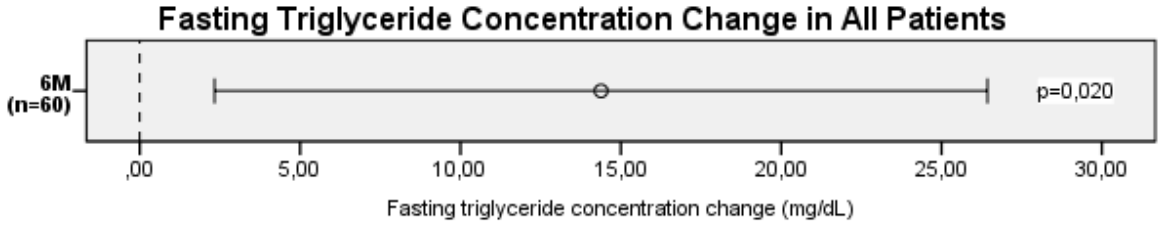



*Figure 46. Triglyceride concentration change in all patients at 6 months after renal sympathetic denervation. Whiskers indicate 5 and 95 percentile, p-value is for comparison with baseline value. Patients with triglyceride concentrations >400mg/dL (n=4) were excluded from this analysis.*

### 3.4.5.2. Fasting Triglyceride Concentration in RSD Non-/Responders

The modified dataset excluding all patients with fasting triglyceride concentration values >400mg/dL was used in this analysis. Mean triglyceride concentration among patients considered RSD responders was 144,9 mg/dL (61,7) at baseline (n=29) and 155,5mg/dL (79,6) at six months (n=28). Non-responders displayed average concentrations of 140,7mg/dL (59,5) at baseline (n=31) and 157,6mg/dL (61,5) at six months (n=29).

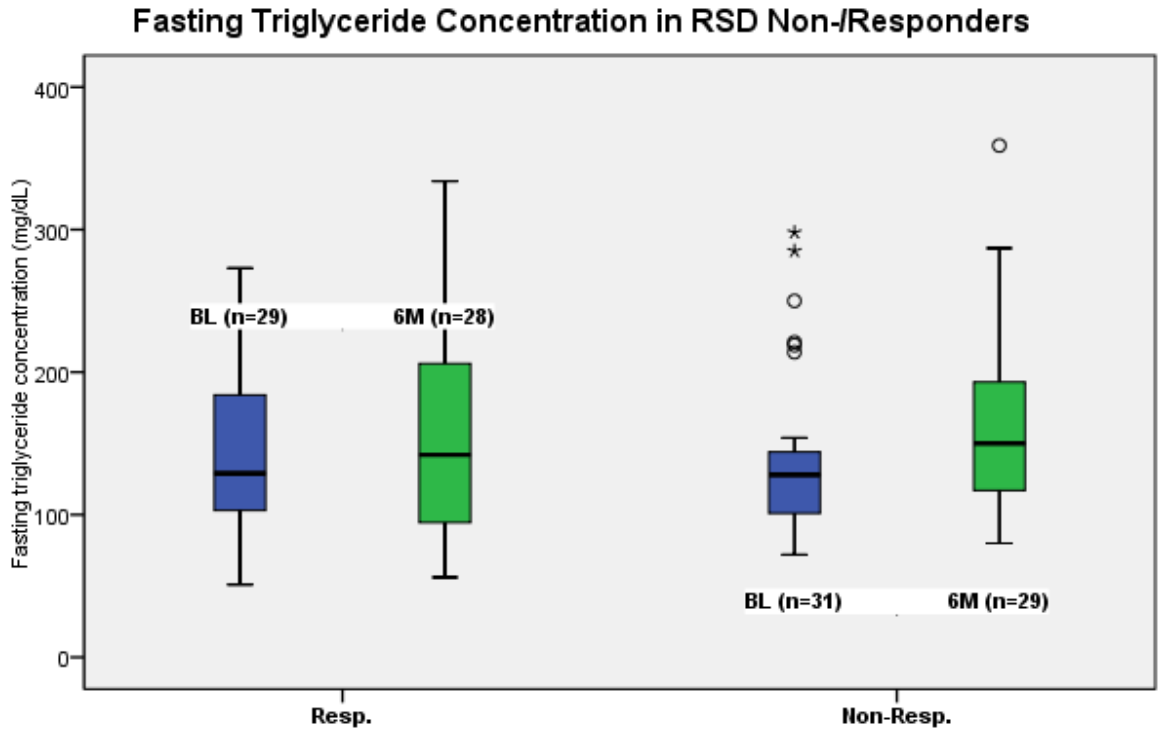



*Figure 47. Triglyceride concentration at baseline and 6 months after renal sympathetic denervation in the RSD responder and the non-responder group, respectively. Whiskers indicate 5 and 95 percentile. Patients with triglyceride concentrations >400mg/dL (n=4) were excluded from this analysis.*

Paired follow up data was available in n=27 RSD responders, who witnessed a mean triglyceride concentration change by 11,9mg/dL (-7,1;30,9 p=0,210). Among RSD non-responders (n=29), average difference amounted to 16,6mg/dL (-1,6;34,7 p=0,073).

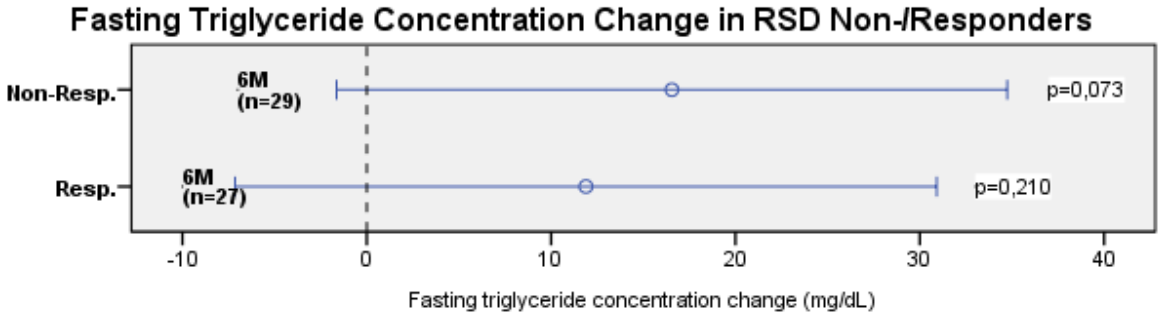



*Figure 48. Triglyceride concentration changes at 6 months after renal sympathetic denervation in the RSD responder and non-responder group, respectively. Whiskers indicate 5 and 95 percentile, p-values are for comparison with baseline values. Patients with triglyceride concentrations >400mg/dL (n=4) were excluded from this analysis.*

Neither baseline values (p=0,767), nor mean changes at six months (p=0,549) differed significantly between the groups.

#### 3.4.5.3. Correlations of Fasting Triglyceride Concentration and Blood Pressure Values

Graphical and mathematical analysis of correlations between fasting triglyceride concentration, ABP and OBP values at baseline and the differences at six months was conducted using the modified dataset for fasting triglyceride concentration and ABP values. Analysis revealed no significant correlation between those values.

## 3.5. Inflammatory Parameters

### 3.5.1. C-Reactive Protein (CRP)

#### 3.5.1.1. C-Reactive Protein Concentration in All Patients

Mean overall CRP-concentration in plasma was 0,47mg/dL (0,72) at baseline (n=78), 0,44mg/dL (0,46) at six months (n=70) and 0,44mg/dL (0,44) at twelve months (n=59). Among the patients, who completed follow up of values, CRP concentration had changed by a mean difference of 0,03mg/l (-0,11;0,16 p=0,720) at six months (n=70) and by -0,004mg/dL (-0,13;0,12 p=0,465) at one year (n=59).

In order to arrange the statistics more clearly, all patients with values >1,5mg/dL (n=5) were excluded for further analyses, resulting in the following development of mean CRP concentration values: 0,36mg/dL (0,27) at baseline (n=73), 0,37mg/dL (0,29) at six months (n=66) and 0,40mg/dL (0,36) at twelve months (n=55).

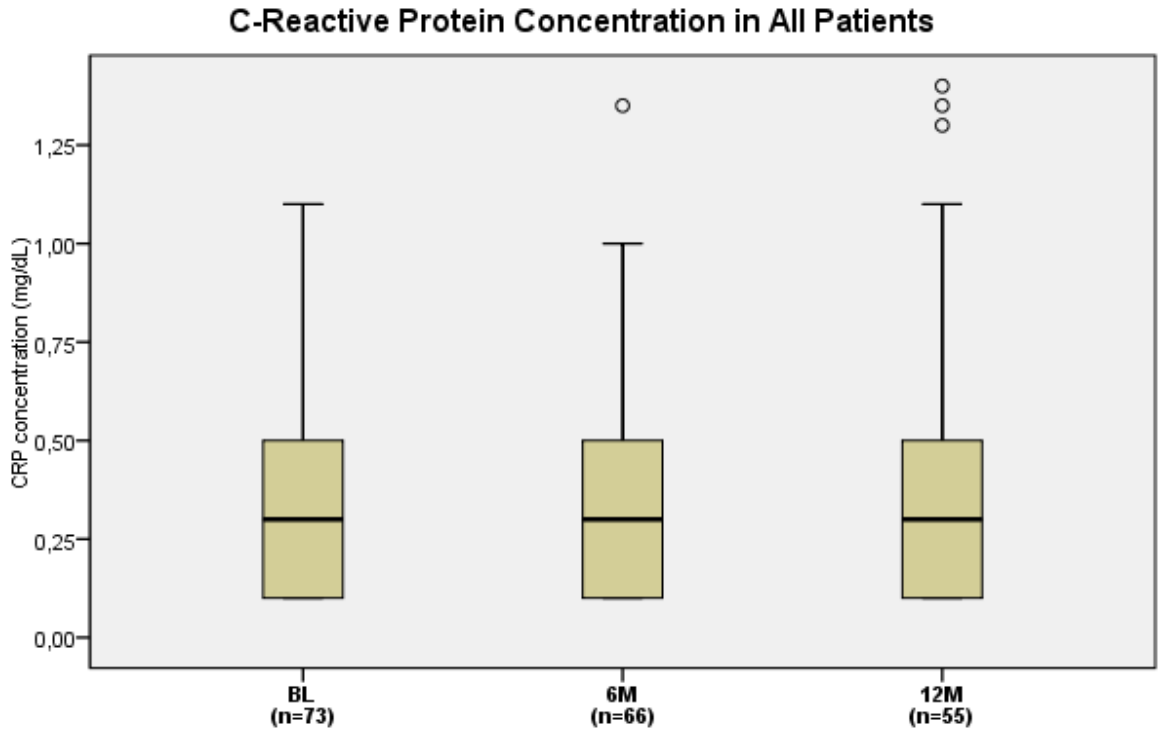



*Figure 49. C-reactive protein (CRP) concentration in all patients at baseline, 6 and 12 months after renal sympathetic denervation. Whiskers indicate 5 and 95 percentile. Patients with CRP concentrations >1,5mg/dL (n=5) were excluded from this analysis.*

Mean change in CRP-concentration was 0,01mg/dL (-0,05;0,07 p=0,891) among the patients, who completed six months follow up of values (n=66) and 0,02mg/dL (-0,07;0,11 p=0,646) in the one year follow up group (n=55).

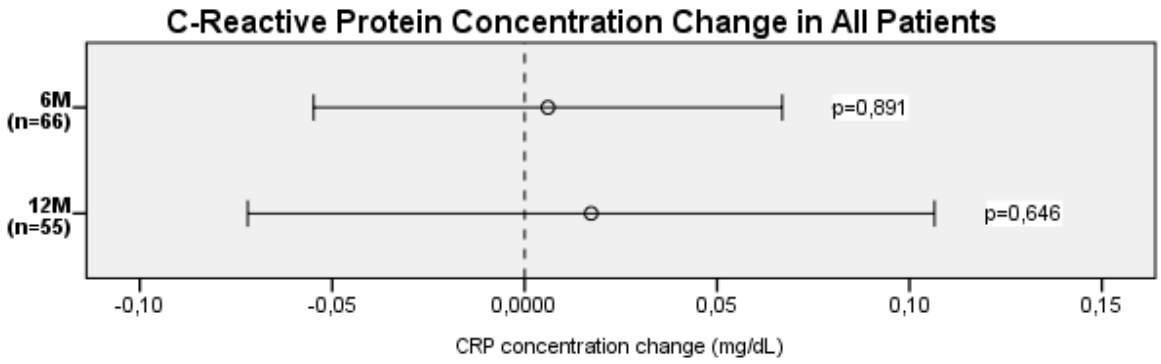



*Figure 50. C-reactive protein (CRP) concentration changes at 6 and at 12 months after renal sympathetic denervation. Whiskers indicate 5 and 95 percentile, p-values are for comparison with baseline values. Patients with CRP concentrations >1,5mg/dL (n=5) were excluded from this analysis.*

### 3.5.1.2. CRP Concentration in RSD Non-/Responders

The modified dataset excluding all patients with CRP concentration values >1,5mg/dL was used in this analysis. In the group of patients considered RSD responders, average CRP

concentration was 0,37mg/dL (0,26) at baseline (n=30), 0,34mg/dL (0,30) at six months (n=30) and 0,35mg/dL (0,34) at twelve months (n=27). Among RSD non-responders, average concentration developed from 0,36mg/dL (0,29) at baseline (n=31) to 0,38mg/dL (0,30) at six months (n=31) and 0,44mg/dL (0,39) at twelve months (n=25).

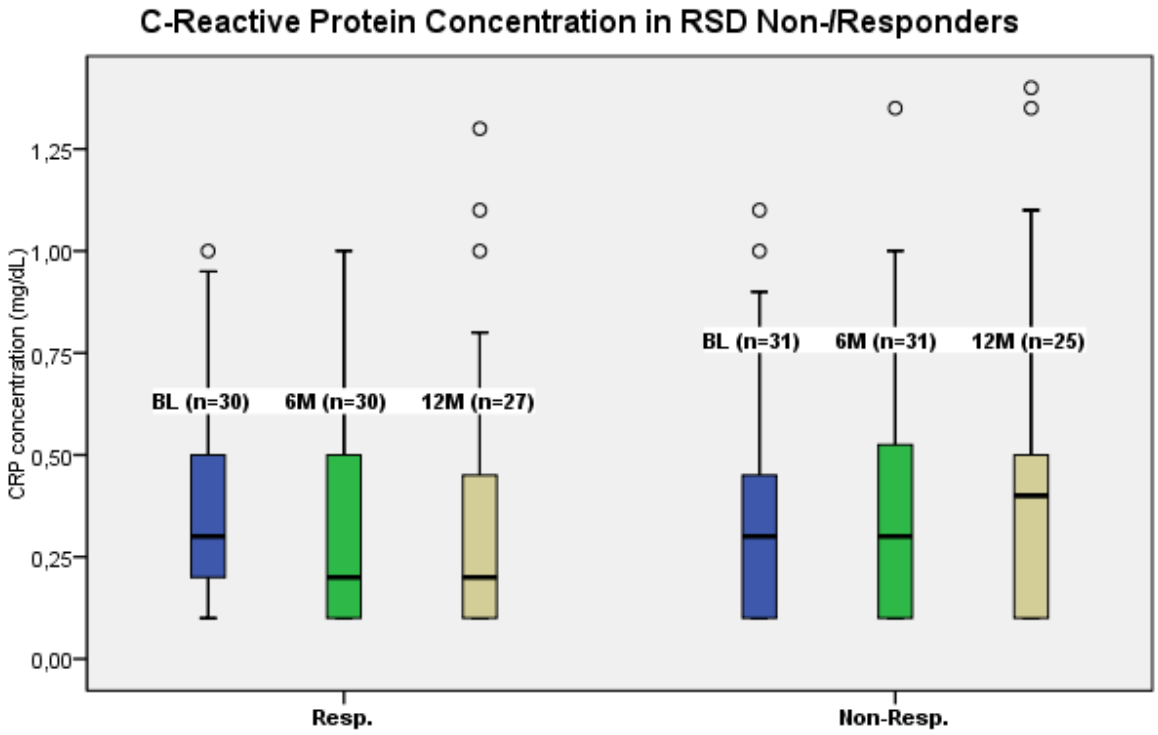



*Figure 51. C-reactive protein (CRP) concentration at baseline, 6 and 12 months after renal sympathetic denervation in the RSD responders and non-responders group, respectively. Whiskers indicate 5 and 95 percentile. Patients with CRP concentrations >1,5mg/dL (n=5) were excluded from this analysis.*

Among patients considered RSD responders, mean difference at six months (n=30) averaged out at -0,02mg/dL (-0,10;0,06 p=0,211), mean change at twelve months (n=27) amounted to -0,01mg/L (-0,13;0,12 p=0,355). RSD non-responders (n=31) had a mean CRP concentration change by 0,01mg/L (-0,09;0,12 p=0,861) and by 0,03mg/L (-0,12;0,18 p=0,938), at six and twelve months, respectively.

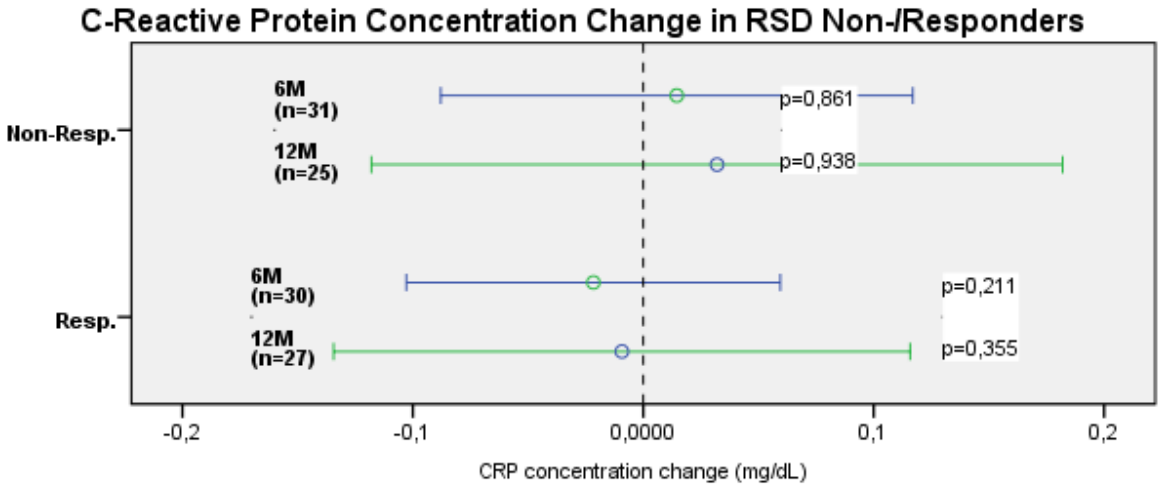



*Figure 52. C-reactive protein (CRP) concentration changes at 6 and 12 months after renal sympathetic denervation in the RSD responder and non-responder group, respectively. Whiskers indicate 5 and 95 percentile, p-values are for comparison with baseline values. Patients with CRP concentrations >1,5mg/dL (n=5) were excluded from this analysis.*

Neither baseline values (p=0,803), nor changes at six (p=0,231) and twelve months (p=0,302) could be shown to differ statistically significant between the two groups.

#### 3.5.1.3. Correlations of CRP Concentration and Blood Pressure Values

Graphical and mathematical analysis of correlations between CRP concentrations values, ABP and OBP at baseline and the differences at six months was conducted using the modified datasets both for CRP concentrations and for ABP values.

Analysis revealed no significant correlation between those values. CRP concentration differences at six months were significantly correlated with baseline CRP concentrations (r=-0,362; p=0,003).

### 3.5.2. Interleukin-6

#### 3.5.2.1. Interleukin-6 Concentration in All Patients

Interleukin-6 in plasma was measured in all n=78 patients at baseline, n=66 at six months and n=56 at twelve months. Average Il-6 levels were 5,3pg/mL (3,0), 5,0pg/mL (3,4) and 3,9pg/mL (3,5) at the follow up examinations.

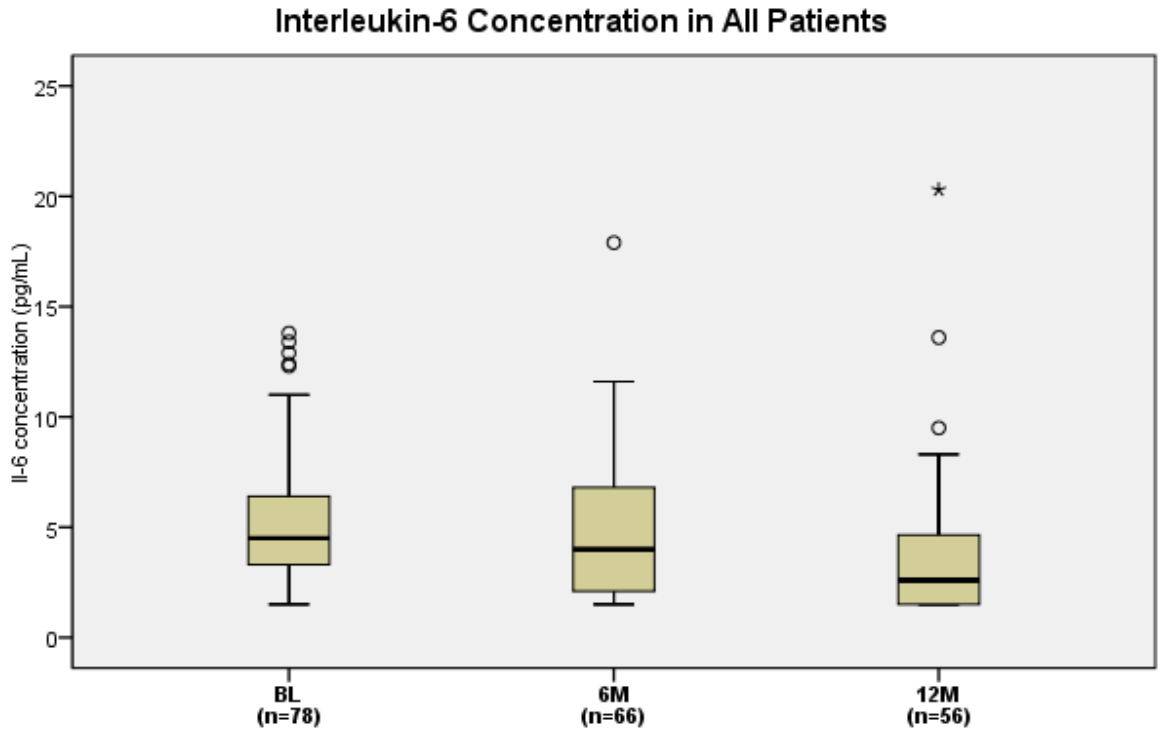



*Figure 53. Interleukin-6 (Il-6) concentration in all patients at baseline, 6 and 12 months after renal sympathetic denervation. Whiskers indicate 5 and 95 percentile.*

Patients, in whom complete six months (n=66) and twelve months (n=56) follow up of values was available, had significant decreases in Il-6 concentrations by -0,5pg/mL (-1,3;0,2 p=0,042) and by-1,7pg/mL (-2,7;-0,7 p<0,001) on average, respectively.

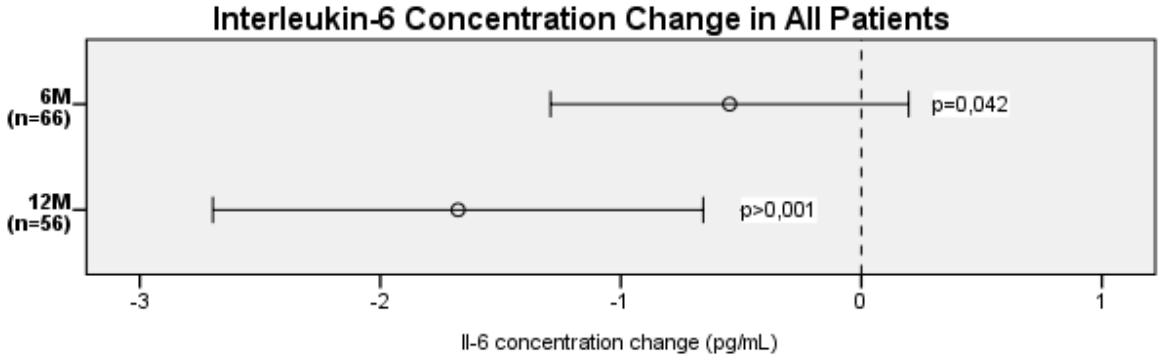



*Figure 54. Interleukin-6 (Il-6) concentration changes in all patients at 6 and at 12 months after renal sympathetic denervation. Whiskers indicate 5 and 95 percentile, p-values are for comparison with baseline values.*

### 3.5.2.2. Il-6 Concentration in RSD Non-/Responders

RSD Responders had average Il-6 concentrations of 5,7pg/mL (2,9) at baseline (n=33), 4,7pg/mL (3,5) at six months (n=32) and 4,2pg/mL (2,9) at twelve months (n=29). Among

the patients considered RSD non-responders, Il-6 levels amounted to 5,0pg/mL (3,2) at baseline (n=32), 5,4pg/mL (3,4) at six (n=29) and 3,8pg/mL (4,2) at twelve months (n=24).

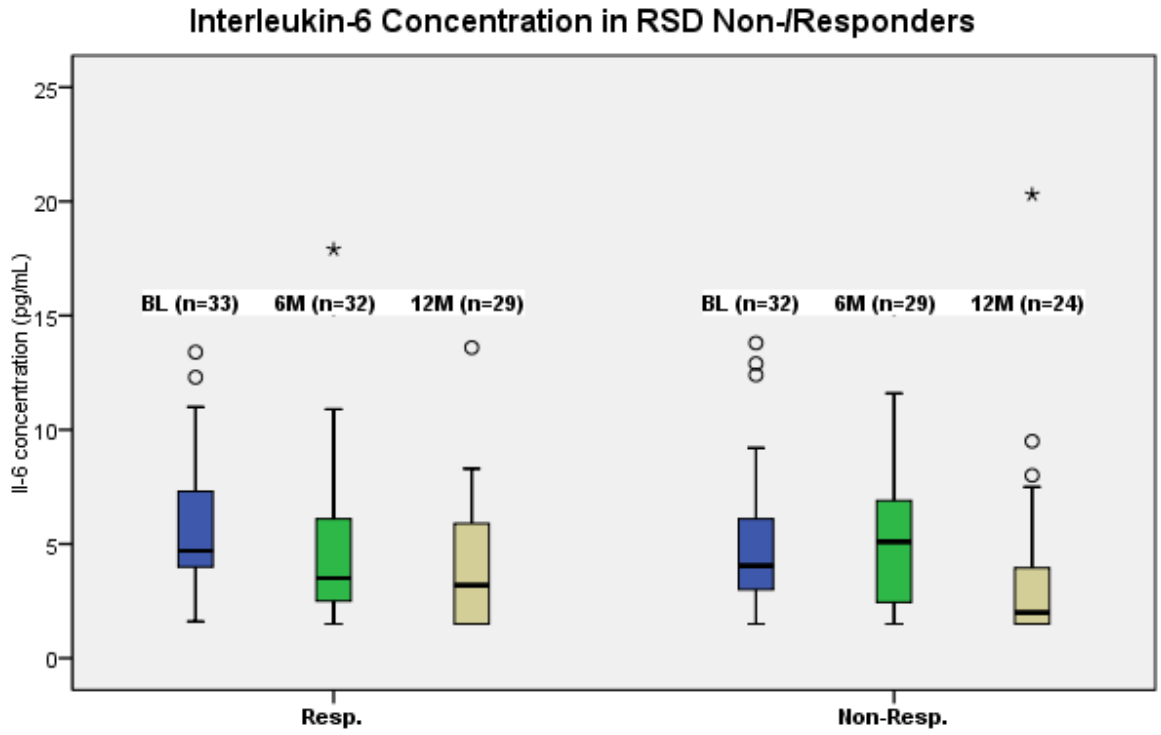



*Figure 55. Interleukin-6 (Il-6) concentration at baseline, 6 and 12 months after renal sympathetic denervation in the RSD responders and non-responders group, respectively. Whiskers indicate 5 and 95 percentile.*

Patients considered RSD responders, who attended the six months follow up visit (n=32), had an average decrease in Il-6 concentration by -1,1pg/mL (-2,4;0,1 p=0,012). Among the responders with complete one year follow up available (n=29), values further decreased by -1,7pg/mL (-2,9;-0,5 p=0,002). RSD non-responders, who completed six months (n=29) or twelve months of follow up (n=24) of values, displayed a mean change by 0,2pg/mL (-0,7;1,1 p=0,776) and a significant decrease by -1,4pg/mL (-3,4;0,5 p=0,004), respectively.

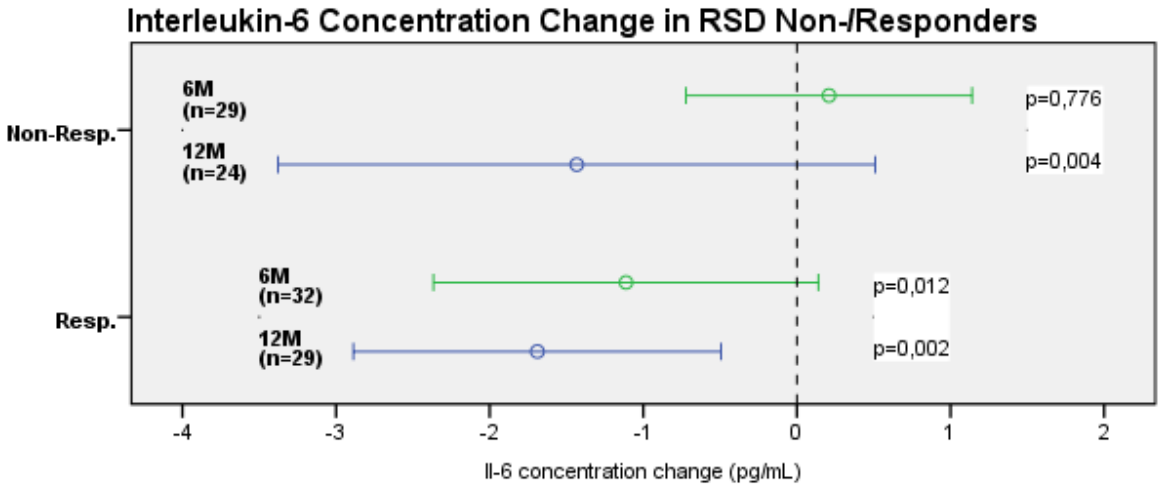



*Figure 56. Interleukin-6 (Il-6) concentration changes at 6 and 12 months after renal sympathetic denervation in the RSD responder and non-responder group, respectively. Whiskers indicate 5 and 95 percentile, p-values are for comparison with baseline values.*

The between-group difference in Il-6 concentration changes at six months deemed statistically significant (p=0,024), while changes at one year (p=0,514) and baseline concentrations (p=0,143) did not differ significantly.

### 3.5.2.3. Correlations of Il-6 Concentrations and Blood Pressure Values

Graphical and mathematical analysis of correlations between Il-6 concentrations, ABP and OBP values at baseline and the differences at six months was conducted using the modified dataset for ABP values.

Analysis revealed statistically significant correlations between baseline Il-6 concentration and baseline ABP values (r=0,255; p=0,027), as well as with ABP differences at six months (r=-0,295; p=0,020). Changes in Il-6 concentrations at six months were significantly correlated with baseline ABP (r=-0,321; p=0,010) and with baseline Il-6 levels (r=-0,367; p=0,002).

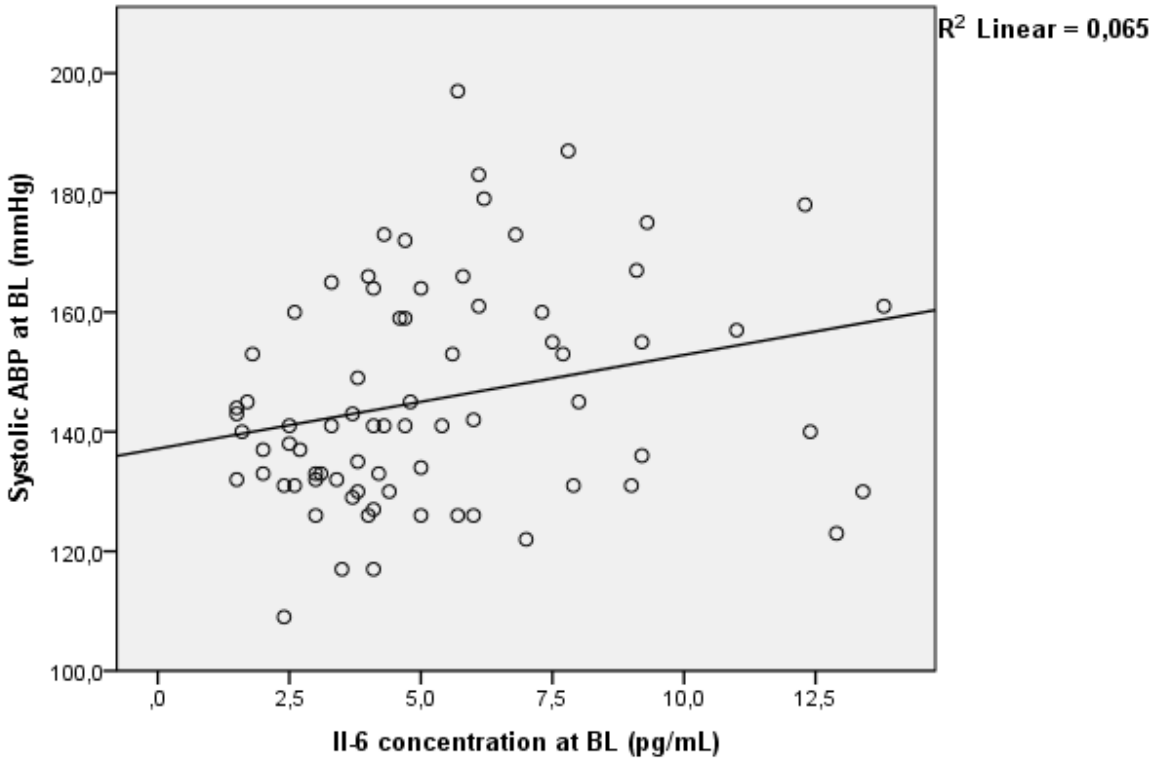



*Figure 57. Relationship between Il-6 concentration and ABP values at baseline (n=75). Modified dataset was used for ABP values.*

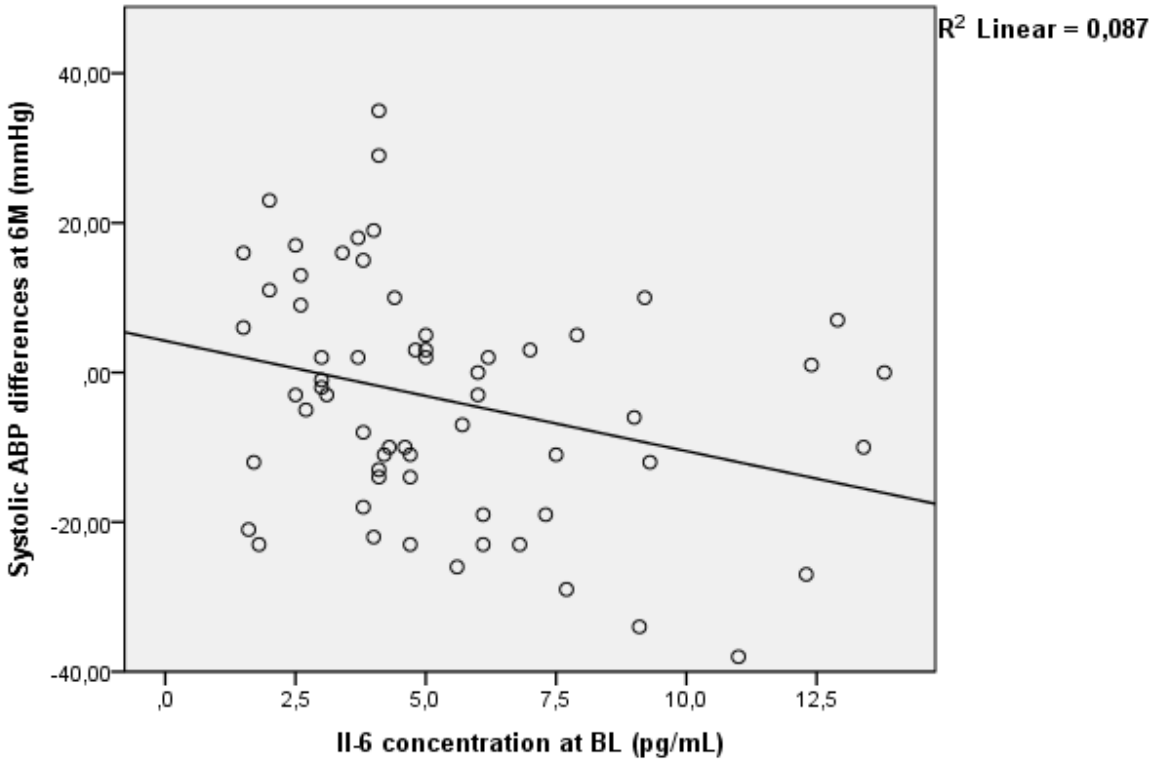



*Figure 58. Relationship between Il-6 concentration at baseline and ABP changes at six months (n=62). Modified dataset was used for ABP values.*

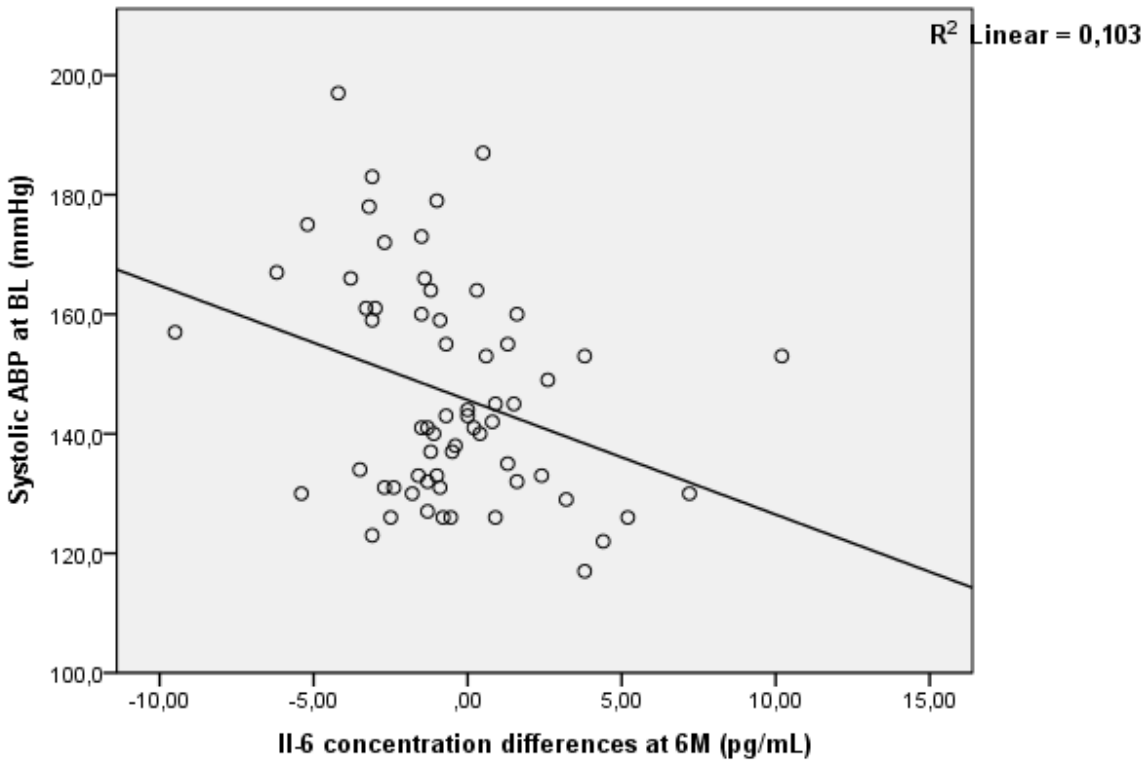



*Figure 59. Relationship between Il-6 concentration changes at six months and ABP values at baseline (n=62). Modified dataset was used for ABP values.*

## 3.5.3. White Blood Cell Count

### 3.5.3.1. White Blood Cell Count in All Patients

Leukocyte counts were conducted in all n=78 patients at baseline, in n=69 at six months and n=58 at one year. Developing of mean WBC count was $8,1*10^3/\mu l$ (2,1), $7,7*10^3/\mu l$ (1,7) and $7,3*10^3/\mu l$ (2,3) at the baseline assessment and the follow up examinations.

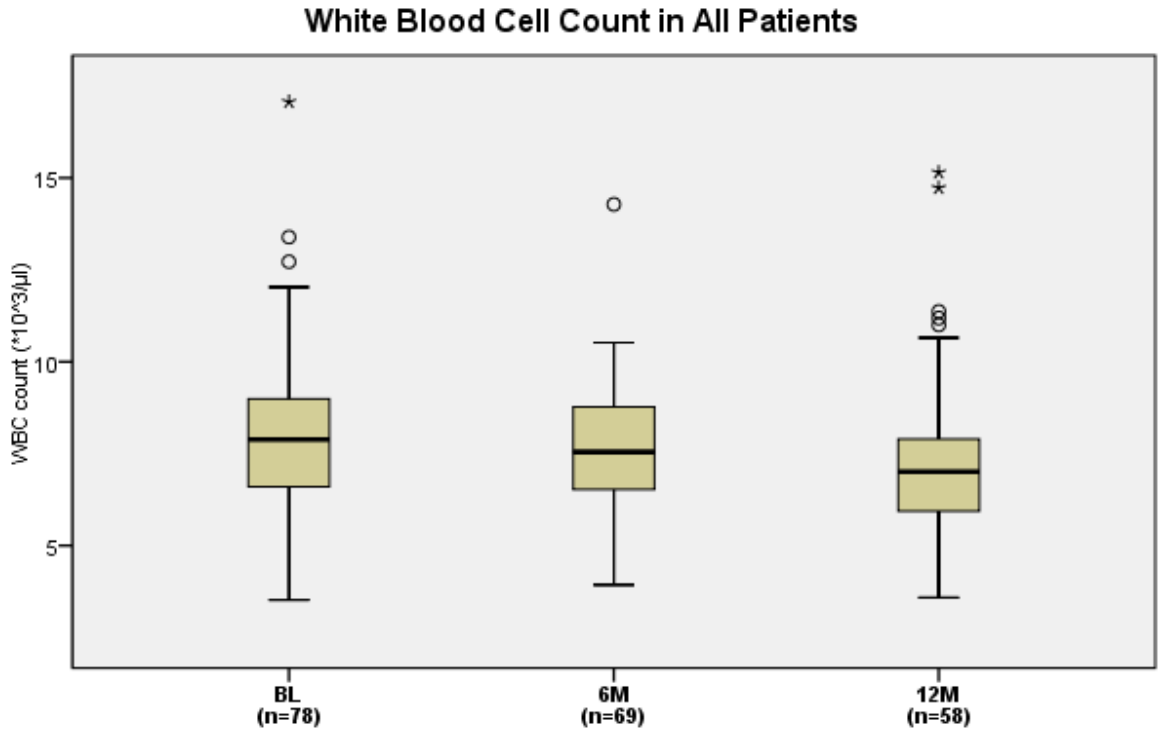



*Figure 60. White blood cell (WBC) count in all patients at baseline, 6 and 12 months after renal sympathetic denervation. Whiskers indicate 5 and 95 percentile.*

Six months follow up of WBC counts was completed by n=69 patients, whose values significantly decreased by an average of -0,5*$10^3$/µl (-0,8;-0,1 p=0,017). In the one year follow up group (n=58), a highly significant (p<0,001) mean decrease by -0,8*$10^3$/µl (-1,3;-0,3 p<0,001) was seen.

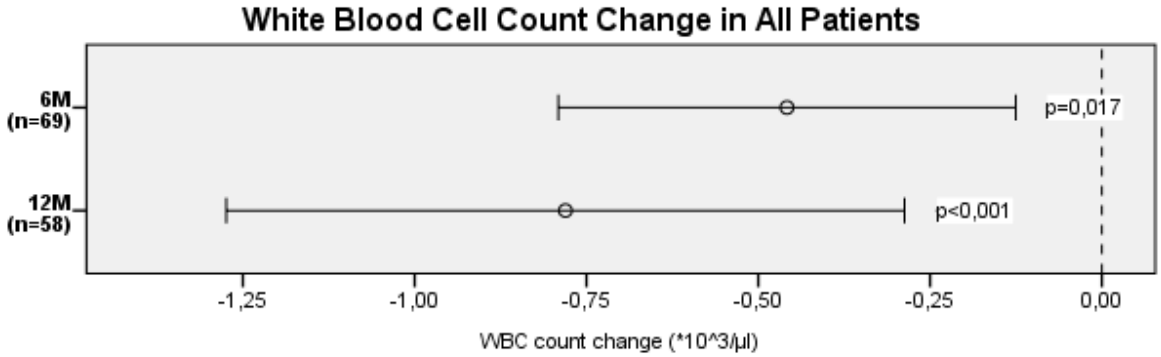



*Figure 61. White blood cell (WBC) count changes in all patients at 6 and at 12 months after renal sympathetic denervation. Whiskers indicate 5 and 95 percentile, p-values are for comparison with baseline values.*

### 3.5.3.2. White Blood Cell Count in RSD Non-/Responders

Average WBC count in the group of patients considered RSD responders was 8,2*$10^3$/µl (1,7) at baseline (n=33), 7,8*$10^3$/µl (1,4) at six months (n=33) and 7,2*$10^3$/µl (2,1) at the

one year follow up examination (n=30). Among RSD non-responders, values amounted to 8,0*$10^3$/µl (2,6) at baseline (n=32), 7,6*$10^3$/µl (2,1) at six months (n=31) and 7,4*$10^3$/µl (2,5) at twelve months (n=26).

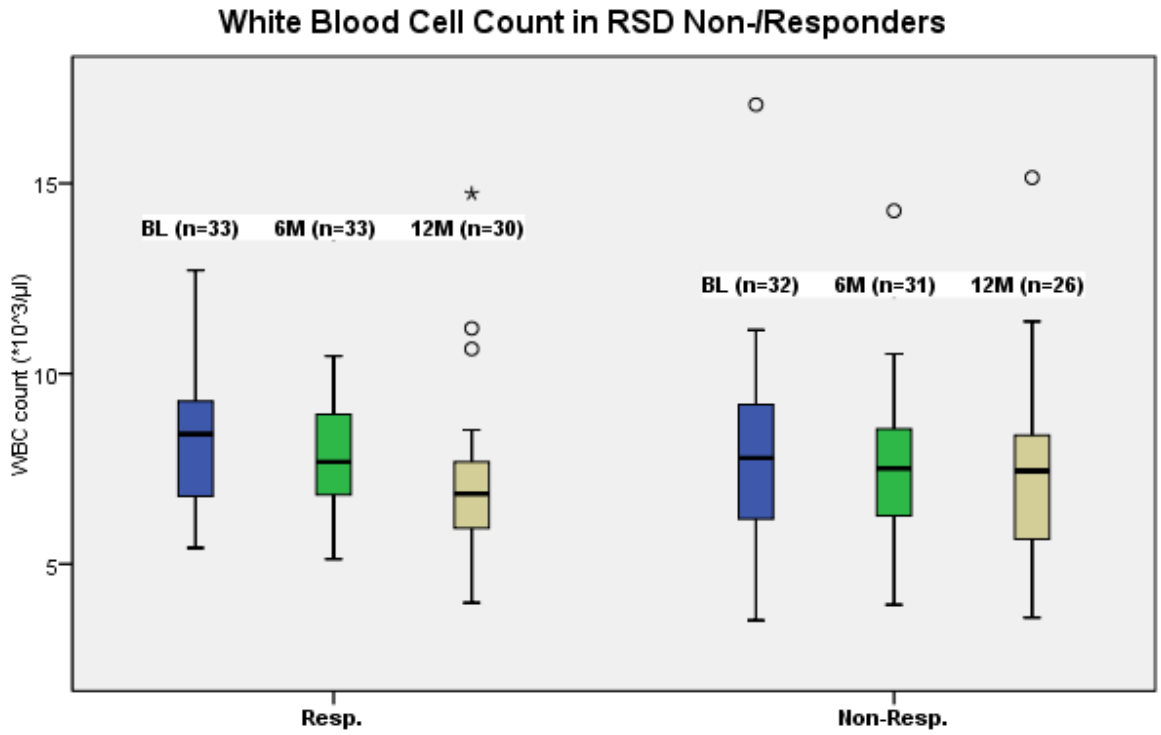



*Figure 62. White blood cell (WBC) counts at baseline, 6 and 12 months after renal sympathetic denervation in the RSD responders and non-responders group, respectively. Whiskers indicate 5 and 95 percentile.*

Among the patients considered RSD responders, n=33 patients attended the six-, and n=30 patients the twelve months follow up visit. Average WBC count changed by -0,5*$10^3$/µl (-1,0;0,1 p=0,098) and significantly by -1,0*$10^3$/µl (-1,8;-0,1 p=0,001), respectively. Non-responders displayed a mean change by 0,3*$10^3$/µl (-0,7;0,1 p=0,388) at six months (n=31) and a significant decrease by -0,6*$10^3$/µl (-1,1;-0,1 p=0,025) at one year (n=30).

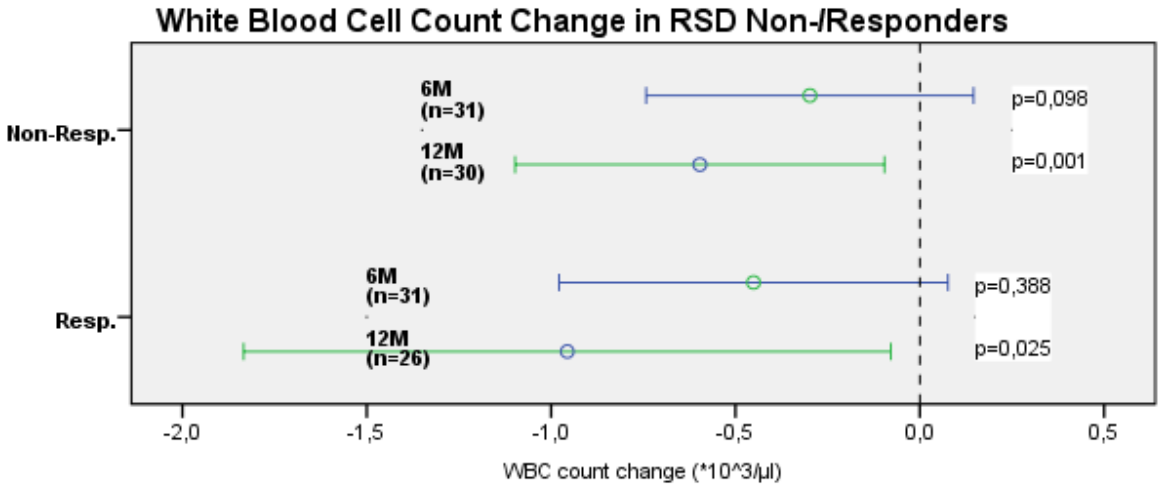



*Figure 63. White blood cell (WBC) counts at 6 and 12 months after renal sympathetic denervation in the RSD responder and non-responder group, respectively. Whiskers indicate 5 and 95 percentile, p-values are for comparison with baseline values.*

Statistical testing revealed no significant difference in baseline values (p=0,427) and mean changes at six (p=0,643) or twelve months (p=0,237) between the groups.

#### 3.5.3.3. Correlations of White Blood Cell Count and Blood Pressure Values

Graphical and mathematical analysis of correlations between WBC count, ABP and OBP values at baseline and the differences at six months was conducted using the modified dataset for ABP values.

Analysis revealed no significant correlation between those values. WBC count changes at six months were significantly correlated with baseline WBC counts (r=-0,619; p<0,001).

## 3.6. Renal Function Parameters

### 3.6.1. Serum Creatinine Concentration

#### 3.6.1.1. Serum Creatinine Concentrations in All Patients

Serum creatinine concentration was assessed among all n=78 patients at baseline, n=70 at six months and n=60 at one year. Overall mean creatinine concentrations at the follow up examinations were 1,00mg/dL (0,38), 1,01mg/dL (0,45) and 1,03mg/dL (0,46).

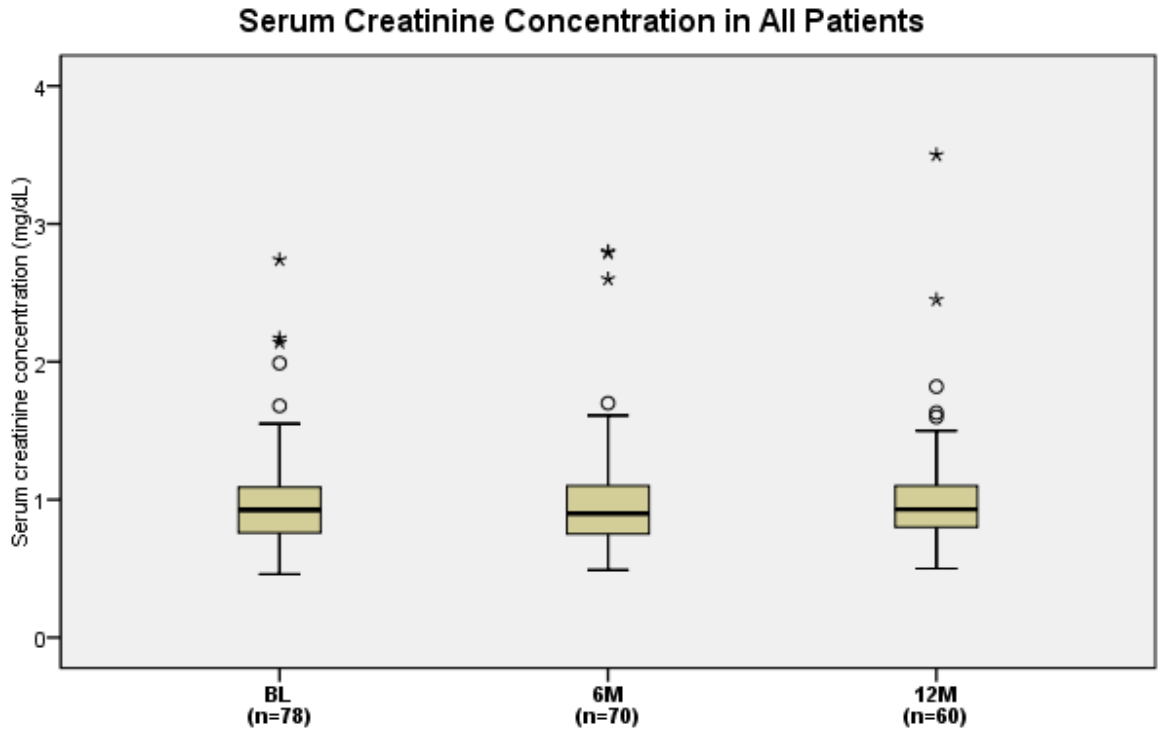



*Figure 64. Serum creatinine concentration in all patients at baseline, 6 and 12 months after renal sympathetic denervation. Whiskers indicate 5 and 95 percentile.*

In the six months follow up group (n=70), mean serum creatinine concentration difference was 0,01mg/dL (-0,03;0,06 p=0,765) on average. In the patients with complete one year follow up available (n=60), concentrations changed by 0,06mg/dL (-0,01;0,13 p=0,114).

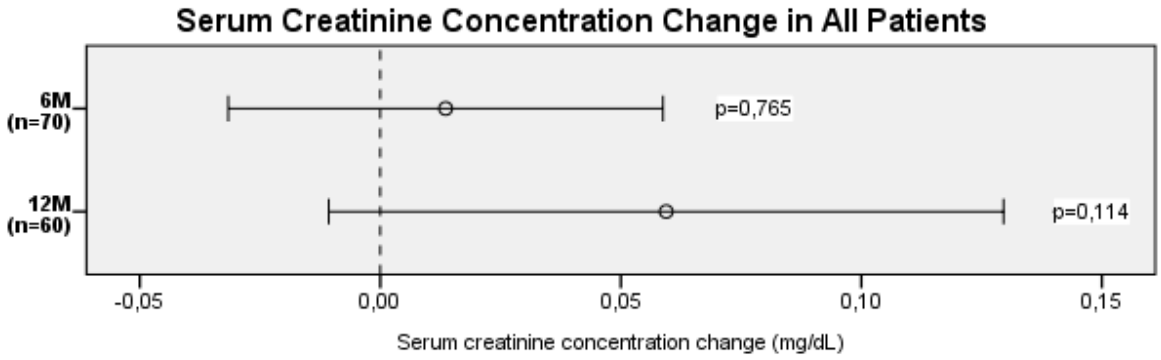



*Figure 65. Serum creatinine concentration change in all patients at 6 and at 12 months after renal sympathetic denervation. Whiskers indicate 5 and 95 percentile, p-values are for comparison with baseline values.*

### 3.6.1.2. Serum Creatinine Concentration in RSD Non-/Responders

Patients considered RSD responders had an average creatinine concentration amounting to 0,97mg/dL (0,34) at baseline (n=33), 1,00mg/dL (0,44) at six months (n=33) and 1,06mg/dL (0,42) at one year (n=30).In the RSD non-responders group, paired creatinine follow up data

was available in n=32 patients at baseline, n=32 at six months and n=27 at twelve months, mean values were 0,98mg/dL (0,38), 0,96mg/dL (0,39) and 0,91mg/dL (0,17).

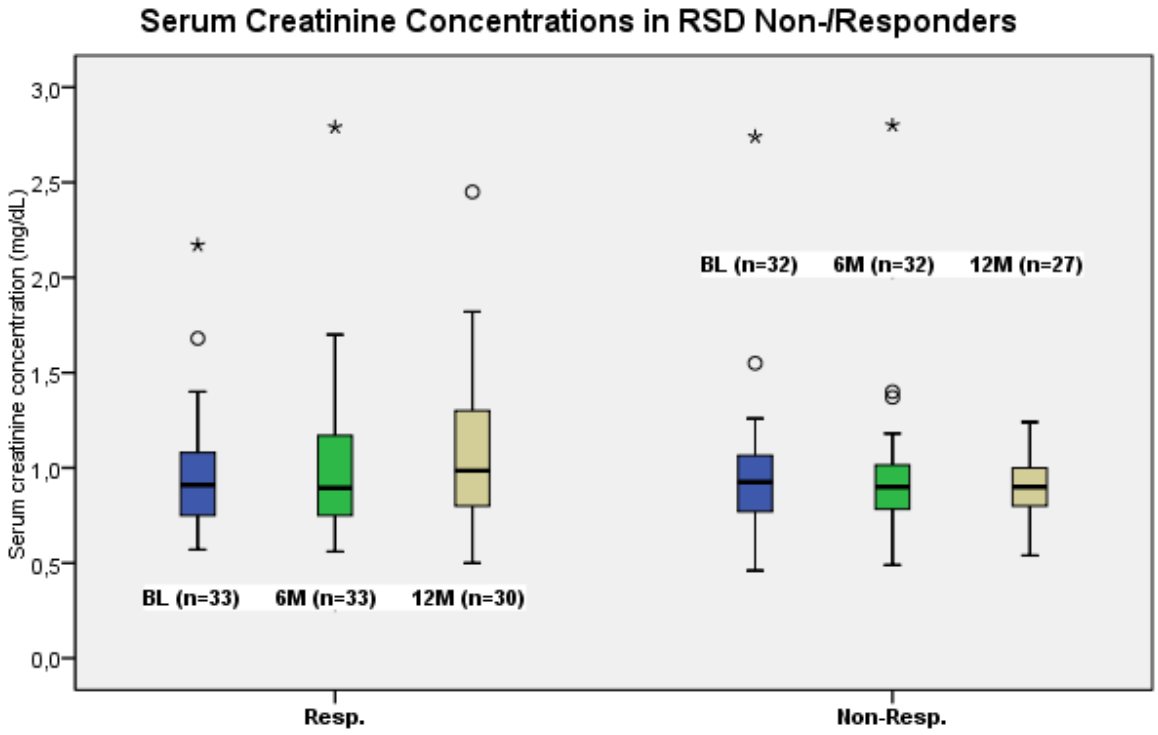



*Figure 66. Serum creatinine concentrations at baseline, 6 and 12 months after renal sympathetic denervation in the RSD responders and non-responders group, respectively. Whiskers indicate 5 and 95 percentile.*

In the RSD responders group, complete follow up of serum creatinine values was available in n=33 patients at six months and in n=30 at twelve months, mean changes were 0,03mg/dL (-0,03;0,09 p=0,562) and 0,08mg/dL (0,00;0,16 p=0,060), respectively. In the RSD non-responders group, six months follow up was completed by n=32 patients, who had a mean change by -0,02mg/dL (-0,08;0,04 p=0,134). Average difference in the one year follow up group (n=28) amounted to -0,01mg/dL (-0,08;0,05 p=0,829).

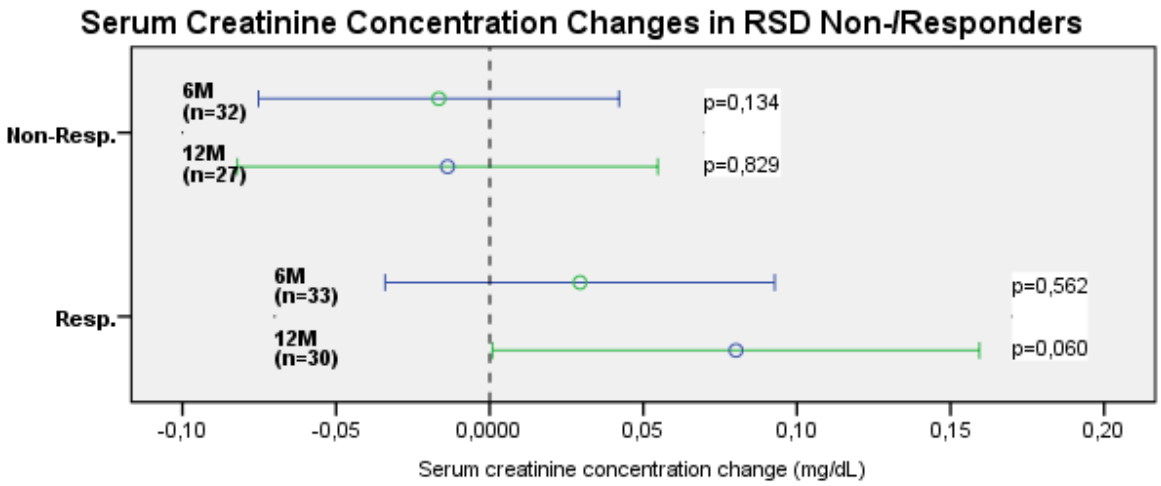



*Figure 67. Serum creatinine concentration changes at 6 and 12 months after renal sympathetic denervation in the RSD responder and non-responder group, respectively. Whiskers indicate 5 and 95 percentile, p-values are for comparison with baseline values*

Differences between the groups concerning baseline values (p=0,890), differences at six (p=0,215) and at twelve months (p=0,150) were tested non-significant.

#### 3.6.1.3. Correlations of Serum Creatinine Concentration and Blood Pressure Values

Graphical and mathematical analysis of correlations between serum creatinine concentration, ABP and OBP values at baseline and the differences at six months was conducted using the modified dataset for ABP values.

Analysis revealed no statistically significant correlation between those values.

### 3.6.2. Glomerular Filtration Rate

#### 3.6.2.1. Glomerular Filtration Rate in All Patients

Mean GFR amounted to 79,8mL/min/1,73m$^2$ (22,0) at baseline (n=78), 80,3mL/min/1,73m$^2$ (23,5) at six (n=70) and 76,1mL/min/1,73m$^2$ (23,7) at twelve months (n=60).

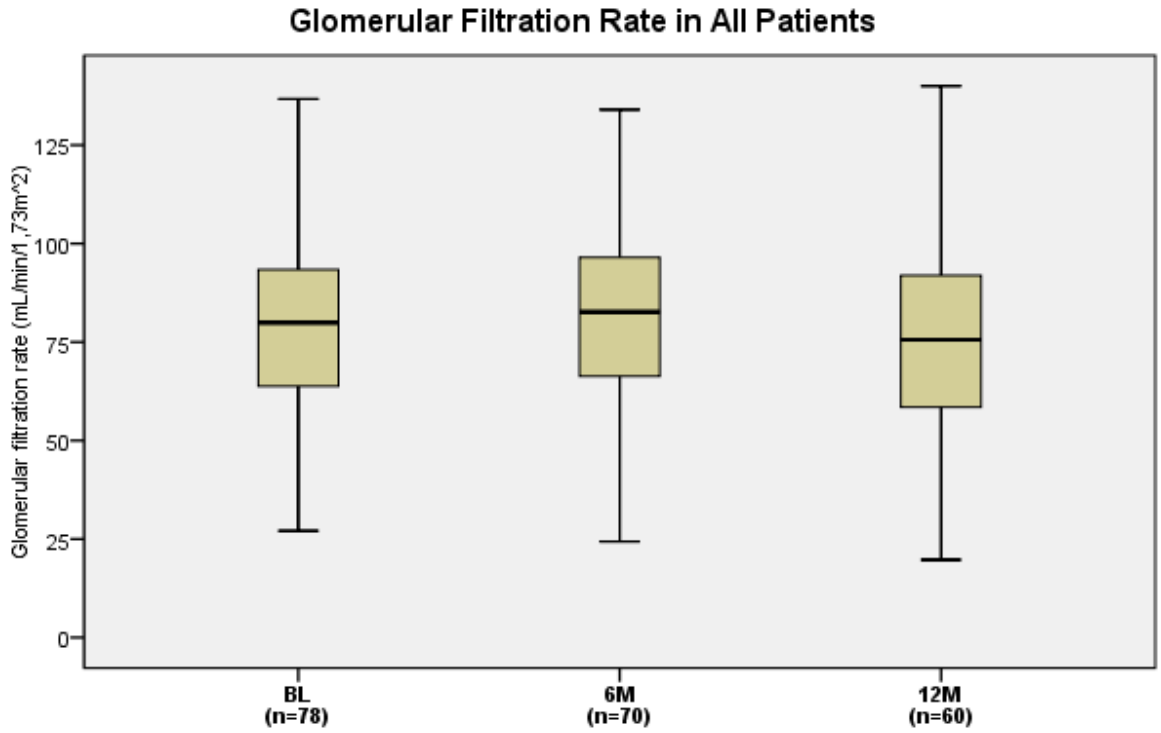



*Figure 68. Glomerular filtration rate (GFR) in all patients at baseline, 6 and 12 months after renal sympathetic denervation. Whiskers indicate 5 and 95 percentile.*

Mean difference between baseline and six months GFR values (n=70) was 1,29ml/min/1,73m$^2$ (-1,6;4,2 p=0,372), changes at one year (n=60) averaged out at -3,2mL/min/1,73m$^2$ (-6,9;0,57 p=0,095).

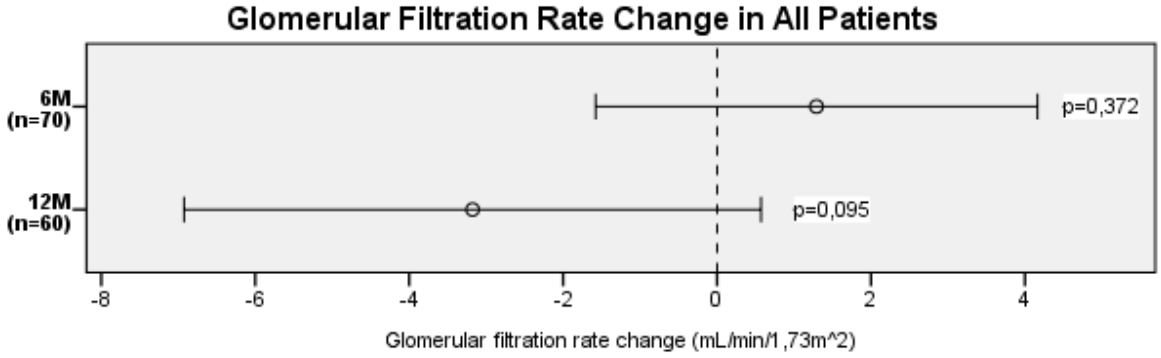



*Figure 69. Glomerular filtration rate (GFR) change in all patients at 6 and at 12 months after renal sympathetic denervation. Whiskers indicate 5 and 95 percentile, p-values are for comparison with baseline values.*

### 3.6.2.2. Glomerular Filtration Rate and RSD Non-/Responders

Among RSD responders, GFR was assessed in n=33 cases at baseline, n=33 at six months and n=30 at twelve months, mean values were 81,9ml/min/1,73m$^2$ (23,2), 81,5mL/min/1,73m$^2$ (25,6) and 74,0mL/min/1,73m$^2$ (26,7). RSD non-responders had a mean

GFR amounting to 78,0mL/min/1,73m$^2$ (18,7) at baseline (n=32), 81,1mL/min/1,73m$^2$ (18,4) at six months (n=32) and 79,0mL/min/1,73m$^2$ (16,4) at twelve months (n=27).

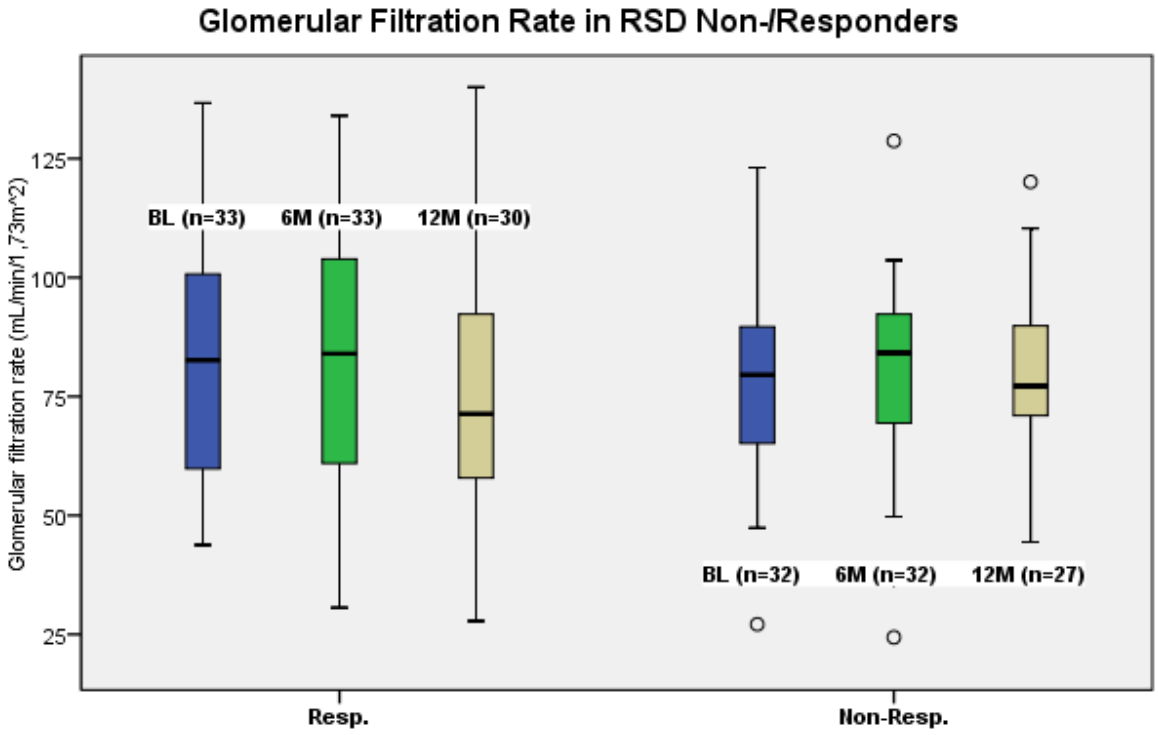



*Figure 70. Glomerular filtration rate (GFR) at baseline, 6 and 12 months after renal sympathetic denervation in the RSD responders and non-responders group, respectively. Whiskers indicate 5 and 95 percentile.*

In the RSD responders group, mean GFR change averaged out at -0,4mL/min/1,73m$^2$ (-5,5;4,8 p=0,889) at six months (n=33) and at -5,7mL/min/1,73m$^2$ (-11,7;0,3 p=0,060) at twelve months (n=30). RSD non-responders, in whom paired follow up data was available, witnessed a mean change by 3,1ml/min/1,73m$^2$ (0,2;6,1 p=0,036) at six months (n=32) and by -0,3mL/min/1,73m$^2$ (-5,4;4,7 p=0,556) at one year (n=27).

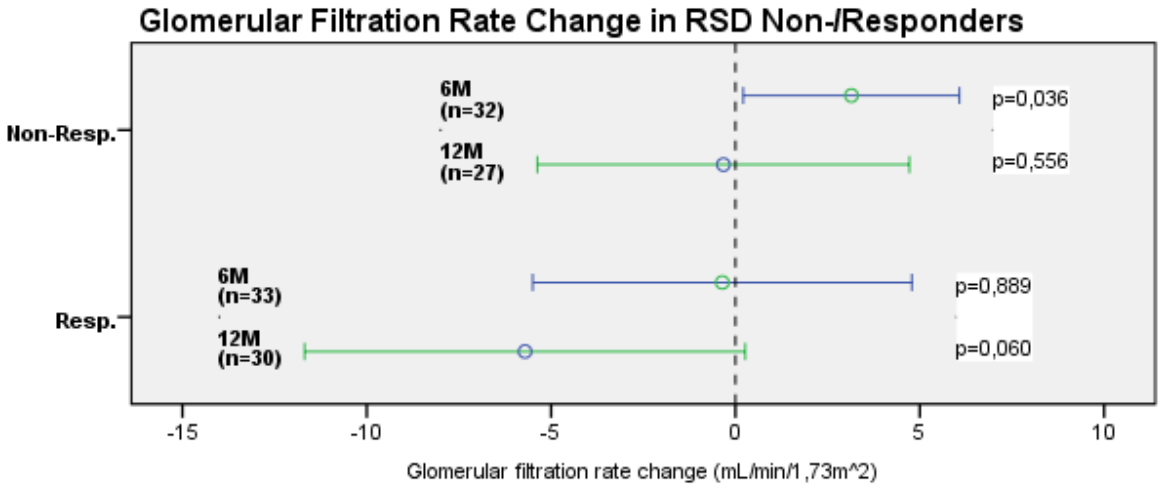



*Figure 71. Glomerular filtration rate (GFR) changes at 6 and 12 months after renal sympathetic denervation in the RSD responder and non-responder group, respectively. Whiskers indicate 5 and 95 percentile, p-values are for comparison with baseline values*

No significant difference between the two group could be found, concerning baseline GFR (p=0,458), six month (p=0,236) or twelve month differences (p=0,164).

#### 3.6.2.3. Correlations of Glomerular Filtration Rate and Blood Pressure Values

Graphical and mathematical analysis of correlations between glomerular filtration rate, ABP and OBP values at baseline and the differences at six months was conducted using the modified dataset for ABP values.

Analysis revealed no statistically significant correlation between those values.

### 3.6.3. Cystatin C

#### 3.6.3.1. Cystatin C Concentration in All Patients

Cystatin C levels were assessed in n=76 patients at baseline and averaged out at 1,11mg/L (0,33). Values at six months (n=66) amounted to 1,17mg/L (0,34) and to 1,25mg/L (0,37) at one year (n=56).

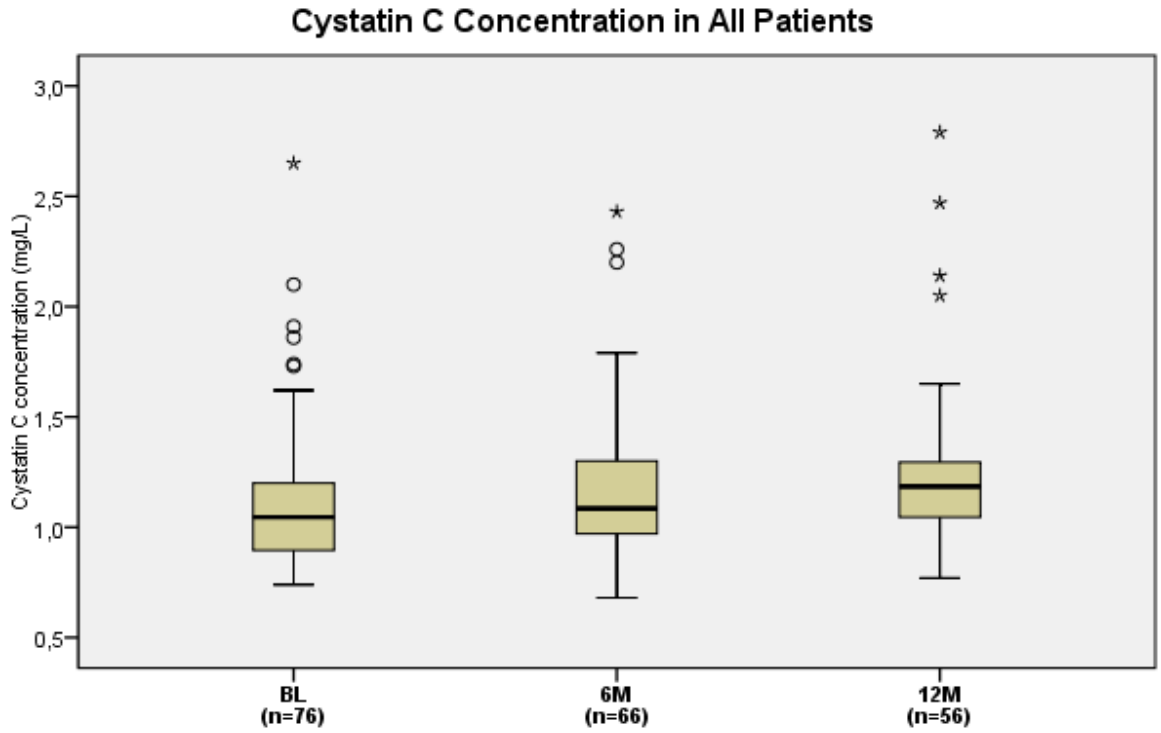



*Figure 72. Cystatin C concentration in all patients at baseline, 6 and 12 months after renal sympathetic denervation. Whiskers indicate 5 and 95 percentile.*

Among patients, who attended the follow up visits, mean Cystatin C concentration significantly increased by an average of 0,04mg/L (0,01;0,08 p=0,026) in the six months (n=64) and by 0,14mg/L (0,08;0,20 p<0,001) in the one year follow up group (n=54).

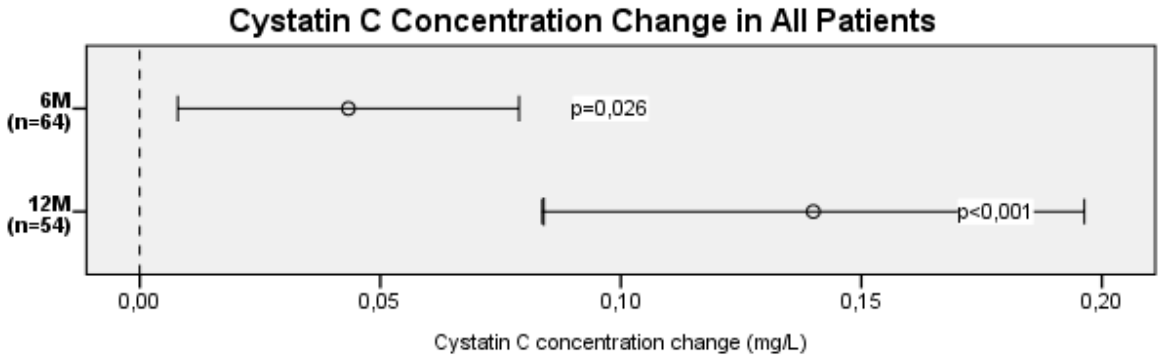



*Figure 73. Cystatin C concentration change in all patients at 6 and at 12 months after renal sympathetic denervation. Whiskers indicate 5 and 95 percentile, p-values are for comparison with baseline values.*

### 3.6.3.2. Cystatin C Concentration and RSD Non-/Responders

Among RSD responders, mean Cystatin C concentration amounted to 1,14mg/L (0,32) at baseline (n=32), 1,19mg/L (0,33) at six months (n=32) and 1,31mg/L (0,38) at twelve

months (n=29). Non-responders had a baseline (n=31) concentration of 1,07mg/L (0,34), increasing to 1,10mg/L (0,31) at six (n=29) and to 1,12mg/L (0,15) at twelve months (n=24).

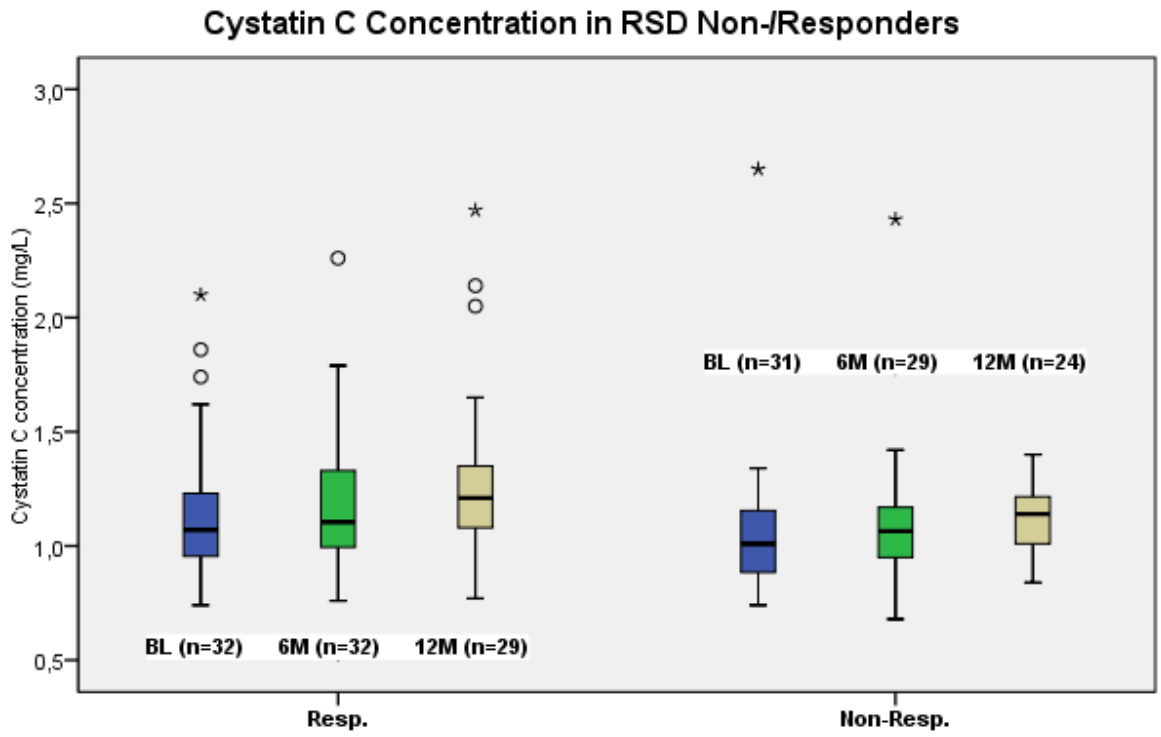



*Figure 74. Cystatin C concentrations at baseline, 6 and 12 months after renal sympathetic denervation in the RSD responders and non-responders group, respectively. Whiskers indicate 5 and 95 percentile.*

Among RSD responders, Cystatin C concentration had changed significantly by 0,05mg/L (-0,00;0,09 p=0,103) at six months (n=31) and by 0,14mg/L (0,06;0,21 p<0,001) at one year (n=28). Among RSD non-responders with complete follow up available, mean change amounted to 0,02mg/L (-0,03;0,06 p=0,250) at six months (n=28) and to 0,11mg/L (0,06;0,16 p<0,001) at one year (n=23).

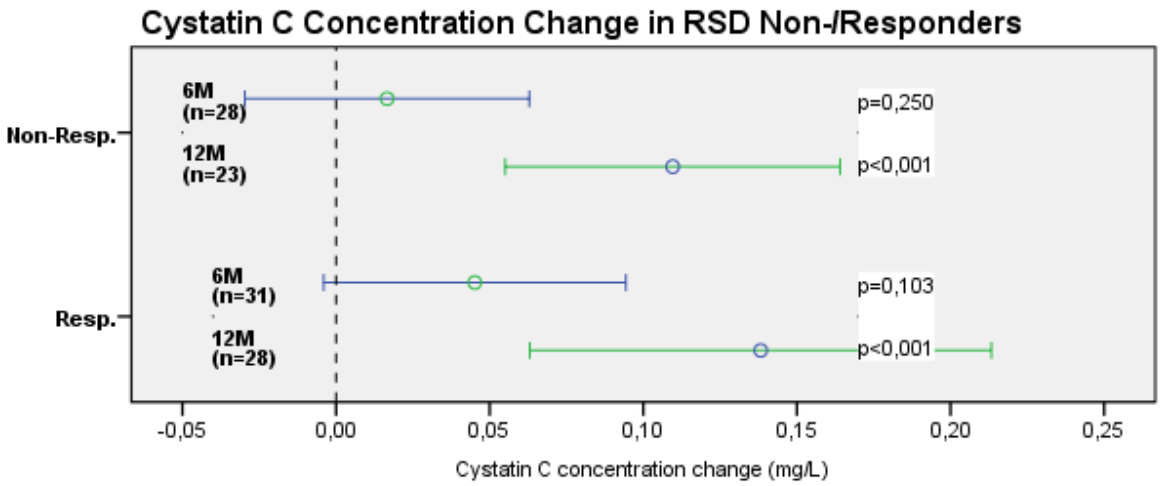



*Figure 75. Cystatin C concentration changes at 6 and 12 months after renal sympathetic denervation in the RSD responder and non-responder group, respectively. Whiskers indicate 5 and 95 percentile, p-values are for comparison with baseline values.*

Neither baseline Cystatin C concentration (p=0,286), nor differences at six (p=0,461) or twelve months (p=0,857) differed statistically significant between the groups.

#### 3.6.3.3. Correlations of Cystatin C Concentration and Blood Pressure Values

Graphical and mathematical analysis of correlations between Cystatin C concentration, ABP and OBP values at baseline and the differences at six months was conducted using the modified dataset for ABP values.

Analysis revealed no statistically significant correlation between those values.

## 3.7. Endocrine Parameters

### 3.7.1. Free Plasma Cortisol Concentration

#### 3.7.1.1. Free Plasma Cortisol Concentration in All Patients

Free cortisol concentrations in plasma were measured in n=67 patients at baseline, n=60 patients at six months and n=56 at twelve months. Among those, average concentrations amounted to 11,9µg/dL (5,2), 14,2µg/dL (4,7) and 14,7µg/dL (5,2), respectively.

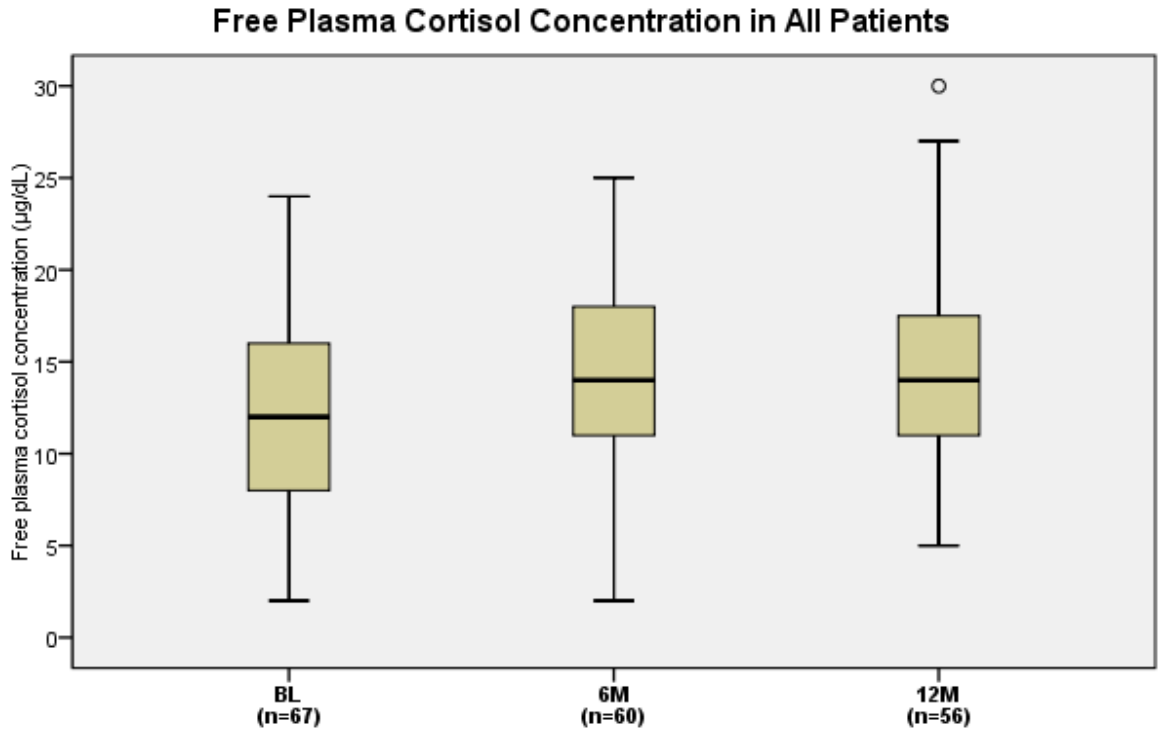



*Figure 76. Free plasma cortisol concentration in all patients at baseline and 6 months after renal sympathetic denervation. Whiskers indicate 5 and 95 percentile.*

Among the patients, who attended the six month follow up visit (n=56), mean cortisol concentration significantly increased by 2,3µg/dL (0,5;4,2 p=0,016). In the one year follow up group (n=50), values significantly rose by 2,9µg/dL (0,8;5,0 p=0,008) on average.

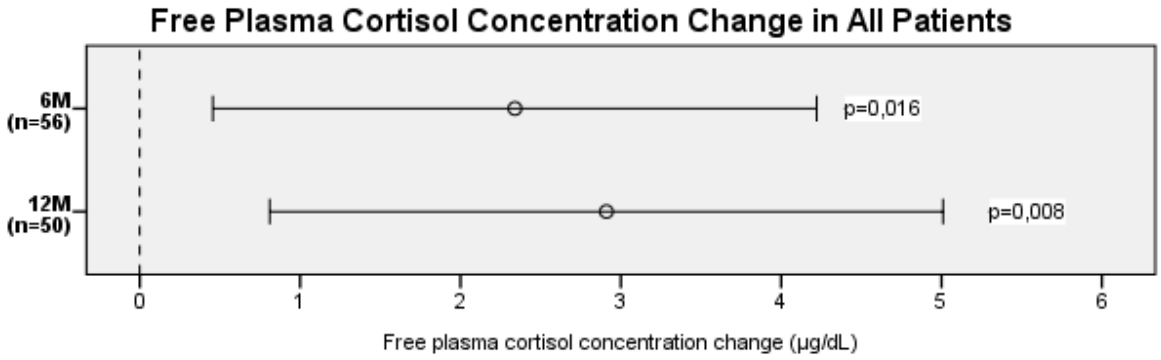



*Figure 77. Free plasma cortisol concentration change in all patients at 6 months after renal sympathetic denervation. Whiskers indicate 5 and 95 percentile, p-value is for comparison with baseline value.*

### 3.7.1.2. Free Plasma Cortisol Concentration in RSD Non-/Responders

In the RSD responders group, free plasma cortisol concentration was measured in n=30 patients at baseline, n=31 at six months and n=29 at twelve months. Values at those

examinations were 11,7µg/dL (5,6), 13,9µg/dL (5,0) and 15,7µg/dL (5,9), respectively. Among RSD non-responders, mean concentration amounted to 12,6µg/dL (4,7) at baseline (n=27), 14,7µg/dL (4,7) at six months (n=25) and 13,4µg/dL (4,4) at one year (n=24).

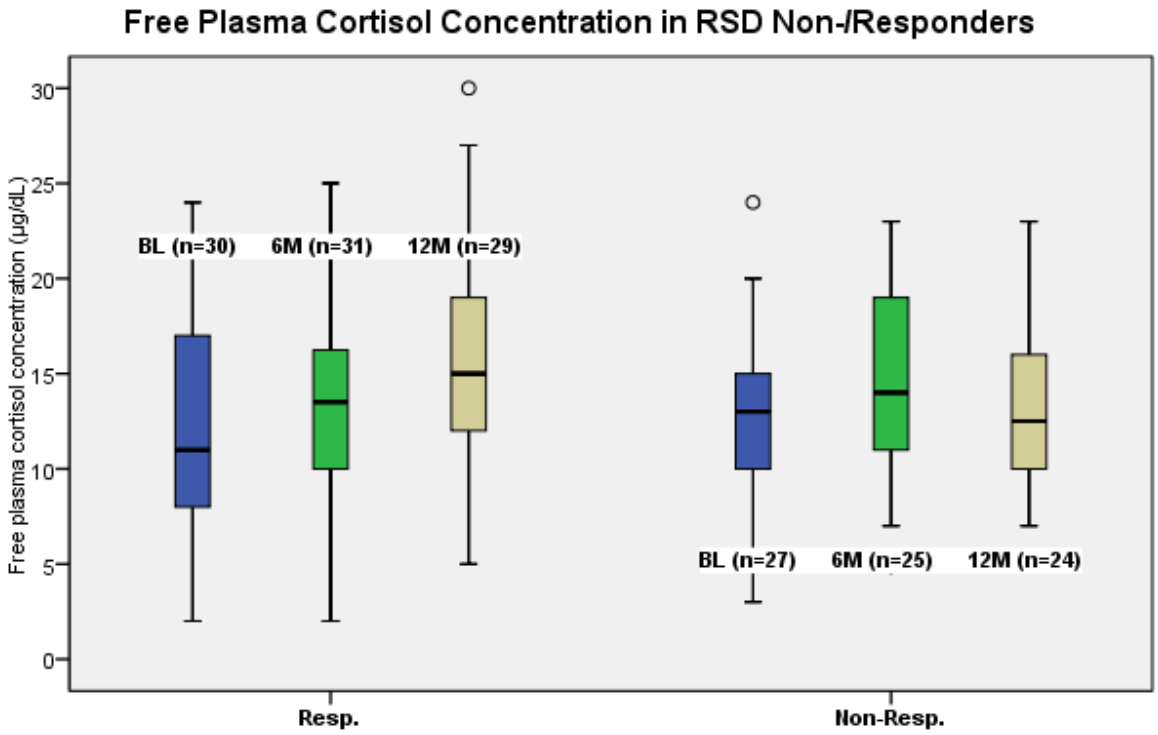



*Figure 78. Free plasma cortisol concentration at baseline and 6 months after renal sympathetic denervation in the RSD responder and the non-responder group, respectively. Whiskers indicate 5 and 95 percentile.*

RSD responders, who attended the follow up examinations, had a mean change in free plasma cortisol concentration by 2,6µg/dL (-0,4;5,6 p=0,088) at six months (n=29) and a significant increase by 3,5µg/dL (0,1;6,8 p=0,044) at one year (n=27). RSD non-responders witnessed an change by 2,0µg/dL (-0,5;4,5 p=0,109) at six months (n=24) and by 1,8µg/dL (-0,9;4,5 p=0,187) at twelve months (n=21).

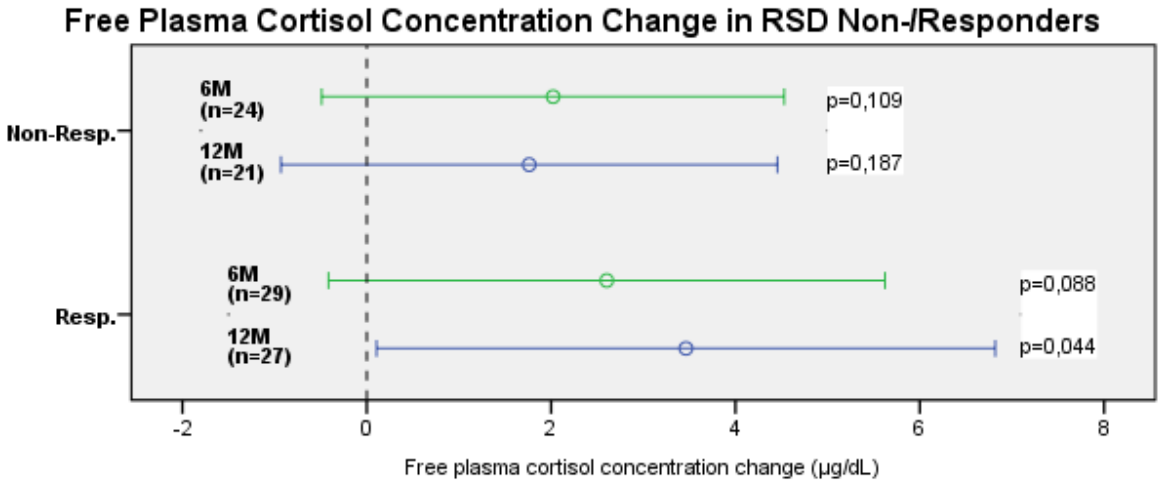



*Figure 79. Free plasma cortisol concentration changes at 6 months after renal sympathetic denervation in the RSD responder and non-responder group, respectively. Whiskers indicate 5 and 95 percentile, p-values are for comparison with baseline values.*

Neither baseline concentrations (p=0,505), nor differences at six (p=0,768) or twelve months (p=0,438) were proven to differ in a statistically significant way between the two groups.

#### 3.7.1.3. Correlations of Free Plasma Cortisol Concentration and Blood Pressure Values

Graphical and mathematical analysis of correlations between free plasma cortisol concentration, ABP and OBP values at baseline and the differences at six months was conducted using the modified dataset for ABP values.

Analysis revealed no statistically significant correlation between those values. Differences in cortisol concentration at six months were significantly correlated with baseline cortisol values (r=-0,749; p<0,001).

### 3.7.2. Free Triiodothyronine ($fT_3$)

#### 3.7.2.1. $fT_3$ Concentration in All Patients

$FT_3$ concentration was measured in n=14 patients at baseline and n=11 at six months, overall mean concentrations changed from 3,04pg/mL (0,32) to 2,96pg/mL (0,34) during follow up.

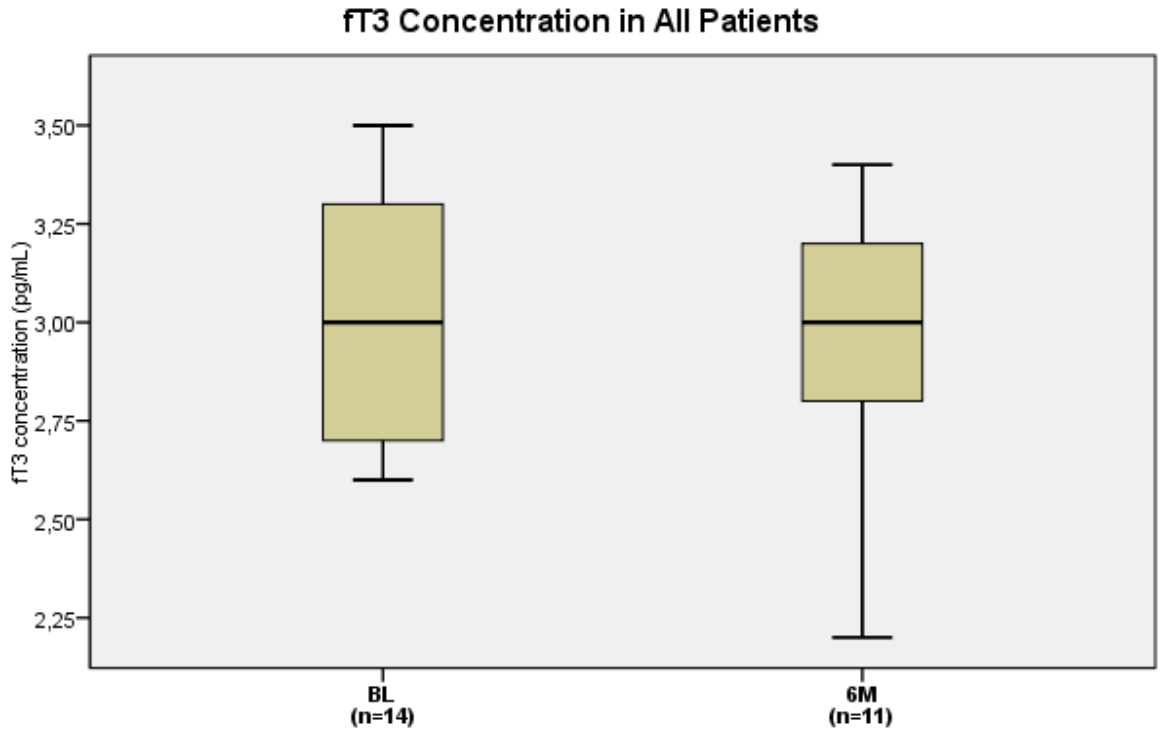



*Figure 80. Free $T_3$ concentration in all patients at baseline, 6 and 12 months after renal sympathetic denervation. Whiskers indicate 5 and 95 percentile.*

In n=5 patients with complete six month follow up of $fT_3$ levels available, values changed by a mean difference of -0,20pg/mL (-0,70;0,30). Due to the lack of paired follow up data, no allocation to groups or further statistical testing was conducted.

## 3.7.3. Free Thyroxine ($fT_4$)

### 3.7.3.1. $FT_4$ Concentration in All Patients

$FT_4$ was measured among n=34 patients at baseline with a mean concentration amounting to 12,6pg/mL (1,9), that changed to 12,1pg/mL (1,8) within six months (n=29).

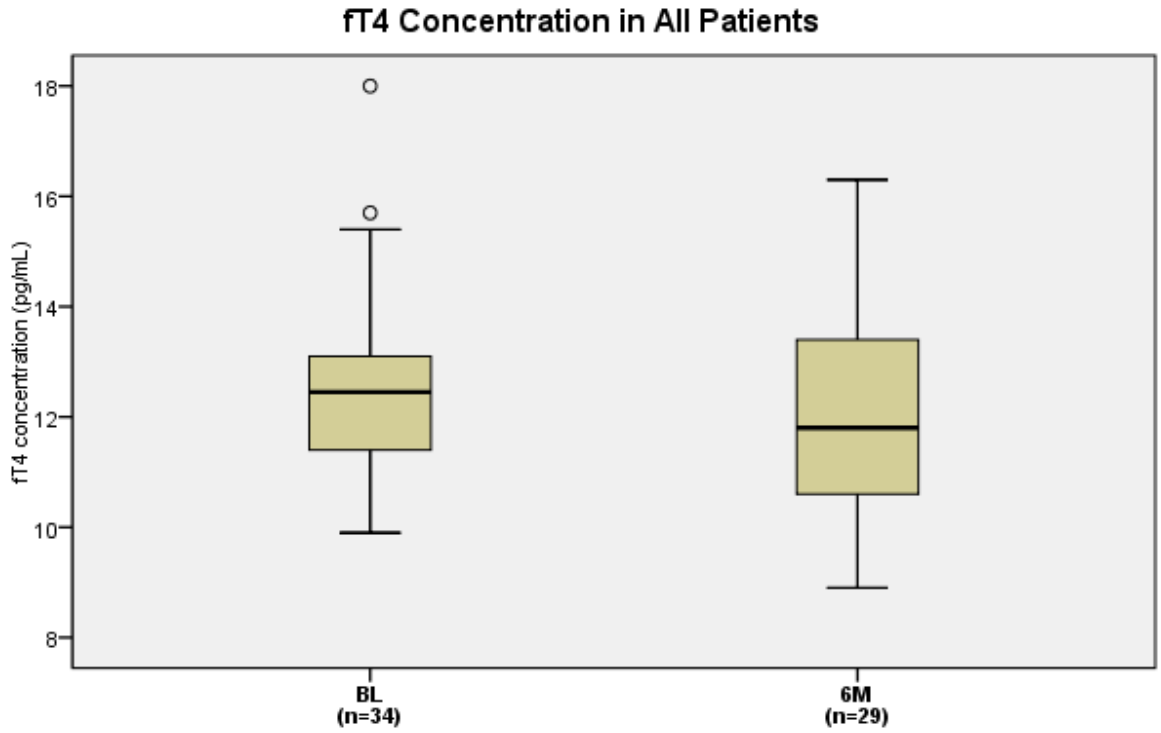



*Figure 81. $FT_4$ concentration in all patients at baseline and 6 months after renal sympathetic denervation. Whiskers indicate 5 and 95 percentile.*

Six months follow up of values was available in n=23 patients, in whom $fT_4$ concentrations significantly decreased by a mean difference of -0,8pg/mL (-1,5;-0,1 p=0,029).

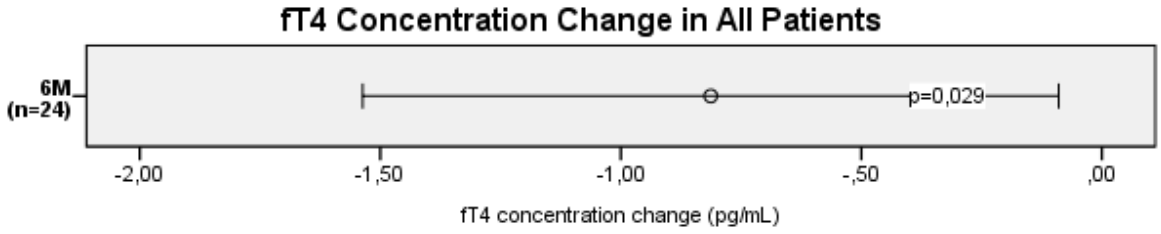



*Figure 82. $FT_4$ concentration change in all patients at 6 months after renal sympathetic denervation. Whiskers indicate 5 and 95 percentile, p-value is for comparison with baseline value.*

### 3.7.3.2. Free Thyroxine and RSD Responders at 6 Months

$FT_4$ concentration in the RSD responders group averaged out at 13,2pg/mL (2,0) at baseline (n=15) and 12,0pg/mL (1,6) at six months (n=14). Among RSD non-responders, values developed from 12,1pg/mL (1,7) at baseline (n=13) to 12,2pg/mL (2,1) at six months (n=13).

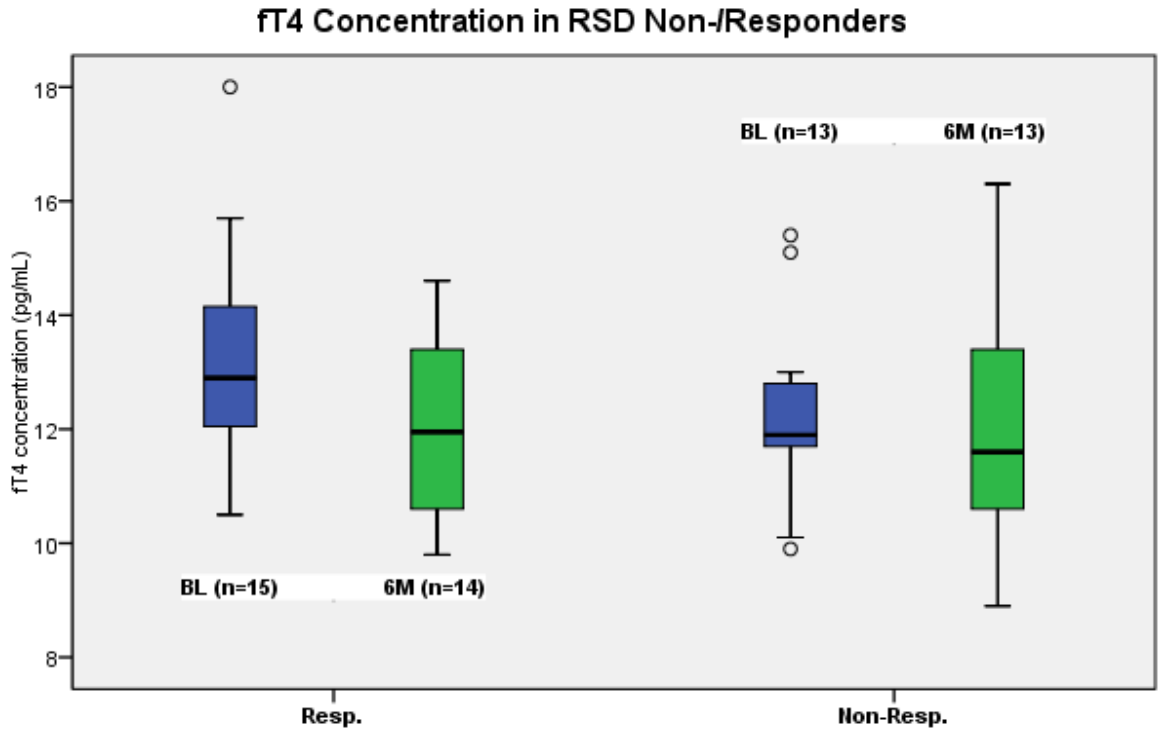



*Figure 83. $FT_4$ concentration at baseline and 6 months after renal sympathetic denervation in the RSD responder and the non-responder group, respectively. Whiskers indicate 5 and 95 percentile.*

Among RSD responders, in whom complete follow up of values was available (n=12), mean $fT_4$ concentration significantly decreased by -1,4pg/mL (-2,6;-0,3 p=0,019), while non-responders (n=9) had a non-significant mean change by -0,02pg/mL (-0,8;0,7 p=0,946).

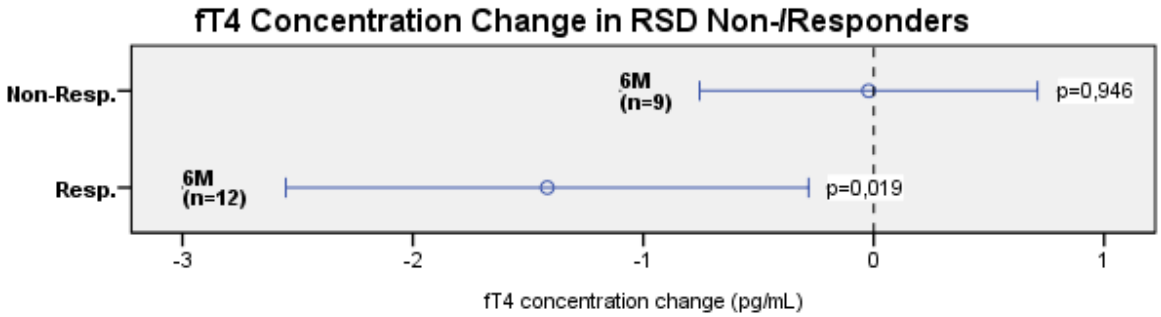



*Figure 84. $FT_4$ concentration changes at 6 months after renal sympathetic denervation in the RSD responder and non-responder group, respectively. Whiskers indicate 5 and 95 percentile, p-values are for comparison with baseline values.*

A statistically significant difference between the RSD responders and non-responders group could be detected for mean $fT_4$ concentration differences (p=0,048) at six months, but not for baseline $fT_4$ concentrations (p=0,128).

### 3.7.3.3. Correlations of $fT_4$ Concentration and Blood Pressure Values

Graphical and mathematical analysis of correlations between $fT_4$ concentration, ABP and OBP values at baseline and the differences at six months was conducted using the modified dataset for ABP values.

Analysis revealed a statistically significant correlation between $fT_4$ concentration differences at six months and office systolic BP at baseline (r=0,432; p=0,040). $FT_4$ concentration differences at six months were significantly correlated to baseline thyroxine concentrations (r=-0,539; p=0,008).

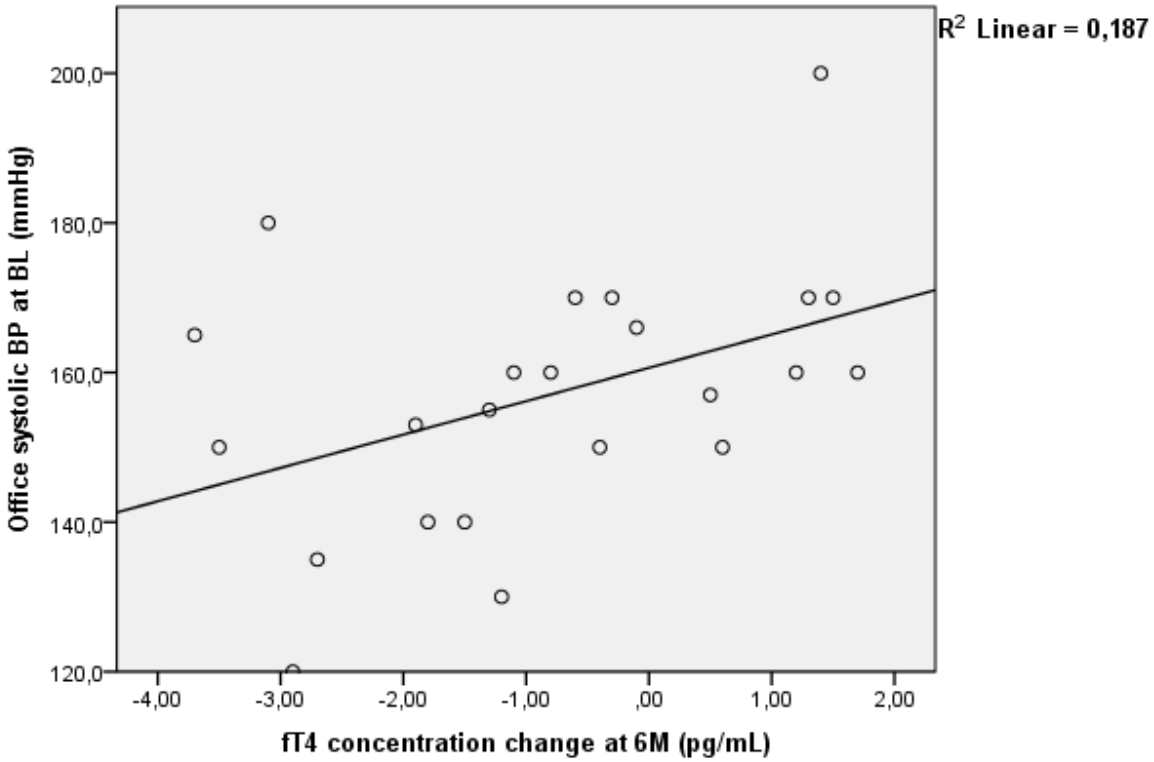



*Figure 85. Relationship between office systolic BP at baseline and $fT_4$ concentration change at six months (n=23). The modified dataset was used for ABP values.*

## 3.7.4. Thyroid-Stimulating Hormone (TSH)

### 3.7.4.1. TSH Concentration in All Patients

TSH concentrations were measured in n=35 patients at baseline, amounting to an average of 1,80µU/mL (1,15), and to 1,88µU/mL (1,55) at six months (n=30).

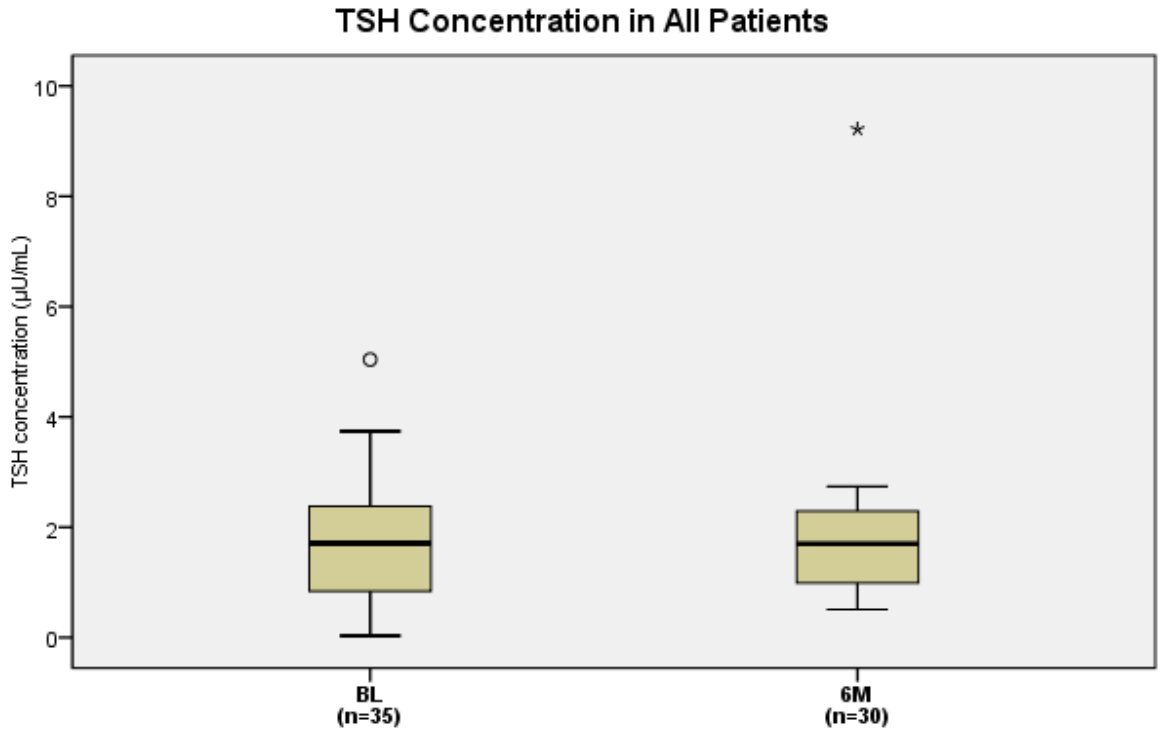



*Figure 86. TSH concentration in all patients at baseline and 6 months after renal sympathetic denervation. Whiskers indicate 5 and 95 percentile.*

In the group of patients, in whom paired six months follow up data was available (n=23), mean TSH concentration changed by 0,39 µU/mL (-0,10;0,87 p=0,140) on average.

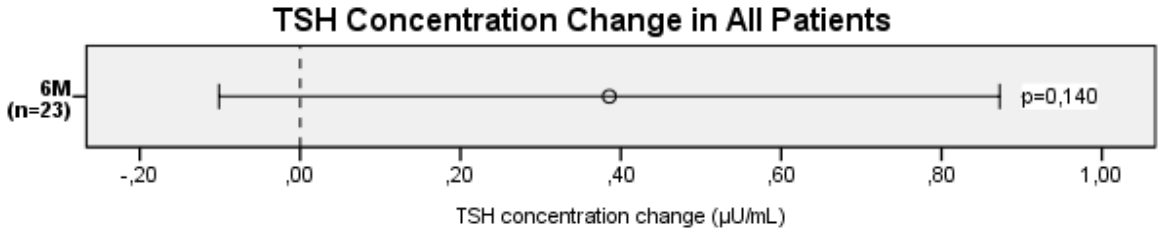



*Figure 87. TSH concentration change in all patients at 6 months after renal sympathetic denervation. Whiskers indicate 5 and 95 percentile, p-value is for comparison with baseline value.*

### 3.7.4.2. TSH Concentration in RSD Non-/Responders

The RSD responders group covered n=15 patients at baseline, with a mean TSH concentration of 1,22µU/mL (0,74), that had increased to 1,67µU/mL (0,64) at six months (n=14). Among the RSD non-responders, baseline TSH concentration (n=14) amounted to 2,32µU/mL (1,34) on average and to 2,10µU/mL (2,18) at six months (n=14).

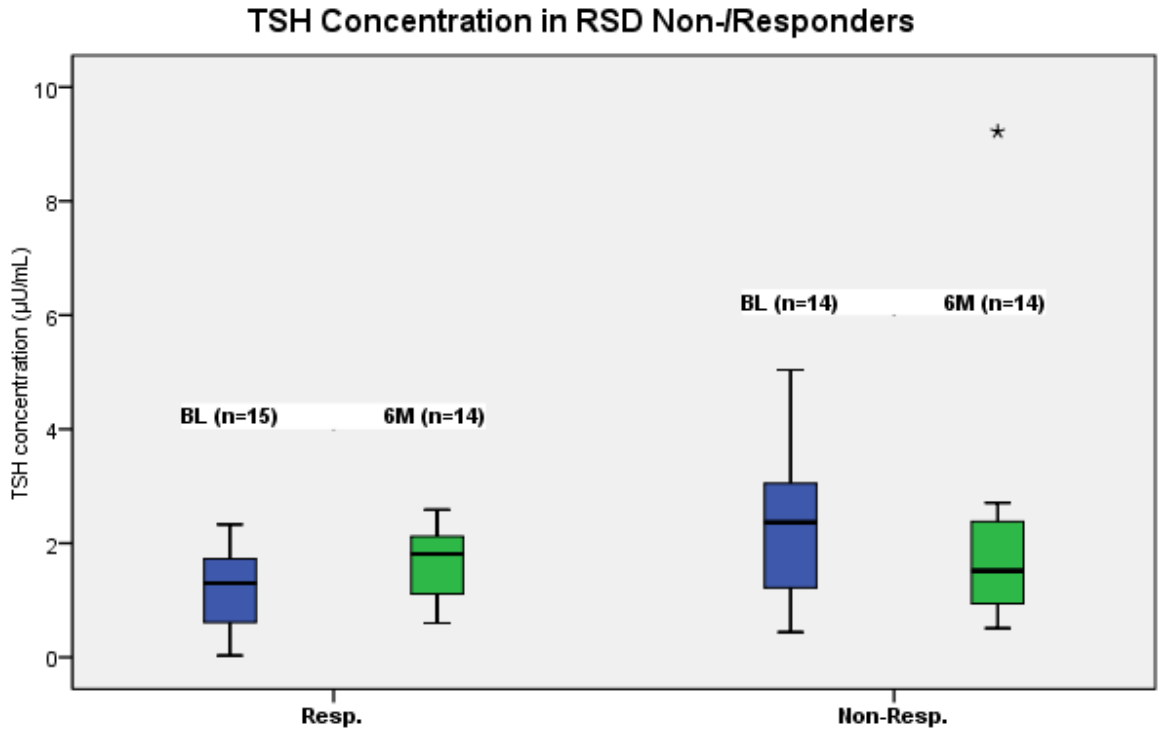



*Figure 88. TSH concentration at baseline and 6 months after renal sympathetic denervation in the RSD responder and the non-responder group, respectively. Whiskers indicate 5 and 95 percentile.*

Among patients considered RSD responders, who completed six months follow up of values (n=12), mean TSH concentration significantly increased by an average difference of 0,56µU/mL (0,05;1,07 p=0,015). RSD non-responders (n=9) witnessed a non-significant mean change by 0,27µU/mL (-0,93;1,47 p=0,813).

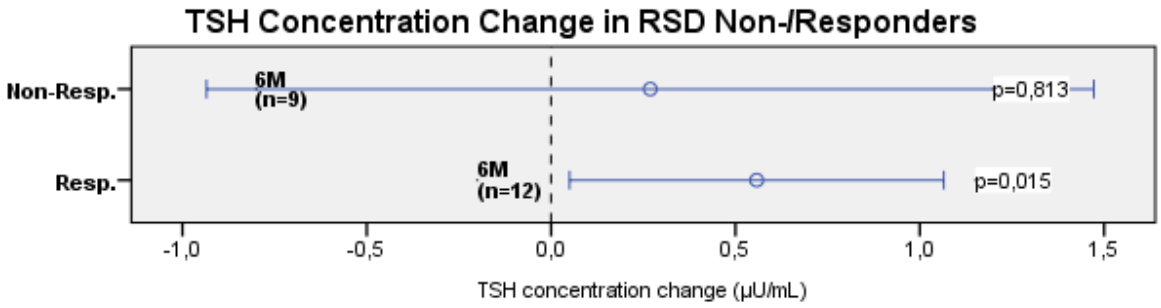



*Figure 89. TSH concentration changes at 6 months after renal sympathetic denervation in the RSD responder and non-responder group, respectively. Whiskers indicate 5 and 95 percentile, p-values are for comparison with baseline values.*

Baseline TSH concentrations were shown to differ significantly (p=0,020), between-group differences for the changes at six months were tested non-significant (p=0,111).

### 3.7.4.3. Correlations of TSH Concentration and Blood Pressure Values

Graphical and mathematical analysis of correlations between TSH concentration, ABP and OBP values at baseline and the differences at six months was conducted using the modified dataset for ABP values.

Analysis revealed a statistically significant correlation between baseline TSH concentration and systolic ABP changes at six months (r=0,399; p=0,039).

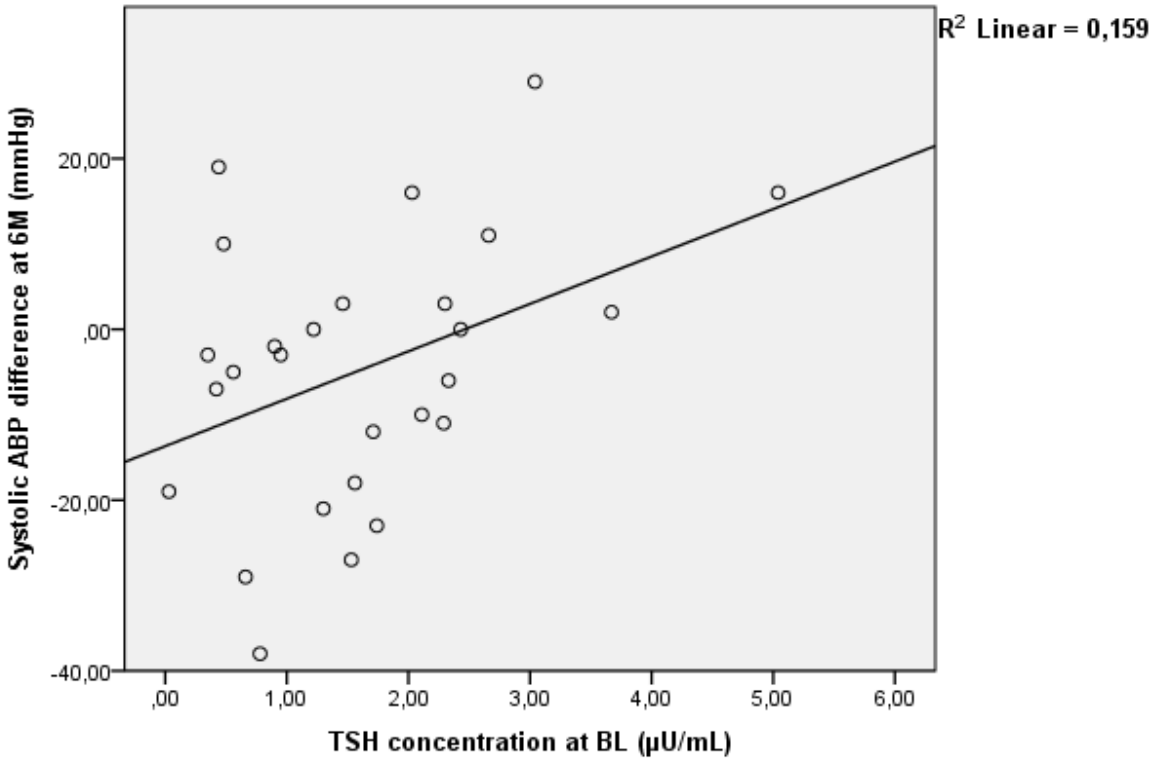



*Figure 90. Relationship between ambulatory systolic BP differences at 6M and TSH concentration at BL (n=27). The modified dataset was used for ABP values.*

# 4. Discussion

## 4.1. Interpretation

### 4.1.1. Interpretation of Office and Ambulatory Blood Pressure Results

We found, that OBP had significantly decreased by -16,0/-6,4mmHg (p<0,001/p=0,002) at the six months follow up visit, however, these reduction were not sustained up to one year. This finding is in contrast to most previously published studies, where significant reductions of OBP were shown to be sustained during follow up to three years after RSD.[290-294] Still, our results do not stand isolated among other studies, as also Brinkmann et al. found office BP levels to be unchanged after RSD in a small, prospective case series and the SYMPLICITY HTN-3 study reported no significant difference in OBP changes between the sham intervention and renal denervation group.[285,296]

ABP values were, despite the changes being statistically non-significant at six months, shown to be significantly reduced by -8,2/-3,8mmHg (p=0,001/p=0,005) after one year. The extent of ABP-reduction was found to be similar to that reported in other studies.[291,293,298]

As suggested by Mahfoud et al., we conducted an analysis separating all patients with baseline ABP levels <130mmHg from those with higher BP.[298] We found "pseudo-resistant HT" in n=13 patients, indicating, that >16% of patients enrolled, may not have had resistant HT at all, but white coat hypertension, which is a common finding, as previously discussed.[19] Upon this, we saw statistically significant decreases in ABP levels by -6,3/-2,9mmHg (p=0,006/p=0,015) at six and by -11,5/-4,6mmHg (p<0,001/p=0,009) at twelve months after RSD, while the "pseudo-resistant HT" group displayed a significant increase in systolic ABP at six months and a trend towards an increase at twelve months. Differences between "true resistant" and "pseudo-resistant" HT patients were regarded significant for systolic and diastolic baseline values and the differences during follow up, except for diastolic changes at twelve months. The same analysis was conducted for office BP levels, but neither baseline values, nor differences at six or twelve months differed statistically between the two groups.

Ambulatory blood pressure monitoring is generally considered superior to office measurement concerning prediction of cardiovascular risk, as it comprises a more detailed survey of BP regulation throughout the day and especially during the night.[1,11,15,16] Our findings implicate, that ABP measurement may not only be the better choice in screening

patients' eligibility for RSD, but also to judge outcomes in follow up. This notion is further supported by the finding that, although ABP and OBP values were significantly correlated to each other at baseline, no such correlation could be found for the BP changes at six months after RSD. In line with that we saw, that nearly all baseline values and significant changes of metabolic parameters were, if at all, correlated to ABP values, but only very rarely with office BP values. Baseline ABP values were significantly correlated with baseline FPG concentration, HbA1c fraction, Il-6 concentration and Il-6 concentration changes at six months. ABP changes at six months were significantly associated with BMI changes at six months, as well as with baseline Il-6 and TSH concentration. Baseline OBP was only found to be correlated with baseline $fT_4$ concentration.

Importantly, our results suggest, that the extent of ABP reduction by RSD could be related to the levels of Il-6 and TSH before the intervention. Thus, screening of inflammatory and thyroid function parameters before RSD should be considered in future studies, to further elucidate the role of these parameters, possibly predicting response to RSD.

Further analyses were conducted for RSD responders, defined as those patients with a decrease in ABP levels by ≥5mmHg at six months after RSD. Approximately 51% of all patients, who attended the six months follow up visit were regarded RSD responders according to this definition. In those, ABP values had significantly decreased at six and twelve months ($p<0,001$ for all), while there was a significant increase at six months in the non-responders group. Baseline values and differences during follow up differed significantly between the groups. When applying this classification to the OBP data, no statistically significant difference between RSD responders and non-responders was visible. OBP decreases at six months deemed significant ($p<0,005$), while changes at twelve months tended towards zero in both groups. These findings again suggest, that ABP measurement is superior to assessing OBP values in patients treated with RSD.

Our results concerning responder status to the intervention seem to be in blatant contrast to those reported in the SYMPLICITY studies, where office BP measurement based classification of responder status, applying a threshold at ≥10mmHg OBP reduction, resulted in a percentage of 81% responders at six months and even 93% at three years.[293] Compared to the fraction of 51% regarded RSD responders at six months in our collective, it seems unlikely, that such dimensions can be reached, when using ABP results for that classification. However, in our study, substantial beneficial effects on especially inflammatory parameters were also seen in RSD non-responders, although they were usually

less distinct than among RSD responders. This suggests, that RSD could have effects apart from, and also in the absence of BP reductions. Hypothetically, one may assume, that increased renal and central SNS activity, primarily targeted by renal denervation, is not the cause of elevated blood pressure in all patients displaying resistant hypertension. The fact, that only about half of the patients responded to RSD in terms of a BP reduction, but anti-inflammatory effects were also seen in non-responders, therefore leads to two hypotheses: First, the other half of patients may have causes underlying their resistant hypertension other than increased SNS activity. Second, also in those patients, RSD may still exert influences on various parameters.

### 4.1.2. Interpretation of BMI, Glucose- and Lipid Metabolism Results

BMI was significantly reduced by -0,5kg/m$^2$ (p=0,004) or -1,4kg in body weight (p=0,006). The decrease had a trend to be stronger in RSD responders and was significantly correlated to ABP changes at six months.

Glucose metabolism parameters were analysed, excluding all patients on insulin treatment (n=5). Among those, FPG did not change at six months after RSD, but had increased significantly at one year. These changes did not differ between RSD responders and non-responders, however, among responders, FPG had a trend towards a decrease at six months, but had increased significantly at one year. HbA1c values indicated a small, but significant increase at six months, which was -non-significantly- sustained at twelve months after RSD. HbA1c results did not differ between RSD responders and non-responders. Baseline FPG concentration and HbA1c fraction were positively correlated with baseline systolic ABP.

Of all lipid metabolism parameters, HDL-C and triglyceride levels increased significantly by 1,1mg/dL (p=0,031) and 7,7mg/dL (p=0,033), respectively, while there were no significant changes in LDL-C, non-HDL-C and total cholesterol concentrations. Baseline values and differences in blood lipid concentration did not differ significantly between responders and non-responders. RSD non-responders displayed a trend towards higher changes in triglyceride concentrations, while HDL-C concentration increased significantly among RSD responders, but not in non-responders.

Numerous studies suggest a close relationship between the sympathetic nervous system and insulin resistance,[77,88-90] mainly at a microvascular level.[42,45,46,172-174] Thus, it could be expected, that an intervention, which has been reported to reduce central sympathetic

outflow to the periphery,[283,284] should have positive effects on insulin resistance and glucose metabolism. However, as reported in a smaller study, MSNA at rest and baroreflex control of MSNA did not change significantly upon RSD, raising the question, whether a decrease in efferent SNS activity was rather an exception than the rule.[285] A study by Mahfoud et al. demonstrated, that FPG, insulin and C-peptide levels decreased within three months after renal denervation. Also 2-hour glucose tolerance test values, HOMA-IR and $IS_{QUICKI}$ were reported to have improved, while HbA1c fraction remained unchanged.[301] Unchanged HbA1c levels were also reported in the SIMPLICITY HTN-3 study.[296]

Our results concerning glucose metabolism seem inconclusive at first glance: The solitary large increase in FPG at the twelve months follow up visit, while there was hardly any change at all at six months, suggests, that patients probably were simply not fasting, as the twelve months follow up examination was conducted in the outpatient clinic, while patients stayed overnight for the six months visit. This assumption is confirmed by the finding, that the alterations in FPG concentration at twelve months were not paralleled by a further increase in HbA1c fraction, like seen at six months. Altogether, though the results seem inconclusive, one can at least state with relative certainty, that glucose metabolism parameters did improve among the patients in this study. However, as our glucose metabolism data seems to comprise methodological limitations additional to the general statistical restrictions in an uncontrolled retrospective registry study, these results should be only interpreted cautiously.

Results concerning lipid metabolism parameters revealed no fundamental changes apart from the mentioned significant increases in HDL-C and triglyceride concentrations. Low HDL levels are a common feature in dyslipidaemia marking the metabolic syndrome.[108,129,130,135] Besides, high fasting triglyceride concentration in visceral obesity, referred to as the "hypertriglyceridaemic waist" is associated with the risk of cardiovascular disease.[131-134] Weight loss and physical activity are known to improve blood lipid metabolism.[108,129,130] Patients in this study actually displayed significant weight loss, which may explain the significant increase in HDL-C, but not the increase in fasting triglycerides, as the opposite would have been expected.

Although speculative, it seems, that among RSD responders, there was a trend towards an increase in LDL-C, non-HDL-C, total cholesterol compared to non-responders. The opposite seemed to be the case for fasting triglycerides and HDL-C, where RSD responders tended to have higher increases in HDL and lower increases in triglyceride concentrations. If those

alterations might have been caused by factors related to RSD and sympathetic deactivation, the decrease in blood pressure itself or by external biases like non-fasting by the patients cannot be elucidated in retrospect. However, as already emphasised, this is only subject to speculation and cannot be clarified in a retrospective study.

Dyslipidaemia is reported to be closely associated with the RAAS,[27] as blockade of the RAAS by ACE-I or ARB has been demonstrated to improve lipid profile, whereas improvements especially in triglyceride and HDL-C levels have been reported among patients meeting the criteria for the metabolic syndrome.[144,145]

The observed significant weight loss may pose one of the most surprising findings in this study. Weight loss was found to be significantly correlated with ambulatory BP changes at six months, which indicates, it may in fact be related to the RSD procedure. Still, due to possible methodological flaws, interpretation should done only carefully, as described in the chapter on possible limitations below.

Nevertheless, weight loss caused by RSD seems possible and even plausible. For instance, the appetite- and energy-household regulation hormone leptin is closely linked with the SNS.[101-103,112-119] Changes in central and peripheral sympathetic nerve activity by RSD may therefore exert influence on the actions of leptin in the CNS, in adipose tissue or in insulin sensitive tissues. Furthermore, as increased SNS activity might be involved in the emergence and perpetuation of insulin resistance,[77,88-93] sympathetic deactivation may improve insulin resistance and thereby promote weight loss.

These assumptions are, in line with the findings reported for α-blockers and centrally acting imidazoline-derivatives like Moxonidine or Rilmenidine: The α-blocker Doxazosin has been demonstrated to deploy better antihypertensive effects in obese subjects, probably due to higher SNS activity in those.[271] Also, this substance has been reported to exert beneficial effects on insulin sensitivity and lipid metabolism, reducing plasma LDL-C and triglyceride concentrations and increasing HDL-C.[270] Rilmenidine increased HDL-C and decreased FPG concentration in patients with the metabolic syndrome,[272] has further been reported to decrease noradrenaline spillover to plasma,[274] and could significantly increase adiponectin concentration in hypertensive patients.[110] Finally, body weight has been demonstrated to be reduced in patients treated with Moxonidine in two relatively large-scale studies, by -1,4kg within eight weeks and by -2,1kg within six months, respectively.[275,276] Interestingly, this reported weight loss was of a similar extent, as in the group of patients examined here.

Although evidence is limited to few studies, centrally acting sympathoinhibitory substances display striking similarities concerning their beneficial metabolic side-effects to the alterations found after RSD in this study. Intuitively, those substances may act in a way similar to RSD and therefore also have the same metabolic effects. Nevertheless, the exact mechanisms of RSD and the interactions between SNS and various metabolic pathways remain largely unclear. Importantly, the power of this study is tightly limited due to its retrospective design, the extraction of data from a patient registry and the relatively low case numbers. No causality between RSD, BP reduction and the metabolic alterations can be proven. Still, these results may give an overview of possible metabolic effects linked with RSD and inspire future trials.

### 4.1.3. Interpretation of Inflammatory Parameter Results

Results indicate, that Il-6 concentration and WBC count were significantly reduced in follow up of patients treated with RSD. CRP concentration however, did not change significantly, which may be due to the lack of hs-CRP measurement, posing an important limitation to this study. CRP changes were small and regarded non-significant, still, a trend towards a decrease in responders and an increase in non-responders may be visible.

Il-6 concentration differences at six months were significantly higher in the RSD responders group, but changes at one year appeared to be similar in both groups. This suggests an anti-inflammatory effect generated by RSD, unrelated to blood pressure reduction or, as previously discussed, renal denervation may have failed to significantly reduce BP in certain patients, but still have caused other effects leading to the observed decrease in inflammatory parameters. Similarly, also the decreases in leukocyte counts were significant for RSD responders and non-responders at one year.

Another interesting finding regarding Il-6 was, that baseline Il-6 values were significantly correlated with ABP differences at six months. This finding implies, that the success of RSD may be predictable by the degree of systemic proinflammatory activity.

A great body of evidence highlights the presence of inflammation and its important role in pathophysiological processes underlying a variety of diseases like visceral obesity,[155-157] the metabolic syndrome[107,108,147-149] or atherosclerosis.[27] Inflammation is also involved in the pathogenesis of hypertension in the kidney,[4,61,62] in microvascular control and insulin resistance,[42,48,49] in the pancreas in diabetes,[195-197] and in deregulations of the endocrine system.[214] Importantly, it is well known, that increases in proinflammatory markers like CRP are an independent risk factor for cardiovascular disease, death and diabetes.[150-152] One may

therefore speculate, that the decreases in inflammatory marker concentrations upon RSD may reduce CV risk.

However, the underlying mechanisms, linking the SNS to inflammation are complicated, and only incompletely understood by now. Increased SNS activity may up-regulate RAAS activity,[120,121] and consecutively increase circulating or local levels of angiotensin II, which is known to be involved in numerous inflammatory processes, probably by causing oxidative stress.[27,142,192] Furthermore, angiotensin II is known to be able to cause activation to immune cells like macrophages,[142,143] which is an important contributor to atherosclerosis,[142] diabetes,[194,195] or kidney injury.[59,61,62] This also explains the protective effect of ACE-I or ARB against diabetes[144,187-189], chronic kidney disease and renal failure.[31,32]

Furthermore, the ARB telmisartan has been recently reported to reduce Il-6 and TNF-α concentrations.[264] Few studies also suggest, that centrally acting imidazoline receptor binding agents like Moxonidine may display anti-inflammatory properties, as TNF-α levels have been shown to be reduced by such medication compared to atenolol.[303] This suggests a direct or indirect effect of central sympathetic blockade on inflammatory pathways and may therefore be a possible explanation for the putative anti-inflammatory effects of RSD seen in our study.

### 4.1.4. Interpretation of Renal Function Parameter Results

Our results suggest, that creatinine concentration and mean glomerular filtration rate did not change in a statistically significant way after RSD. Cystatin C concentrations significantly increased during follow up ($p=0,026$ and $p<0,001$, respectively).

The findings concerning creatinine and GFR are line with those reported in most studies on renal denervation, where also no deterioration of renal function could be found after RSD either.[283,290,291,296] However, the three year follow up report of the SYMPLICITY HTN-1 study described a significant decrease in GFR and an increase in serum creatinine concentrations.[293]

Renal function parameters at baseline and during follow up did not differ significantly between RSD responders and non-responders, but creatinine concentration showed a trend towards an increase and GFR seemed to decrease among responders, while non-responders tended to have changes to the opposite direction. This may suggest a connection of renal function parameter changes with blood pressure reduction. Glomerular hyperfiltration in hypertension, possibly caused by obesity,[70,71] sympathetic nerve- and RAAS activity,[120]

could thus have been reversed by weight loss or decreased SNS activity, causing decreases in GFR and increases in creatinine concentration.

Our results concerning Cystatin C however, are contrary to those reported in the SYMPLICITY HTN-2 study, where no changes were reported at six months after RSD.[291] Cystatin C levels had significantly increased among RSD responders and non-responders at one year ($p<0,001$ for both), which suggests effects apart from alterations in blood pressure. Indeed, increased Cystatin C levels are frequently found in obesity and the metabolic syndrome, independent of alterations in other renal function parameters.[304,305] Recent studies demonstrated, that Cystatin C is also produced in fat cells, whereupon in obesity, subcutaneous and visceral adipose tissue display an increased expression of Cystatin C mRNA.[305] Still, reduction of fat mass by bariatric surgery has been reported not to result in significant decreases of Cystatin C concentrations.[306] Importantly, Cystatin C also seems to be involved in inflammatory processes, as its plasma concentrations have been shown to be inversely correlated with TNF-α and Il-6 levels in hypertensive patients.[307] This seems to be in line with the finding in our study, whereupon Cystatin C levels significantly increased, while Il-6 concentration was reduced after RSD. An association between weight loss and Cystatin C seems unlikely in this study, as patients lost weight but Cystatin C levels increased.

However, the underlying mechanisms are not understood up to now. In any case, Cystatin C seems to play an important role in inflammation, especially in obesity. The findings in our patients, may possibly be a sign of anti-inflammatory properties of Cystatin C, but a direct impact of changed SNS activity on Cystatin C levels also seems possible.

Concluding, further scientific effort is needed to increase our knowledge in this field.

### 4.1.5. Interpretation of Endocrine Parameter Results

We found, that free plasma cortisol concentrations had significantly increased at six and twelve months after RSD. Intuitively, blood pressure reduction should rather be accompanied by a decrease in HPA-axis activity and thus cortisol concentrations. Still, a possible explanation for this finding exists:

States of chronic inflammations, like IBD or rheumatoid arthritis, are known to be associated with impaired responsiveness of the HPA-axis.[216-218] It is unclear, what ultimately causes those alterations, leading to actual or functional hypocortisolism,[220] but the glucocorticoid receptor may play a crucial role.[220,222] As the metabolic syndrome, diabetes and hypertension are known to be accompanied by increased measures of inflammation,[107,108,147-149] it seems

likely, that those alterations of the HPA-axis may also occur in those states. Thus, it seems possible, that the decrease in inflammatory parameters seen in this study upon RSD may be paralleled by an increase in cortisol concentration, as inhibitory effects on the HPA axis might have been reduced.

The HPA-axis may also be closely linked with the SNS,[223] and noradrenaline is known to exert modulatory effects on immune cells.[223,224] Studies in patients with rheumatoid arthritis suggest, that chronic inflammation may influence the response to adrenergic stimuli in immune cells,[226] as well as local and systemic sympathetic innervation, whereupon SNS activity has been shown to be increased in RA patients.[227-229] TNF-α blockers, used in the treatment of rheumatoid arthritis have been reported to significantly reduce ABP, as well as plasma noradrenaline levels and plasma renin activity.[230] Apart from classic inflammatory diseases, an impaired HPA-axis reactivity was also found in hypertension, coronary artery disease and obesity,[231-233] and low plasma cortisol levels were reported in hypertension and OSAS.[234-235] This suggests, that an increase in plasma cortisol levels upon RSD can be plausible. However, those findings should be interpreted only carefully, as singular measurements of plasma cortisol levels are not a very reliable way to judge HPA-axis function, as those results may have been influenced by a great number of other factors next to RSD. Still, as the increase in cortisol concentration parallels a decrease in Il-6 and leukocyte levels, an association with a decrease in SNS activity by RSD seems likely.

Analysis of thyroid function parameters in a smaller subset of patients revealed a significant decrease in $fT_4$ concentration and trend towards an increase in TSH levels, which was tested significant for RSD responders. Baseline TSH and TSH changes were significantly higher among RSD responders, as well as the decrease in $fT_4$. This suggests a close relationship between alterations in thyroid function and changes in blood pressure. Indeed, ABP changes at six months were significantly correlated with baseline TSH concentration, suggesting a possible prediction of ABP response to RSD by thyroid function parameters.

Obesity is reported to be associated with higher levels of TSH, which decrease with weight loss,[238,240-242] while results concerning free thyroid hormones vary between the studies.[240-242] Weight loss may especially influence the peripheral conversion of $T_4$ to $T_3$.[243] The HPT-axis might also be linked with the actions of leptin at some level. However, as weight decreased and thus probably also leptin levels, a decrease in TSH concentration would have been expected.

Inflammation and oxidative stress, for example caused by Il-6,[248,256] may exert similar influences in the HPT-axis, than those describe for the HPA-axis.[246] This may affect both the CNS regulation of thyroid hormones, as well as their peripheral metabolism.[247-251]

The observed decrease in inflammatory parameters would therefore suggest, that the inhibitory effects on the HPT-axis might have been at least partly reversed. As TSH tended to rise, the increase being significant among RSD responders, a decrease of suppression to the HPT-axis by inflammation seems possible. However, in this case, alterations in TSH would have been expected to be paralleled by an increase in free hormone concentrations. Thus, it seems more likely, that rather a primary decrease in $fT_4$ consecutively caused a reactive increase of TSH through the HPT axis feedback system. A decrease in $fT_4$ could be explained by periphery effects involving the conversion and inactivation of free thyroid hormones, which may have been be influenced by weight loss or changes in SNS activity.

Nevertheless, it has to be emphasised, that thyroid hormone parameters were only collected in a small subset of patients in this study and results may have been biased by various factors, as discussed below. Still, changes in thyroid parameters upon RSD seem possible and may intuitively contribute to blood pressure and metabolic effects.

Further studies are required to allow a clearer view of those alterations and the possible underlying causes.

## 4.2. Possible Limitations of this Study

It has to be emphasised, that this is an uncontrolled, retrospective study based on a patient registry. Due to the retrospective character of the study, no sample-sized calculation could be conducted, as the number of patients enrolled in this analysis resulted from the number of patients treated. Importantly, due to the lack of a control group and its statistical limitations, this study can neither give evidence on any causalities of alterations in the examined parameters, nor can it rule out other factors possibly having biased the measures. Due to those mentioned statistical restrictions and the small size of the study sample, only simple statistical analysis, including descriptive statistics, calculation of correlation coefficients, t-tests and non-parametric tests were conducted.

The retrospective character of this analysis also limits the evidence that can be drawn from this study, as in some cases it is not possible to re-enact afterwards, how and when exactly the different measures were taken. Also, the collected parameters themselves may limit the quality of this analysis, as the available measures not always represent the gold standard in

the assessment of the various examined functions of the cardiovascular, metabolic, endocrine or immune systems.

This thesis refers to the "metabolic syndrome" as primary point of interest. However, not all patients in this dataset may have met all criteria necessary for this denotation, as some patients had baseline BMI values <25kg/$m^2$. Also, this dataset may not represent a typical cross-sectional collective of individuals with the metabolic syndrome, because of a pre-selection of patients displaying resistant hypertension, but hypertension itself constitutes an important diagnostic feature of the metabolic syndrome. However, average baseline BMI among all patients was 30,4kg/$m^2$ and a large fraction exhibited impairments in glucose- and lipid metabolism, as well as in renal function or inflammation. Together, these facts suggest, that despite a possible bias due to pre-selection of resistant hypertensive patients, the majority of patients have actually met the diagnostic criteria for the metabolic syndrome.

BMI values were based on documentation of body weight in the patient charts. Although body weight is usually measured by nursing staff upon admission, there is no proof, that weight was really measured in all cases. It is possible, that some patients were only asked about their approximate weight. Also, the accuracy of the measurement was not defined, so that in some patients, weight was approximated to full kilograms, while more exact documentation was done in others. Still, it should be noted, that several admission notes and outpatient reports on the follow up examinations gave account of patients, who themselves reported they had experienced considerable weight loss.

Another possible methodological limitation to this dataset is, that according to most guidelines on the MetS, abdominal obesity should be judged by measuring waist circumference, as it is the best correlate for all other MetS parameters.[129,130,131] Still, guidelines suggest, that if -as it was the case here- BMI was over 30kg/$m^2$, no measurement of WC was necessary, as abdominal obesity would be definitely present in this state.[129]

Glucose metabolism was examined by analysing FPG concentration and HbA1c fraction. As the name fasting plasma glucose implies, it should be assessed in a state of fasting. As discussed before, it is important to remark, that the baseline and the six months examination was done, while the patients stayed in hospital overnight, while the twelve months follow up visit took place at the outpatient clinic. It suggests itself, that fasting discipline may therefore have been higher among inpatients than outpatients, which is likely to represent a considerable bias in this study.

Concerning inflammatory parameters, the greatest limitation to our results is the lack of the measurement of high-sensitivity-CRP, which should be used for the assessment of subclinical inflammatory processes in diseases like hypertension and coronary artery disease. The "normal" CRP assessment, used in our study may be suitable for detection of major inflammations, however, small changes will remain undetectable.

Free plasma cortisol was measured in a relatively large part of the study patients. Most measures were taken before noon but still, the circadian rhythm of cortisol may have biased the results, as the exact time of assessment was not standardized in this study. Also, singular measurement of blood cortisol levels does certainly not represent the best way to assess HPA-axis function. Conduction of dexamethasone-suppression tests or salivary cortisol measuring several times a day would have been desirable, but were not conducted among the study patients.

Measures of thyroid function were only assessed in a minor part the patient collective. Patients with thyroid disorders or on thyroid hormone substitution may have been included in our study sample and have thereby biased the results. This and low patient numbers considerably limit the reported results concerning thyroid hormones, which should therefore only interpreted very cautiously.

## 4.3. Conclusion

Patients treated with renal sympathetic denervation displayed significant decreases in ABP levels up to one year after the intervention, while OBP values did not change significantly versus baseline. Exclusion of patients considered "pseudo-hypertensive" resulted in significantly improved BP results, which suggests, that ABP measurement should be performed in all patients screened for eligibility for RSD. Also, the finding, that metabolic and inflammatory parameters at baseline and the changes in those were, if at all, correlated to ABP values, raises the question about the actual value of OBP measurement in assessing RSD results.

Interestingly, we observed a significant decrease in body weight at six months after RSD. Changes in parameters concerning glucose and lipid metabolism were mostly small and non-significant. However, we conclude, that most glucose and lipid parameters did at least not improve, but partly displayed a trend towards a deterioration of values. As an exception, HDL-C levels significantly increased in all patients and more markedly among RSD responders.

A major finding in this study is the decrease we found among the inflammatory parameters Il-6 and WBC count. Although those alterations were of a higher extent among RSD responders, also non-responders had witnessed significant improvements after one year of follow up. Although it is speculative, our results may suggest, that effects of RSD concerning inflammatory activity could also be seen in non-responders in terms of blood pressure changes. Therefore, non-response to RSD may putatively not be due to a lack in reduction of SNS activity, but to causes underlying resistant hypertension apart from increased SNS activity. Given the possible connections between SNS, RAAS and inflammation, it would be interesting to learn more about changes in measures of SNS activity after RSD, as evidence of decreased noradrenaline spillover rate is based on only very few patients, and results concerning MSNA are not conclusive.

Changes in endocrine parameters should be interpreted only cautiously, as low patient number and methodological constraints may limit the results. Still, it seems highly interesting, that both the HPA-axis and thyroid parameters may have been influenced by RSD. Whether these alterations have been caused by concomitant changes in inflammation, body weight, SNS activity or blood pressure itself, remains to be elucidated by future studies.

Concluding, it should be mentioned once more, that this study may be limited by the uncontrolled, retrospective design, low patient numbers and methodological weaknesses. Especially the findings from the previously published SIMPLICITY HTN-3 study should teach us a lesson, not to prematurely accept and apply novel scientific findings before they have been proven evidence in randomized controlled trials.

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