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Eeftinck Schattenkerk, D.W.

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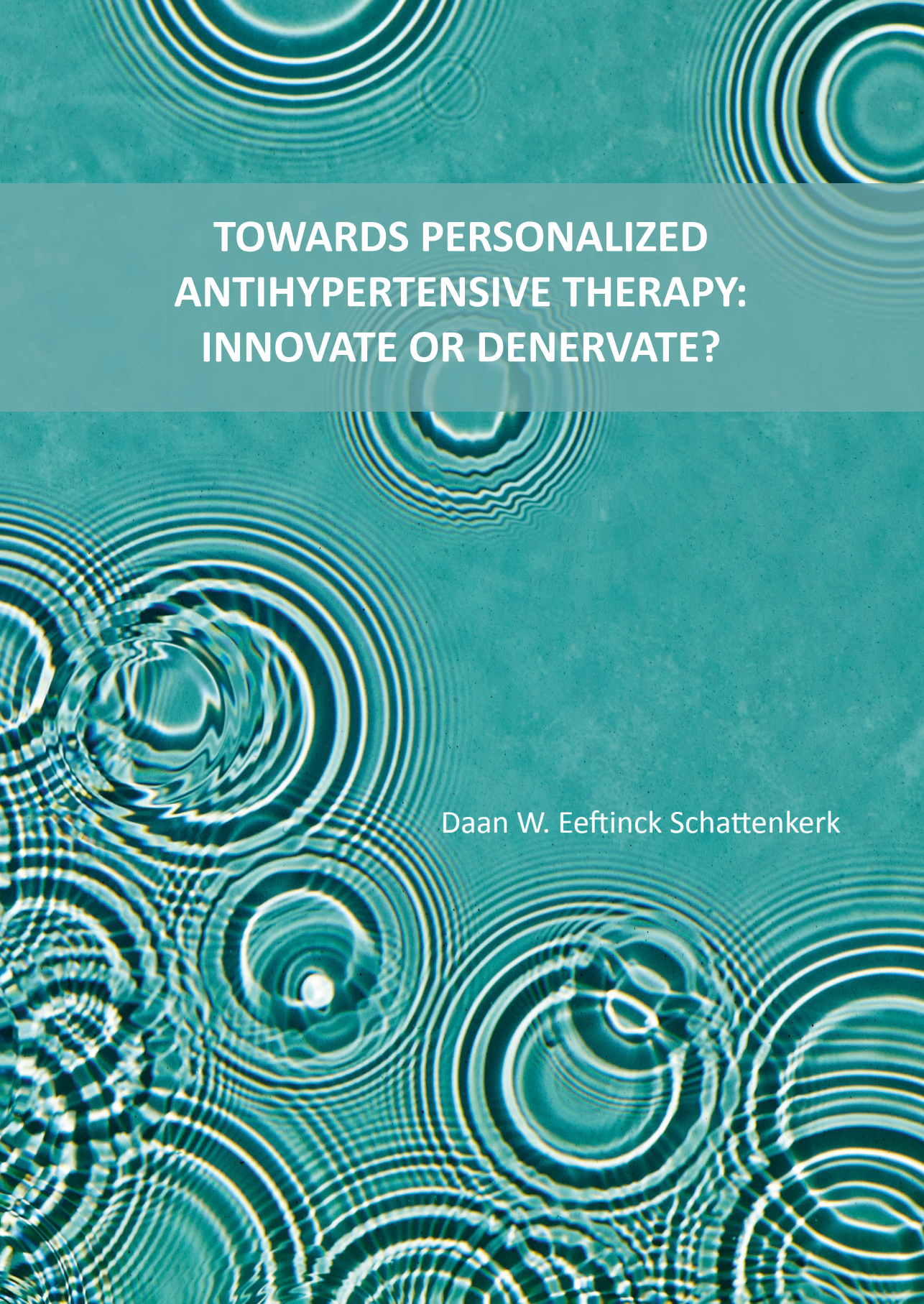

TOWARDS PERSONALIZED ANTIHYPERTENSIVE THERAPY: INNOVATE OR DENERVATE?

Daan W. Eeftinck Schattenkerk

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Daan Wouter Eeftinck Schattenkerk

Author: Daan W. Eeftinck Schattenkerk
Cover: Nikki Vermeulen - Ridderprint
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TOWARDS PERSONALIZED ANTIHYPERTENSIVE THERAPY: INNOVATE OR DENERVATE?

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Chapter 01

General introduction and outline of the thesis

General introduction

Cardiovascular disease and risk assessment

Cardiovascular disease (CVD) is the leading cause of death globally. Each year approximately 17,5 million people suffer from the fatal consequences of CVD, accounting for nearly one third of annual worldwide deaths.[1, 2] The number of fatalities due to CVD rises and by the year of 2030, more than 23 million people are expected to die from CVD annually, posing a major global health threat.[3]

The foundation for combatting CVD was laid with the identification of cardiovascular risk factors in the Framingham Heart study, which was initiated in the 1940's and is still ongoing.[4] Prior to Framingham, little was known about CVD and it was generally considered an inevitable consequence of ageing.[5] Since the first identification of cardiovascular risk factors in Framingham, various large prospective and cross-sectional population-based studies have extended our knowledge on cardiovascular illnesses and its risk factors. Of the potentially modifiable cardiovascular risk factors, hypertension is generally considered to be the most important.

Hypertension: a common but elusive condition

Recently the World Health Organization has identified hypertension as the major cause for global morbidity and mortality in their Global Burden of Disease Study.[6] While already a major health issue, the worldwide prevalence of hypertension and related disease rises, in particular in the developing world.[6] In Westernized countries, such as the United States of America, but also in the Netherlands, an estimated one third of its inhabitants currently suffer from hypertension.[7, 8]

Despite its high prevalence and relevance to global health, hypertension is a condition that is difficult to grasp. Not only because it is often asymptomatic and hard to assess due to its variable nature, but also because much is still not known regarding its pathophysiology.[9] While blood pressure is conventionally measured at the level of the brachial artery, it has been recognised that substantial differences in blood pressure exist along the arterial tree. Central or aortic blood pressure may be a more accurate risk factor and improve cardiovascular risk assessment. Antihypertensive therapy is also lacking, since less than half of patients with hypertension have their blood pressure regulated adequately, despite a variety of available blood pressure lowering drugs.[10, 11] The effectiveness of antihypertensive treatment is with great variation and a differential impact on brachial versus aortic blood pressure may also be important in this respect.

To improve cardiovascular healthcare, a better understanding and treatment of hypertensive disorders is of utmost importance. Personalization of antihypertensive strategies may aid as such: by identification of subjects who need treatment the most, for example by assessment of central hemodynamics, and diverting treatment to those that will benefit most, to increase its effectiveness. Novel measurement techniques as well as new treatment modalities are paramount to further improve hypertension control and reduce the global burden of cardiovascular disease.

Blood pressure measurement 2.0: from Riva-Rocci to central hemodynamics

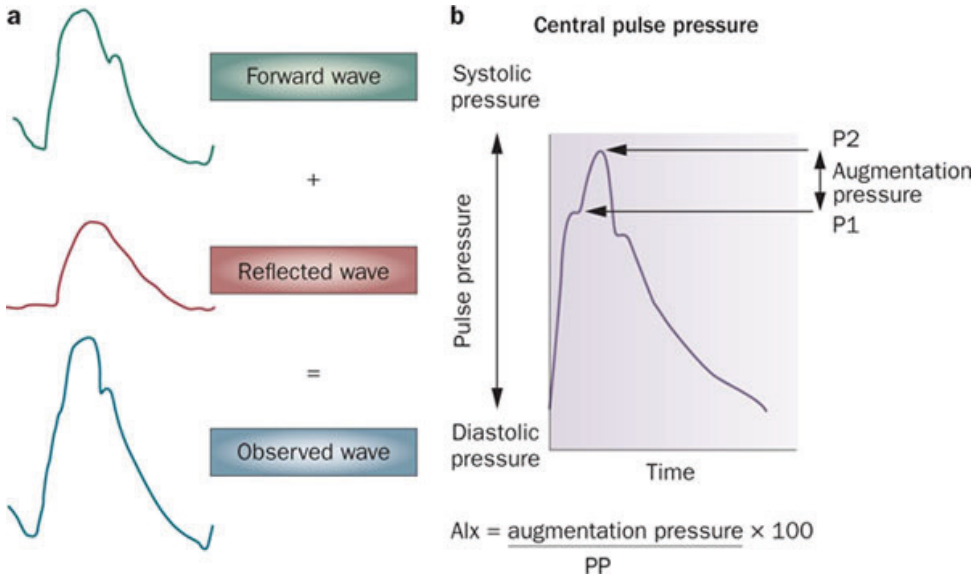
Our present understanding of hypertension originates from the groundwork of physician William Harvey, who first described the cardiovascular system in "e*xercitatio anatomica de motu cordis et sanguinis in animalibus"* in 1628. One decade later Stephen Hales was the first to measure "*the force of blood*" (i.e. blood pressure) in 1733 using a glass tube inserted into a horses' artery (figure 1 [12]). However, it was not until 1896 that the measurement of blood pressure came into clinical practice, thanks to the invention of the sphygmomanometer by Scipione Riva Rocci . Ten years later Nicolai Korotkoff improved this technique by use of a stethoscope with the identification of the Korotkoff sounds in 1905. At present, the gold standard for the non-invasive assessment of blood pressure remains the combined use of a sphygmomanometer and a stethoscope according to Riva-Rocci Korotkoff. To aid us in our understanding and thereby the treatment of hypertensive disorders in individuals at risk, technological advancements now enable us to non-invasively assess the distinct components involved in blood pressure regulation. In addition, measurement of blood pressure itself has also improved, as we are now able to measure it where it matters the most: central blood pressure.

FIGURE 1 - Assessment of "the force of blood" by Stephen Hales**.**

For more than a century blood pressure has been measured using an inflatable cuff around the upper arm. The thereby obtained brachial blood pressure values have proven their worth in cardiovascular risk assessment and management. However, while diastolic blood pressure is relatively constant, substantial differences in systolic blood pressure are known to exist along the vessels of the arterial tree. Intra-arterial blood pressure measurements have shown that systolic blood pressure in the brachial artery may be up to 40 mmHg higher than in the aorta.[13] These differences in blood pressure seem relevant, as aortic blood pressure more closely resembles the burden imposed to the organs at risk (i.e. the heart, brain and kidneys) than brachial blood pressure, it may thereby improve CVD risk assessment.

Differences between central and peripheral blood pressure are thought to be caused by arterial wave reflection and pressure augmentation.[14-16] As the heart pumps, blood pressure waves are generated that travel forward from the heart towards the periphery. Impedance mismatch results in reflection of these forward travelling waves, giving rise to their reflected counterparts: backward pressure waves, that travel back from the periphery towards the heart. Coinciding of these oppositely travelling pressure waves results in augmentation of systolic blood pressure, which is often quantified as the augmentation index (figure 2 [17]).[14-16] Several factors may influence the process of arterial wave reflection and pressure augmentation, and affect differences between central and peripheral blood pressure, these include, large artery stiffness, heart rate, systemic vascular resistance, stroke volume and body height.[18-22]

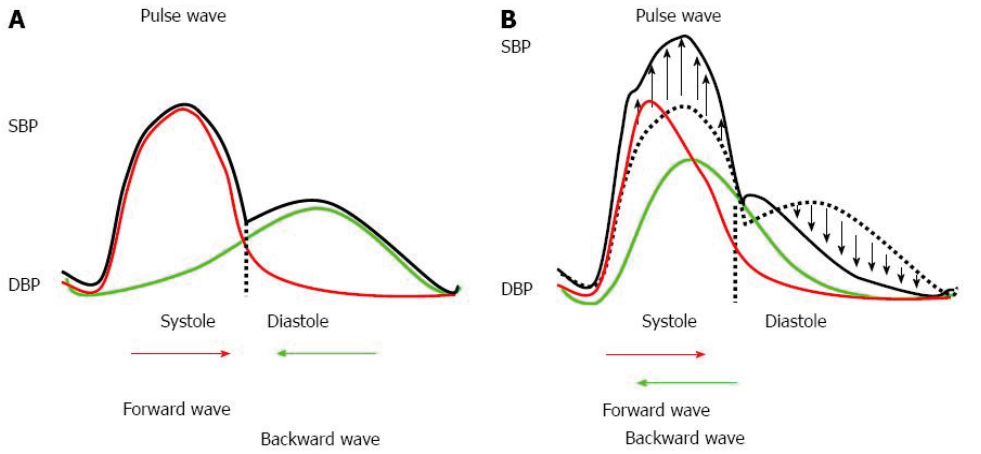
Figure 2 - Arterial wave reflection and pressure augmentation.



Amounting evidence suggests that central (i.e. aortic) blood pressure is indeed a stronger predictor for future CVD than conventionally measured peripheral (i.e. brachial) blood pressure.[23-26] Furthermore, indices of wave reflection, such as augmentation index, have also been independently associated with CVD in various populations, possibly by detrimental effects on myocardial ejection and perfusion.[27, 28] Depending on the timing and morphology of forward and reflected pressure waves the systolic load imposed on the heart may be increased, while in the meantime beneficial effects on coronary perfusion during diastole may be lost (figure 3 [29]). Given the association

with cardiovascular risk, the assessment of central hemodynamics (including blood pressure and wave reflection) may be clinically relevant and its physiology important to comprehend. Yet, little is known regarding differences in central versus peripheral blood pressure among subjects with different hypertensive phenotypes, or ethnic backgrounds. This may be important to distinct hypertensive conditions, such as isolated systolic hypertension of the young[30], or shed light on ethnic disparities in CVD that traverse conventional risk assessment.[31-34] Furthermore, pharmacological interventions aimed at lowering blood pressure have also been shown to be able to differentially affect central versus peripheral systolic blood pressure, by mechanisms that are incompletely understood.[35, 36]

FIGURE 3 - Adverse effects of changes in wave reflection.

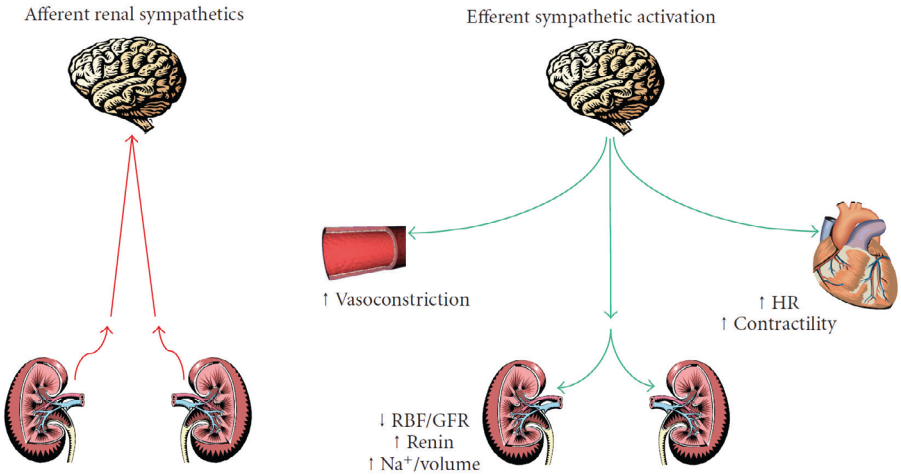


Blood pressure treatment 2.0: Renal sympathetic denervation

Recently, a major breakthrough in anti-hypertensive therapy was renal sympathetic denervation, a promising new treatment modality for refractory hypertension.[37-39] This minimally invasive, catheter-based technique aimed at lowering blood pressure by disrupting the sympathetic nervous crosstalk between the kidneys and the central nervous system using radiofrequency ablation. As evidenced in animal studies, renal denervation would theoretically lower blood pressure by inhibiting central sympathetic activity (thereby lowering heart rate and reducing vascular resistance), attenuate renin release and reduce water and salt retention (figure 4 [40]). The introduction of renal sympathetic denervation as a proof-of-principle in the Simplicity-HTN 1 trial was quickly followed by the promising results of the Simplicity-HTN 2 trial that showed a blood pressure reduction following renal denervation of

more than 30 mmHg compared to baseline. In both Simplicity-HTN trials, a single point ablation catheter produced by Medtronic™ was used.[38, 39] Following Simplicity-HTN, several other large biotech companies sprang into action and each developed their own catheters, spinning off several other renal denervation studies. Yet, the lack of a sham-controlled treatment arm in the Simplicity-HTN 1 and 2 trials was much debated. Despite the encouraging results of the Simplicity-HTN trials, it was clear that the blood pressure lowering effects of renal denervation were highly variable. The reason for this variability in blood pressure lowering effect is unclear, but could be related to inter-individual differences in (renal) sympathetic activity or variations in ablation effectiveness. At present, the only available technique to assess effectiveness of the ablation involved cumbersome nor-adrenaline spill-over measurements, that due to a lack of radioisotopes could not be performed in many countries. Therefore, an easily applicable, readily available non-invasive technique to determine who will benefit from renal denervation and to assess therapeutic effectiveness is warranted.

FIGURE 4 - Sympathetic crosstalk of the kidneys.



Outline of the thesis

This thesis addresses several aspects that are important to our current understanding of hypertensive disorders and improve care for the hypertensive patient. Both (patho)physiological aspects, making use of innovative techniques to assess central hemodynamics, as well as a new treatment modality: renal sympathetic denervation, are discussed.

In **part I,** the emphasis lies with the assessment and physiology of central hemodynamics.

Chapter 2 includes the validation of a novel non-invasive continuous blood pressure monitor: the Nexfin®, compared to conventional Riva-Rocci Korotkoff assessment of blood pressure. The Nexfin® device applies volume-clamp plethysmography, according to the Finapres method, to derive an arterial pressure waveform and determine blood pressure and various derived hemodynamics on a beat-to-beat basis. This measurement technique has proven invaluable in numerous research settings, including several studies as performed in this thesis. In **Chapter 3,** we study determinants of vascular baroreflex sensitivity in a random population. While baroreflex sensitivity commonly addresses the quickly occurring changes in heart rate in response to changes in blood pressure as sensed by the carotid baroreceptors, the vasomotor arm of the arterial baroreflex is neglected. By using data of the above mentioned validation study we sought out to compare determinants of cardiac and vascular baroreflex sensitivity. **Chapter 4** focuses on the role of the heart in arterial wave reflection and central blood pressure augmentation. While traditionally wave reflection and the effects on central hemodynamics are considered to be principally determined by the properties of the arterial system, we demonstrated important effects of changes in the cardiac properties, including stroke volume and contractility, on indices of wave reflection.

Part II involves central hemodynamics, individual differences and effects of pharmacotherapy.

In **Chapter 5,** we study differences in arterial wave reflection and central blood pressure among subjects from various ethnic backgrounds with the aim to provide a potential explanation for the inequities in cardiovascular disease burden among ethnic groups beyond conventional risk factors. To do so, we gratefully used the data provided by HELIUS: a large scale, multi-ethnic cohort study, conducted in Amsterdam, the Netherlands. In **Chapter 6,** we studied the nature of isolated systolic hypertension in the young, a special form of hypertension which is associated with large differences in peripheral and central blood pressure. At present the pathophysiology of isolated

systolic hypertension in young individuals is incompletely understood as is its association with cardiovascular risk. We used the HELIUS dataset to explore the determinants of isolated systolic hypertension in the young. In **Chapter 7** we study the effects of nebivolol - a third generation beta blocker with vasodilatory properties - compared to metoprolol on central hemodynamics. While beta-blockers generally have deleterious effects on central blood pressure and pressure augmentation by lowering heart rate and prolonging ejection duration, it was postulated that a reduction in wave reflection due to vasodilation with nebivolol could potentially circumvent this issue.

Part III focuses on renal sympathetic denervation as a novel treatment option for hypertension.

In **Chapter 8,** we assessed the effects of renal sympathetic denervation on cardiac sympathetic nerve activity and function in subjects with therapy resistant hypertension. We used 123-I mIBG scintigraphy, a nuclear imaging technique that uses a radioactively labelled nor-adrenaline analogue that is taken up post-synaptically, to evaluate sympathetic nerve activity and non-invasively measured central hemodynamics prior to and after renal denervation . In **Chapter 9** we used the same technique to quantify the effects of renal sympathetic denervation on kidney sympathetic innervation. While 123I-mIBG imaging has been extensively used on the heart, we aim to use this technique to assess effectiveness of nerve ablation by renal sympathetic denervation. Renal 123I-mIBG imaging could thereby provide a non-invasive alternative to nor-adrenalin spill-over studies that require venous and arterial catheterisation of the organ of interest. **Chapter 10** illustrates the viability of renal 123I-mIBG scintigraphy in a case report of a subject undergoing a kidney auto-transplantation because of refractory fibromuscular dysplasia of the renal artery.

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Part I

ASSESSMENT AND PHYSIOLOGY OF CENTRAL HEMODYNAMICS

Chapter 02

Nexfin noninvasive continuous blood pressure validated against Riva-Rocci / Korotkoff

Daan W Eeftinck Schattenkerk
Johannes J van Lieshout
Anton H van den Meiracker
Karel R Wesseling
Stéphane Blanc
Wouter Wieling
Gert A van Montfrans
Jos J Settels
Karel H Wesseling
Berend E Westerhof

Am J Hypertens. 2009 Apr; 22 (4): 378-383

Abstract

Background: The Finapres methodology offers continuous measurement of blood pressure in a non-invasive manner. The latest development using this methodology is the Nexfin™ monitor. The present study evaluated the accuracy of Nexfin noninvasive arterial pressure (NAP) compared with auscultatory blood pressure measurements (Riva-Rocci / Korotkoff, RRK).

Methods: In supine subjects NAP was compared to RRK, performed by two observers using an electronic stethoscope with double earpieces. Per subject three NAP – RRK differences were determined for systolic (SBP) and diastolic (DBP) blood pressure and bias and precision of differences were expressed as median (25^{th}, 75^{th} percentiles). Within-subject precision was defined as the (25^{th}, 75^{th} percentiles) after removing the average individual difference.

Results: A total of 312 data sets of NAP and RRK for SBP and DBP from 104 subjects (aged 18 to 95 years, 54 males) were compared. RRK SBP was 129 (115, 150), and DBP was 80 (72, 89), NAP – RRK differences were 5.4 (–1.7, 11.0) mmHg and –2.5 (–7.6, 2.3) mmHg for systolic and diastolic BP respectively; within-subject precisions were (–2.2, 2.3) and (–1.6, 1.5) mmHg, respectively.

Conclusion: Nexfin provides accurate measurement of blood pressure with good within-subject precision when compared to RRK.

Introduction

In the late 1970s and early 1980s Wesseling *et al.* developed the Finapres method for continuous and non-invasive measurement of finger arterial pressure.[1] This method is based on the volume-clamp methodology of Peňáz[2] and the Physiocal criteria of Wesseling.[1] Since then, measurement of blood pressure (BP) on a continuous basis became possible without the necessity of cannulating an artery. Presently, the non-invasive Finapres method has become an established substitute for invasive intra-arterial BP measurements in both clinical[3,4] and research settings.[5-15]

Volume-clamping keeps the artery at its "unloaded" volume, where transmural pressure is zero, with a fast inflatable cuff system around the finger. Arterial blood volume is assessed by an optical plethysmograph mounted in the cuff. The Physiocal criteria establish the unloaded volume of the artery by analyzing the plethysmogram during short periods of steady cuff pressure levels. The Physiocal is regularly repeated during the measurement to ensure accuracy in the presence of changing physiological states of the vasculature. With the correct implementation of these techniques, the finger cuff pressure waveform equals finger arterial pressure throughout the cardiac cycle and can be measured for hours on end.[8,16]

The Nexfin™ monitor (BMEYE B.V. Amsterdam, The Netherlands) uses an updated implementation of the Finapres method that was used originally in the TNO Finapres model 5 and Ohmeda Finapres 2300. These devices have been clinically validated earlier and are being used in a variety of settings.[3-15,17,18] The Nexfin, using the same basic principles, has been redesigned to allow the use of present-day hardware, software and physiological models. The finger cuffs have been redesigned with modern optical components for better signal to noise ratio.

Nexfin, as opposed to Finapres, provides reconstructed brachial arterial BP.[16,19-21] While traveling from the aorta to the periphery, the arterial pressure-wave changes shape. As a result of wave reflection systolic pressure increases whereas diastolic pressure decreases due to resistance to flow. By applying a physiological model and a regression based level correction, brachial BP is obtained.[16,19-21]

The present study aimed to evaluate the accuracy of the Nexfin reconstructed brachial arterial BP as compared to classical sphygmomanometry (Riva-Rocci / Korotkoff, RRK) BP measurements.

Methods

Subjects

For this study, 116 subjects (59 males) were included. BPs spanned the range from hypotensive (<100 mmHg systolic and <60 mmHg diastolic) to hypertensive (>140 mmHg systolic and > 90 mmHg diastolic) values. The Medical Ethics Committee of the Academic Medical Center of the University of Amsterdam approved the study and all participants gave informed consent.

Measurements

Measurements were performed in a quiet and temperature-controlled room. The subjects lay supine to minimize hydrostatic errors and to limit the magnitude of spontaneous variations in blood pressure, interfering with a correct comparison of the non-simultaneous measurements. The subjects refrained from speaking or moving during measurements.

An appropriate size Nexfin finger cuff was applied to the mid-phalanx of the left middle finger and an appropriate size RRK cuff was applied to the upper left arm. Arm and cuffed finger were positioned alongside the body, facilitating access to the brachial artery for RRK measurement. The cuffed finger and the manometric arm cuff were positioned at mid-thorax level to avoid hydrostatic pressure differences.

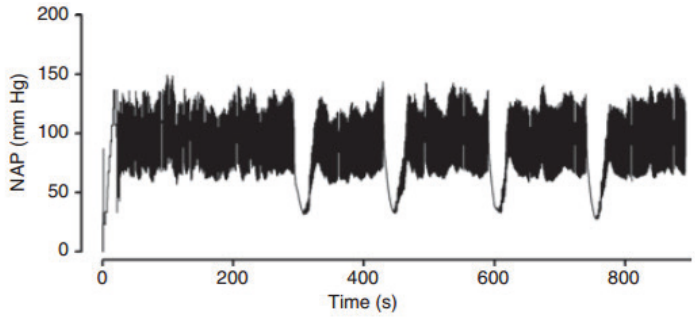
An electronic stethoscope (Littmann stethoscope model 3000, 3M Health Care, St. Paul MN, U.S.A.) with dual earpieces was used for the RRK measurements. The observers were instructed by a qualified hypertension specialist (GAvM) and successfully completed the online training program of the British Hypertension Society (http://www.abdn.ac.uk/medical/bhs/tutorial/tutorial.htm).

Since mercury sphygmomanometry is no longer allowed for use in clinical practice or research in the Netherlands, auscultatory RKK measurements were performed using an automated arm cuff in- and deflation system with built-in manometer (Finometer[22], FMS, Amsterdam, The Netherlands). The manometer displays an analog representation of a mercury column and digital real time values. Deflation rate was 2 mmHg/s. The manometer was calibrated before the study and checked after conclusion of the study. Two trained observers performed RRK measurements simultaneously in each subject. During RRK measurements, both observers independently recorded the Korotkoff I and V sounds, reflecting systolic and diastolic BP. The observers were blinded from each other's measurement and entered their measurements online via separate computers into a database. To ensure that measurements from both observers fell

within an acceptable range, data were checked at once for inter-observer differences. In case differences for systolic or diastolic BP were ≥ 4 mmHg, the observers were alerted and measurements were repeated. After acceptance, pairs of measurements from both observers were averaged for further analysis. The RRK measurement procedure was repeated at least 3 times for each subject. The first RRK measurement was intended to accustom the subject to the procedure. The last 3 observer-averaged RRK measurements were used as reference BPs.

During the supine resting period BP was allowed to stabilize. Physiocal was switched off as soon as the Physiocal interval had reached the proper clamping set-point.[23] Subsequently, a RRK measurement procedure was performed. After complete deflation of the manometric cuff and subsiding of initial BP swings caused by the post-occlusive hyperemic response (typically lasting ~30 s), recording was resumed with Physiocal calibration active for 1 min followed by the next RRK determination. An example of a measurement is given in Figure 1.

FIGURE 1 - Nexfin noninvasive blood pressure.



Continuous non-invasive arterial pressure (NAP) during the validation procure. After a period of supine rest, a first Riva-Rocci / Korotkoff (RRK) is performed and NAP temporarily drops to low values. The first RRK is not used in the analysis. NAP systolic and diastolic pressures are averaged over 30 s preceding the RRK. RRK served as a reference for the preceding and following NAP average. The variability in the continuous pressure recording is prominent. Continuous recording can give a reliable description of pressure, while RRK depends on the moment at which it is determined.

Analysis

The currently available protocols for the evaluation of BP measuring devices consider intermittent rather than continuous measuring devices and therefore are not directly applicable to continuous non-invasive measurement of blood pressure.[24,25] In this study a protocol resembling the guidelines of the Association for the Advancement of Medical Instrumentation (AAMI) was followed.[24]

Nexfin noninvasive arterial pressure (NAP) was averaged over 30 s to account for the effects of BP variability due to respiration and other physiological and psychological influences. Simultaneous RRK and NAP on one and the same arm is not possible and therefore RRK served as a reference for both the preceding and following NAP 30 s average. Differences are reported as "NAP before RRK" and "NAP after RRK". The smallest and largest values of adjacent NAP – RRK differences are also given. The AAMI protocol[24] prescribes two methods of data evaluation. For Method 1, the mean of 255 (3 observations in 85 subjects, possibly more) NAP – RRK differences must be ± 5 mmHg or less, with a standard deviation (SD) of 8 mmHg or less. Bland-Altman plots are given for the "NAP before RRK" differences. For Method 2, the data of the 3 observations are averaged before further data analysis. The allowed mean and SD of NAP – RRK differences of exactly 85 subjects are defined in a table.[24] Larger SDs are allowed for smaller mean differences.

In addition, the within-subject precision was calculated. To that end, the average of the three NAP – RRK differences in each subject was subtracted from those three differences and then the SD over the group was calculated. The mean difference will always be zero, and the SD is a measure of precision without the systematic component of the differences.

Results

Nexfin NAP was compared in a group of 116 volunteers. In 3 subjects, phase V of the RRK measurement could not be detected, 6 subjects were excluded due to technical problems, and 3 more subjects were excluded because of the presence of arrhythmias rendering the RRK data unreliable.

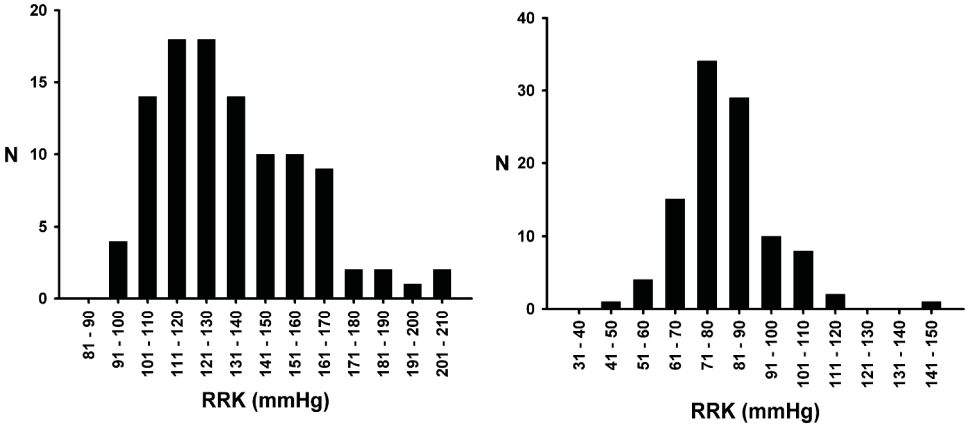
TABLE 1 - Characteristics for study subjects.

	Range	**Median (P25, P75)**	**Mean ± SD**
Age (years)	18-95	45 (31, 59)	46 ± 18†
Height (cm)	153-203	172 (167, 180)	173 ± 10†
Weight (kg)	45-124	72 (65, 85)	76 ± 16†
Heart rate (bpm)	42-95	66 (59, 74)	67 ± 11†
RRK Systole (mmHg)	94-208	129 (115, 150)	134 ± 24†
RRK Diastole (mmHg)	46-141	80 (72, 89)	81 ± 14†

P25 and P75 give the 25th and 75th percentiles. Mean and SD are given although data is non-normally distributed as indicated by †. Systolic and diastolic pressures as determined by Riva-Rocci / Korotkoff (RRK), averaged over the two observers and the three measurements.

Thus, data of measurements in 104 subjects (54 males) were available for analysis (Table 1). Systolic and diastolic pressures for the group as determined by RRK, averaged over the two observers and over the three measurements, are given in Table 1; the distribution of pressures is shown in Figure 2.

FIGURE 2A AND FIGURE 2B. - RRK BP distribution.



Distribution of blood pressures as determined by average of three RRK measurements per subject. Systolic (a) and diastolic (b) BP respectively.

In table 2, both median and 25th and 75th percentiles are reported as well as mean and SD, to allow comparison to criteria as proposed by the AAMI.[24] When data was not normally distributed, this is indicated (Table 2). NAP – RRK differences for systolic and diastolic pressures equal (non-parametrically tested) for "NAP before RRK" and "NAP after RRK" (Table 2). Figure 3 shows systolic and diastolic Bland-Altman plots of the NAP and RRK ("NAP before RRK") values and their differences. The scatterplots with correlations can be found in supplemental Figure 1.

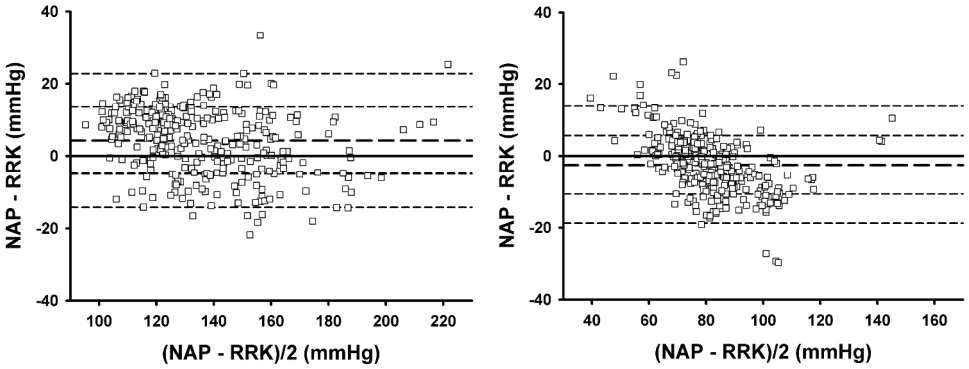
The within-subject precisions were better than group precisions and show high percentages of differences falling within the 5, 10 and 15 mmHg limits (Table 2).

TABLE 2 - NAP and RRK, Method 1: 312 data points in 104 subjects.

	Median (P25, P75)	Mean ± SD	% < 5 mmHg	% < 10 mmHg	% <15 mmHg
Systole					
NAP before RRK	5.4 (–1.7, 11.0)	4.3 ± 9.3†	30.1	61.9	87.8
within-subject precision	*0.1 (–2.2, 2.3)*	*0.0 ± 3.8*†	*85.3*	*97.1*	*100*
NAP after RRK	5.6 (–1.5, 11.7)	4.6 ± 9.3†	31.1	59.9	85.8
within-subject precision	*0.0 (–2.6, 2.4)*	*0.0 ± 4.1*†	*77.6*	*97.1*	*100*
Smallest difference	3.9 (–0.8, 9.4)	3.8 ± 7.5	45.2	73.1	93.9
within-subject precision	*0.0 (–1.9, 1.8)*	*0.0 ± 2.8*	*92.6*	*100*	*100*
Largest difference	7.8 (–4.3, 12.8)	5.0 ± 10.7†	16.0	48.7	79.8
within-subject precision	*0.8 (–3.7, 3.5)*	*0.0 ± 5.0*†	*70.2*	*94.2*	*100*
Diastole					
NAP before RRK	–2.5 (–7.6, 2.3)	–2.5 ± 8.1	46.8	76.3	93.3
within-subject precision	*–0.1 (–1.6, 1.5)*	*0.0 ± 2.4*	*96.8*	*100*	*100*
NAP after RRK	–2.7 (–8.0, 1.9)	–2.7 ± 8.1	48.1	76.3	92.9
within-subject precision	*0.1 (–1.9, 1.7)*	*0.0 ± 2.9*	*92.3*	*100*	*100*
Smallest difference	–1.8 (–6.5, 1.6)	–2.3 ± 7.2†	55.1	80.8	95.5
within-subject precision	*–0.1 (–1.2, 1.4)*	*0.0 ± 2.2*	*98.7*	*100*	*100*
Largest difference	–3.5 (–8.9, 3.4)	–2.9 ± 8.9†	39.7	71.8	90.7
within-subject precision	*0.3 (–2.6, 2.5)*	*0.0 ± 3.3*†	*90.4*	*100*	*100*

Pressures in mmHg. "RRK" is Riva-Rocci / Korotkoff, "NAP" is Nexfin noninvasive arterial pressure. "NAP before RRK" and "NAP after RRK" give the differences between RRK and preceding and following NAP, respectively. "Smallest difference" and "Largest difference" give the smallest and largest difference between RRK and the two adjacent NAPs. "W*ithin-subject precision*" is the SD over the group after removing the individual mean NAP – RRK differences. P25 and P75 give the 25th and 75th percentiles of the differences. Mean and SD are given to allow comparison to AAMI criteria; non-normally distributed data is indicated by †. The columns labeled "% < 5 mmHg", "% < 10 mmHg" and "% <15 mmHg" give the percentages of the differences within the specified range.

FIGURE 3 A AND FIGURE 3B - Bland-Altman analysis of Nexfin NAP and RRK BP measurements.



Systolic (a) and diastolic (b) BP respectively. Drawn lines represent mean differences (bias), dashed lines show ±SD and ±2SD; 312 data points (NAP before RRK) of 104 subjects.

Minimal differences (smallest difference of "NAP before RRK" and "NAP after RRK") were within AAMI limits (Method 1), i.e. not larger than 5 ± 8 mmHg.[24]

For Method 2, the three differences of each subject were averaged before further analysis.[24] Results of the first 85 subjects[24] are given in Table 3. The findings are similar to those for Method 1 (Table 2).

Prior to the recording, patients were supine while the cuffs were being applied and remained in that position for 337.5 (245, 450) s expressed as median (25th, 75th percentiles) from the start of recording with Nexfin until the first RRK. Thus, the reported supine resting period is somewhat underestimated. The average time between successive RRKs was 130 (110, 145) s.

Nexfin cuffs for finger circumferences ranging from 37-43 mm (XS), 43-51 mm (S), 51-60 mm (M) and 60-71 mm (L) were used 1, 35, 56 and 12 times, respectively. A standard upper arm cuff applicable for arm circumferences ranging from 29-42 cm was used in all cases except one, where a small cuff for arm circumferences ranging from 20-29 cm was used.

The inter-observer differences were –1 (–2, 0) mmHg for systolic and 0 (–1, 1) mmHg for diastolic pressures.

TABLE 3 - NAP and RRK, Method 2: 85 averaged data points in the first 85 subjects.

	Median (P25, P75)	Mean ± SD
Systole		
NAP before RRK	6.3 (–1.4, 11.3)	4.3 ± 8.7†
NAP after RRK	5.5 (–0.6, 11.7)	4.6 ± 8.5
Smallest difference	4.0 (–0.6, 8.9)	3.7 ± 7.2
Largest difference	8.0 (–1.6, 12.8)	5.2 ± 10.2†
Diastole		
NAP before RRK	–1.3 (–6.5, 1.7)	–2.1 ± 7.8
NAP after RRK	–2.2 (–6.4, 2.0)	–2.3 ± 7.5
Smallest difference	–1.2 (–5.7, 1.2)	–2.1 ± 6.9†
Largest difference	–2.6 (–7.3, 2.6)	–2.4 ± 8.5

Pressures in mmHg. "RRK" is Riva-Rocci / Korotkoff, "NAP" is Nexfin noninvasive arterial pressure. "NAP before RRK" and "NAP after RRK" give the differences between RRK and preceding and following NAP, respectively. "Smallest difference" and "Largest difference" give the smallest and largest difference between RRK and the two adjacent NAPs. P25 and P75 give the 25th and 75th percentiles of the differences. Mean and SD are given to allow comparison to AAMI criteria (Method 2; see text); non-normally distributed data is indicated by †.

Discussion

We validated reconstructed brachial pressures obtained by the Nexfin monitor in 104 subjects with a wide range of blood pressures. Nexfin provides accurate pressure measurement with good within-subject precision.

We consider that the guidelines of the Association for the Advancement of Medical Instrumentation (AAMI)[24] are not intended for the evaluation of continuous non-invasive measurement of blood pressure. For instance, simultaneous measurement of the device under assessment and the reference method on the ipsilateral arm is for obvious reasons not possible and the spontaneous variability in blood pressure will thus contribute to the error (see Figure 1). Nonetheless ipsilateral measurements are preferred to contralateral measurements since an unknown left-to-right difference might also afflict the comparison. Another disadvantage is that the RRK reference measurement itself often disturbs the test measurement: with temporal occlusion of the brachial artery the blood pressure distal to the arm cuff becomes strongly reduced (Figure 1). Subsequent release of the arm cuff may affect finger arterial pressure measurements by inducing a post-occlusion hyperemic response. The procedure may be painful as well. However, the similarity in the NAP – RRK differences when using "NAP before RRK" or "NAP after RRK" indicates that the potential disturbance of the

Nexfin measurement by the RRK has abated by the time a next NAP average was determined.

Further, in this study a continuous method was compared to single measurements with RRK. The time over which a continuous measurement should be averaged is not defined in the guidelines. The averaging time should be sufficiently long to encompass spontaneous blood pressure variations due to breathing and for instance Mayer waves. On the other hand, averaging time should not be too long, to minimize the time between successive measurements and to avoid effects of slower blood pressure oscillations. As a compromise, we used 30 s averages.

When sequential measurements are employed, the order of the test and reference measurements should be randomized in the analysis according to the AAMI protocol.[24] Instead, we reported all differences because of our interest in the consistency of the results over time. In addition, we report the within-subject precision, i.e. the SD over the group after the individual mean differences were subtracted.

Also in deviation from the AAMI protocol,[24] we did not include subjects on basis of their arm circumference. The protocol prescribes that 10% of subjects should have an arm circumference < 25 cm and 10% should have an arm circumference > 35 cm. However, we evaluated a blood pressure monitor using a finger cuff rather than an upper arm cuff based device. An important issue in comparison studies is that the RRK is performed with a proper cuff. We did include a range of finger cuff sizes.

The AAMI[24] specifies three blood pressure readings in at least 85 subjects, with 10% of the measured pressures below 100/60 mmHg and 10% above 160/100 mmHg. Although we included 19 subjects over and above the required number of 85, we were not able to acquire sufficient data in the lower pressure range.

Methods 1 and 2 as proposed by the AAMI give comparable results. Where each person provides the same number of measurements, whether or not the differences are averaged for individuals has no effect on the overall mean difference. However, when the individual data are first averaged, the overall standard deviation would be less, assuming the error is randomly distributed. In case of a systematic error (an offset from a reference method which will be maintained throughout the measurement) the standard deviation would be less reduced. When averaging individual data before group averaging (Method 2, in the first 85 subjects), the SD hardly improved (Table 3); according to the AAMI protocol[24], SDs not larger than 5.24 and 6.47 mmHg would be allowed for the found mean differences. The small improvement of the SDs with

averaging of the individual data indicates a large proportion of the difference is of a systematic nature. After elimination of the systematic component, the SDs reduce substantially (see Table 2, within-subject precisions). At least part of the remaining variability can be attributed to the non-simultaneously performed NAP and RRK.

No limits are provided for comparisons with potential systematic components. However, the AAMI protocol[24] Method 2 allows an SD of 6.95 mmHg for mean differences of 0 mmHg.

Systolic pressure is the most difficult part of the pressure wave to reconstruct correctly due to its large variability.[26] Imholz *et al.*[5] reviewed all evaluations of finger arterial blood pressure and found that the systolic part of the finger blood pressure was on average comparable to RRK systolic pressure. However, the precision (SD) of finger arterial systolic pressure was 9.4 mmHg. To improve the systolic precision of the reconstructed brachial pressure, originally the Return to Flow (RTF) calibration was developed.[26] Application of this RTF calibration resulted in a precision for systolic pressure better than 8 mmHg,[22,27,28] although one study showed worse precision with the Return to Flow method than is usually obtained without any calibration.[29]

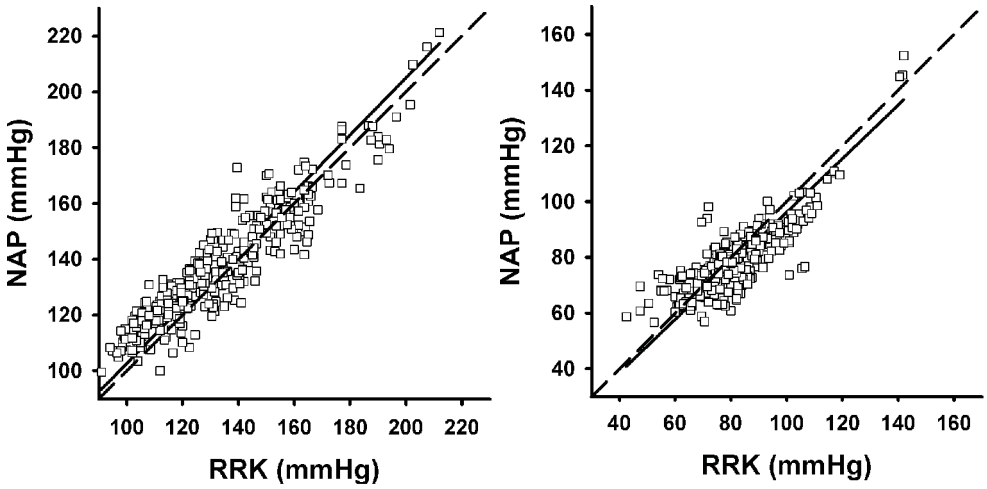
The auscultatory method according to Riva-Rocci / Korotkoff (RRK) still is the non-invasive standard for blood pressure measurement in clinical practice. This method, however, provides not more than a single estimate of intra-arterial blood pressure.[30-36] NAP tends to overestimate RRK systolic and to underestimate RRK diastolic pressure (Table 2) as a consequence of reconstruction of brachial pressure from finger pressure. RRK usually delivers too low systolic and too high diastolic values as compared to intra-arterial pressures.[30-36]

Other caveats for the use of the RRK method for determining blood pressure are related to intra-observer variability and the use of less reliable aneroid manometers as successor of the mercury sphygmomanometers.[37,38] With our measurement set-up including an electronic dual earpiece stethoscope, trained observers, real time data checking, automated arm cuff inflation and deflation at constant rates, and a calibrated manometer, we consider that the obtained RRK measurements were satisfactory.

Against a background demonstrating that the AAMI criteria cannot be directly applied to compare continuous non-invasive arterial pressure with discrete upper arm cuff pressure measurements, we conclude that the Nexfin provides accurate measurements with good within-subject precision.

Additional information can be found in supplementary figure 1

SUPPLEMENTARY FIGURE 1A AND SUPPLEMENTARY FIGURE 1B - Correlation of Nexfin NAP and RRK for systolic (a) and diastolic (b) blood pressure.



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Chapter 03

Determinants of vascular and cardiac baroreflex sensitivity values in a random population sample

Anke J. Borgers
Bert-Jan H. van den Born
Anneke Alkemade
Daan W. Eeftinck Schattenkerk
Johannes J. van Lieshout
Karel H. Wesseling
Peter H. Bisschop
Berend E. Westerhof

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Abstract

The arterial baroreflex regulates blood pressure by modifying heart rate and systemic vascular resistance. Baroreflex sensitivity is expressed as the relation between changes in blood pressure and the resulting changes in reciprocal values of heart rate (cardBRS) and in reciprocal values of vascular resistance (vascBRS). This study investigated determinants of vascBRS and cardBRS and their relationship in a random population sample.

Continuous noninvasive arterial pressure was analyzed in 105 adults (43 males) with a median age of 45 (range 18-95) years and body mass index of 24.5 (range 18.1-39.1) $kg \cdot m^{-2}$. Systolic and diastolic blood pressures were 130 (range 95-205) and 80 (range 47-141) mmHg and heart rate was 66 (range 42-109) $beats \cdot min^{-1}$. Pulse contour (CO-trek) determined vascular resistance was 1.37 (range 0.60-7.75) $mmHg \cdot s \cdot ml^{-1}$. The results of vascBRS and cardBRS were log-transformed; linear regression analysis revealed that age, $resistance^{-1}$, systolic and diastolic blood pressures were major determinants of log(vascBRS) explaining 30.5% of the variance. Determinants of log(cardBRS) were age, body mass index, heart rate, systolic and diastolic blood pressures, explaining 70.4% of the variance. Thus, some established determinants of cardBRS were not correlated with vascBRS. There was no correlation between log(cardBRS) and log(vascBRS) after correction for age, supporting that vascBRS is an independent description of baroreflex regulation.

These findings suggest that vascBRS and cardBRS report different modalities of cardiovascular autonomic function.

Introduction

The cardiac and vasomotor branches of the arterial baroreflexes are important mechanisms for short-term regulation of systemic blood pressure (BP) in humans, modifying heart rate (HR) and systemic vascular resistance (SVR) [35]. When BP rises, the carotid and aortic sinuses are distended, resulting in stretch and therefore activation of the baroreceptors. This baroreceptor signal is relayed to nuclei in the spinal cord and brain stem, causing activation of the parasympathetic nervous system and inhibition of the sympathetic nervous system [9]. Parasympathetic activation slows HR (i.e. cardiac baroreflex, cardBRS) whereas sympathetic inhibition primarily reduces SVR (i.e. vascular baroreflex, vascBRS). These chronotropic and vasomotor effects in concert lower BP, closing the control loop.

Cardiac baroreflex regulation is primarily parasympathetically mediated whereas the vascular baroreflex is a predominantly sympathetically driven mechanism. These two divisions of the autonomic nervous system are in a constant balance with each other and complex interactions exist between them [7]. Modulation of either part may alter its counterpart's activity [1, 27].

The changes in BP and HR needed for estimating cardBRS are easily to obtain and the physiological role of the cardBRS has been extensively studied [22]. In contrast, the vascBRS has thus far received less attention since beat-to beat variations in SVR are difficult to obtain non-invasively. As a consequence data on dual (cardiac and vascular) efferent baroreflex pathways, of importance for BP control are as yet not available [20]. Measurement of cardBRS alone may give an incomplete image of the complete control loop.

Recent developments in arterial pulse wave analysis and modelling have enabled us to obtain beat-to-beat variations in SVR using noninvasive hemodynamic monitoring where SVR is expressed as the ratio of mean BP [10, 24] and cardiac output determined by the pulse contour method [4, 5]. For the assessment of cardBRS we have previously developed a cross-correlation method using BP and HR [41] and determined computational precision in the presence of varying signal-to-noise ratios [40]. In the present study VascBRS was quantified using BP and SVR in a manner similar to cardBRS.

To investigate characteristics of the vascBRS obtained by this new method, we examined its determinants in a random population sample. In addition, we assessed how these determinants compared to predictors of cardBRS. Finally, we evaluated the relation between cardBRS and vascBRS to assess the relationship between both reflexes.

Methods

Subjects

We analyzed the records of 105 subjects who were originally recruited for a BP measurement validation study [10]. This study had ethical approval from the Medical Ethics Committee of the Academic Medical Center of the University of Amsterdam, Amsterdam, the Netherlands, and was conducted in accordance with the guidelines of the Declaration of Helsinki. All participants gave informed consent.

Measurements

Examinations were performed in a quiet room, where ambient temperature was controlled between 20° and 24°C. Subjects were placed in supine position and were requested not to speak or move during measurements.

The examination commenced with a 5 min supine period to bring physiologic functions to resting levels. Beat-to-beat BP was then measured using the Nexfin monitor (BMEYE B.V. Amsterdam, The Netherlands). This monitor uses the volume clamp methodology as proposed by Peňáz [38] and Physiocal criteria developed by Wesseling [39]. An inflatable cuff around the middle phalanx of the left finger with an optical blood volume measuring system clamps the blood volume to a preset level. Clamping was achieved by applying a pressure equal to arterial pressure throughout the cardiac cycle. In combination with Physiocal, calibrated recordings of the entire finger arterial pressure wave are obtained. From finger arterial pressure, brachial arterial BP was reconstructed by accounting for wave shape changes, related to the tapering of the arteries [12] and the drop in mean arterial pressure between brachial and finger artery[13, 42] caused by resistive losses. To avoid hydrostatic pressure differences, the cuffed finger was positioned at mid-thorax level.

In the original study, upper arm BP measurements were also collected [10] and these were used in the regression analysis (see statistics section). We selected 1 min periods of uninterrupted measurements before upper arm cuff inflation. For each subject at least four upper arm BP measurements were performed and the last three periods were included for statistical analysis.

Vascular Baroreflex

A method similar to the xBRS method [41] was developed to determine the sensitivity of the reflex on SVR. The Nexfin CO-trek (BMEYE B.V. Amsterdam, The Netherlands) method gives beat-to-beat values of SVR [4], and the reciprocal values of SVR

(1000·SVR^{-1}, expressed in mMU), called systemic vascular conductance (SVC) were used for further analysis. This is comparable to using interbeat interval (IBI) instead of HR in determining the cardiac BRS, resulting in positive BRS values. After interpolation and resampling at 1 s of the SVC and diastolic BP (DBP) values [33, 37], windows of 10 data points of each were taken into account. The cross-correlation was calculated for delays in SVC of 0 s to 9 s [32]. The combination yielding the highest coefficient of correlation was selected and accepted if $P < 0.05$. VascBRS, determined as the slope of the regression line through SVC versus DBP points was expressed as mMU·$mmHg^{-1}$ and was given together with "vascular delay".

Subsequently, this process was repeated for series of DBP and SVC samples 1 s later. Geometric averages were used to obtain one value per subject.

Cardiac Baroreflex

For the quantification of the cardBRS, we used the xBRS method as previously described [29, 41]. This method establishes the cross-correlation between systolic BP (SBP) variations and subsequent variations in IBI. After interpolation of the beat-to-beat values of SBP and IBI and resampling at 1s, the cross-correlation was calculated for 10 s windows. Delays of 0 s to 5 s were taken into account and the combination giving the highest correlation was accepted when $P < 0.05$. CardBRS was determined as the slope of the regression line, expressed in ms·$mmHg^{-1}$ and given together with the "cardiac delay". Subsequently, the process was repeated for a series of SBP and IBI samples 1 s later. Distributions of individual cardBRS values were best described as log-normal [41]. Therefore geometric averages were used to obtain one value per subject.

Statistics

Data were analyzed with SPSS for Windows, version 16.0 (SPSS Inc. Chicago, Illinois, USA). Normal distribution of the parameters was evaluated using the Kolmogorov-Smirnov test. VascBRS and cardBRS were log-transformed to improve normality and goodness-of-fit for statistical testing.

Univariate linear regression analyses were performed to assess potential determinants of log(vascBRS) and log(cardBRS). Included variables are respectively age, gender, body mass index (BMI), HR, SBP, DBP, SVR and SVC. Variables from these univariate analyses with $P < 0.20$ were included in the multivariate model. For each regression model we checked the assumptions for linearity and constant variance. These were assessed by plotting residuals against predicted values, and investigating deviations for linearity and inconsistent variance.

Simple bivariate correlation between log(vascBRS) and log(cardBRS) was performed using the Pearson product-moment correlation. Partial correlation after adjusting for age was performed to determine the strength of this association. The statistical significance level for all analyses was set at $P < 0.05$ (two-sided) unless otherwise mentioned.

Results

We identified a study sample of 105 adults (Table 1). Male comprised 50.9% of the cohort. The median age of the population was 45 years (range 18 – 95) and their median BMI was 24.5 kg·m^{-2} (range 18.1 – 39.1). The median SBP was 130 mmHg (range 95 – 205), the median DBP was 80 mmHg (range 47 – 141) as determined by upper arm cuff, the median HR was 66 beats·min^{-1} (range 42 – 109) and the median SVR was 1.37 mmHg·s·ml^{-1} (range 0.60 – 7.75).

TABLE 1 - Subject characteristics (n = 105, 54 males / 51 females)

parameter	Data median (min – max)
Age [years]	45 (18 - 95)
BMI [kg·m^{-2}]	24.5 (18.1 - 39.1)
SBP [mmHg]	130 (95 - 205)
DBP [mmHg]	80 (47 - 141)
MAP [mmHg]	96 (66 - 162)
HR [beats·min^{-1}]	66 (42 - 109)
SVR [MU]	1.37 (0.60 - 7.75)
SVC [mMU]	732 (129 - 1656)

BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; MAP: mean arterial blood pressure; HR: heart rate; SVR: systemic vascular resistance; SVC: systemic vascular conductance. MU: medical units. SBP and DBP according to Riva-Rocci / Korotkoff measurements.

Vascular baroreflex sensitivity (vascBRS)

The vascBRS method provided BRS values for 97 subjects; in eight cases vascBRS method found no significant correlations in the analysed periods. Per subject 12.1 ± 7.2 individual vascBRS values per minute were determined. Mean ± SD were 19.1 ± 8.2 mMU·mmHg^{-1}; median and interquartile range were 18.1 (13.1, 24.9) mMU·mmHg^{-1}; mode of the vascular delays was 7 s. Log transformed data of vascBRS were used for further analysis. Univariate analysis (Figure 1 and Table 2) showed that age, SBP, DBP and SVC were significantly associated with log(vascBRS). Additionally, HR met the criteria to be included in the multivariate model ($P = 0.182$). In contrast, gender, BMI and SVR

were not significantly associated with log(vascBRS) and therefore excluded from the multivariate model. Thus introducing age, HR, SBP, DBP and SVC in a multivariate linear regression analysis model (Table 3), SBP and SVC remained individual predictors of vascBRS. A total goodness-of-fit of 30.5% was found.

FIGURE 1 - Linear regressions of log(vascBRS) and log(cardBRS).

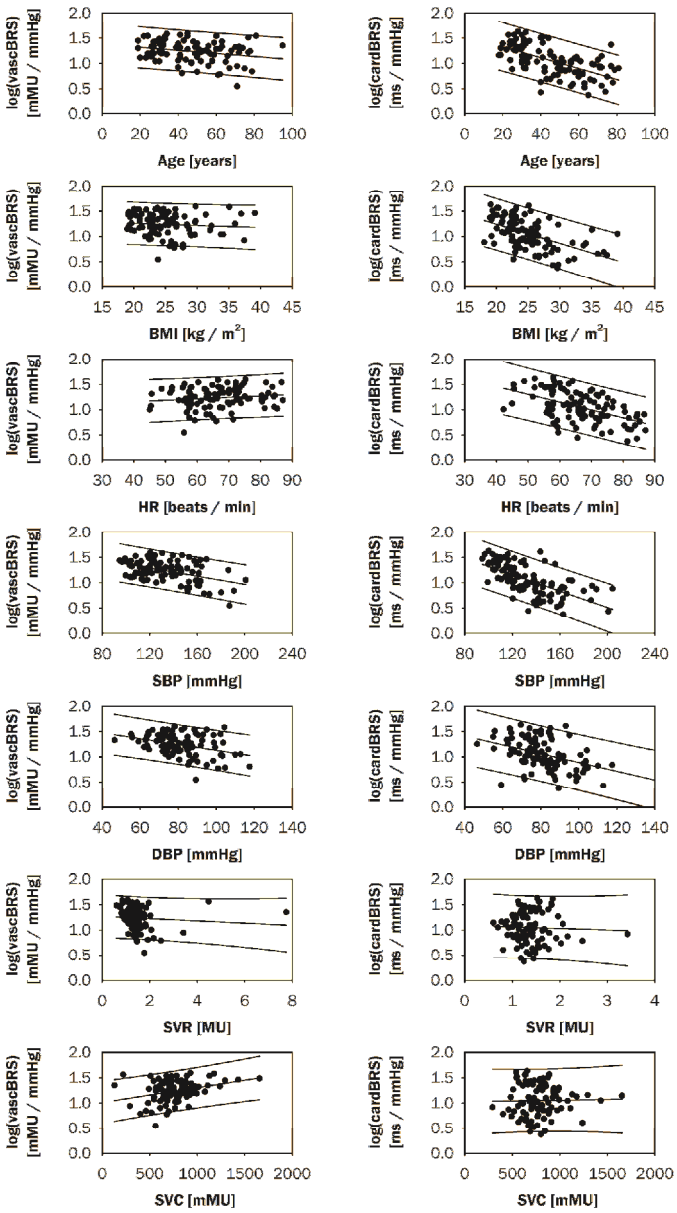

TABLE 2 - Univariate linear regression analysis of log(vascBRS)

Variable	Adjusted r^2	SE	P-value
Age [years]	0.054	0.001	0.012
Female (Male)	0.001	0.042	0.299
BMI [kg·m^{-2}]	-0.003	0.005	0.413
SBP [mmHg]	0.196	0.001	0.000
DBP [mmHg]	0.124	0.002	0.000
HR [beats·min^{-1}]	0.009	0.002	0.182
SVR [MU]	-0.003	0.026	0.3910
SVC [mMU]	0.090	0.000	0.002

BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; HR: heart rate; SVR: systemic vascular resistance; SVC: systemic vascular conductance. MU: medical units. SBP and DBP according to Riva-Rocci / Korotkoff measurements.

TABLE 3 - Multiple linear regression analysis of log(vascBRS)

Variable	Estimate	SE	*P*-value
Intercept	1.337	0.166	0.000
SBP [mmHg]	-0.004	0.001	0.005
SVC [mMU]	0.000	0.000	0.008
HR [beats·min^{-1}]	0.003	0.002	0.139
Age [years]	0.000	0.001	0.749
DBP [mmHg]	0.000	0.002	0.869

SBP: systolic blood pressure; DBP: diastolic blood pressure; HR: heart rate; SVC: systemic vascular conductance. MU: medical units. SBP and DBP according to Riva-Rocci / Korotkoff measurements.

Cardiac baroreflex sensitivity (cardBRS)

The cardBRS provided values for 103 subjects, since in two subjects the cardBRS method found no significant correlations in the analysed periods. Per subject 20.2 ± 9.3 individual cardBRS values per minute were determined. Mean ± SD was 14.1 ± 9.5 ms·mmHg^{-1}; median and interquartile range were 10.8 (7.2, 19.7) ms·mmHg^{-1}; mode of the cardiac delays was 0 s. For further analysis log transformed data of cardBRS were used. Age, BMI, SBP, DBP and HR were major determinants of the log(cardBRS) in univariate linear regression (Figure 1 and Table 4). In a multivariate regression analysis (Table 5), the model retained age, BMI, SBP and HR as predictors of the log(cardBRS). This model explained 70.4% of the variance.

TABLE 4 - Univariate linear regression analysis of log(cardBRS)

Variable	Adjusted r^2	SE	P-value
Age [years]	0.366	0.001	0.000
Female (Male)	-0.004	0.059	0.461
BMI [kg·m^{-2}]	0.282	0.006	0.000
SBP [mmHg]	0.412	0.001	0.000
DBP [mmHg]	0.167	0.002	0.000
HR [beats·min^{-1}]	0.269	0.002	0.000
SVR [MU]	0.000	0.000	0.850
SVC [mMU]	0.000	0.082	0.716

BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; HR: heart rate; SVR: systemic vascular resistance; SVC: systemic vascular conductance. MU: medical units. SBP and DBP according to Riva-Rocci / Korotkoff measurements.

TABLE 5 - Multiple linear regression analysis of log(cardBRS)

Variable	Estimate	SE	P-value
Intercept	2.877	0.137	0.000
Age [years]	-0.007	0.001	0.000
HR [beats·min^{-1}]	-0.012	0.002	0.000
SBP [mmHg]	-0.003	0.001	0.006
BMI [kg·m^{-2}]	-0.012	0.004	0.010
DBP [mmHg]	0.000	0.002	0.902

SBP: systolic blood pressure; DBP: diastolic blood pressure; HR: heart rate. MU: medical units. SBP and DBP according to Riva-Rocci / Korotkoff measurements.

Relation between cardBRS and vascBRS

There was a weak correlation between log(cardBRS) and log(vascBRS) (r^2 = 0.069, P = 0.010), which was lost after correction for age (r^2 = 0.013, P = 0.280).

Discussion

In this study we investigated determinants of vascular baroreflex sensitivity in an unselected population sample. A cross-correlation method was introduced for continuous noninvasive assessment of vascBRS. SBP, DBP and SVC were the major determinants of vascBRS, whereas age, SBP, HR and BMI were the strongest predictors of cardBRS, in line with previous studies [14, 16, 19]. Thus, established determinants of cardBRS such as HR and BMI were not correlated with vascBRS. Interestingly, cardBRS and vascBRS were not correlated after correction for age. We interpret this as an indication that vascBRS assesses a different aspect of baroreflex regulation.

The vascular aspect of the baroreflex may, apart from changes in sympathetic tone, also be influenced by other determinants of vasomotor tone, both systemic and local. For example BMI, a well-known predictor of sympathetic activity [8, 14, 15, 18], was not correlated with vascBRS. However, previous studies have demonstrated that the increase in sympathetic activity is not necessarily related to an increase in SVR [2, 3]. It is conceivable that local and systemic vasodilation related to changes in metabolic demand and insulin mediated vasodilatory responses may counteract the sympathetically mediated increase in SVR [3, 34]. It is likely that both systemic (i.e. cardiovascular reflexes, angiotensin II) and local vasodilatory mechanisms such as autoregulation may attenuate sympathetically mediated changes in SVR during vascular homeostasis [6, 30]. In that sense, it is conceivable that vascBRS could act as an indicator of disrupted vascular homeostasis.

We did not find a significant contribution of SVR in the linear regression model of vascBRS. This suggests that dynamic changes in SVR in response to changes in DBP are not per se related to the level of SVR. There was however a relation between SVC, expressed as $1000 \cdot SVR^{-1}$ and vascBRS. From our cross-sectional data it is not possible to explain this relation; studies with physiological interventions may perhaps give further insight in this finding.

SVR was determined from the ratio of beat-to-beat calculation of cardiac output with a noninvasive pulse contour method and mean arterial pressure and not by direct measurement [4]. The strong points of pulse contour methods are that data on cardiac output and SVR can be obtained noninvasively and continuously and that changes in CO can be reliably followed [36]. Beat-to-beat changes in CO may also be assessed noninvasively with echo-Doppler. Mukkamala et al. have previously described a method to identify the arterial and cardiopulmonary vascular resistance baroreflex using cardiac ultrasound [28]. Acquiring stable measurements with all beats accurately

determined over longer periods may be challenging and measurements during interventions such as standing and exercise may be difficult.

Another method to gauge the vascular resistance regulation in response to changes in DBP uses muscle sympathetic nerve activity (MSNA) as surrogate of changes in SVR. [8]. The practical application of this invasive technique may be limited, as this method requires an electrode placed in the peroneal nerve at the fibular head [11]. Moreover, one muscle group is the assumed to represent SVR and indeed SVR is positively related to MSNA in young healthy men, suggesting that MSNA is a good index of net whole body vasoconstrictor tone [7, 17]. However, hormonal fluctuations occurring during the normal menstrual cycle may alter sympathetic outflow of sympathetic activity into SVR [25, 26]. The implication is that in young women, MSNA does not determine SVR [17]. Finally, the relationship between MSNA and SVR is also modified by the variable transduction of MSNA to vasomotor tone, for instance an elevation of MSNA may not be translated to vasoconstriction in small muscle group exercise (functional sympatholysis) [31], thus, sympathetic vasoconstriction in contracting skeletal muscle is metabolically modulated. It is conceivable that the vascBRS as an estimate of the association between sympathetic activity and changes in SVR may provide additional information on the functional control of the sympathetic nervous system on SVR in real-life situations.

There are some limitations to our study. First, measurements were not performed after an overnight fast and subjects were included randomly without consideration of used medication. One could argue that random inclusion and absence of control on medication or fasting may have introduced a larger grade of variance in our results. However, our data of cardBRS corresponds to median levels in a normal population [23] and even with this possibly higher level of variance we identified significant determinants of the vascBRS and cardBRS. In addition, it was recently shown that the reproducibility of cardiovascular autonomic function tests under non-standardized conditions corresponds well with standardized test conditions [21], suggesting that implementation of our method in a clinical setting may be feasible. Second, because vascBRS is calculated from changes in SVR in relation to changes in BP, alterations in sympathetic nervous activity by mechanisms that involve both variables (e.g. vasodilatory agents) cannot be correctly assessed.

Conclusions

We introduced a new noninvasive method to continuously assess the influence of sympathetic tone on SVR. The method is easily applicable in population studies. Interestingly, established determinants of cardBRS were not correlated with vascBRS, which raises the possibility that vascBRS and cardBRS report different modalities of cardiovascular autonomic function. To what extent the values may be influenced by local and other systemic mediators of vasomotor tone needs requires further research.

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Chapter 04

Effects of sublingual nitroglycerin and head-up-tilt on indices of wave reflection principally result from changes in myocardial performance

D.W. Eeftinck Schattenkerk
H.J. Best
B. van den Bogaard
W.J. Stok
B.E. Westerhof
B.J.H. van den Born

Manuscript in preparation

Abstract

Indices of arterial wave reflection predict cardiovascular events in various populations. The mechanisms driving changes in wave reflection are not fully understood. We hypothesized that myocardial performance is an important contributor to changes in indices of wave reflection and central pressure augmentation. We examined the effects of sublingual nitroglycerin (NTG) in 25 subjects (14 males, aged 18-26 years) and head-up-tilt (HUT) in 12 subjects (6 males, aged 22-29 years) with and without unilateral thigh-cuff inflation on indices of wave reflection and myocardial performance. Non-invasive continuous monitoring of blood pressure (BP) and reconstructed central hemodynamics allowed to determine the effects of NTG and HUT on left ventricular contractility (dp/dt_{max}), stroke volume (SV), augmentation index (AIx) and augmentation pressure (AP). Wave separation analysis gave forward and backward pressure waves. AIx progressively decreased after NTG by -17.9±9.9% at 20 minutes, while AP and central systolic BP remained similar. HUT significantly reduced AIx by -17.7±10.4% and AP by -7.0±8.7mmHg. Unilateral thigh-cuff inflation attenuated the fall in AIx, while AP was preserved. In both experiments, changes in AIx principally resulted from changes in forward pressure waves. Changes in AIx were associated with changes in dP/dt_{max} ($r=-0.80$, $p<0.001$) and SV ($r=0.32$, $p<0.001$) after NTG, but not with heart rate (HR) or total peripheral resistance (TPR). Predictors of changes in AIx during HUT were HR ($r=-0.53$, $p<0.001$) and SV ($r=0.39$, $p=0.006$), but not dP/dt_{max} or TPR. In conclusion, changes in myocardial performance may contribute to changes in indices of wave reflection and differently impact central pressure augmentation.

Introduction

Blood pressure (BP) at heart level (central BP) is determined by the interplay between forward waves, which are generated by left ventricular ejection into the elastic aorta, and backward travelling waves that augment central BP and the late systolic BP load imposed on the heart.(13; 21; 33; 34) Indices of wave reflection and pressure augmentation as quantified by the Augmentation Index (AIx) have been associated with cardiovascular disease in various populations.(3; 15; 31) Differences in wave reflection and pressure augmentation are generally attributed to changes in large artery stiffness,(20) changes in reflection coefficient related to altered diameter of peripheral muscular arteries (14; 18) and heart rate (HR).(36-38) Since the morphology of the aortic pressure wave is determined by the interaction of pressure waves generated by the heart and the arterial system, myocardial performance may be an important contributor to changes in wave reflection. Interventions that alter myocardial performance by either preload reduction,(4; 12; 16; 27; 29) preload enhancement,(10) or stimulation of left ventricular contractility,(23; 25) have reported marked effects on the aortic pressure waveform and indices of wave reflection, but have primarily focused on functional aspects of the arterial tree. The effects of nitroglycerin (NTG) on central pressure augmentation, for example, have been attributed to reduced wave reflection by arterial smooth muscle cell relaxation and attenuation of total peripheral resistance (TPR).(8; 9) However, NTG in anti-anginal doses has an important effect on cardiac preload and myocardial contractility,(1; 11; 30; 35; 39) which suggests that heart related effects rather than arterial vasodilatation may be responsible for the change in AIx after NTG. This is further supported by a recent study showing that after NTG central pressure augmentation is associated with changes in forward pressure waves.(6) In addition, changes in cardiac preload during postural stress have been shown to decrease AIx, despite an increase in TPR and large artery stiffness,(4; 12; 27; 29) suggesting that the gravitational translocation of thoracic blood to the legs,(24) may be pivotal for the decrease in AIx.

We therefore investigated the effects of changes in myocardial performance following administration of a single dose of NTG and HUT with and without application a unilateral thigh-cuff to preserved cardiac preload, on central pressure augmentation and arterial wave reflection in healthy volunteers.

Subjects and Methods

For both the NTG and the HUT protocol young volunteers were recruited. The volunteers were fasting and free of medication during the experiments. Continuous noninvasive arterial BP was recorded using volume-clamp photoplethysmography (Nexfin®, Edwards Lifesciences BMEYE, Amsterdam, the Netherlands).(5) A finger-cuff was placed around the mid-phalanx of the third finger of the right hand, which remained at heart-level throughout the measurements. Experiments were carried out in accordance with the Declaration of Helsinki (2008) and were approved by the Medical Ethical Committee of the Academic Medical Center, Amsterdam, the Netherlands. All participants gave written informed consent.

The effects of NTG were tested in 25 subjects (14 males), aged 18-26 years in supine position. A single sublingual dose of NTG-spray (0.4 mg/dose) was administered after 10 minutes of supine rest. Data were acquired using 30 second time windows of the recordings at baseline and at 5, 10, 15 and 20 minutes following NTG.

The effects of HUT were tested in 12 subjects (6 males), aged 22-29 years. Patients were passively tilted head up to 70° following 10 min. of supine rest. After 5 min. in head-up position data were sampled using a time windows of 30 seconds, after which subjects were tilted back to supine position. Next, a unilateral thigh cuff was inflated to a pressure 60 mmHg above previously determined (seated) brachial systolic BP. After 5 min. of stabilization, data were sampled followed by passive HUT to 70° with the thigh-cuff inflated and data were again sampled after 5 min. of HUT. Then patients were tilted back and the thigh cuff was deflated. The side of placement of the thigh cuff was randomized using a computerized random number generator.

Calculations were programmed in Mathematica (Wolfram Research, Inc., Mathematica, Version 4.0, Champaign, IL).(13; 21; 33; 34)

Statistics

Statistical analyses were performed using the Statistical Package for Social Sciences version 19.0.0.1 (SPSS Inc., Chicago, Illinois, USA). Data are expressed as mean ± SD for continuous variables and as n (%) for categorical variables. A general linear model for repeated measurements was used to analyze changes in wave reflection and hemodynamics following NTG and HUT with and without unilateral thigh-cuff. Post-hoc two-sided pair-wise comparisons, based on estimated marginal means and corrected according to least significant difference, were used to explore differences in central pressure augmentation, wave reflection and hemodynamics at maximal mean

response in AIx vs. baseline in the NTG experiment. Likewise, we performed post-hoc comparisons in the HUT experiment for supine vs. HUT data, both with and without thigh-cuff. Additional analysis was performed to compare HUT with- vs. without thigh-cuff with correction for differences in supine position (i.e. supine with thigh-cuff – supine without thigh-cuff) as covariate. Pearson's correlation was used to explore the association between central pressure augmentation, forward and backward waves, and dP/dt, SV, TPR and HR. For all statistical analyses a two-sided p value <0.05 was considered significant.

Results

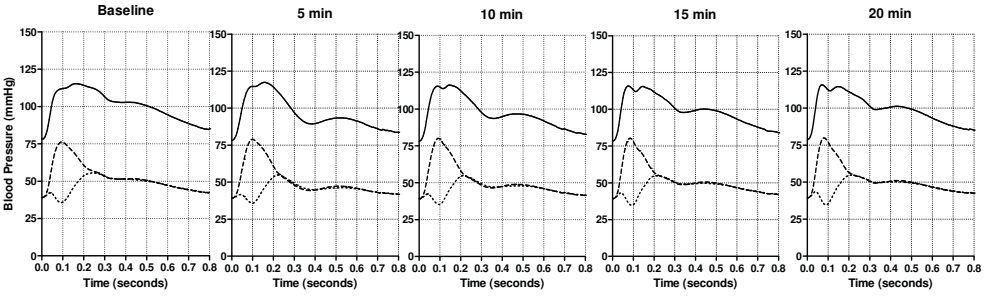
The effects of NTG on wave reflection, central pressure augmentation and hemodynamics are listed in table 1. The effects on aortic pressure wave morphology are shown in figure 1. NTG resulted in a progressive decrease in AIx from 10.4±12.9% to -6.6±14.0% after 20 min. ($p<0.001$), while AP, central SBP and HR remained similar. Changes in the aortic pressure waveform resulted primarily from changes in forward waves. Forward wave pressure was significantly increased by 11.3% ($p<0.001$) after 20 min., while backward wave pressure increased by only 5.0% ($p=0.06$) at 20 min., resulting in a significantly lower RM (-6.3%, $p<0.001$). The $TPfw_{max}$ shortened by a maximum of -15.9% ($p<0.001$) as dP/dt_{max} increased significantly, with a maximum rise in dP/dt_{max} of 21.2% ($p<0.001$) after 20 min. CCT was shorter (-6.3%, $p<0.001$) and central systolic BP and central PP were significantly higher (2.8%, $p=0.02$ and 6.3%, $p=0.01$) 20 min. after NTG, while SV, HR and TPR were not significantly different compared to baseline. We observed a transient increases in HR (6.5% at 5 min. and 4.8% at 10 min.) and a decrease in TPR (-5.8% at 5 min.) and SV (-3.0%, at 10 min.) following NTG, all $p<0.05$. Despite the variations in TPR and HR, there was a strong association between dP/dt_{max} and AIx ($r=-0.80$) and AIx@hr75 ($r=-0.75$), both $p<0.001$ (figure 2). SV was also associated with AIx ($r=0.32$) and AIx@hr75 ($r=0.44$), both $p<0.001$, but not HR ($r=0.15$, $p=0.09$) or TPR ($r=-0.07$, $p=0.46$). In the wave separation analysis both dP/dt_{max} and SV were strongly associated with changes in both forward ($r=-0.89$ for dP/dt_{max} and 0.68 for SV, $p<0.001$) and backward wave reflection ($r=-0.89$ and $r=0.71$, $p<0.001$).

TABLE 1 - Results of the NTG Experiment

Parameter, units	Baseline	+5 min	+10 min	+15 min	+20 min	P
AIx, %	10.4±12.9	7.4±8.1	-0.8±10.7*	-3.8±14.5*	-6.6±14.0*	<0.001
AIx@hr75, %	4.0±15.0	2.9±10.3	-5.8±11.8*	-10.5±15.6*	-12.9±16.4*	<0.001
AP, mm Hg	5.1±2.6	3.7±1.9*	3.8±1.7*	5.5±2.8	5.7±3.8	0.03
P1, mm Hg	110.3±12.8	113.3±10.4*	113.4±9.6*	112.8±10.2	112.9±9.3	0.07
cSBP, mm Hg	115.4±12.1	117.1±9.8	117.2±10.2	118.3±11.3	118.6±11.3*	0.02
cDBP, mm Hg	77.4±7.1	77.4±6.9	77.4±6.7	77.6±6.2	78.3±6.2	0.77
cPP, mm Hg	37.9±6.8	39.7±4.5*	39.8±5.2*	40.7±6.2*	40.3±7.1*	0.003
PPfw, mm Hg	38.3±8.2	40.8±4.9*	42.4±5.5*	43.0±6.8*	42.7±7.4*	<0.001
PPbw, mm Hg	20.1±3.6	19.7±2.6	20.4±2.8	21.3±3.3*	21.1±3.5	0.003
RM	0.53±0.03	0.48±0.02*	0.48±0.02*	0.50±0.03*	0.50±0.02*	<0.001
$TPfw_{max}$, ms	101±19	102±11	96±12	90±15*	86±15*	<0.001
CCT, ms	126±9	112±5*	114±6*	116±7*	118±6*	<0.001
HR, beats·min^{-1}	61.8±9.9	65.8±12.0*	64.7±10.4*	61.5±10.2	62.2±10.2	<0.001
SV, ml	109.9±16.4	108.7±15.2	106.6±14.1*	107.8±12.9	107.1±12.3	0.12
CO, l·min^{-1}	6.7±1.1	7.0±1.3*	6.8±1.1	6.6±1.0	6.5±0.9	0.001
TPR, dyn s·cm^{-5}	1.162±0.16	1.094±0.20*	1.130±0.16	1.183±0.17	1.185±0.14	0.001
dP/dt_{max}, mm Hg / s	1141±296	1138±203	1261±220*	1372±294*	1383±327*	<0.001

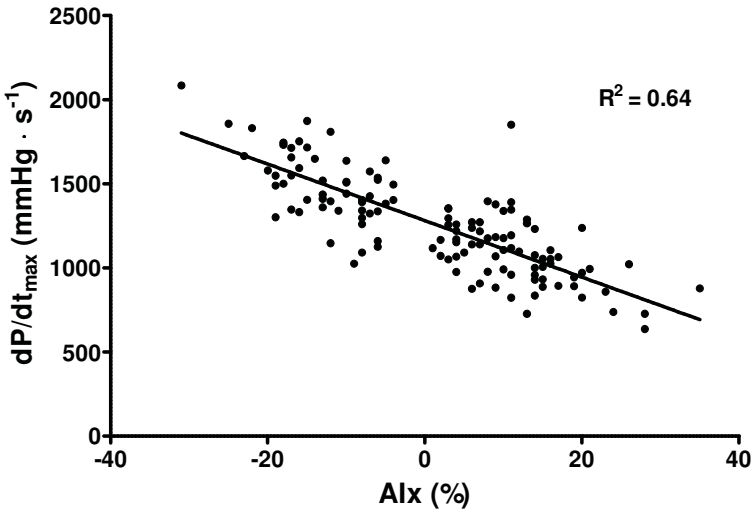
AIx: augmentation index; AIx@hr75: heart-rate-corrected AIx; AP: augmentation pressure; P1: inflection point pressure ; cSBP: central systolic blood pressure (BP); cDBP: central diastolic BP; cPP: central pulse pressure; PPfw: forward wave pulse pressure (PP); PPbw: backward wave PP; RM: reflection magnitude; T_{Pfwmax}: Time until P_{max} of the forward wave; CCT: cross-correlation return time of the reflected wave; HR: heart rate; SV: stroke volume; CO: cardiac output; TPR: total peripheral resistance; dP/dt_{max}: left ventricular contractility. Values are mean±SD. * p<0.05 two-sided pairwise comparison vs. baseline.

FIGURE 1 - Effects of NTG – Wave Separation Analysis



Correlation of dP/dt_{max} (estimate of left ventricular contractility) and AIx (augmentation index). Data consist of combined measurements at baseline, 5 min., 10 min., 15 min. and 20 min. following sublingual NTG in n=25 subjects corresponding with a total of 125 data points.

FIGURE 2 - Correlation of Left Ventricular Contractility and Augmentation Index



Correlation of dP/dt_{max} (estimate of left ventricular contractility) and AIx (augmentation index). Data consist of combined measurements at baseline, 5 min., 10 min., 15 min. and 20 min. following sublingual NTG in n=25 subjects corresponding with a total of 125 data points.

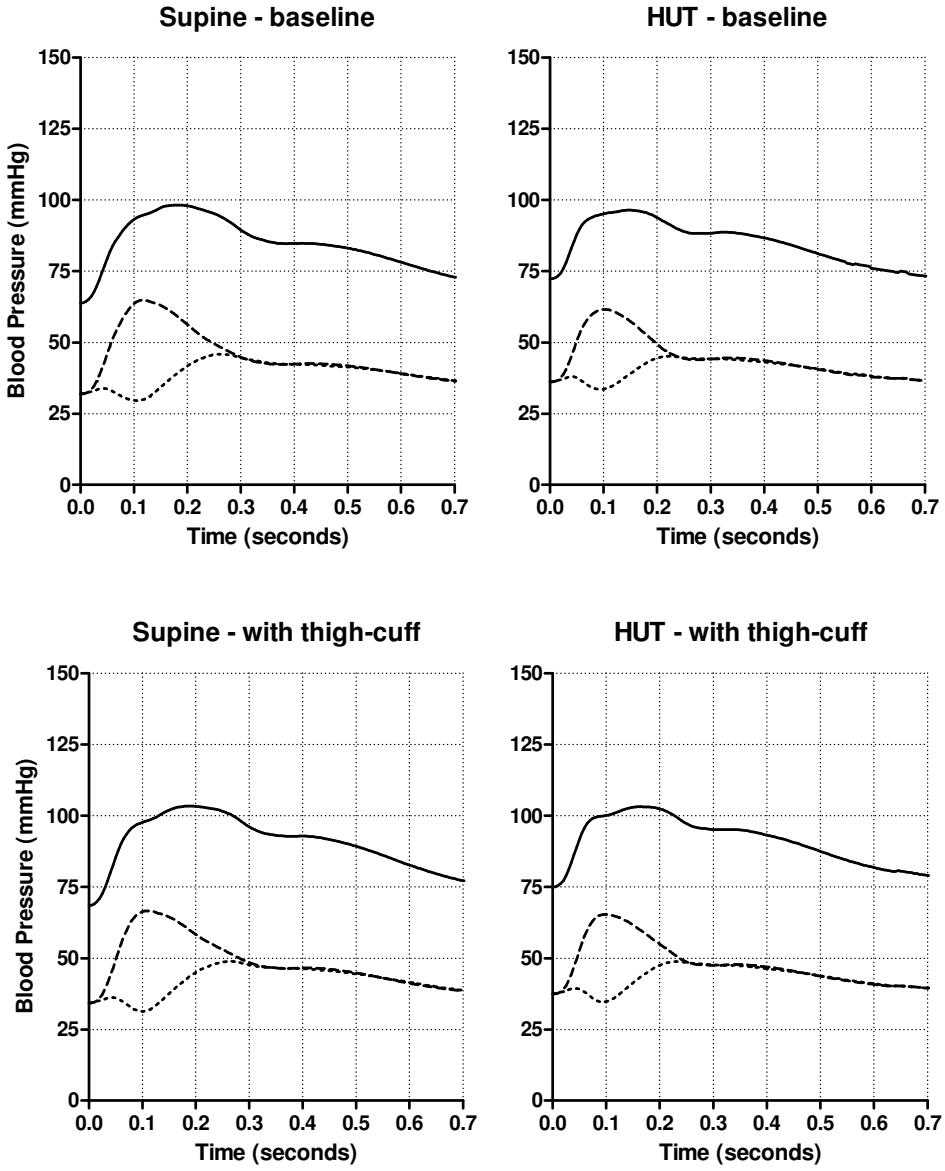
The effects of HUT with and without unilateral thigh-cuff on central pressure waveform, wave reflection and hemodynamics are shown in table 2. The effects on aortic pressure wave morphology are shown in figure 3. HUT lowered AIx by -17.7±10.4% (AIx@hr75 -7.5±11.1%) and AP by -7.0±8.7mmHg. This was caused by a reduction in both forward wave pressure (-7.2±5.1 mmHg) and backward wave pressure (-4.8±3.2 mmHg) and associated with a decrease in CCT (all $p<0.05$). Central systolic BP and PP decreased after HUT, while TPR increased. SV and CO decreased in the presence of an increase in HR (all $p<0.05$). HUT with inflated unilateral thigh-cuff attenuated the lowering in AIx by 36% (AIx@hr75 32%), and central PP, while AP and central systolic BP remained similar. These changes were mainly caused by preservation of forward wave pressure, while CCT ($p=0.80$) was not significantly affected. HUT with thigh-cuff also attenuated the rise in HR and fall in SV (all $p<0.05$), while TPR was unchanged ($p=0.45$). Correction for differences in supine position did not influence the significance of the changes in wave reflection and hemodynamics (data not shown). AIx was significantly associated with SV ($r=0.39$, $p=0.006$) and HR ($r=-0.53$, $p<0.001$), but not with dP/dt_{max} ($r=-0.27$, $p=0.07$) or TPR ($r=-0.09$ $p=0.53$). Correction for HR (AIx@75) attenuated the association between AIx and SV ($r=0.25$, $p=0.08$). SV was strongly associated with forward wave pressure ($r=0.86$ for SV) and backward wave pressure ($r=0.83$ for SV, $p<0.001$), while HR was only associated with changes in forward wave pressure ($r=-0.31$, $p=0.03$).

TABLE 2 - Results of the HUT Experiment

Parameter, units	Supine (Sup)	Head-up-tilt (HUT)	Cuff Supine ($_c$Sup)	Cuff HUT ($_c$HUT)	$_c$HUT vs. HUT, P
AIx, %	22.2±11.0	4.5±15.0*	20.8±13.5	13.8±11.7#	0.008
AIx@hr75, %	14.7±11.0	7.3±14.3*	14.0±12.5	13.7±11.4	0.027
AP, mm Hg	7.7±3.8	0.7±10.3*	6.0±6.5	9.4±7.8	0.088
P1, mm Hg	91.1±12.9	96.4±11.3*	98.2±10.0	94.6±7.0	0.56
cSBP, mm Hg	98.8±12.6	97.1±9.1	104.2±10.5*	104.0±8.4	0.011
cDBP, mm Hg	63.8±7.5	72.1±6.2*	68.5±5.2*	75.0±5.1#	0.080
cPP, mmHg	35.0±7.1	24.9±4.5*	35.7±7.2	29.0±5.0#	0.005
PPfw, mm Hg	33.6±7.0	26.3±5.1*	33.7±7.0	29.1±4.6#	0.014
PPbw, mm Hg	17.3±3.7	12.5±2.2*	18.5±4.2	14.9±2.1#	0.002
RM	0.52±0.04	0.48±0.04*	0.55±0.06*	0.51±0.04#	0.02
TPfw$_{max}$, ms	125±18	101±18*	116±21	103±19	0.55
CCT, ms	135±8	127±10	135±10	127±9	0.80
HR, b.p.m.	59.8±5.7	80.8±10.1*	61.1±7.0	74.9±8.6#	0.004
SV, ml	106.9±17.0	75.2±12.3*	107.5±14.6	83.7±10.8#	<0.001
CO, l·min^{-1}	6.4±1.4	6.1±1.4	6.5±1.1	6.3±1.2	0.32
TPR, dyn s·cm^{-5}	1039±182	1162±231*	1081±183	1182±211#	0.45
dP/dt_{max}, mm Hg / s	861±211	788±196	919±220	865±161	0.11

AIx: augmentation index; AIx@hr75: heart-rate-corrected AIx; AP: augmentation pressure; P1: inflection point pressure; cSBP: central systolic blood pressure (BP); cDBP: central diastolic BP; cPP: central pulse pressure; PPfw: forward wave pulse pressure (PP); PPbw: backward wave PP; RM: reflection magnitude; T_{Pfwmax}: Time until P_{max} of the forward wave; CCT: cross-correlation return time of the reflected wave; HR: heart rate; SV: stroke volume; CO: cardiac output; TPR: total peripheral resistance; dP/dt_{max}: left ventricular contractility; Values are mean±SD. * p<0.05 for HUT vs. Sup (without cuff). # p<0.05 for $_c$HUT vs. $_c$Sup (with cuff)

FIGURE 3 - Effects of HUT and Thigh-cuff – Wave Separation Analysis



Changes in aortic pressure wave (solid lines) morphology and separated forward (dotted lines) and backward (interrupted lines) pressure waves, during supine rest and head-up-tilt (HUT), without (baseline) and with inflated unilateral thigh-cuff.

Discussion

In the present study we show that dP/dt_{max} and SV have important effects on arterial wave reflection and central pressure augmentation after NTG and during HUT with and without unilateral thigh cuff. In both experiments the observed differences in central waveform resulted primarily from changes in the forward pressure waves and resulted primarily from an increased dP/dt_{max} following NTG and from changes in SV after HUT.

Our experiment shows for the first time that changes in AIx with low dose NTG are predominantly caused by changes in myocardial performance. Previous studies have shown that sublingual doses of 0.6-0.9 mg NTG exert mainly arterial vasodilatory effects,(17) whereas lower NTG doses have been shown to primarily affect venous capacitance vessels, cardiac preload,(19) and left ventricular contractility.(11) In our study, AIx progressively decreased while forward -and to a lesser extent backward- wave pressure *increased* resulting in a relative preservation of AP. This apparent discrepancy resulted from a widening and deepening of the dicrotic notch caused by an increase and shortening of the time until peak pressure of the forward wave ($TPfw_{max}$). This suggests that conditions or agents that affect dP/dt_{max} may differently impact indices of wave reflection and central pressure augmentation.

The magnitude of arterial wave reflection is considered to depend heavily on impedance mismatching in high resistance arteries and arterioles. However, the positive association between TPR and indices of wave reflection (14; 22) is not present in all physiological circumstances. We and others have shown that during postural stress, wave reflection and AIx decrease despite an increase in TPR.(4; 12; 27; 29) In the present study, we show that the decrease in AIx after HUT results from a decrease in both forward and backward pressure waves. In analogy with previous experiments, HUT led to a significant fall in SV and an increase in HR and TPR. In contrast to the NTG experiment, dP/dt_{max} did not significantly change, possibly because the increase in sympathetic activity was accompanied by a significant decrease in SV. Preservation of cardiac preload by unilateral thigh-cuff inflation attenuated the decrease in AIx and SV compared to HUT without thigh-cuff, and also reduced the increase in HR. Also after correction for HR the difference in AIx remained significant before and after unilateral thigh cuff inflation suggesting that the effects on wave reflection are, at least in part, independent of changes in HR. In addition, AIx and both forward and backward pressure waves were associated with changes in SV.

The impact of HR on AIx and central BP has been well recognized.(36; 38) Next to the established chronotropic effects on wave reflection, we show that changes in

preload and dP/dt_{max} are also an important determinant of wave morphology. This is in line with previous cross-sectional studies showing that measures of left ventricular contractility and filling pressure are associated with AIx independent of HR.(32) Still, inotropic and chronotropic effects on central pressure augmentation are difficult to disentangle because of the intertwining of cardiac auto-regulatory mechanisms,(23) including the Bowditch effect, involving increased myocardial contractility at higher HR,(2) Frank-Starling mechanisms and baroreflex control of contractility and HR. These mechanisms may obscure individual associations with indices of wave reflection such as AIx. In our NTG experiment we show that, in absence of changes in HR, enhanced dP/dt_{max} as measure of left ventricular contractility resulted in lower AIx. In addition, after correction for HR, dP/dt_{max} remained strongly associated with central pressure augmentation.

Our study is subject to several potential limitations. Aortic pressure and flow were derived from distal, noninvasive pressure measurements, both in supine position and after HUT. The flow wave calculation depends on the pressure wave and may have some degree of inherent redundancy as they are not independent. In a previous study however we demonstrated that from supine to standing the bias for systolic finger pressure and flow wave calculations did not change significantly.(26) In addition, measurement of SV has been validated both in supine and standing position, whereas peripheral acquired assessment of dP/dt_{max} has been shown to a reproducible criterion for left ventricular systolic performance.(7; 28) Second, the observed effects in both the NTG and HUT experiment may be partly attributable to arterial effects of the interventions of interest. In particular, an effect on aortic compliance and on changes in flow of peripheral conduit arteries cannot be fully excluded since this was not assessed directly. However, previous studies have shown that the reduction in AIx after NTG is independent of changes in pulse wave velocity and backward wave reflection.(6; 14) In addition, we observed a shortening in CCT after NTG, corresponding with reduced aortic compliance that would be expected to cause an increase (and not decrease) in AIx. In the HUT experiment, a potential effect on aortic compliance is likely to have been small as there was no effect of thigh-cuff inflation on the return time of the reflected wave. A potential limitation of the HUT experiment is the lack of randomization in the order of the performed study procedures. It is however unlikely that carry-over-effects have caused substantial bias, since ample time was allowed for stabilization of hemodynamics before data were collected for analysis, as demonstrated by the concordance in baseline measurements. In addition, correction for baseline differences did not affect the observed changes in central pressure waveform or hemodynamics. Finally, the experiments were performed in young healthy individuals, which limits extrapolation to other populations. Nonetheless, the effects of NTG and on AIx occur

both in healthy and subjects with coronary artery disease (8) and the effects of HUT on AIx are comparable between younger and older persons.(4)

Conclusion

Our results extend previous observations by showing that changes in forward pressure waves can be the driving force behind changes in indices of wave reflection following NTG and during HUT. The association of forward wave pressure with dP/dt_{max} and SV suggests that changes in myocardial performance may have important effects on wave reflection and central pressure augmentation. In addition, we show that low-dose NTG may progressively lower AIx by diverging forward and backward pressure waves, while leaving indices of central pressure augmentation relatively unaffected, suggesting that the myocardium determines, at least in part, the effect of wave reflection and pressure augmentation. Since indices of wave reflection have been shown to independently predict cardiovascular events, these results may be relevant for interventions targeting left ventricular contractility and cardiac preload.

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Part II

CENTRAL HEMODYNAMICS IN HYPERTENSIVE PHENOTYPES AND TREATMENT EFFECTS

Chapter 05

Ethnic differences in arterial wave reflection are mostly explained by differences in body height: cross-sectional analysis of the HELIUS study

Daan W. Eeftinck Schattenkerk
Jacqueline van Gorp
Marieke B. Snijder
Aeilko H. Zwinderman
Charles O. Agyemang
Ron J.G. Peters
Bert-Jan H. van den Born

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Abstract

Background: Differences in arterial wave reflection and central blood pressure (BP) have been associated with cardiovascular disease (CVD) in various populations and may contribute to ethnic differences in CVD. Whether ethnic differences in wave reflection and central BP can be explained by conventional risk factors for CVD or may result from physiological differences remains undetermined.

Methods: We examined ethnic differences in augmentation index (AIx) and central systolic BP and their determinants in a large multi-ethnic cohort study in Amsterdam, the Netherlands. A total of 8812 (46% male) participants aged 18-70 years of Dutch, South-Asian Surinamese, African Surinamese and Ghanaian origin were included. AIx and central BP were measured in duplicate using the Arteriograph system.

Results: AIx and central systolic BP were significantly higher in South-Asian Surinamese (35±17%, 126±22 mmHg), African Surinamese (33±17%, 129±23 mmHg) and Ghanaian (33±16%, 135±24 mmHg) as compared with Dutch (27±17%, 118±20 mmHg, all $p<0.001$). Correction for cardiovascular risk factors only slightly reduced the difference in AIx, whereas correction for body height attenuated age and gender corrected ethnic differences in AIx the most. Differences in central systolic BP were primarily determined by differences in AIx for South-Asian Surinamese and by differences in peripheral systolic BP for subjects of African origin.

Conclusions: Substantial differences in AIx and central BP exist across different ethnic groups that cannot be explained by differences in conventional risk factors for CVD. These findings may explain part of the underestimation of cardiovascular risk observed in populations of African and South-Asian descent.

Introduction

Ethnic disparities in the burden of cardiovascular disease (CVD) are well recognized, but incompletely understood.[1–3] These disparities may be related to differences in arterial wave reflection and central blood pressure (BP). Independent of BP indices of wave reflection have been associated with CVD in different populations.[4–6] In addition, mounting evidence suggests that central (i.e. aortic) BP is a stronger predictor for future CVD than brachial BP as measured in daily practice and may be affected by arterial wave reflection.[7–10] Previous studies have reported higher augmentation index (AIx) and central BP among different ethnic groups compared to subjects of Western-European descent.[11–14] However, the contribution of conventional risk factors and the physiological origin of differences in AIx and central BP remain undetermined. Hypertension, smoking and diabetes have been independently associated with ethnic differences in AIx.[11,15] However, physiological factors including body height,[16,17] heart rate,[18,19] large artery stiffness,[20] peripheral resistance,[21] and stroke volume [22] have been shown to affect arterial wave reflection and could contribute to ethnic differences in AIx and central BP.

In the present study, we investigated differences in AIx and central BP in a large multi-ethnic population in Amsterdam, the Netherlands. Our principal aim was to assess the contribution of conventional cardiovascular risk factors and physiological determinants on ethnic differences in AIx as a BP independent measure of wave reflection, and secondary to assess the influence of differences AIx on variations in central BP.

Methods

Study population

We used baseline data from the HEalthy LIfe in an Urban Setting (HELIUS) study, a large multi-ethnic population study carried out by the Academic Medical Center, Amsterdam, and the Public Health Service on health and health-care utilization among the six major ethnic groups residing in Amsterdam, the Netherlands. Details of the HELIUS study have been previously described in detail.[23] The HELIUS study is conducted in accordance with the Declaration of Helsinki and has been approved by the Ethical Review Board of the Academic Medical Center, Amsterdam. All participants provided written informed consent. For the present analysis, data collected from January 2011 until December 2014 were used from participants of Dutch (n=2838), South-Asian Surinamese (n=2383), African Surinamese (n=2938) and Ghanaian (2189) origin, totaling 10.348 individuals who underwent a physical examination and in whom

questionnaire data were available. Within this group we only included participants with available data on AIx and central BP, which resulted in a dataset of 8812 participants aged 45.5±13.0 years (49% male) for the current analyses. This included 2431 Dutch, 1928 South-Asian Surinamese and 2501 African-Surinamese (both first and second generation), and 1952 participants of Ghanaian descent (exclusively first generation). Missing data were primarily due to logistic reasons or failure to obtain valid readings.

Study procedures

Information on demographics, smoking behaviour, alcohol intake, and history of diseases was obtained by questionnaire. Participants were asked to bring their prescribed medications to the research location, which were coded according to the Anatomical Therapeutic Chemical (ATC) classification. Diabetes was defined as a fasting glucose level ≥7.0 mmol/l, or the use of glucose lowering medication. Hypertension was defined as brachial BP ≥140 systolic or ≥90 mmHg diastolic while seated, or the use of blood pressure lowering medication. Height and weight were recorded and body mass index (BMI) was calculated as weight (kg) divided by height squared (m^2).

Augmentation Index, Central Blood Pressure and Pulse Wave Velocity

Study participants visited the research location in the morning after an overnight fast and were asked to refrain from smoking the morning prior to the visit. Measurements were performed after at least 10 minutes of rest in supine position. The Arteriograph system (Tensiomed Kft., Budapest, Hungary) was used to assess AIx, central BP, brachial BP and pulse wave velocity (PWV). The Arteriograph system is an operator-independent non-invasive device that applies an oscillometric, occlusive technique by use of an upper-arm cuff to register brachial pressure curves. Its methodology and validation is described in detail elsewhere.[24–26] Arteriograph AIx has close correlation with the widely applied Sphygmocor (AtCor Medical Pty Ltd, West Ryde, Australia) AIx ($r=0.89$, $p<0.001$);[26] invasively measured AIx ($r=0.90$, $p<0.001$), central BP ($r=0.95$, $p<0.001$) and PWV ($r=0.91$, $p<0.001$).[25] All Arteriograph measurements were performed in duplicate and the results were averaged for further analysis.

Systemic Vascular Resistance, Stroke Volume and Heart Rate

Hemodynamics were assessed by volume-clamp photoplethysmography with the Nexfin™ device (Edwards Lifesciences BMEYE, Amsterdam, the Netherlands).[27] This device uses the Finapres method [28] to continuously and non-invasively record finger arterial BP and reconstructed brachial BP.[29,30] Recordings were made at 200Hz using a finger-cuff placed around the mid-phalanx of the third finger. Mean arterial pressure (MAP) was calculated from the true integral of the arterial pressure wave over

one beat divided by the corresponding inter-beat interval. Heart rate was the inverse of the inter-beat interval. Stroke volume (SV) was determined by the pulse contour method (Nexfin CO-trek).[31] Cardiac output (CO) was SV divided by the inter-beat interval. Systemic vascular resistance (SVR) was the ratio of MAP and CO. All Nexfin hemodynamic parameters were calculated from the average of a 1 minute period of stable recording.

Statistical analyses

Estimates of AIx and central systolic BP according to age were depicted for the four ethnic groups by spline interpolation using four degrees of freedom. A general linear model was used to compare AIx and central systolic BP between ethnic groups, pairwise comparisons were made according to least-significant-difference (LSD) and differences were reported as mean±SE. To assess the potential effects of antihypertensive therapy on ethnic differences in AIx and central systolic BP, we additionally performed a sensitivity analysis including antihypertensive drug naïve subjects exclusively. To explore the contribution of peripheral BP and AIx to ethnic differences in central BP, differences in central BP across ethnic groups were corrected for differences in peripheral BP and AIx with Dutch acting as reference. AIx was selected as a BP independent determinant of ethnic differences in central BP to explore the contribution of different covariates using three models. Model 1 was used to correct for age and gender. In model 2 differences in AIx were additionally corrected for systolic BP, BMI, total cholesterol, smoking and diabetes to explore the contribution of conventional CVD risk factors. Model 3 was used to additionally correct for body height, PWV, heart rate, SV and SVR. The physiological factors from model 3 were also individually included next to age and gender, to assess their relative contribution to ethnic differences in AIx. Furthermore, linear regression analysis was performed to test the contribution of covariates from model 3 to variations in AIx within each ethnic subgroup. Because of potential multicollinearity in regression analyses we assessed variance inflation factors for the various models and ethnic subgroups, in all instances these were <5 which indicates an acceptable level of potential multicolinearity.[32] Finally, we compared AIx between ethnic groups in young (≤30 years) and elderly (≥60 years) subjects to further explore the contribution of ageing to differences in AIx. In these subgroups we assessed the effects of physiological factors (model 3) on ethnic differences in AIx.

A p-value <0.05 was considered statistically significant. Statistical analyses were performed using SPSS (Version 20.0, IBM corp., Armonk, NY, USA). R (*V*ersion 2.15, *R Foundation for Statistical Computing, Vienna, Austria*) and GraphPad Prism (Version 5.00, GraphPad Software, San Diego, California, USA) were used for graphical representation of data.

Results

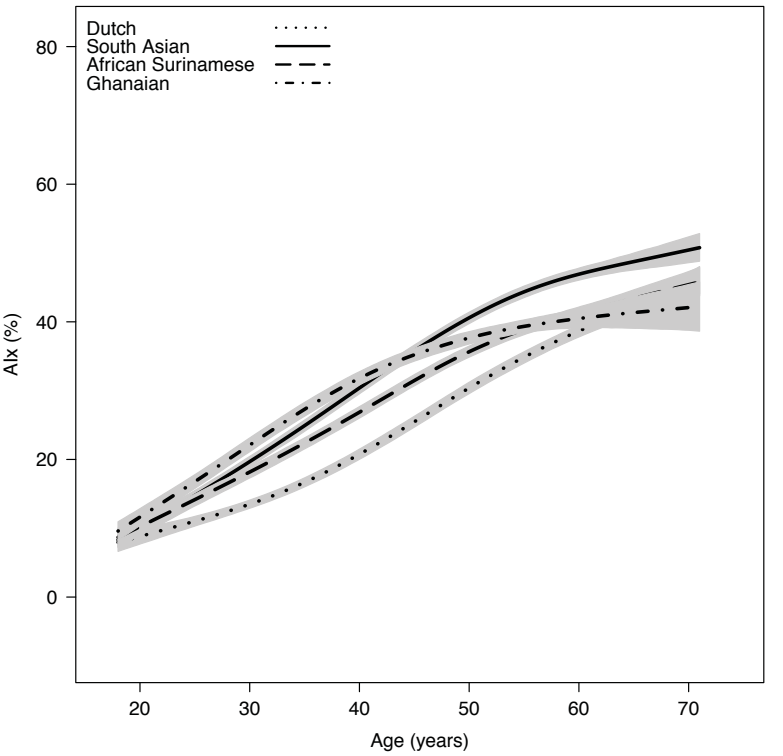
General characteristics and hemodynamics of the study participants stratified by ethnicity are shown in table 1. AIx and central BP for the different ethnic groups are shown in Figs 1 and 2, respectively. Both AIx and central systolic BP were markedly higher in_South-Asian Surinamese (35±17%, 126±22 mmHg), African Surinamese (33±17%, 129±23 mmHg) and Ghanaian (33±16%, 135±24 mmHg) as compared with Dutch (27±17%, 118±20 mmHg, all pairwise p<0.001). In antihypertensive drug naïve subjects (n=6952) AIx and central BP were lower in all groups, yet ethnic differences remained comparable: South-Asian Surinamese (32±17%, 121±20 mmHg), African Surinamese (30±16%, 125±21 mmHg) and Ghanaian (30±16%, 129±23 mmHg) versus Dutch (25±16%, 116±18 mmHg, all pairwise p<0.001). Figs 1 and 2 show the crude estimates of AIx and central systolic BP according to age for the four ethnic groups using spline interpolation. The age-related increase in AIx was most pronounced for South-Asian Surinamese and Ghanaians followed by African Surinamese. The difference in AIx with African Surinamese and Ghanaians became smaller after the age of 50, decreasing to values comparable with Dutch. Likewise, central systolic BP was higher in all ethnic minority groups compared to Dutch, showing similar curves in South-Asian and African Surinamese. Differences in central systolic BP as compared to Dutch were primarily explained by differences in AIx for South-Asian Surinamese and by differences in peripheral systolic BP for subjects of African descent. Correction for AIx resulted in a central systolic BP difference of 0.1±0.5 mmHg for South-Asian Surinamese (p=0.93), 5.6±0.5 mmHg for African Surinamese (p<0.001) and 10.2±0.5 mmHg for Ghanaians (p<0.001) compared to Dutch. While with correction for peripheral systolic BP differences in central systolic BP were 3.1±0.2 mmHg for South-Asian Surinamese, 1.4±0.2 mmHg for African Surinamese and 1.1±0.2 mmHg for Ghanaians compared to Dutch (all p<0.001).

TABLE 1 - Characteristics of the study population stratified by ethnicity.

	Dutch (n=2431)	South-Asian Surinamese (n=1928)	African Surinamese (n=2501)	Ghanaians (n=1952)	p-value
Age, y	46 ± 14	46 ± 13	48 ± 13	46 ± 11	
Men, %	51	53	41	44	
Height, cm	176 ± 9	166 ± 9	169 ± 9	166 ± 8	
BMI, kg/m^2	25 ± 4	26 ± 4	27 ± 5	28 ± 5	
Hypertension, %	25	40	45	55	
Smoking, %	26	28	32	5	
Diabetes, %	3	19	11	13	
HbA1c, mmol/mol	36 ± 5	43 ± 10	40 ± 11	40 ± 10	All p<0.001
AIx, %	26 ± 17	34 ± 17	32 ± 17	33 ± 16	
SBPao, mmHg	118 ± 20	126 ± 22	132 ± 18	134 ± 24	
SBPbr, mmHg	125 ± 16	130 ± 18	139 ± 22	136 ± 19	
DBPbr, mmHg	77 ± 10	80 ± 10	82 ± 11	85 ± 11	
HR, beats/min	60 ± 9	63 ± 9	63 ± 10	64 ± 10	
SV, ml	93 ± 21	89 ± 20	88 ± 22	88 ± 17	
SVR, dynes/sec/cm5	1484 ± 585	1487 ± 567	1544 ± 605	1542 ± 548	
PWV, m/sec	7.9 ± 2.1	8.7 ± 2.6	8.5 ± 2.3	8.7 ± 2.2	

BMI: body mass index; AIx: augmentation index; SBPao: aortic (central) systolic blood pressure; SBPbr: brachial systolic blood pressure; DBPbr: brachial diastolic blood pressure. HR: heart rate; SV: stroke volume; SVR: systemic vascular resistance; PWV: pulse wave velocity. Values are presented as mean ± standard deviation.

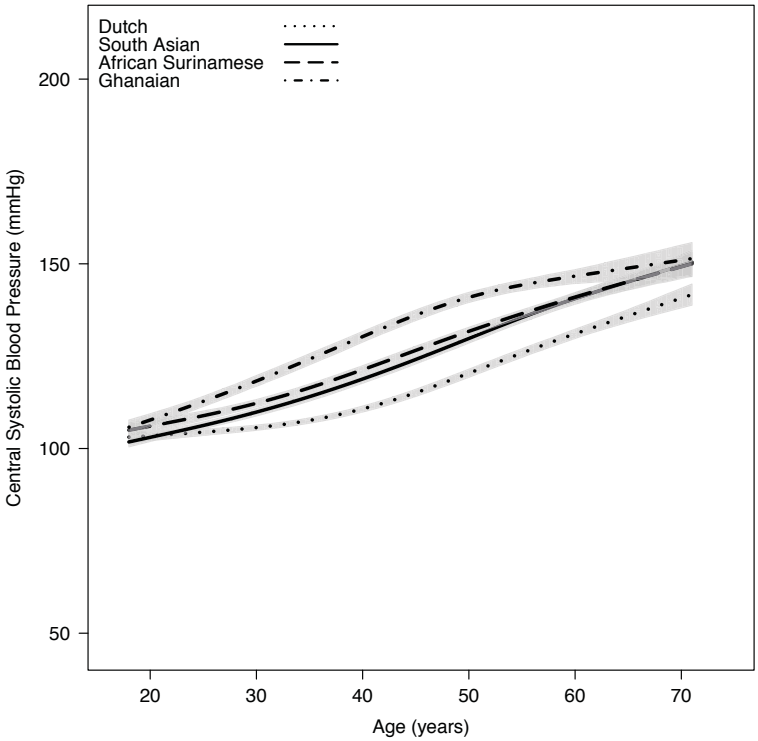
FIGURE 1 - Augmentation index (AIx) for the different ethnic groups according to age.



Gray shaded area denotes 95% CI of the regression line.

In linear regression analysis, age and gender explained 46% of the variation in AIx in the total study sample. Adding conventional CVD risk factors increased this to 54%. Next to age and gender, addition of body height, heart rate, SVR, SV and PWV explained 72% of the variations in AIx. Within each ethnic group, the contribution of the different physiological determinants to the variation in AIx was comparable (supplement added as S1 Table). Table 2 shows differences in estimates of AIx among ethnic groups with correction for various covariates. Age and gender adjusted AIx was 8.2±0.4% higher in South-Asian Surinamese, 3.4±0.4% higher in African Surinamese and 6.3±0.4% higher in Ghanaians as compared with Dutch, all $p<0.001$. Additional correction for cardiovascular risk factors only slightly reduced the difference in AIx (model 2), whereas correction for physiological factors next to age and gender significantly attenuated ethnic differences in AIx (model 3). Correction for body height attenuated age and gender corrected ethnic differences in AIx the most to 4.4±0.4% for South-Asian Surinamese, 1.3±0.4% for African Surinamese, and 3.0±0.3% for Ghanaians, while PWV corrected differences in AIx to 6.1±0.3% for South-Asian Surinamese, 2.5±0.3% for

FIGURE 2 - Central BP for the different ethnic groups according to age.



Gray shaded area denotes 95% CI of the regression line.

African-Surinamese and 4.4±0.3% for Ghanaians as compared to Dutch. Correction for SV and SVR marginally attenuated ethnic differences in AIx, while correction for heart rate increased the differences in AIx between ethnic groups (data not shown). Additional correction for the use of antihypertensive therapy in both models 1 and 2 had a minor effect (<0.1%) on ethnic differences in AIx (data not shown).

Irrespective of ethnicity, females (36±17%) had significantly higher AIx than males (26±16%), p<0.001. Correction for additional covariates (as in included in model 2 and 3) next to age revealed that height had the largest impact on gender differences in AIx. With correction for age and height the estimated gender-difference AIx was reduced by approximately 50%, to 4.9±0.3%, p<0.001.

TABLE 2 - Difference in augmentation index (AIx) between ethnic groups.

		Difference ± SE	P-value
Model 1	**Dutch**	**(reference)**	
Age, Gender	South-Asian Surinamese	8.2 ± 0.4	p<0.001
	African Surinamese	3.4 ± 0.5	p<0.001
	Ghanaians	6.3 ± 0.4	p<0.001
Model 2	**Dutch**	**(reference)**	
Age, Gender, SBPbr, BMI, TC, smoking, DM	South-Asian Surinamese	7.9 ± 0.4	p<0.001
	African Surinamese	2.9 ± 0.3	p<0.001
	Ghanaians	5.5 ± 0.4	p<0.001
Model 3	**Dutch**	**(reference)**	
Age, Gender, Height, PWV, HR, SV, SVR	South-Asian Surinamese	3.9 ± 0.4	p<0.001
	African Surinamese	2.1 ± 0.3	p<0.001
	Ghanaians	3.0 ± 0.3	p<0.001

SBPbr: (seated, brachial) systolic blood pressure; BMI: Body Mass Index; TC: total cholesterol; DM: diabetes mellitus; PWV: pulse wave velocity; HR: heart rate; SV: stroke volume; SVR: systemic vascular resistance.

Table 3 shows differences in AIx stratified by age category. In the young, AIx was significantly lower in Dutch (11.8±8.5%), compared to South-Asian Surinamese (14.5±8.7%), African Surinamese (14.2±10.3%) and Ghanaians (14.3±10.5%), all p<0.001. Stepwise, forward linear regression revealed height as the most important determinant of differences in AIx. After correction for height differences in AIx were all <1% in participants aged ≤30 years of age and no longer significant. In the elderly (age ≥60 years) AIx was lower in Dutch (37.9±14.9%) compared to South-Asian Surinamese (45.7±13.3%, p<0.001), African Surinamese (39.9±14.9%, p=0.001) and Ghanaians (39.4±14.7%, p=0.027). In South-Asian Surinamese, differences in height remained the most important contributor to differences in AIx, whereas differences in PWV were the most important determinant of AIx in elderly African Surinamese and Ghanaians.

TABLE 3 - Ethnic difference in AIx in younger (age ≤ 30 years) and older (age ≥60 years) individuals.

		Difference ± SE	P-value
	Age ≤ 30 years		
Crude	Dutch	(reference)	
	South-Asian Surinamese	2.7 ± 0.7	p<0.001
	African Surinamese	2.4 ± 0.7	p<0.001
	Ghanaians	2.6 ± 0.7	p=0.001
Correction for:	Dutch	(reference)	
Height	South-Asian Surinamese	0.6 ± 0.6	p=0.38
	African Surinamese	0.1 ± 0.6	p=0.82
	Ghanaians	0.7 ± 0.7	p=0.31
Correction for:	Dutch	(reference)	
PWV	South-Asian Surinamese	2.9 ± 0.6	p<0.001
	African Surinamese	2.4 ± 0.7	p<0.001
	Ghanaians	2.4 ± 0.7	p=0.001
	Age ≥ 60 years		
Crude	Dutch	(reference)	
	South-Asian Surinamese	7.7 ± 0.7	p<0.001
	African Surinamese	2.0 ± 0.6	p=0.001
	Ghanaians	1.5 ± 0.7	p=0.027
Correction for:	Dutch	(reference)	
Height	South-Asian Surinamese	0.4 ± 0.7	p=0.54
	African Surinamese	2.5 ± 0.6	p<0.001
	Ghanaians	4.5 ± 0.7	p<0.001
Correction for:	Dutch	(reference)	
PWV	South-Asian Surinamese	4.1 ± 0.6	p<0.001
	African Surinamese	1.2 ± 0.5	p=0.021
	Ghanaians	0.7 ± 0.6	p=0.219

PWV: pulse wave velocity.

Discussion

In the present study we show that there are substantial differences in AIx and central systolic BP between subjects from various ethnic backgrounds. In addition, we demonstrate for the first time that the differences in AIx and central BP between ethnic groups are largely independent of differences in traditional cardiovascular risk factors and are predominantly influenced by physiological determinants, particularly body height.

Our data are in agreement with previous studies that found higher AIx and central BP in subjects from African and South-Asian descent compared to people of Western-European descent.[11–14] Interestingly, ethnic differences in central systolic BP were entirely accounted for by differences in peripheral BP for African subjects, and by differences in AIx for South-Asian subjects. This implies that in particular in South-Asian subjects peripheral BP readings may underestimate the actual BP burden imposed to target organs because of increased wave reflection.

Body height and arterial stiffness explained most of the ethnic differences in AIx, while heart rate, which is inversely related to AIx,[18] increased ethnic differences in AIx because of the lower heart rate in Dutch. In line with our findings, Chirinos et al.[11] recently demonstrated that ethnic differences in AIx remained present after correction for a limited number of physiological determinants known to be associated with AIx, including height. By contrast we also performed concomitant assessment of the known hemodynamic determinants of AIx, (including PWV HR, SVR and SV) thereby enabling comparison of their relative contribution and found body height and large artery stiffness to be most important determinants. Body height and large artery stiffness affect arterial wave reflection by altering the timing between forward and backward travelling pressure waves.[16,20,33–35] Increased arterial stiffness causes faster propagation of pressure waves, while shorter stature may reduce both the travel path of the reflected pressure wave and travel time resulting from a decrease in aortic diameter.[36,37] In both instances the resultant is an earlier return of reflected pressure waves in the proximal aorta, leading to increased wave reflection and central BP augmentation. Although adult height has been shown to independently predict coronary artery disease and stroke,[38,39] the mechanisms leading to a higher risk of CVD are still undetermined. An increase in wave reflection and central BP could be one of the potential mechanisms linking body height to CVD risk.

In the present study, we demonstrate a divergent pattern of age-dependent rise in AIx among different ethnic groups. In young subjects, ethnic differences in AIx were

principally driven by differences in body height, while PWV was not a predictor of ethnic differences in wave reflection. In subjects aged ≥60 differences in body height remained the strongest predictor followed by PWV. Because increased vascular stiffness was the driving force behind the age-dependent increase in AIx, ethnic differences in AIx that emerge with age most likely result from disparities in vascular ageing. By contrast, younger subjects may not have developed significant vascular remodelling as evidenced by the much smaller absolute difference in AIx. This suggests that disparate gene-environment interactions relevant to arterial stiffening, may be responsible. In addition, potential age dependent interactions between determinants of wave reflection could be different for subjects of different ethnic backgrounds thereby affecting disparities in AIx by age for various ethnic subgroups. Although we studied subjects from the same geographical area, migration history may have contributed to selection of participants. Differences in socio-economic, geographic and environmental factors have been shown to influence central BP and AIx.[40,41] In addition, there was a relative underrepresentation of elderly subjects of Ghanaian descent, which may have contributed to the apparent decrease in differences in AIx and central BP at older age. Otherwise, there was a remarkable concordance in the differences and determinants of wave reflection and central BP between African-Surinamese and Ghanaians. As both populations share a common West-African ancestry, this may imply a similar contribution of gene-environment interactions.

Strengths and Limitations

The strengths of our study are the large scale, the fact that subjects were studied under identical geographical circumstances, and the concomitant investigation of various potential determinants of wave reflection. Potential limitations of the study are its cross sectional nature and the fact that differences in ethnicity and migration history may not be generalizable to other migrant populations. Non-invasive techniques were used to assess central hemodynamics and wave reflection. These techniques have been validated mostly in populations of West-European origin. Because the physiological determinants of the outcomes of interest were homogenous across different ethnic groups, we assume that the physiological principles are also generalizable to other ethnic groups. Arterial stiffness measured with the Arteriograph system shows comparable values as obtained by invasive measurements and by MRI.[42] However, in contrast to AIx, assessment of arterial stiffness using a one-point estimate has moderate correlation with the foot-to-foot method rendering extrapolation of absolute values difficult.[24] For the present analyses we used relative differences to assess the contribution of PWV to changes in AIx, although differences in body height

remained the strongest predictor of ethnic differences in AIx, also in the higher age categories.

Perspectives

Our study shows that AIx and central systolic BP are substantially increased in subjects from African and South-Asian origin compared to persons of West-European origin. Peripheral BP was the most important determinant of differences in central BP in African Surinamese and Ghanaians, whereas differences in AIx was the most important determinant of differences in central BP in South-Asian Surinamese. Body height contributed most to the ethnic differences in AIx. In contrast, conventional cardiovascular risk factors had a small contribution to ethnic differences in wave reflection. Because of the contribution of height to ethnic differences in AIx, our findings suggest that body height may be an important risk factor to explain part of the underestimation of cardiovascular risk observed in populations of African and South-Asian descent.

SUPPLEMENTARY TABLE 1 - Associations of physiological determinants with AIx for the total study sample and stratified by ethnicity.

	Total study sample		**Dutch**		**South-Asian Surinamese**		**African Surinamese**		**Ghanaians**	
Model fit	$R^2 = 0.716$		$R^2 = 0.747$		$R^2 = 0.763$		$R^2 = 0.678$		$R^2 = 0.682$	
	β*	**Sig.**	**β***	**Sig.**	**β***	**Sig.**	**β***	**Sig.**	**β***	**Sig.**
Age, years	0.312	<0.001	0.413	<0.001	0.334	<0.001	0.289	<0.001	0.280	<0.001
Female, gender	0.072	<0.001	0.135	<0.001	0.049	0.01	0.098	<0.001	0.180	<0.001
Height, cm	-0.276	<0.001	-0.228	<0.001	-0.219	<0.001	-0.182	<0.001	-0.172	<0.001
PWV, m/sec	0.441	<0.001	0.434	<0.001	0.426	<0.001	0.438	<0.001	0.423	<0.001
HR, beats/min	-0.297	<0.001	-0.275	<0.001	-0.258	<0.001	-0.345	<0.001	-0.318	<0.001
SVR, dynes/sec/cm^5	0.134	<0.001	0.091	<0.001	0.161	<0.001	0.134	<0.001	0.229	<0.001
SV, ml	0.031	0.001	0.035	0.042	0.064	0.005	-0.010	0.55	0.086	<0.001

*β: indicates standardized regression coefficients. PWV: pulse wave velocity ; HR: heart rate ; SVR: systemic vascular resistance ; SV: stroke volume.

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Chapter 06

Ethnic and gender differences in central hemodynamics among subjects with ISH of the young – the HELIUS study

D.W. Eeftinck Schattenkerk
M. Snijder
L. Vogt
R.J.G. Peters
B.J.H. van den Born

Manuscript in preparation

Abstract

Background: Isolated systolic hypertension (ISH), defined as systolic blood pressure (SBP) ≥140 and diastolic blood pressure <90 mmHg of the young is an elusive condition with debated pathophysiology, which has been associated with both high and normal cardiovascular risk.

Methods: We compared differences in central SBP and hemodynamic characteristics between ISH and other hypertensive phenotypes in a randomly-selected multi-ethnic cohort of 5400 subjects (40% males), aged <40 years, participating in the HEalthy Life In an Urban Setting (HELIUS) study that included subjects of Dutch, South-Asian Surinamese, African-Surinamese, Turkish, Moroccan and Ghanaian descent.

Results: ISH was more prevalent in males (5.2%) than in females (1.0%). Ghanaian subjects had a higher prevalence of ISH compared to other ethnic subgroups. Subjects with ISH had lower central SBP and pulse wave velocity (PWV) than Isolated Diastolic Hypertension (IDH), or Systolic Diastolic Hypertension (SDH). ISH was associated with a larger difference between central and peripheral blood pressure (BP), lower augmentation index (AIx), larger stroke volume and taller stature compared to IDH, SDH or normal BP. Males with ISH had lower central SBP, AIx and PWV, than females. Larger differences between central and peripheral BP in ISH were associated with male gender, taller stature, lower AIx, lower PWV and larger stroke volume.

Conclusion: ISH of the young is a heterogeneous condition associated with both normal and high central BP. The assessment of central hemodynamics may aid to differentiate high from low CVD risk and help to understand gender and ethnic differences in cardiovascular risk associated with ISH and other hypertensive phenotypes.

Introduction

Isolated systolic hypertension (ISH), defined as systolic blood pressure (BP) ≥140 mmHg and diastolic BP <90 mmHg, is the most common hypertensive phenotype above age 50,[1] but is also observed in adolescents and younger adults.[2,3] In contrast to the elderly, where ISH is considered a result of vascular ageing,[4,5] and associated with a two- to fourfold increased risk of cardiovascular disease (CVD) and mortality,[6,7] the pathophysiology and cardiovascular risk associated with ISH in the young remains elusive. Whether ISH in young subjects is a mere physiological phenomenon caused by increased pulse pressure amplification with increased brachial, but normal aortic systolic BP (i.e. "spurious hypertension")[8-10], or results from increased arterial stiffness and larger stroke volume, that may evolve in sustained hypertension[11-13] is subject to debate. Besides controversies regarding the pathophysiological basis, initiation of BP lowering therapy in ISH of the young is also disputed.[14,15] The Chicago Heart Association study demonstrated that ISH was associated with an increased risk of CVD and mortality compared to optimal BP during a 31-year follow-up period in young and middle-aged subjects.[16] Particularly young and middle-aged women with ISH were at higher risk of CVD than their male counterparts. This may be related to gender differences in aortic (i.e. central) BP, which is a potentially better predictor for CVD than brachial BP as it more accurately reflects the burden imposed on the organs at risk (i.e. heart, brain and kidneys).[17] Besides gender differences, age-dependent differences in pulse pressure amplification likely contribute to CVD risk.[8,18] This is supported by data from the Framingham heart study showing that, in contrast to the elderly, pulse pressure is not associated with coronary events below age 50[19] and even inversely related to coronary disease in men aged <40 years.[20] The inverse association with pulse pressure below age 40 possibly results from increased pulse pressure amplification in the setting of low central SBP.[21] Finally, while ethnic differences in CVD are well recognised,[22-24] data regarding differences in hypertensive phenotypes, including ISH, in young to middle-aged subjects from different ethnic groups are lacking. We previously identified substantial ethnic differences in blood pressure and central hemodynamics.[25,26] This may affect the prevalence and pathophysiology of hypertensive phenotypes, particularly of ISH in the young. In the present study we compared differences in the prevalence of ISH and other hypertensive phenotypes in subjects aged <40 years across different ethnic groups and examined differences in central BP and its determinants in an attempt to further elucidate the potential implications for CVD risk.

Methods

Study population

This study was performed using baseline data of the HEalthy LIfe in an Urban Setting (HELIUS) study. HELIUS is a large-scale, multi-ethnic prospective cohort study that focusses on public health among six major ethnic groups residing in Amsterdam, the Netherlands. Details of the HELIUS study have been previously described.[27] HELIUS is conducted in accordance with the Declaration of Helsinki and has been approved by the Ethical Review Board of the Academic Medical Center, Amsterdam. All participants gave written informed consent. We used data of participants aged <40 years who were included between January 2011 and December 2014, and underwent a physical examination (including all required hemodynamics assessments) and had a complete questionnaire. This resulted in a dataset of 5400 subjects (2180 males), including 1008 Dutch, 728 South-Asian Surinamese, 706 African-Surinamese. 1120 Turkish and 1263 Moroccan (all both first and second generation) and 575 participants of Ghanaian descent (exclusively first generation).

Study Procedures

Study participants visited the research location in the morning after an overnight fast and refrained from smoking the morning prior to the visit. Information regarding demographics, smoking behaviour and history of disease was obtained by questionnaire. Prescribed medications were coded according to the Anatomical Therapeutic Chemical (ATC) classification. BP was calculated as the average of two seated brachial BP readings, after 5 minutes of rest, using a semi-automated, validated sphygmomanometer (Microlife WatchBP Home, Microlife AG, Switzerland). Blood samples were taken while fasting. Diabetes was defined as a fasting glucose level ≥ 7.0 mmol/l, or the use of glucose lowering medication.

Blood pressure stratification

All subject were stratified in exclusive BP categories according to their systolic BP (SBP) and diastolic BP (DBP). We defined normal blood pressure as SBP <140 and DBP <90 mmHg, ISH as SBP ≥140 and DBP <90 mmHg, isolated diastolic hypertension (IDH) as SBP <140 and DBP ≥ 90 mmHg and combined systolic and diastolic hypertension (SDH) as SBP ≥ 140 and DBP ≥ 90 mmHg.

Central Blood Pressure and Augmentation Index

The Arteriograph system (Tensiomed Kft., Budapest, Hungary) was used to assess AIx, central BP, brachial BP and pulse wave velocity (PWV) in supine position after at least 10

minutes of rest in supine position. The Arteriograph system is an operator-independent non-invasive device that applies an oscillometric, occlusive technique by use of an upper-arm cuff to register brachial pressure curves. Its methodology and validation are described in detail elsewhere.[28-30] Arteriograph has a close correlation with the widely applied Sphygmocor system (AtCor Medical Pty Ltd, West Ryde, Australia) for AIx (r=0.89, p<0.001),[30] and a close correlation with invasively measured AIx (r=0.90, p<0.001), central BP (r=0.95, p<0.001) and PWV (r=0.91, p<0.001).[29] Arteriograph PWV values are also comparable to values obtained by MRI.[31] All Arteriograph measurements were performed in duplicate and the results were averaged for further analysis.

Hemodynamics

Hemodynamics were assessed by volume-clamp photoplethysmography using the Nexfin™ device[32] (Edwards Lifesciences BMEYE, Amsterdam, the Netherlands). This device applies the Finapres method[33-35] to continuously and non-invasively record finger arterial BP and reconstructed brachial BP.[36,37] Finapres recordings were made at 200Hz with a finger-cuff placed around the mid-phalanx of the third finger, with subjects in a supine position. Mean arterial pressure (MAP) was the true integral of the arterial pressure wave of one beat divided by the corresponding inter-beat interval. Stroke volume (SV) was determined by the pulse contour method (Nexfin CO-trek).[38] Cardiac output (CO) was SV divided by the inter-beat interval. Systemic vascular resistance (SVR) was the ratio of MAP and CO. Left ventricular contractility was estimated as dP/dt_{max} of the brachial pressure pulse. All Nexfin hemodynamic parameters were calculated from the average of a one minute period of stable recording.

Statistical analyses

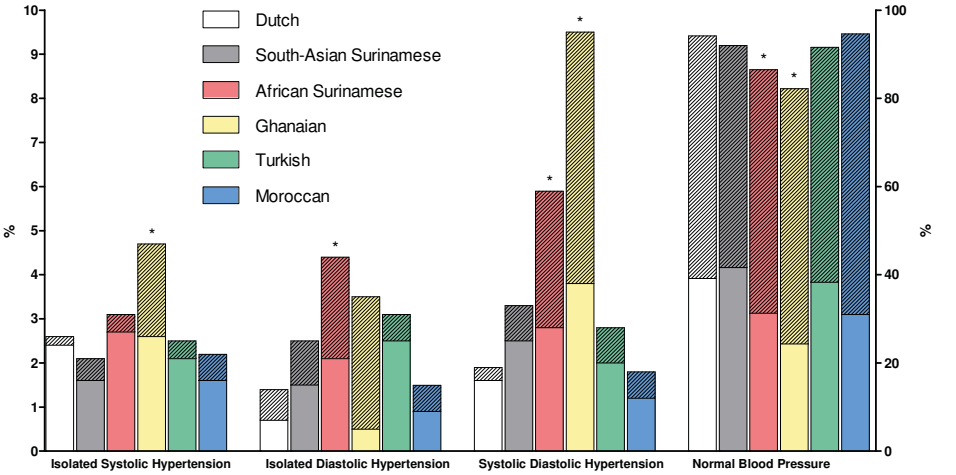
All baseline information used in statistical analysis was provided by HELIUS. Statistical analyses were performed using SPSS (Version 20.0, IBM corp., Armonk, NY, USA). Baseline differences between different hypertensive phenotypes were compared using chi-square for categorical variables and analysis of variance for continuous variables. Pair-wise comparisons were performed according to least-significant difference. To further study differences in central and peripheral BP in subjects with ISH, we performed linear regression analyses and additionally subdivided subjects with ISH into tertiles of central versus peripheral SBP differences (ΔSBP). Finally we studied central SBP differences among ISH subjects by subdivision into tertiles of central SBP. We considered a two-sided p-value <0.05 of statistical significance.

Results

We studied data of 5400 randomly selected subjects (40.3% males), mean age 29.2±6.1 years from six major ethnic subgroups, residing in Amsterdam, the Netherlands, that participated in HELIUS between January 2011 and December 2014. Our study population included 1008 Dutch, 728 South-Asian Surinamese, 706 African-Surinamese, 1120 Turkish, 1263 Moroccan and 575 participants of Ghanaian descent.

Figure 1 shows the prevalence of the different hypertensive phenotypes by gender and ethnicity. Males had a higher overall prevalence of hypertension (13.8%) compared to females (5.4%, p<0.001). Of all hypertensive phenotypes, the largest differences between males and females were observed in ISH with a prevalence of 5.2% in males and 1.0% in females. In subjects of African Surinamese and Ghanaian descent the prevalence of hypertension was more than twice as high as in all other ethnic groups (13.5% and 17.7% respectively, versus 6.8% average of other groups). The highest prevalence of ISH was observed in Ghanaians (4.7%), while IDH was most frequent in African-Surinamese (4.4%). Combined systolic and diastolic hypertension (SDH) had the highest prevalence in Ghanaians (9.6%), followed by African-Surinamese (5.9%), compared to the other ethnic groups where the average prevalence was 2.5%.

FIGURE 1 - Prevalence of Hypertensive phenotypes by gender and ethnicity



Baseline characteristics of study subjects by hypertensive category are depicted in table 1. Subjects with SDH and IDH were older compared to subjects with ISH or normal BP. Subjects with SDH and IDH had the highest BMI and total cholesterol levels, while BMI and cholesterol levels in subjects with ISH were in between. Diabetes was more prevalent in any of the hypertensive phenotypes compared to subjects with normal BP.

TABLE 1 - Baseline characteristics hypertensive phenotypes

	Normal BP	**ISH**	**IDH**	**SDH**	**p**
N=	4923	146	137	194	
Males, %	38.2%	77.4%	54.7%	58.2%	
Age, yrs	28.9±6.2	29.8±6.4	32.6±5.1*#	33.6±4.7*#	<0.001
Systolic BP, mmHg	116.8±10.4	144.8±5.3*	133.1±4.7*#	154.3±12.7*#	<0.001
Diastolic BP, mmHg	72.4±7.5	82.1±5.8*	92.6±2.4*#	99.1±7.7*#	<0.001
BMI, kg/m2	25.0±4.6	28.7±6.2*	28.5±6.0*	30.5±5.6*#	<0.001
Smoking, %	26.4	26.7	30.7	22.7	ns
Diabetes, %	0.8	3.4*	5.1*	5.2*	<0.001
Cholesterol, mmol/L	4.5±0.9	4.7±0.9*	4.9±1.0*	5.0±0.9*#	<0.001

Systolic and diastolic BP are the average two consecutive, seated measurements.
* P<0.05 pairwise comparison versus normal BP.
# P<0.05 pairwise comparison versus ISH

Table 2 shows the hemodynamic variables by hypertensive phenotype. Supine SBP and DBP was highest in subjects with combined hypertension (table 3). Subjects with ISH had lower aortic SBP (119.6±13.1 mmHg) than subjects with IDH (126.0±11.8 mmHg) or SDH (144.9±18.5 mmHg, all p<0.05). Differences between aortic and brachial SBP were larger in subjects with ISH (Δ11.9±7.0 mmHg) compared to those with IDH (Δ5.3±7.0 mmHg) or SDH (Δ5.3±7.0 mmHg and also compared to subjects with normal BP (Δ9.3±5.6 mmHg). Subjects with ISH had significantly lower AIx, larger stroke volume and height compared to subjects with IDH, SDH or normal BP, while SVR was significantly higher in subjects with SDH and IDH than in those with ISH or normal BP. PWV was lower in subjects with ISH (7.0±1.3 m/s) than IDH (7.7±1.7m/s) or SDH (8.5±1.8m/s), yet higher than in subjects with normal BP (6.6±0.9m/s), all p<0.05.

TABLE 2 - Hemodynamics by hypertensive phenotype

	Normal BP	ISH	IDH	SDH	p
Sup. brSBP, mmHg	115.8±10.4	131.6±11.2*	131.4±10.3*	147.6±14.4*#	
Sup. aoSBP, mmHg	106.5±10.7	119.6±13.1*	126.0±11.8*#	144.9±18.5*#	
Δ SBP, mmHg	9.3±5.6	11.9±7.0*	5.3±7.0*#	5.3±7.0*#	
Sup. DBP, mmHg	69.3±8.0	77.0±8.0*	84.9±7.3*#	94.8±10.1*#	
Height, cm	169±10	176±10*	170±9#	171±11*#	All
Heart rate, beats/min	61.3±8.8	60.3±8.1	69.0±9.9*#	67.3±10.0*#	<0.001
PWV, m/sec	6.6±0.9	7.0±1.3*	7.7±1.7*#	8.5±1.8*#	
AIx, %	16.2±10.6	13.6±11.9*	24.2±13.2*#	30.5±15.8*#	
Stroke volume, ml	100±18	113±15*	99±15#	103±19#	
SVR, dynes/sec/cm^5	1208±355	1162±280	1255±367#	1322±416*#	
dp/dt$_{max}$, mmHg/sec	829±302	958±269*	844±243#	950±284*	

Sup: supine. brSBP: brachial systolic BP. aoSBP: aortic systolic BP. Δ SBP = sup. brSBP – sup. aoSBP. PWV: pulse wave velocity. AIx: augmentation index. SVR: systemic vascular resistance.
* P<0.05 pairwise comparison versus normal BP.
# P<0.05 pairwise comparison versus ISH.

Table 3 shows linear regression analysis of brachial minus aortic SBP (ΔSBP) in subjects with ISH. In univariate linear regression analysis ΔSBP was significantly correlated with the following factors: age (r=-0.470, p<0.001), gender (r=-0.564, p<0.001), height (r=0.577, p<0.001), PWV (r=-0.364, p<0.001), SV (r=0.362, p<0.001), SVR (r=-0.440, p<0.001), dp/dt$_{max}$ (r=0.238, p=0.006) and total cholesterol (r=-0.213, p=0.008). Additionally, subjects with ISH were divided into tertiles of ΔSBP (online supplement). The majority of females with ISH (78.3%) were in the lowest ΔSBP tertile. Likewise, Ghanaian subjects more frequently (60.9%) had a lower ΔSBP, while Dutch and Moroccan subjects more often had a large difference between central and brachial SBP (52.2% and 45.8% respectively). Supine brachial SBP was significantly higher, while aortic SBP was lower in subjects from the upper tertile compared to the lower tertile. Subjects from the upper tertile of Δ SBP were younger, taller and had a larger stroke volume than those from the lower tertile of Δ SBP. The augmentation index and SVR were lower in subjects from the upper compared to the lower tertile of ΔSBP, while PWV was not different. There was no differences in BMI, smoking, diabetes, nor total cholesterol.

TABLE 3 - Linear regression analysis of systolic blood pressure amplification

	Pearson's r	**p**
Age	-0.435	<0.001
Gender	-0.537	<0.001
Height, cm	0.577	<0.001
BMI, kg/m2	-0.148	ns
Heart rate, beats/min	0.073	ns
PWV, m/sec	-0.364	<0.001
Stroke volume, ml	0.362	<0.001
SVR, dynes/sec/cm^5	-0.440	<0.001
dp/dt$_{max}$, mmHg/sec	0.238	0.006
Smoking, %	-0.017	ns
Diabetes, %	-0.009	ns
Total cholesterol, mmol/l	-0.206	0.016

PWV: pulse wave velocity. SVR: systemic vascular resistance

In table 4 gender differences in subjects with ISH are shown. Aortic SBP was significantly lower in males than in females with ISH, and accompanied by a higher ΔSBP in males. Males with ISH were significantly taller, had lower AIx, PWV, and heart rate and had larger stroke volume and higher dp/dt$_{max}$ compared to females. Smoking was more prevalent in males than females, while females were older, suffered from diabetes more frequently and had a higher BMI.

TABLE 4 - Gender differences in subjects with ISH

	Males (n=107)	Females (n=23)	p
Age	29.0±6.1	32.6±6.8	0.004
Height, cm	180.2±7.1	162.7±6.5	<0.001
BMI, kg/m2	27.1±4.2	34.2±8.5	<0.001
Sup. aoSBP, mmHg	117.4±10.5	129.5±18.7	<0.001
Sup. brSBP, mmHg	131.2±10.4	133.0±14.3	ns
Δ SBP, mmHg	13.7±5.0	3.4±8.8	<0.001
Sup. DBP, mmHg	76.1±7.6	81.2±9.6	0.005
Heart rate, beats/min	59.4±7.9	64.5±7.5	0.005
PWV, m/sec	6.9±1.0	7.9±2.0	<0.001
AIx, %	10.6±8.5	27.9±15.1	<0.001
Stroke volume, ml	116.6±12.4	101.1±16.8	<0.001
SVR, dynes/sec/cm^5	1141±256	1235±349	ns
dp/dt_{max}, mmHg/sec	984±220	868±388	0.045
Smoking, %	31	12	0.03
Diabetes, %	1.8	9.1	0.04
Total cholesterol, mmol/l	4.7±1.0	4.8±0.8	ns

Sup.: supine. brSBP: brachial systolic BP. aoSBP: aortic systolic BP. Δ SBP = sup. brSBP – sup. aoSBP. PWV: pulse wave velocity. AIx: augmentation index. SVR: systemic vascular resistance.

Discussion

Our study shows heterogeneity in the prevalence and pathophysiology among subjects with ISH of the young. The assessment of central hemodynamics may help to differentiate ISH with likely low from high CVD risk. We provide a potential explanation for previously observed gender differences in the CVD risk associated with ISH in young and middle-aged subjects[16] by demonstrating higher central SBP and AIx in women compared to males with ISH. In addition, we show that the prevalence of ISH differs among ethnic groups with the highest prevalence in Ghanaians. However, their hemodynamic profile was less favourable, showing higher central SBP and AIx compared to other ethnic groups, whereas subjects with ISH because of exaggerated systolic pressure amplification were more frequently of Dutch descent. Subjects with increased systolic pulse pressure amplification were mostly male, younger, taller and had a larger stroke volume compared to subjects with smaller differences between peripheral and central BP.

Our findings are in line with previous studies that have reported a prevalence ranging from 2% to 8% in young and middle-aged subjects.[2,13,39] Variations in previously observed prevalence of ISH of the young may originate from differences in age, gender and ethnicity, among the studied populations as well BP measurement methodology. In our population we found higher prevalence of ISH of the young in males (5.2%) compared to females (1%), which is also consistent with previous findings[2,16], while data regarding ethnic differences in the prevalence of ISH of the young are limited, although higher AIx and central BP has been reported among African descent populations.[40]

The pathophysiology of ISH in the young as well as its clinical relevance is much debated.[14,15] In the recently published results of the Chicago Heart Association study an increased risk of CVD mortality was observed in young and middle aged subjects that suffered from ISH.[41] Interestingly, the relative risk associated with ISH was considerably higher in females than in males. The higher central SBP, PWV and AIx in women with ISH of the young as observed in our study provide a potential explanation for this discrepancy in cardiovascular risk. Mounting evidence suggests that central BP is a stronger predictor for future CVD than brachial BP.[17] In addition, indices of wave reflection, such as AIx, have been associated with CVD independent of BP in various populations, possibly by detrimental effects on myocardial perfusion.[42,43] The raised PWV in women with ISH may reflect a pathophysiological substrate for the closer central to peripheral SBP values as well as a marker for preclinical organ damage. Apart from providing a possible explanation for gender differences in CVD risk associated with ISH of the young, our data also suggest that the physiological basis for ISH of the younger differs according to gender and among different ethnic groups.

The usual pattern of blood pressure (BP) change through life is considered to result from the progressive loss of large arterial compliance and elasticity,[18,44] accelerated by cardiovascular risk factors. This process of arterial stiffening leads to a steady rise in SBP —due to changes in arterial wave reflection and increased systolic pressure augmentation— and a fall in DBP —due to the loss of Windkessel-function and increased diastolic runoff of stroke volume—. Therefore, as a consequence of vascular ageing, the prevalence of ISH rises steadily with age and is associated with a two- to fourfold increased risk of cardiovascular events, renal dysfunction and mortality in elderly subjects.[6,7] By contrast at younger age, the pathophysiology of ISH is unclear and it is much debated whether ISH is to be considered an innocuous phenomenon (i.e., spurious hypertension) or a potentially harmful condition.[14,15] Two major views are supported. First, ISH in the young is considered a 'spurious' condition caused by exaggerated aortic pressure wave amplification that typically occurs in tall, fit males

and is accompanied by much lower aortic SBP,[8-10] whereas others advocate that ISH in the young results from increased arterial stiffness and larger stroke volumes which is more likely to transform into sustained hypertension and is already accompanied by higher than normal aortic SBP.[11,13]

In the present study we demonstrate a heterogeneity within subjects with ISH of the young, providing support for both concepts. Subjects with the largest systolic pressure amplification were taller, younger, almost exclusively male, and had a larger stroke volume and lower AIx compared to ISH with low systolic pressure augmentation. Arterial stiffness in these subjects was comparable to those with low systolic pressure augmentation. Women with ISH of the young, compared to men, had higher central SBP, PWV and AIx, with aortic SBP values being closer to brachial SBP, resembling ISH at higher age. Likewise, in subjects of Ghanaian descent, in whom the highest prevalence of ISH was observed, we found similar differences in central hemodynamics as in women, as well as a shorter stature, which offers a potential explanation for a smaller ΔSBP and higher central SBP.[25] An additional subgroup analysis (online supplement) of subjects with ISH of the young showed that lower central SBP was accompanied by lower PWV, AIx and BMI and a taller stature compared to the upper tertile of aortic SBP. Subjects with ISH of the young from the lower tertile of aortic SBP, had aortic SBP and PWV values comparable to normotensive subjects, yet they were taller, had a larger stroke volume and large difference between central and peripheral BP, also in line with so-called 'spurious hypertension'.

Our study has some potential limitations including its cross-sectional design and external validity due to the ethnic makeup of our population. We made use of non-invasive techniques to assess central hemodynamics. Although validated, these techniques have predominantly been studied in West-European populations. We assume that their physiological principles are generalizable to other ethnic groups. In addition while relative differences are likely accurate, the extrapolation of absolute values should be done with caution. Since ISH of the young in women was rare, this may have resulted in type 1 errors. To classify the hypertensive phenotypes we used seated brachial BP readings, while the hemodynamics investigations were performed in supine position. However, as seated brachial BP readings guide antihypertensive therapy in daily practice, we consider this discrepancy to be justified. Migration effects may also have affected ethnic differences in ISH as Ghanaians are almost exclusively first generation migrants that likely had a poorer socio-economic status prior to migration.

Perspectives

We identified a substantial heterogeneity within a population of subjects with ISH of the young, showing both a favourable and potentially detrimental hemodynamic profile. By extent the implication of ISH in the young for future CVD may have a significant within group variation. The assessment of central hemodynamics may prove valuable to distinguish benign from harmful ISH in young subjects, yet prospective studies that includes such investigations are called for. Within HELIUS, subjects will be followed up at 5 year intervals. This could yield valuable information regarding the course of central hemodynamics in subjects with ISH.

ONLINE SUPPLEMENT - ISH tertiles of systolic blood pressure amplification

	Lower Tertile	Middle Tertile	Upper Tertile	p
Males (n=107)	23.4%	37.4%	39.3%	<0.001
Females (n=23)	78.3%	17.4%	4.3%	
Dutch (n=23)	21.7%	26.1%	52.2%	
South-Asian Sur (n=12)	33.3%	66.7%	0%	
African Sur (n=20)	30.0%	35.0%	35.0%	0.015
Ghanaian (n=23)	60.9%	21.7%	17.4%	
Moroccan (n=28)	25.0%	29.2%	45.8%	
Turkish (n=24)	28.6%	39.3%	32.1%	
Age	33.1±5.7	28.9±6.2*	29.8±6.4*	<0.001
Height, cm	171±10	179±8*	182±7*	<0.001
BMI, kg/m2	28.3±5.5	27.2±4.9	28.5±5.3	ns
Sup. aoSBP, mmHg	125.4±17.3	116.7±10.1*	116.7±8.5*	0.001
Sup. brSBP, mmHg	129.5±13.7	129.9±10.1	134.9±8.8*#	0.047
Δ SBP, mmHg	4.1±6.4	13.3±1.2*	18.2±2.0*#	<0.001
Sup. DBP, mmHg	79.3±9.3	76.5±8.2*	75.1±6.8*	ns 0.054
Heart rate, beats/min	60.0±8.7	60.4±8.0	62.6±9.1	ns
PWV, m/sec	7.4±1.6	6.5±0.7*	7.2±1.3#	0.005
AIx, %	26.3±11.6	10.9±4.0*	3.8±3.7*#	<0.001
Stroke volume, ml	108±19	116±13*	117±12*	0.014
SVR, dynes/sec/cm^5	1275±331	1130±226*	1092±275*	0.015
dp/dt_{max}, mmHg/sec	878±278	1011±270*	1028±251*	0.034
Smoking	30%	27%	23%	ns
Diabetes	2.3%	4.6%	2.3%	ns
Total cholesterol	4.9±0.9	4.6±0.9	4.6±0.8	ns

Sup.: supine. brSBP: brachial systolic BP. aoSBP: aortic systolic BP. Δ SBP = sup. brSBP – sup. aoSBP. PWV: pulse wave velocity. AIx: augmentation index. SVR: systemic vascular resistance.
* P<0.05 pairwise comparison versus lower tertile.
# P<0.05 pairwise comparison versus middle tertile

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Chapter 07

Lack of difference between nebivolol/HCTZ and metoprolol/HCTZ on aortic wave augmentation and central blood pressure

D.W. Eeftinck Schattenkerk
B. van den Bogaard
M. Cammenga
B.E. Westerhof
E.S.G. Stroes
B.J.H. van den Born

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Abstract

The vasodilating beta blocker nebivolol is thought to be superior in lowering wave reflection and central blood pressure (BP) compared to non-vasodilating beta blockers. The results from studies comparing nebivolol with either metoprolol or atenolol, with or without hydrochlorothiazide (HCTZ), are not unequivocal. We examined the effects of nebivolol 5 mg and metoprolol 100 mg with HCTZ 12.5 mg on aortic wave augmentation, central BP and hemodynamics using a randomized, double-blind, crossover design. We included 22 subjects (17 males, age 59.9±6.4 years) with office systolic BP of 155±16 mmHg and diastolic BP of 93±10 mmHg. Radial applanation tonometry and non-invasive, continuous finger arterial BP measurement was performed at baseline and after four weeks of treatment with either drug regimen, separated by a 4 week washout period. Neither treatment affected aortic wave augmentation significantly. Augmentation index increased 1.0±7.8% (p=0.5) for nebivolol/HCTZ and 2.4±6.6% (p=0.07) for metoprolol/HCTZ. Nebivolol/HCTZ lowered central systolic BP by 15.8±14.9 mmHg and diastolic BP 10.5±8.4 mmHg and with metoprolol/HCTZ by 13.5±12.3 mmHg for systolic and 9.5±6.8 mmHg for diastolic BP (all $p<0.001$). Heart rate was lowered 8.1±5.4 beats·min^{-1} by nebivolol/HCTZ and 8.6±4.9 beats·min^{-1} by metoprolol/HCTZ. Peripheral BP was reduced to a similar extent as central BP. Peripheral BP decreased by 16.3±14.9 mmHg systolic and 10.1±8.2 mmHg diastolic with nebivolol/HCTZ and by 15.2±13.0 mmHg systolic and 9.1±6.9 mmHg diastolic with metoprolol/HCTZ. Both treatment modalities had a similar effect on stroke volume, cardiac output, left ventricular contractility and peripheral resistance. In conclusion, nebivolol was not superior to metoprolol in reducing aortic wave augmentation or central BP when combined with HCTZ.

INTRODUCTION

Beta-blockers are widely used for the treatment of hypertension and have been shown to reduce cardiovascular disease.[1] The use of beta-blockers as first-line therapy for hypertension is debated because they appear less effective in preventing cardiovascular disease compared to other anti-hypertensive drugs.[1-3] This has been partially attributed to the inability of beta-blockers to lower aortic wave augmentation and central blood pressure (BP) compared to other anti-hypertensive drugs.[4] In the Anglo-Scandinavian Cardiac Outcomes Trial (ASCOT) atenolol with hydrochlorothiazide (HCTZ) as add-on was associated with higher all-cause mortality and cardiovascular events, particularly stroke, compared to perindopril based therapy,[5] and resulted in a smaller reduction in aortic wave augmentation and central BP.[6] In support, observational studies show that central BP may be superior over peripheral BP in predicting cardiovascular disease.[6-9] Most of the available data on the efficacy of beta-blockers in lowering central BP and aortic wave augmentation has been obtained with atenolol.[1-3, 6] This has led to a preference for other beta-blocking agents including metoprolol and vasodilating beta-blockers such as nebivolol. Nebivolol, a beta-1 selective receptor blocker, has been shown to stimulate endothelial nitric oxide synthase (eNOS) activity.[10, 11] Like other vasodilating agents this may offer potential benefit on wave reflection, aortic wave augmentation and central BP by inducing vasorelaxation.[12-14] In a previous study, nebivolol was superior to atenolol in decreasing wave reflection and central pulse pressure.[15] A recent randomized parallel group study comparing nebivolol and metoprolol with a non-fixed dose of HCTZ, demonstrated that nebivolol was superior to metoprolol in reducing central BP.[16] This suggests that nebivolol may be more effective in reducing wave reflection and lowering central BP compared to metoprolol, with or without HCTZ.

Our aim was to compare the effect of nebivolol and metoprolol in combination with HCTZ on aortic wave augmentation. Secondly, we investigated the effects of nebivolol/HCTZ and metoprolol/HCTZ on central BP, hemodynamic parameters and metabolic profile.

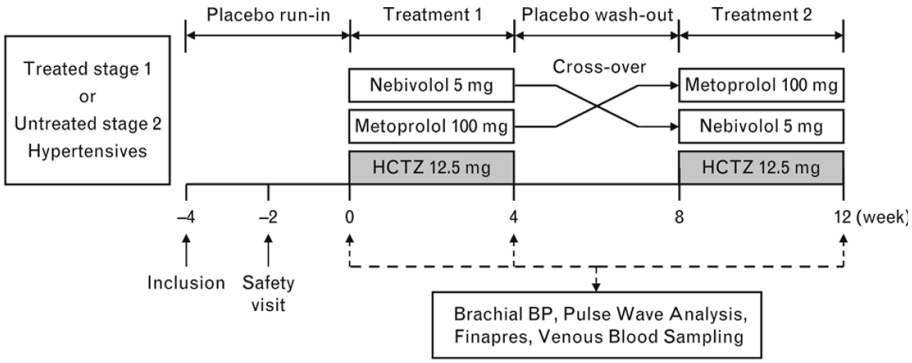
METHODS

We used a double-blind, randomized crossover study-design to compare nebivolol/ HCTZ and metoprolol/HCTZ in twenty-two otherwise healthy subjects of European descent, aged 40-70 years, with untreated stage 2 hypertension (office brachial BP 160-179/ 100-109 mmHg), or stage 1 (140-159/ 90-99 mmHg) hypertension while using one or two BP lowering drugs. Subjects were excluded from participation if they had a contraindication for, or previous intolerance to, a beta-blocker or thiazide diuretic, chronic atrial fibrillation, diabetes mellitus (fasting glucose ^{3}7.0 mmol/l or use of glucose-lowering therapy), dyslipidemia (fasting total cholesterol ^{3}6.5 mmol/l or cholesterol lowering drugs), renal dysfunction (serum creatinine ^{3}150 µmol/l), secondary hypertension, previous or current cardiovascular disease, or were not willing or had a contraindication to temporarily discontinue BP lowering medication. Subjects could withdraw from this study at any time for any reason. Specific criteria for withdrawal from this study were: BP ≥180 mmHg systolic and/ or ≥110 mmHg diastolic, side-effects related to the study medication necessitating termination of the study, asymptomatic bradycardia with a pulse rate <50 bts/min, abnormalities on the ECG consisting of 2nd or 3rd degree atrioventricular block or sick sinus syndrome. Eligibility for participation was assessed during a screening visit which included a medical history, physical examination and fasting venous blood sample. Patients included in the study started a 4-week placebo run-in phase. If applicable, concomitant anti-hypertensive medication would be discontinued. After two-weeks of placebo run-in a safety visit was planned to check for possible BP elevations above acceptable limits (BP ≥180 mmHg systolic and/ or ≥110 mmHg diastolic).

The protocol outline is shown in figure 1. After completion of the 4-week placebo run-in phase, study participants were randomized to receive either metoprolol 100 mg (as 95 mg metoprolol succinate extended release) in combination with HCTZ 12.5 mg once daily or nebivolol 5 mg (as nebivolol hydrochloride: 2.5mg d-nebivolol and 2.5 mg l-nebivolol) in combination with HCTZ 12.5 mg once daily for 4 weeks. The first active treatment period was followed by a 4-week placebo wash-out. Hereafter subjects crossed over to the alternate treatment arm; those having received nebivolol/ HCTZ were to receive metoprolol/HCTZ and vice versa. All measurements were performed at the end of the 4 week placebo run-in phase, after the first and after the second treatment period. The study was conducted in accordance with Good Clinical Practice Guidelines at the Academic Medical Center, Amsterdam, the Netherlands, between February 2010 and October 2011, and approved by the local medical ethics committee. EudraCT number: 2009-015824-27. Written informed consent was given by all study participants. The primary endpoint of this study was differences in

central aortic wave augmentation between nebivolol/HCTZ versus metoprolol/HCTZ as assessed by applanation tonometry. Secondary endpoints were differences in central BP, hemodynamic parameters and metabolic profile for nebivolol/HCTZ versus metoprolol/HCTZ.

FIGURE 1 - Study Protocol Outline



Study procedures

Office brachial blood pressure

Office brachial BP was measured using a validated semi-automated oscillometric device (Omron 705it, Omron Healthcare Europe BV, Hoofddorp, the Netherlands), while seated and after 5 minutes rest in a quiet room, 3 times at 1-minute intervals. The mean of the last 2 measurements was recorded as representative of office brachial BP. During the screening visit BP was recorded three times with a 1-minute interval at both arms. If the difference in mean systolic BP of the last two out of three readings was >10mmHg, the arm with the highest pressure was taken for consecutive readings. In other cases the non-dominant arm was used for further measurements.

Pulse wave analysis

Pulse wave analysis was performed using the SphygmoCor system (AtCor Medical Pty Ltd, West Ryde, Australia). The SphygmoCor system was calibrated using systolic and diastolic brachial BP, defined as the mean of three consecutive readings, obtained after fifteen minutes of supine rest from the same arm and just prior to radial tonometry. Radial artery pressure waveforms were sampled over 10 seconds with a Millar tonometer (SPC-301, Millar Instruments, Texas, USA). Radial arterial measurements were performed twice in each patient and processed using dedicated software (SphygmoCor version 7, AtCor). Radial artery waveforms were averaged and corresponding central aortic pressure waveforms were derived using a validated transfer function.[17, 18] Central

systolic BP, diastolic BP, augmentation index (AIx), heart rate adjusted AIx standardized to 75 beats per minute (AIx@hr75), ejection duration and return time of the reflected wave were determined from the obtained central aortic pressure waveform.

Continuous finger arterial blood pressure measurement

Continuous non-invasive finger arterial BP and derived hemodynamic parameters were obtained with the Nexfin™ monitor (Edwards Lifesciences BMEYE, Amsterdam, The Netherlands).[19] This device applies volume-clamp plethysmography according to the Finapres method to measure finger arterial BP[20] and reconstruct brachial BP.[21-23] The finger cuff was placed around the mid-phalanx of the third finger of the hand on the side opposite to applanation tonometry. Hemodynamic parameters were calculated from the average of a thirty second stable recording period after fifteen minutes of supine rest. Mean arterial pressure (MAP) was calculated from the true integral of the arterial pressure wave over one beat divided by the corresponding beat interval. Stroke volume (SV) was determined by the pulse contour method (Nexfin CO-trek).[24] Heart rate (HR) was the inverse of the interbeat interval. Cardiac output (CO) was defined as SV divided by the interbeat interval. Systemic vascular resistance (SVR) was calculated as the ratio of MAP and CO. Left ventricular contractility was estimated as dP/dt_{max} of the pressure pulse. For determination of baroreflex sensitivity, cross-correlation time-domain baroreflex sensitivity (xBRS) was derived from spontaneous BP fluctuations as previously described,[25, 26] a six minute window of stable recordings in supine rest was used for calculation.

Laboratory analysis

Fasting venous blood samples were taken and processed within 1 hour, stored at minus 70° C and analyzed in a central laboratory using standard clinical analytical procedures. Homeostatic model assessment (HOMA) index was calculated as previously described. [27]

Sample size calculation and statistical analysis

Our primary aim was to assess the effects of nebivolol/HCTZ and metoprolol/HCTZ on aortic wave augmentation by comparing differences in AIx as the most important determinant of differences in central and peripheral BP. AIx has the advantage that it is independent of BP, accounting for possible differences in BP lowering effect between the two treatment groups. Previous studies have shown that non-invasive calculation of AIx by applanation tonometry has good reproducibility with a standard deviation of the difference (SDD) of 4%.[28] In a randomized parallel study comparing nebivolol with atenolol, treatment with nebivolol resulted in a 7% lower AIx compared

to baseline, while atenolol tended to increase AIx slightly.[15] We expected to find a difference of at least 3% between metoprolol/ HCTZ and nebivolol/ HCTZ. Assuming a SDD of 4.5%, we calculated that 20 subjects would be needed to detect this difference with a power of 80% and alpha of 0.05. To account for withdrawal and measurement errors our aim was to enroll 24 subjects. Statistical analyses were performed using the Statistical Package for Social Sciences version 19.0.0.1 (SPSS Inc.,Chicago, Illinois, USA). Data are expressed as mean ± standard deviation (SD) for continuous variables and as n (%) for categorical variables. Differences in outcome measures between the two treatments were tested for statistical significance using a general linear model for repeated measurements with age, gender, BMI and the first allocated active treatment as covariates. Pairwise comparisons based on estimated marginal means, corrected according to least significant difference, were performed for Nebivolol/HCTZ and Metoprolol/HCTZ vs. baseline, and for Nebivolol/HCTZ vs. Metoprolol/HCTZ. A p-value<0.05 was considered statistically significant.

RESULTS

Patient Characteristics

Patient recruitment is shown in figure 2. Thirty-two patients were screened for participation. Twenty-four subjects fulfilled all in- and exclusion-criteria and were subsequently enrolled. One patient was excluded during the safety visit in the placebo run-in phase due to unacceptable BP elevation (BP ≥180 mmHg systolic and/ or ≥110 mmHg diastolic) and one patient withdrew consent thirteen days after randomization during the active treatment phase with metoprolol/HCTZ because of neurological complaints, unlikely related to the study medication, leaving twenty-two patients for analysis. Baseline patient characteristics are shown in table 1.

FIGURE 2 - Flow-chart Participants

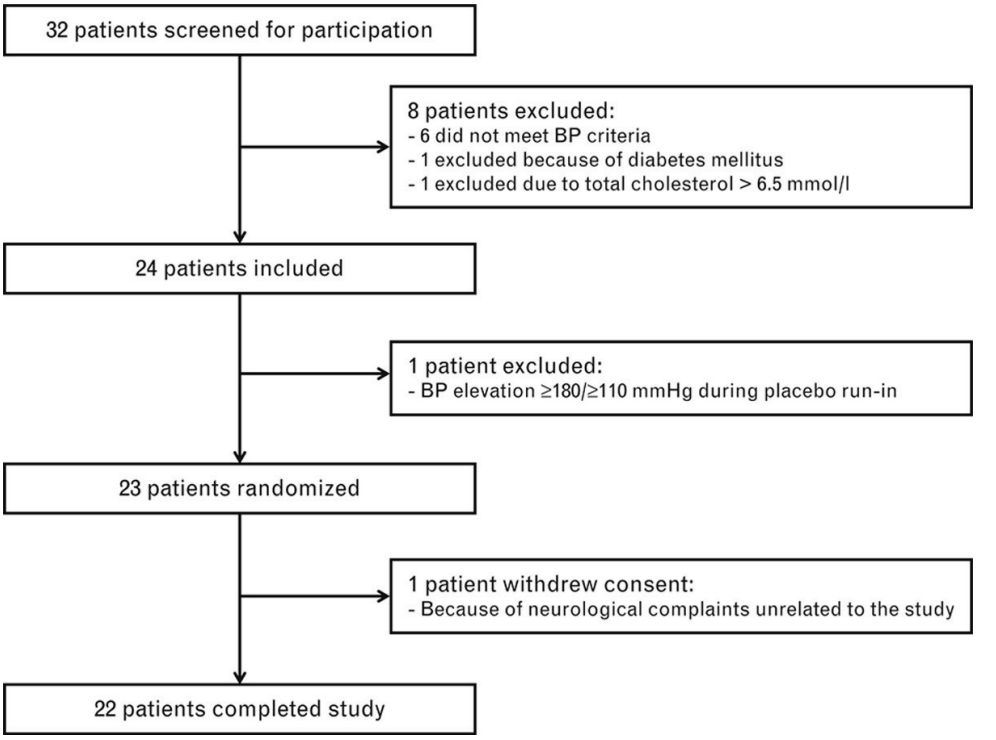


TABLE 1 - Baseline Characteristics

Parameter, units	
Completed study, n	22
Males, n (%)	17 (77%)
Age, years	59.9 ± 6.4
BMI, $kg \cdot m^{-2}$	27.5 ± 3.5
Waist circumference, cm	98.0 ± 10.8
Hip circumference, cm	108.5 ± 7.6
Office Brachial Systolic BP, mmHg	154.9 ± 16.3
Office Brachial Diastolic BP, mmHg	92.5 ± 9.8
Office heart rate, $beats \cdot min^{-1}$	66.9 ± 8.2
On prior antihypertensive therapy, n (%)	15 (68%)
Total cholesterol, mmol/L	5.4 ± 0.8
HDL cholesterol, mmol/L	1.5 ± 0.5
LDL cholesterol, mmol/L	3.4 ± 0.4
Triglycerides, mmol/L	1.2 ± 0.5
Glucose, mmol/L	4.7 ± 0.7

Data are expressed as mean ± SD for continuous variables and n (%) for categorical variables. BMI: body-mass-index. BP: blood pressure.

Pulse wave analyses

Results of the pulse wave analyses are shown in table 2. Unadjusted AIx increased with 1.0±7.8% (p=0.53) for nebivolol/HCTZ and 2.4±6.5% (p=0.07) for metoprolol/HCTZ compared to baseline, while AIx corrected for heart rate decreased with 2.8±6.9% (p=0.08) for nebivolol/HCTZ and 1.8±5.7% (p=0.14) for metoprolol/HCTZ. Central BP was lowered by 15.8±14.9 mmHg systolic and 10.5±8.4 mmHg diastolic for nebivolol/HCTZ compared to 13.5±12.3 mmHg systolic and 9.5±6.8 mmHg diastolic for metoprolol/HCTZ (all p<0.001). Peripheral BP was lowered by 16.3±14.9 mmHg systolic and 10.1±8.2 mmHg diastolic for nebivolol/HCTZ versus 15.2±13.0 mmHg systolic and 9.1±6.9 mmHg diastolic for metoprolol/HCTZ (all p<0.001). Heart rate was lowered with 8.1±5.4 beats·min^{-1} for nebivolol/HCTZ and 8.6±4.9 beats·min^{-1} for metoprolol/HCTZ (both p<0.001). Ejection duration increased 17.2±13.8 ms for nebivolol/HCTZ and 16.0±21.5 for metoprolol/HCTZ. Return time of the reflected wave was not affected by either treatment regimen. There were no significant differences between nebivolol/HCTZ and metoprolol/HCTZT in effects on aortic wave augmentation, central BP, peripheral BP, heart rate or ejection duration.

TABLE 2 - Pulse Wave Analysis Results

	Baseline	**Neb/HCTZ**	**Met/HCTZ**	**Neb/HCTZ vs. Baseline**	**Met/HCTZ vs. Baseline**	**Neb/HCTZ vs. Met/HCTZ**
Parameter, units				p-value	p-value	p-value
Brachial SBP, mmHg	153.6 ± 17.7	137.3 ± 10.4	138.4 ± 12.7	<0.001	<0.001	0.50
Brachial DBP, mmHg	91.9 ± 9.8	81.8 ± 8.0	82.8 ± 8.6	<0.001	<0.001	0.39
Heart rate, beats·min^{-1}	61.0 ± 7.2	52.9 ± 7.6	52.4 ± 7.3	<0.001	<0.001	0.64
Central SBP, mmHg	144.9 ± 17.5	129.0 ± 9.6	131.4 ± 13.4	<0.001	<0.001	0.22
Central DBP, mmHg	92.8 ± 10.0	82.3 ± 8.3	83.3 ± 8.6	<0.001	<0.001	0.39
Central PP, mmHg	52.1 ± 14.6	46.7 ± 9.1	48.1 ± 11.0	0.01	0.03	0.27
AIx, %	30.3 ± 6.8	31.4 ± 9.5	32.7 ± 9.6	0.53	0.07	0.43
AIx@Hr75, %	23.7 ± 5.8	20.9 ± 8.3	21.9 ± 8.5	0.08	0.14	0.51
Ejection duration, ms	334.1 ± 16.0	351.3 ± 15.4	350.1 ± 18.2	<0.001	0.004	0.77
Return time, ms	146.3 ± 12.2	147.0 ± 16.4	148.2 ± 15.7	0.77	0.51	0.72

Results shown are mean ± SD calculated with a general linear model for repeated measurements with correction for age, gender, BMI and first allocated active treatment. Measurements were performed in supine position after 15 minutes of rest.
SBP: systolic blood pressure. DBP: diastolic blood pressure. PP: pulse pressure (SBP-DBP). AIx: augmentation index. AIx@Hr75: heart rate corrected AIx (standardized to 75 beats beats·min^{-1}).

Volume clamp finger plethysmography

The effects of nebivolol/HCTZ and metoprolol/HCTZ on hemodynamics are shown in table 3. SV significantly increased with metoprolol/HCTZ 3.5±6.8 (p=0.03) and tended to increase with nebivolol/HCTZ (3.4±9.1 ml, p=0.11). Both treatments decreased HR and CO, while leaving SVR unaffected. Left ventricular contractility (dp/dt) decreased by 165±251 mmHg·s^{-1} (p=0.003) for nebivolol/HCTZ and 237±260 mmHg·s^{-1} (p<0.001) for metoprolol/HCTZ. Nebivolol/HCTZ and metoprolol/HCTZ increased baroreflex sensitivity by 2.6±3.4 ms·mmHg^{-1} (p=0.001) and 2.5±3.1 ms·mmHg^{-1} (p=0.002), respectively. There were no differences between nebivolol/HCTZ and metoprolol/HCTZ on hemodynamics and baroreflex sensitivity.

TABLE 3 - Continuous Noninvasive Finger Arterial Blood Pressure Measurement Results

	Baseline	**Neb/HCTZ**	**Met/HCTZ**	**Neb/HCTZ vs. Baseline**	**Met/HCTZ vs. Baseline**	**Neb/HCTZ vs. Met/HCTZ**
Parameter, units				p-value	p-value	p-value
Systolic BP, mmHg	153.9 ± 19.0	137.6 ± 13.5	137.9 ± 13.9	<0.001	<0.001	0.92
Diastolic BP, mmHg	83.5 ± 8.0	75.2 ± 6.6	75.5 ± 7.4	<0.001	<0.001	0.80
MAP, mmHg	111.0 ± 10.8	98.4 ± 8.7	99.6 ± 9.7	<0.001	<0.001	0.53
PP, mmHg	70.0 ± 15.0	62.0 ± 10.2	61.9 ± 10.1	0.009	0.001	0.97
Heart rate, beats·min^{-1}	62.5 ± 7.9	54.5 ± 6.9	53.4 ± 7.3	<0.001	<0.001	0.29
Stroke Volume, ml	85.4 ± 11.8	88.8 ± 12.2	88.9 ± 13.4	0.11	0.027	0.98
Cardiac Output, L·min^{-1}	5.3 ± 0.8	4.8 ± 0.8	4.7 ± 0.9	<0.001	<0.001	0.25
SVR, dyn s·cm^{-5}	1726 ± 330	1686 ± 281	1749 ± 358	0.46	0.68	0.18
dP/dt_{max}, mmHg·s^{-1}	1183 ± 290	1018 ± 280	946 ± 224	0.003	<0.001	0.14
xBRS, ms·mmHg^{-1}	7.1 ± 3.9	9.7 ± 4.1	9.6 ± 4.9	0.001	0.002	0.89

Results shown are mean (SD) calculated with a general linear model for repeated measurements with correction for age, gender, BMI and first allocated active treatment. Hemodynamic and baroreflex measurements were calculated from continuous finger arterial blood pressure measurements and performed in supine position after 15 minutes of rest.
BP: blood pressure. MAP: mean arterial pressure. PP: pulse pressure. SVR: systemic vascular resistance. DP/DT: index for left ventricular contractility. xBRS: cross correlation time-domain baroreflex sensitivity.

Metabolic Profile

The effects of metoprolol/HCTZ and nebivolol/HCTZ on metabolic parameters are shown in table 4. Both nebivolol/HCTZ and metoprolol/HCTZ significantly decreased HDL-cholesterol levels by 0.13±0.13 mmol/L and 0.16±0.16 mmol/L respectively (both p<0.001). Triglyceride levels increased by 0.29±0.34 mmol/L for nebivolol/HCTZ and by 0.34±0.42 mmol/L for metoprolol/HCTZ (both p=0.02). LDL-cholesterol levels did not significantly change with either treatment. HOMA-IR was not affected by nebivolol/HCTZ treatment, whereas metoprolol/HCTZ tended to increase HOMA-IR by 0.6±1.1 (p=0.07). There was no significant difference on metabolic parameters between the two treatment modalities.

TABLE 4 - Fasting Venous Blood Sampling Results

	Baseline	**Neb/HCTZ**	**Met/HCTZ**	**Neb/HCTZ vs. Baseline**	**Met/HCTZ vs. Baseline**	**Neb/HCTZ vs. Met/HCTZ**
				p-value	p-value	p-value
Creatinine, µmol/L	78.5 ± 10.0	81.6 ± 11.6	81.3 ± 11.6	0.094	0.026	0.86
Total cholesterol, mmol/L	5.4 ± 0.8	5.3 ± 0.9	5.4 ± 0.9	0.35	0.89	0.35
HDL cholesterol, mmol/L	1.48 ± 0.5	1.31 ± 0.4	1.35 ± 0.4	<0.001	<0.001	0.21
LDL cholesterol, mmol/L	3.40 ± 0.4	3.26 ± 0.8	3.34 ± 0.7	0.12	0.49	0.35
Triglycerides, mmol/L	1.13 ± 0.5	1.41 ± 0.9	1.46 ± 0.7	0.02	0.02	0.29
Glucose, mmol/L	4.7 ± 0.7	5.1 ± 1.1	5.1 ± 0.9	0.20	0.21	0.79
Insulin, pmol/L	59.6 ± 29.7	60.5 ± 27.1	70.3 ± 37.6	0.54	0.082	0.35
HOMA-IR	1.9 ± 1.2	1.9 ± 0.9	2.5 ± 1.8	0.72	0.070	0.26
HOMA-β	133.8 ± 50.8	155.2 ± 121.4	122.7 ± 65.8	0.47	0.54	0.29

Results shown are mean (SD) calculated with a general linear model for repeated measurements. Samples are obtained after a 12-hour overnight fasting period.
HOMA-IR: homeostatic model assessment of insulin resistance.
HOMA-β: homeostatic model assessment of beta-cell function

DISCUSSION

The principal finding of this study is that treatment with the vasodilating beta-blocker nebivolol was not superior to metoprolol in reducing aortic wave augmentation and central BP when combined with hydrochlorothiazide. Both treatments had comparable effects on hemodynamics with a similar reduction in heart rate and left ventricular contractility, while leaving SVR unaffected. Nebivolol/HCTZ and metoprolol/HCTZ both lowered HDL-cholesterol and increased triglyceride levels, but only metoprolol/HCTZ tended to increase HOMA-IR.

BP lowering drugs that induce relaxation of vascular smooth muscle cells have been shown to lower central BP more than peripheral BP.[4, 6] This has been attributed to a decrease in aortic wave augmentation through attenuation of pulse wave reflection by peripheral vasodilatation.[12-14] Because nebivolol has been demonstrated to exert vasodilating properties it may offer potential benefit over non-vasodilating beta-blockers such as atenolol and metoprolol.[11, 15, 16, 29] Clinical evidence for the vasodilating properties of nebivolol in humans results primarily from forearm blood flow studies.[11, 30-33] Intra-arterial nebivolol infusion increases forearm blood flow in a dose dependent matter in both healthy subjects[30] and patients with essential hypertension.[31] In addition, nebivolol has been shown to improve flow-mediated-dilatation, an indicator for shear-mediated NO release, compared to atenolol.[11, 32]

Our data are in disagreement with previous reports that showed superiority of nebivolol compared to metoprolol and atenolol, with or without HCTZ, in reducing wave reflection[15] and central BP.[16, 29] The EXPLOR study showed that despite the addition of the potent vasodilator amlodipine to an atenolol based regimen aortic wave augmentation increased compared to baseline, whereas central BP remained higher compared to combination therapy with valsartan/amlodipine.[34] In light of the present study, there are a number of possible explanations for the discrepancy as opposed to previous reports on the beneficial actions of nebivolol. First, an inverse linear relation exists between heart rate and central wave augmentation,[35, 36] possibly through an effect on ejection duration.[37] Two previous studies comparing wave reflection between nebivolol and atenolol showed a differential effect on HR. In a randomized, double-blind crossover study Dhakam et al. found a larger reduction in central PP and a less pronounced rise in AIx for nebivolol compared to atenolol.[29] However, HR was significantly higher during treatment with nebivolol (61±2 beats·min^{-1}) than with atenolol (57±1 beats·min^{-1}). In a different, parallel group, study nebivolol reduced central PP and AIx more than atenolol. However, HR was also differentially reduced: -8 beats·min^{-1} for nebivolol and -14 beats·min^{-1} for atenolol.

[15] When comparing beta-blockers, it is paramount to achieve equipotency in HR-reduction in order to make sound conclusions regarding aortic wave augmentation and central BP. Differences in HR are likely to have attributed to the observed differential effects of nebivolol versus atenolol on AIx and central PP. In our study the reduction in HR and increase in ejection duration were identical for both treatment modalities and therefore did not differentially affect aortic wave augmentation or central BP.

Second, adding a diuretic could have exerted differential effects on aortic wave augmentation and central BP when combined with nebivolol and metoprolol. Although diuretics as monotherapy have a neutral effect on peripheral and central BP reduction,[4] the effect of combined treatment with a vasodilating beta-blocker and diuretic is not well established. Nevertheless, when combined with bendrofluazide nebivolol improved flow-mediated-dilatation compared to atenolol/bendrofluazide. [11] The vasodilating properties of nebivolol could, in theory, enhance the reactive rise in sympathetic activity by HCTZ treatment, thereby mitigating differences in AIx. However, our hemodynamic data show a similar increase in baroreflex sensitivity for nebivolol and metoprolol, indicative for a non differential effect on autonomic balance.

Recently, Kampus and colleagues performed a randomized, double-blind parallel trial comparing nebivolol (5mg) and metoprolol (50 or 100 mg) in combination with a non-fixed dose of HCTZ (12.5 to 25 mg) if target BP (<140/90 mmHg) was not achieved. [16] Consistent with our findings they found no differential effect between drug arms on aortic wave augmentation after one year of treatment. Yet central BP and brachial PP decreased only in the nebivolol, but not in the metoprolol treated group when compared to baseline. Patients randomized to nebivolol more often received add-on treatment with 12.5 to 25 mg of hydrochlorothiazide compared to patients receiving metoprolol. This may have caused differences in peripheral and central BP between both groups. When correcting central BP for the observed difference in peripheral BP, the additional central BP lowering effect of nebivolol would be 2.5 mmHg in systolic BP, which is consistent with the difference of 2.4 mmHg observed in our study.

Third, differences in results of our study compared to previous studies may have emerged from differences in study populations. It is possible that patients with more extensive inward vascular remodeling are less susceptible to the vasodilating properties of nebivolol and thus the effects on aortic wave augmentation and central BP may be less profound. The population studied by Kampus et al. was relatively healthy and younger than our study population. However, in both young healthy volunteers[30] and middle-aged patients with essential hypertension[31] nebivolol infusion induces

similar increases in forearm blood flow, suggesting that the vasodilatory actions of nebivolol do not depend on age or hypertension status.

Finally, apart from the effect of vasodilation on aortic wave augmentation, it is conceivable that small differences in SV, HR and left ventricular contractility may have affected differences in AIx in our and previous studies. For example, it has been shown that upon standing or passive head-up-tilt central aortic augmentation decreases despite an increase in SVR.[38, 39] Alternatively, the well-established changes in forearm blood flow and flow mediated dilatation induced by nebivolol may not necessarily translate in reduced peripheral resistance as indicated by the absence in effect on SVR as found in our study. The physiological mechanisms by which vasomodulation affects aortic wave augmentation and central BP are still subject to debate and require further elucidation.

Strenghts and Limitations

The main strengths of our study are the randomized double-blind crossover design and the achieved equipotent effects on HR for both treatment modalities. By applying a cross-over design we aimed to detect a 3% difference in AIx between treatments. The observed SDD in aortic wave augmentation between nebivolol/HCTZ metoprolol/HCTZ in our study was larger than expected (±7.8% vs. ±4.5%). Given the observed SDD our study was sufficiently powered to detect a difference in mean AIx of ±4.8% between treatment arms. This is considerably smaller than previously reported.[15] Our study was not powered to detect differences in central BP generating the possibility for a type II error for differences between peripheral and central BP between the two treatment arms. However, if true differences exist in the potency to lower central BP between nebivolol and metoprolol these differences are likely to be much smaller than previously reported. Still a small difference in central BP could be relevant from a clinical perspective and should therefore be investigated in sufficiently powered and well designed trial.

Perspectives

In contrast to previous reports we did not find a differential effect for the third-generation beta-blocker nebivolol versus the second-generation beta-blocker metoprolol on aortic wave augmentation when used in addition to HCTZ. Third generation beta-blockers, such as nebivolol, have been proposed to more effectively reduce pulse wave reflection due their vasodilating capabilities. However, our study does not support the supposed superiority of nebivolol compared to non-vasodilating beta-blocker metoprolol. Nonetheless, previous studies suggest that nebivolol may

improve endothelial function and elicit antiproliferative effects. Whether nebivolol has superior efficacy on outcome compared to other beta-blockers, and how this compares to other BP lowering drugs remains to be determined in large prospective trials.

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Part III

RENAL SYMPATHETIC DENERVATION

Chapter 08

Renal sympathetic nerve activity after catheter-based renal denervation

Daan W. Eeftinck Schattenkerk¤
Linn C. Dobrowolski¤
C.T. Paul Krediet
Peter M. van Brussel
Liffert Vogt
Frederike J. Bemelman
Jim A. Reekers
Bert-Jan H. van den Born
Hein J. Verberne

¤ *both authors contributed equally*

Submitted

ABSTRACT

Objectives: We hypothesized that variation in the reported RDN efficacy might be explained by incomplete nerve disruption as assessed by renal ^{123}I-*meta*-iodobenzylguanidine (^{123}I-*m*IBG) scintigraphy.

Background: Catheter-based renal sympathetic denervation (RDN) has been considered a potential treatment for therapy resistant hypertension (RHT). However, in a randomized placebo-controlled trial, RDN did not lead to a substantial blood pressure (BP) reduction.

Methods: In 21 RHT patients (median age 60 years) we performed ^{123}I-*m*IBG scintigraphy before and 6 weeks after RDN. Additionally, we assessed changes in BP (24 h day, night and average), plasma- and urinary-catecholamines and plasma renin activity (PRA) before and after RDN. Planar scintigraphy was performed at 15 min and 4 h after ^{123}I-*m*IBG administration. The ratio of the mean renal (specific) counts vs. muscle (non-specific) counts represented ^{123}I-*m*IBG uptake. Renal ^{123}I-*m*IBG washout was calculated between 15 min and 4 h.

Results: After RDN office-based systolic BP decreased from 172 to 153 mmHg (p=0.036), while diastolic office BP (p=0.531), mean 24 h systolic and diastolic BP (p=0.602, p=0.369, respectively), PRA (p=0.409) and plasma catecholamines (p=0.324) did not significantly change post-RDN. Following RDN, ^{123}I-*m*IBG renal uptake at 15 min was 3.47 (IQR 2.26-5.53) compared to 3.08 (IQR 2.79-4.95) before RDN (p=0.289). Renal ^{123}I-*m*IBG washout did not change post-RDN (p=0.230). In addition there was no significant correlation between the number of denervations and the renal ^{123}I-*m*IBG parameters.

Conclusions: No changes were observed in renal ^{123}I-*m*IBG uptake or washout at 6 weeks post-RDN. These observations support incomplete renal denervation as a possible explanation for the lack of RDN efficacy.

INTRODUCTION

Reduction of sympathetic nerve activity by catheter based renal sympathetic denervation (RDN) has raised considerable attention as a new treatment modality for resistant hypertension (RHT). This interest was fuelled by the promising results of RDN in the initial open label studies Symplicity HTN-1 and HTN-2.(1-3) However, the recent randomized sham-controlled Symplicity HTN-3 trial did not show a difference in blood pressure (BP) lowering efficacy between RDN and sham treatment.(4) One of the potential causes for the lack of efficacy might be the failure of the RDN procedure to sufficiently ablate renal sympathetic nerves. Yet, a routine technique to measure the extent of renal denervation is lacking and potential causes of insufficient denervation remain hypothetical.

^{123}I-*meta*-iodobenzylguanidine (^{123}I-*m*IBG) scintigraphy offers the possibility to evaluate organ specific sympathetic nerve activity. *m*IBG is an analogue of the 'false' neurotransmitter guanetidine, a potent neuron blocking agent that acts selectively on sympathetic nerves. *m*IBG follows similar uptake mechanisms as norepinephrine: as such *m*IBG-uptake enables assessment of the intactness and density of the neural tissue. Radiolabelling of *m*IBG with ^{123}Iodide enables scintigraphic assessment. ^{123}I-*m*IBG organ uptake and washout reflect sympathetic activity.(5,6) Previously, we validated this technique for visualizing renal sympathetic innervation by showing its ability to detect changes in sympathetic innervation during kidney allograft reinnervation.(7)

Based on the inter-individual variation in BP response after RDN, we hypothesized that there is a wide variability in kidney sympathetic denervation following RDN. Secondly, we hypothesized that changes in renal sympathetic activity would relate to changes in BP and neurohormonal activity following RDN. Against this background, we examined changes in renal ^{123}I-*m*IBG uptake and washout in RHT patients before and after RDN treatment.

METHODS

From July 2011 to December 2013, we performed a prospective observational study using ^{123}I-*m*IBG scintigraphy as a parameter of renal sympathetic activity in patients with RHT undergoing RDN.

Objectives were to compare measures of renal ^{123}I-*m*IBG uptake (uptake at 15 min and washout between 15 min and 4 h) on planar and single photon emission computed tomography-CT (SPECT-CT) images, changes in office based BP and ambulatory BP measurements (ABPM) and neurohormonal activation before and 6 weeks after RDN.

Patients

In the present study, we enrolled 21 consecutive patients aged 40–70 years with a clinical indication for RDN because of therapy resistant hypertension defined as a mean daytime BP ≥150/ 100 mmHg despite the use of 3 or more anti-hypertensive drugs including or with intolerance to a diuretic.(8) Secondary causes of hypertension (e.g., renal artery stenosis, pheochromocytoma, primary aldosteronism and hyper- or hypothyroidism) and abnormal renal artery anatomy, including the presence of accessory renal arteries, were ruled out prior to the intervention. Patients with renal insufficiency (estimated glomerular filtration rate (eGFR) >45 mL/min/1.73 m^2) or proteinuria (<1 g/24 h) or having a pacemaker, implantable cardioverter-defibrillator (ICD), atrial fibrillation or type 1 diabetes mellitus were excluded. Antihypertensive treatment was performed according to international guidelines and included instructions on dietary sodium restriction, physical activity and instructions to remain compliant to antihypertensive medication.(8,9) Six weeks prior to the first measurements patients were screened to assess eligibility for study participation. Patients were deemed eligible for study participation if they were at least 3 weeks on stable BP lowering medication prior to the first study visit. BP lowering medication was kept unchanged throughout the study until the final visit 6 weeks after RDN.

When fully informed and willing to participate, patients were asked to provide written informed consent. Six weeks hereafter, office BP and ABPM was measured. Patients were required to maintain the same antihypertensive drug regimen throughout study participation. All patients provided informed consent before inclusion in the study. This study was a part of a larger effort to assess the sympaticolytic potential of RDN with the predetermined idea to assess the effects of RDN on renal ^{123}I-*m*IBG uptake and washout.

For reference, we used data of 5 patients (aged 39-66 years) in whom ^{123}I-*m*IBG was performed of the kidney allograft after recent kidney transplantation (0.1 to 1.5 years after transplantation), whose detailed characteristics are described elsewhere.(7) In summary, all these surgically denervated kidneys functioned well with creatinine clearance rates (calculated from 24 h urine collections) ranging from 54-128 ml/min. As a negative control we also included ^{123}I-*m*IBG data from a patient with complete renal denervation after autologous kidney transplantation for renal artery stenosis.(10)

Although ^{123}I-*m*IBG is primarily cleared via the kidneys, we have shown that both the cardiac as well as the renal ^{123}I-*m*IBG parameter are not influenced by kidney function.(7,11)

Study Protocol

The study protocol met the ethical guidelines of the Declaration of Helsinki (originally adopted by the 18th WMA General Assembly, Helsinki, Finland, June 1964 and last amended in Fortaleza, Brazil 2013) and was approved by the local ethics committee of the Academic Medical Center at the University of Amsterdam (number NL.36755.018.11). All patients gave oral and written informed consent.

Renal sympathetic denervation procedure

The renal denervation procedure was performed via the femoral artery approach by a single highly experienced interventional radiologist (JAR) with > 5 RDN procedures before this study was initiated. RDN was performed by use of radiofrequency energy delivered by the Symplicity renal-denervation catheter (Medtronic Inc., Santa Rosa, California, USA). Prior to the procedure, midazolam 1.0 mg and metoclopramide 10 mg was given intravenously. After inserting a 6 F introducer in the right femoral artery, the guiding catheter was introduced in the aorta and an aortagram was made. The guiding catheter was advanced in the right and left renal artery in no pre-specified order. The denervation catheter was introduced in the renal artery via the delivery catheter. After nitroglycerine 0.2 mg and fentanyl 0.02 mg intravenously, catheter ablations were performed in a helical pattern with the goal of at least 4-6 ablations per renal artery to cover each short axis transaxial quadrant, according to the user's instruction of the device. No peri-procedural complications occurred.

Blood pressure monitoring

At baseline and 6 weeks after RDN 24 h ABPM was performed using the Spacelabs 90217 ABPM monitoring device (Spacelabs Healthcare, Issaquah, Washington, USA). During day time between 06.00 am- 23.00 pm measurements were performed every 15 min and at night-time (i.e. 23.00 pm - 6.00 am) every 30 min. BP readings were accepted when the success rate of the measurements was minimally 70% per 24 h. Patients were blinded to their BP readings. Instructions were given to continue usual daily activities during 24 h of BP recording, but avoiding strenuous exercise. Office brachial BP using appropriate cuff-sizes was measured with a validated semi-automated oscillometric device (Omron 705it, Omron Healthcare Europe BV, Hoofddorp, The Netherlands), while seated and after 5 min rest in a quiet room, 3 times at 1 min intervals by a trained research assistant or physician. The mean of the last 2 measurements was recorded as representative of office brachial BP. No BP measurements were performed in the kidney transplant recipient group.

Blood and urine analysis

Plasma renin activity (PRA) (µgA1/L/h) was analysed using radioimmunoassays. Urine and plasma epinephrine, norepinephrine (NE), metanephrine and normetanephrine were analysed using liquid chromatography-mass spectrometry. Epinephrine and NE and were obtained in supine as well as after 5 min in standing position. The delta of supine minus standing position was calculated. Urinary sodium excretion (mmol/24 h), urine creatinine (µmol/L), was calculated from 24 h urine collections obtained before and 6 weeks post-RDN.

^{123}I-mIBG scintigraphy

The protocol of the renal ^{123}I-*m*IBG scintigraphy has been previously described. (7) In summary, 2 h prior to the administration of 185 MBq (5 mCi; ± 10%) ^{123}I-*m*IBG (AdreView™, GE Healthcare, Eindhoven, the Netherlands) patients received 100 mg potassium-iodide to block thyroid uptake of 'free' ^{123}I. In addition subjects were given a single oral dose of furosemide retard 60 mg to promote the urinary excretion of ^{123}I-*m*IBG. No specific instructions on fluid intake were given to enhance excretion of ^{123}I-*m*IBG. Anterior and posterior planar semi-whole body images were performed at 15 min and 4 h after administration of ^{123}I-*m*IBG. A vial with a reference amount of radioactivity of ^{123}I was included in the planar images. Additionally, at 4 h post-injection (p.i.), SPECT-CT (low dose) was performed. The CT-images were used for an adequate anatomical registration of ^{123}I-*m*IBG uptake.

Since we recently showed that uptake at 15 min p.i. of ^{123}I-*m*IBG and washout between 15 min and 4 h can detect renal sympathetic reinnervation over time after transplantation, we report in this study the ^{123}I-*m*IBG uptake on the 15 min p.i. images and analysed the mean counts/pixel for calculation of washout between 15 min and 4 h.(7)

^{123}I-mIBG imaging procedures

The planar images were acquired with a 20% energy window centred at 159 keV, using medium-energy collimators. Planar anterior and posterior planar semi-whole body acquisitions were used to create geometrical mean images.

^{123}I-mIBG image analysis

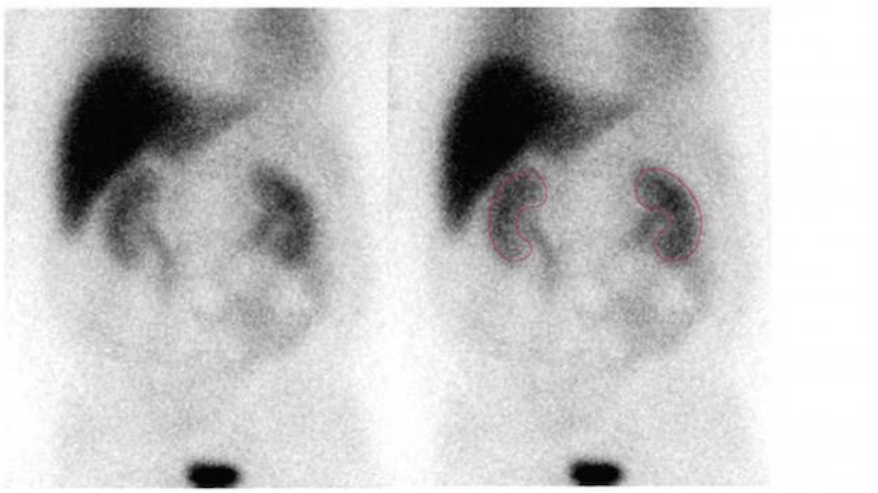
An investigator (LCD) analysed the geometric mean (GM) planar images (Hybrid Viewer™, Hermes Medical Solutions, Stockholm, Sweden) by manually drawing regions of interest (ROI) for kidneys, muscle (m. quadriceps femoris) and the ^{123}I vial. A predefined and fixed ROI for the muscle (50 pixels) was used for all patients.

We analysed the counts of the left kidney only since scatter or overlay of the liver with a high uptake of ^{123}I-*m*IBG resulted in poor delineation of the right kidney. Mean counts per pixel per ROI (figure 1) were used to calculate of ^{123}I-*m*IBG uptake: specific (kidney) to non-specific uptake (muscle). Formulas to calculate uptake and washout were:

$$\text{Relative uptake} = \frac{\text{kidney (specific)} - \text{muscle (non-specific)}}{\text{muscle (non-specific)}}$$

$$\text{Washout} = \frac{\left(\frac{\text{uptake kidney 15 min}}{\text{uptake muscle 15min}}\right) - \left(\frac{\text{uptake kidney 4 h}}{\text{uptake muscle 4 h}}\right)}{\left(\frac{\text{uptake kidney 15 min}}{\text{uptake muscle 15min}}\right)} \text{ x } 100\%$$

FIGURE 1 - Planar ^{123}I-*m*IBG scintigraphy



Planar ^{123}I-mIBG scintigraphy at 15 min post-injection before renal denervation. The right panel shows the regions of interest around the contours of the kidney.

The percentage uptake of the injected dosage of ^{123}I-*m*IBG was calculated using the actual injected dose and mean counts per pixel in relation to the activity in ^{123}I-vial.

Washout (WO) in the left kidney was calculated from 15 min and 4 h images using skeletal muscle as reference.

A secondary analysis was focused on the SPEC-CT images. In this method the transverse CT images were used to optimize anatomical delineation of the kidney contours. The main advantage of this method is the availability of anatomical information obtained from the low dose CT, allowing for a superior delineation of kidneys and a subsequently a potential better estimation of the renal ^{123}I-*m*IBG uptake. ROIs were drawn on the CT-images along the contours of kidney cortices, excluding the calyces. ROIs were then fused into volumes of interest (VOIs) and copied to the co-registered SPECT. Mean counts/voxel expressed ^{123}I-*m*IBG uptake. VOIs in muscle served as background activity.

Based on the difference in ^{123}I-*m*IBG uptake, we divided patients with a positive change in ^{123}I-*m*IBG uptake, i.e. indicating an increase in ^{123}I-*m*IBG uptake or washout and those with a negative change, i.e. a decrease in ^{123}I-*m*IBG uptake or washout after RDN.

Statistical analysis

This study was part of a larger effort to study sympahticolytic effects of RDN. The sample size has been described elsewhere.(12)

Data are presented as medians and interquartile ranges (IQR with 25 and 75 percentiles) and comparisons were performed by non-parametrical tests. P values below 0.05 were considered statistically significant. All analyses were performed using IBM SPSS Statistics software for Windows version 21.0 (IBM Corp. Armonk, New York, USA).

RESULTS

Baseline characteristics

We studied 21 patients with therapy resistant hypertension (Table 1). The majority of patients were male (71% with a median 60 years) and were Caucasian (76%). Median body mass index was 28.0 kg/m^2 (24.8-30.5 kg/m^2). Diabetes mellitus was present in 33% and left ventricular hypertrophy, according to electrocardiography voltage criteria, was present in 29% of the patients. A history of a cardiovascular disease (i.e. coronary artery disease, angina pectoris, heart failure, stroke, peripheral arterial disease) was present in 48% of the study participants.

Renal ^{123}I-mIBG uptake and washout in the left kidney

The planar derived mean relative uptake of ^{123}I-*m*IBG of the left kidney at 15 min p.i. did not change significantly from pre RDN 3.08 (2.79-4.95) to post RDN 3.47 (2.26-5.53), p=0.289 (Table 2). Figure 2 represents pre vs. post RDN ^{123}I-*m*IBG uptake at 15 min p.i. including recently transplanted kidneys as controls. The percentage uptake of the injected dosage of ^{123}I-*m*IBG in the left kidneys showed a non-significant decrease after

RDN from 17.8% to 15.4% (delta -13%, p=0.881). Washout rate between 15 min and 4 h p.i. was 41.5% before and 42.7% after RDN, p=0.230. The SPECT derived uptake at 4 h decreased non-significantly after RDN (1.41 to 1.07, p=0.526). None of the renal uptake or washout parameters were correlated with kidney function (data not shown).

TABLE 1 - Characteristics patients treated with RDN (n=21)

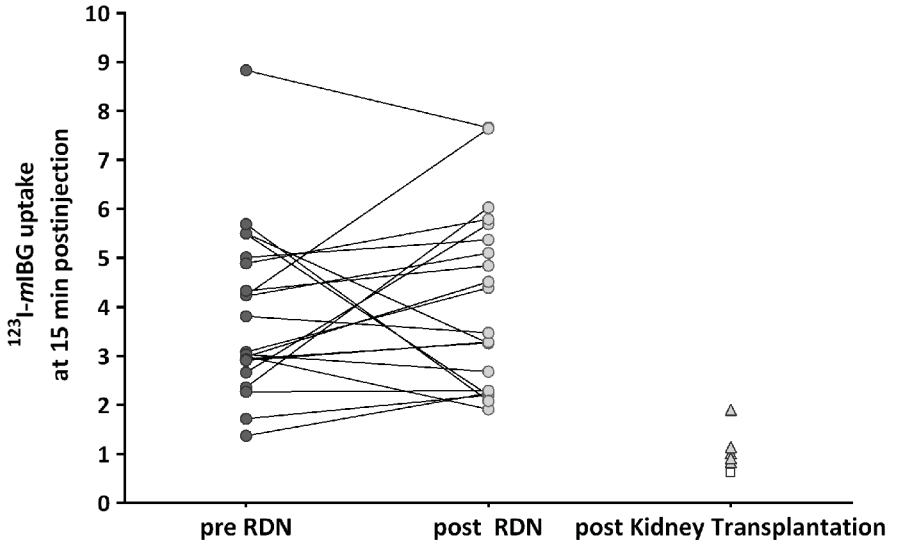
Male, n (%)	15 (71.4)
Age at intervention (yrs)	60 [53-70]
Caucasian ethnicity, n (%)	16 (76.2)
Weight (kg)	88.0 [69.5-99.5]
Body mass index (kg/m2)	28.0 [24.8-30.5]
Diabetes Mellitus, n (%)	7 (33.3)
Left ventricular hypertrophy, n (%)	6 (28.6)
History of any cardiovascular event, n (%)	10 (47.7)
Proteinuria (g/L/24 h)	0.10 [0.07-0.20]
Macroalbuminuria, n (%)	2 (9.5)
N^0 of denervation pulses left renal artery	4.3 ± 0.6
N^0 of denervation pulses right renal artery	4.2 ± 0.5

TABLE 2 - Pre and post RDN differences in quantifications of 123I-mIBG uptake (n=21)

	PRE-RDN	**POST-RDN**	**p-value**
Planar GM Images			
Uptake 15 min	3.08 [2.79-4.95]	3.47 [2.26-5.53]	0.289
Uptake 4 h	1.64 [1.44-1.98]	1.52 [1.12-2.27]	0.876
% Injected dose 15 min*	17.88 [17.88-21.75]	15.43 [13.73-22.13]	0.881
% Injected dose 4 h*	8.91 [8.91-13.52]	9.37 [7.20-12.35]	0.681
Washout 15 min-4 h (%)	41.53 [28.26-56.25]	42.69 [35.02-56.16]	0.230
SPECT-CT Images			
Uptake CT 4 h	1.41 [0.95-1.86]	1.07 [0.73-1.69]	0.526

Data are presented as medians with interquartile ranges (IQR 25-75%). RDN=Renal denervation, GM=geometric mean images, with muscle as background, SPECT= single photon emission computed tomography. n.a.=not available, *data from n=20 patients since in one patient a 123I-vial was not included during the scintigraphy and therefore the percentage of injected dose 123I-mIBG could not be calculated.

FIGURE 2 - Change in renal uptake of ^{123}I-*m*IBG after RDN



Uptake of ^{123}I-mIBG on 15 min post injection images of 21 patients before and after RDN. Five kidney transplant recipients with recent transplantation (diamonds) and one patient with an autograft (square) served as controls (7,12).

Number of denervations and renal ^{123}I-mIBG uptake and washout

No significant correlation was found between the number of denervations (left renal artery (4.3 ± 0.6), right renal artery 4.2 ± 0.5) and renal uptake of ^{123}I-*m*IBG in the left kidney at either 15 min (R= -0.27, p=0.243), 4 hrs p.i. (R= -0.37, p=0.103) or ^{123}I-*m*IBG washout (R=0.05, p=0.837).

Effect of RDN on blood pressure, PRA and catecholamines

Table 3 shows the effect of RDN on blood pressure and catecholamines. RDN resulted in a significant decrease in systolic office BP (p=0.036), without reducing diastolic BP (p=0.531). Systolic and diastolic daytime ABPM were not significantly different after denervation. Neither antihypertensive medication nor sodium intake, as inferred from urinary sodium excretion, were significantly different between pre vs. post-RDN (Table 2).

At baseline, plasma and urine catecholamine levels were within reference values. Plasma epinephrine and NE did not change (p=0.780 and p=0.324 respectively) nor did the 24 h urinary excretion of metanephrine (p=0.51) and normetanephrine (p=0.91) following RDN (Table 2).

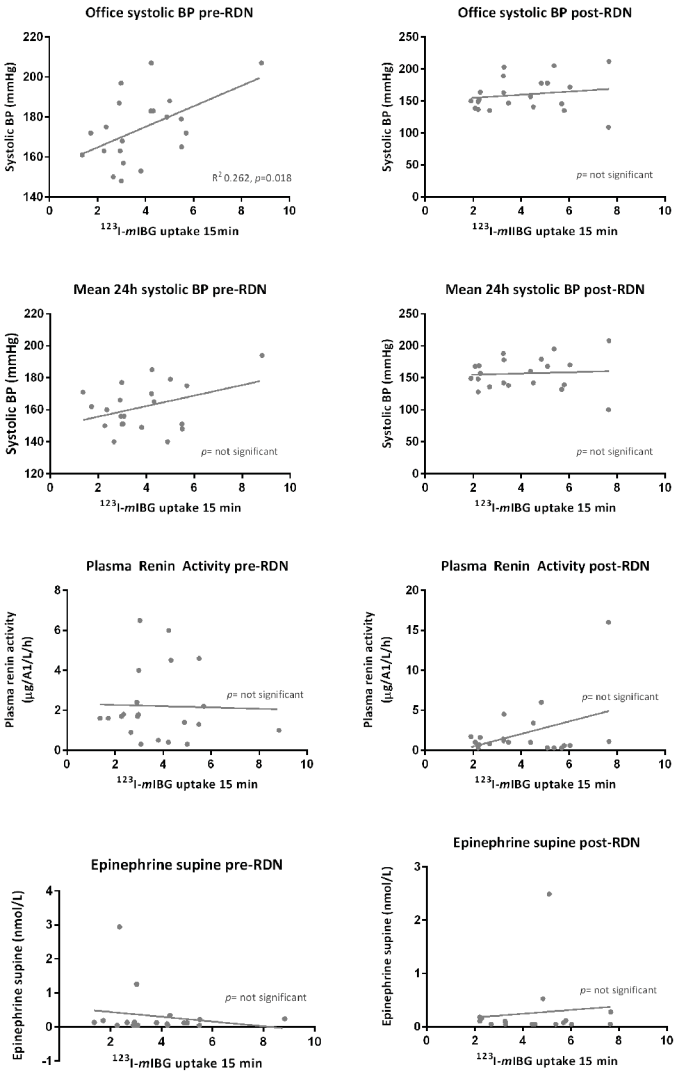
TABLE 3 - Blood pressure, kidney function and catecholamines

	PRE-RDN	POST-RDN	p-value
Parameters			
Blood pressure			
Office based Systolic (mmHg)	172.0 [162.0-185.0]	153.0 [140.0-178.0]	0.036
Office based Diastolic (mmHg)	97 [90.5-112.5]	90.0 [81.5-100.5]	0.531
ABPM Daytime Systolic (mmHg)	166.0 [157.0-179.5]	165.0 [141.5-186.0]	0.578
ABPM Daytime Diastolic (mmHg)	98.0 [87.0-108.0]	93.0 [83.0-99.5]	0.409
ABPM Night time Systolic (mmHg)	151.0 [133.5-158.5]	145.0 [125.0-165.5]	0.490
ABPM Night time Diastolic (mmHg)	84.0 [75.5-90.0]	80.0 [71.0-91.5]	0.640
ABPM Average Systolic (mmHg)	160.0 [150.5-173.0]	157.0 [138.5-174.0]	0.602
ABPM Average Diastolic (mmHg)	93.0 [83.5-100.5]	92.0 [80.0-94.5]	0.369
Antihypertensive drugs			
Number of antihypertensive drugs	4.6 ± 1.3	4.4 ± 1.4	0.157
3 classes, n (%)	5 (23.8)	7 (33.3)	
4 or more classes, n (%)	16 (76.2)	14 (66.7)	
Kidney function			
Creatinine serum (µmol/L)	94.0 [76.5-107.5]	89.0[73.5-113.5]	0.369
eGFR (ml/min/1.73m2)	60.7[48.5-101.9]	64.6 [48.0-99.9]	0.218
Proteinuria (g/L/24 h)	0.10 [0.07-0.20]	0.11 [0.07-0.26]	0.722
Sodium urine (mmol/24 h)	161 [102-203]	128 [90-161]	0.230
(Neuro) endocrine activity			
Plasma renin activity (µg/A1/L/h)	1.70 [0.95-3.20]	1.0 [0.60-1.68]	0.409
Epinephrine supine, plasma (nmol/L)	0.12 [0.05-0.23]	0.10 [0.05-0.17]	0.780
Norepinephrine supine, plasma (nmol/L)	2.43 [1.32-3.78]	2.76 [1.49-4.02]	0.324
Epinephrine urine (nmol/24 h)	27.5 [14.5-33.8]	26.0 [18.0-38.0]	0.551
Norepinephrine urine (nmol/24 h)	268.5 [137.5-495.0]	308.5 [237.5-479.3]	0.245
Metanephrine urine (nmol/24 h)	0.78 [0.49-1.05]	0.68 [0.50-1.02]	0.506
Normetanephrine urine (nmol/24 h)	2.13 [1.73-3.37]	2.53 [1.74-3.02]	0.911

Renal ^{123}I-mIBG uptake and washout and blood pressure, PRA and catecholamines

Except for the correlation between renal ^{123}I-*m*IBG uptake and office systolic BP (p=0.018), no correlations were found between any of the renal ^{123}I-*m*IBG uptake and washout parameters and blood pressure, PRA or catecholamines (figure 3). Subgroup analyses revealed no changes in patients with a BP decrease and their ^{123}I-*m*IBG parameters (supplemental data).

FIGURE 3 - Renal ^{123}I-*m*IBG uptake with blood pressure and biochemistry



DISCUSSION

In the present study we were unable to demonstrate that treatment with RDN results in significant changes in renal ^{123}I-*m*IBG uptake and washout. These data suggest that RDN does not significantly alter renal sympathetic tone and does not sufficiently denervate renal sympathetic nerves. This was further supported by the finding that ABPM and biochemical markers of sympathetic nerve activity remained unchanged after RDN, while the reduction in office BP was similar compared to Symplicity HTN-1 and HTN-2.(2,3)

The absence of consistent changes in ^{123}I-*m*IBG uptake and washout as well as the lack of a sustained BP decrease after RDN suggests that the present RDN technique fails to achieve adequate denervation of the kidneys. The degree of renal sympathetic nerve disruption required for inducing a sustained BP response remains unclear, but likely falls short with the current RDN technique. The lack of efficacy may be related to the number of ablations, since in a subset of patients of Symplicity HTN-3 a more profound BP decrease was observed in patients with more ablations, suggesting a relation between the quantity of ablations and the BP lowering effects.(4) This effect, however, was also observed in patients receiving sham treatment. We found no association between the number of ablations and renal MIBG uptake or washout, while the number of denervations in our study was similar to the Symplicity HTN-1 and HTN-2 trials that demonstrated a significant decrease in office BP. (2,3)

In a recent post-mortem study of a patient who received RDN it was shown that nerves in the (peri-)adventitial parts of the renal artery were unaffected, indicating that interruption of the nerve fibre continuity had not been successful.(13) This suggests that the ablation pulse may not be sufficient to generate adequate denervation of renal sympathetic nerves.(14) A previous study using NE spill-over to assess the effect of RDN on renal sympathetic activity in 10 patients with resistant hypertension showed that RDN reduced NE spill-over by 47% (95% CI 28-65%).(15) In the present study we could not replicate these findings. Besides lack of procedural effectiveness, this discrepancy could also be explained by differences in population characteristics or technical shortcomings of ^{123}I-*m*IBG scintigraphy. The patients in our study were however fully comparable to the populations studied in Symplicity HTN-1 and Symplicity HTN-2. Although, we used ABPM instead of office BP to include patients with resistant hypertension, baseline office BP in our study and the number of BP lowering drugs were comparable to that observed in Symplicity HTN-1 and Symplicity HTN-2. In addition, office BP was reduced to a similar extent with a decrease of 29 mmHg for systolic office BP following RDN and all other baseline parameters of our

study population were similar to that of previous studies.(2,3,4) In kidney transplant recipients we recently showed that uptake at 15 min p.i. of ^{123}I-*m*IBG and washout is correlated with time after transplantation independent of kidney graft function. (7) This suggests that renal ^{123}I-*m*IBG scintigraphy can be used to assess differences in renal innervation. To assess whether our technique is also sufficient to assess changes in sympathetic innervation following RDN, we calculated the difference in renal ^{123}I-*m*IBG-uptake after 15 min that could be detected using the observed SD of our data. We calculated that we were able to demonstrate a difference between -1.21 to +1.21 in renal ^{123}I-*m*IBG-uptake with 95% confidence at an alpha level of 0.05 and with 80% power. Using the baseline difference in renal ^{123}I-*m*IBG uptake in the left kidney and after complete denervation in kidney allograft recipients as reference, we would be able to demonstrate a 44% difference in renal ^{123}I-*m*IBG uptake assuming that background ^{123}I-*m*IBG-uptake is similar. This suggests that our sample size was sufficient to detect a less than 50% reduction in renal sympathetic activity.

We previously showed that cardiac sympathetic activity did not change after RDN. (12) This is also supported by the lack of change in neurohormonal activation following RDN in the present and in previous studies.(16,17) Whether this is caused by insufficient denervation or results from a limited overall contribution of renal nerves in determining efferent sympathetic activity could not be assessed because quality parameters for successful RDN are lacking. In the present study we show that the lack of change in cardiac sympathetic activity may be caused by an inability of RDN to cause a sufficient decrease in afferent sympathetic nerve activity as ^{123}I-*m*IBG-uptake was unchanged.

A few limitations of our study merit discussion. Firstly, it remains possible that the modulation of SNA induced by RDN lies below the detection level of ^{123}I-*m*IBG. However, it may well be that sympathicolysis is achieved by RDN but that this does not influence BP, activity of the renin-angiotensin system and ^{123}I-*m*IBG parameters. Radiotracer dilution NE spill-over for organ specific assessment of sympathetic nerve activity is an alternative to ^{123}I-*m*IBG scintigraphy. Although this technique is considered the gold standard, its application is limited by its invasive nature. Moreover a widespread use of the technique is restricted by the poor availability of the required compounds. Secondly, ^{123}I-*m*IBG is primarily cleared via the kidneys and therefore kidney function may have influenced our data. However we have shown that both cardiac and renal ^{123}I-*m*IBG parameters are not influenced by kidney function.(7,11) Finally, we were aware of the potential influence of antihypertensive medication (calcium blocking agents, beta blocking agents) that may alter sympathetic drive and thereby uptake of ^{123}I-*m*IBG. In 2 patients, BP lowering medication had to be tapered because of

hypotension post RDN. In the remaining patients however BP lowering medication and sodium excretion were unchanged during the study period. We therefore feel that changes in antihypertensive medication do not explain the lack of change in ^{123}I-*m*IBG readouts.

In conclusion, we could not observe significant changes in functional kidney denervation as assessed with ^{123}I-*m*IBG scintigraphy following RDN with the Symplicity Catheter System. Our data suggest that the lack of BP lowering efficacy in the sham-controlled Simplicity HTN-3 study may be related to lack of procedural effectiveness. In comparison to available clinical tools renal ^{123}I-*m*IBG scintigraphy is minimally invasive and more widely available for clinical use. For future studies, renal ^{123}I-*m*IBG scintigraphy may be used as a parameter to assess RDN effectiveness.

Clinical competencies

A potential cause for the lack of efficacy of RDN might be the failure of RDN to sufficiently ablate renal sympathetic nerves. Yet, a routine technique to measure the extent of renal denervation is lacking and potential causes of insufficient denervation remain hypothetical. In this study we show that renal ^{123}I-*m*IBG scintigraphy, a minimally invasive technique to measure sympathetic nerve activity, can be used as a parameter of nerve disruption efficacy. To our knowledge, renal ^{123}I-*m*IBG scintigraphy has not been used for this purpose. Our study underlines the importance of evaluating procedural effectiveness and adds to the discussion whether fairly invasive tools need to have a clear read out of their efficacy.

Translational outlook

Our data suggest that RDN by means of the Symplicity catheter does not result in significant changes in functional kidney denervation as assessed with ^{123}I-*m*IBG scintigraphy. This may explain the lack of BP lowering effect of this technique. Our results are relevant to further delineate the role of RDN in therapy resistant hypertension and create a better understanding of the lack of efficacy of the current RDN techniques. Most of the available tools, however, are invasive and not applicable for broad clinical use. In this study we showed that renal ^{123}I-*m*IBG scintigraphy can be used as a parameter of nerve disruption efficacy. This technique is minimally invasive and is a measure of sympathetic nerve activity. Future RDN catheters could be evaluated for their potential to lower sympathetic activity using readouts such as renal ^{123}I-*m*IBG scintigraphy.

SUPPLEMENTAL DATA

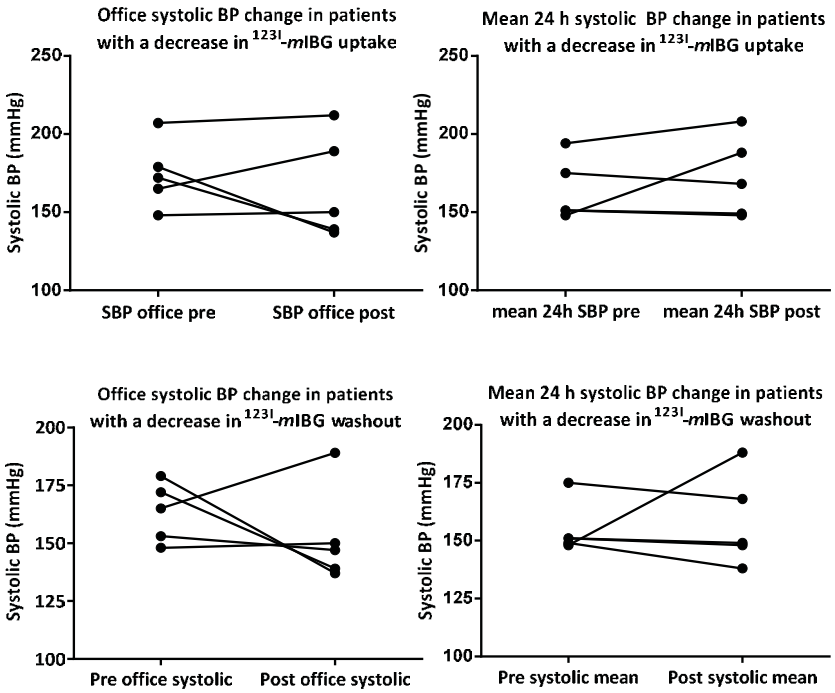
Subgroup analyses

We selected patients with the largest decrease in 123I-mIBG uptake at 15 min, defined as a delta ≤ -1.0 (n=5) and patients with the largest increase in 123I-mIBG uptake at 15 min, defined as patients with a delta of ≥ 1.0 (n=5). In neither of these groups did ABPM, kidney function or catecholamine levels change significantly after RDN (Supplementary file, table 1 and figure). Nor did the patients with the largest change (i.e. both increased and decreased) in 123I-mIBG uptake differ in baseline characteristics from the patients without these changes in 123I-mIBG uptake (data not shown).

In a different sub-selection of 5 patients with the largest decrease in washout (defined as a delta ≤-5.0) we did not find differences in pre- and post BP measurements, neither in catecholamines nor in kidney function (data not shown). In 10 of the patients with the largest increase in 123I-mIBG washout (i.e. delta ≥ 5.0) we found a difference in office systolic BP of p=0.05 (pre vs. post RDN median 181.5 vs. 158.0 mmHg), whereas diastolic BP was not different. In this subgroup no significant changes in ABPM, kidney function or catecholamines were observed after RDN (data not shown). In patients with the largest decrease in 123I-mIBG washout only the 24 h urine metanephrine was significantly higher at baseline compared to patients with the largest increase in washout after RDN (p=0.045).

No correlations were found between any of the renal 123I-mIBG uptake parameters and BP measurements (data not shown).

SUPPLEMENTARY FIGURE 1



Left upper panel: pre- and post-RDN office systolic BP change in patients with the largest decrease in ^{123}I-*m*IBG uptake.

Right upper panel: pre- and post-RDN mean 24 h systolic BP in patients with the largest decrease in ^{123}I-*m*IBG uptake.

Left lower panel: pre- and post-RDN office systolic BP change in patients with the largest decrease in ^{123}I-*m*IBG washout in patients with the largest decrease in ^{123}I-*m*IBG uptake.

Right lower panel: pre- and post-RDN mean 24 h systolic BP change in patients with the largest decrease in ^{123}I-*m*IBG washout in patients with the largest decrease in ^{123}I-*m*IBG washout.

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Chapter 09

Effects of renal sympathetic denervation on cardiac sympathetic activity and function in patients with therapy resistant hypertension

Peter M. van Brussel
Daan W. Eeftinck Schattenkerk
Linn C. Dobrowolski
Robbert J. de Winter
Jim A. Reekers
Hein J. Verberne
Liffert Vogt
Bert-Jan H. van den Born

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ABSTRACT

Background: Renal sympathetic denervation (RSD) is currently being investigated in multiple studies of sympathetically driven cardiovascular diseases such as heart failure and arrhythmias. Our aim was to assess systemic and cardiac sympatholytic effects of RSD by measurement of cardiac sympathetic activity and cardiovascular parameters.

Methods: A total of 21 consecutive patients with refractory hypertension (daytime ambulatory blood pressure (BP) ≥150/100 mmHg despite the use of 3 or more antihypertensive drugs), no evidence for secondary hypertension and normal renovascular anatomy were included. RSD was performed with the Medtronic Symplicity renal denervation catheter with an average of 4.2 (range 3-6) ablations per renal artery. To assess cardiac sympathetic activity, ^{123}I-*m*IBG cardiac scintigraphy was performed before and 6 weeks after. In addition, the effect of RSD on peripheral BP and cardiac hemodynamics were assessed non-invasively.

Results: ^{123}I-*mIBG* uptake before and after RSD was 1.7±0.4% vs. 1.7±0.5% at 15 min. and 1.4±0.4% vs. 1.5±0.5% after 4 hours. As a consequence, washout rate was similar before (33.7 ±11.7%) and after RSD (30.1 ± 12.6%, p=0.27). In line with earlier RSD studies, a significant drop in systolic office BP (-12.2 mmHg, p=0.04) was detected, whereas the decrease in ambulatory BP was not significant. No changes were seen in heart rate, stroke volume or left ventricular contractility, both in supine position and after standing.

Conclusion: In concert with previous reports, RSD leads to a significant drop in office BP. However, a reduction in sympathetic activity could not be demonstrated on a cardiac level.

INTRODUCTION

Renal sympathetic denervation (RSD) is a catheter-based treatment that targets sympathetic nerve fibers in the renal arterial wall. By interrupting the sympathetic outflow to the kidneys and brain, RSD could be an attractive treatment option for diseases that are initiated or sustained by an overshooting sympathetic nervous system. With this in mind, RSD has been the subject of intensive research for the treatment of resistant hypertension, a condition characterized by increased sympathetic activity. Despite initial promising results of the effect of RSD on blood pressure (BP), the results of the recent large sham controlled SYMPLICITY HTN-3 trial were disappointing.(1) The link between increased sympathetic activity and cardiac diseases including heart failure and atrial and ventricular cardiac arrhythmias has been well identified.(2–4) Different studies have shown that RSD is associated with a reduction in renal specific and overall sympathetic activity as demonstrated by a decrease in norepinephrine (NE) spillover and muscle sympathetic nerve activity (MSNA), respectively.(2–4) Therefore, it is conceivable that RSD might also attenuate sympathetic outflow to the heart. In the past, multiple other sympatholytic interventions including beta blockade and left cardiac sympathetic denervation have shown to be beneficial in treating heart failure and arrhythmias. The potential for RSD as a novel tool to treat cardiac anomalies has gained much attention with RSD currently being tested for the treatment of heart failure and ventricular and supraventricular arrhythmias in over 500 clinical studies.(5) At present, the effects of RSD on cardiac sympathetic activity and function are still undetermined.

^{123}I-metaiodobenzylguanidine (^{123}I-*m*IBG) is a radiolabeled false analog of NE and is taken up by presynaptic NE-transporters. By presynaptic internalization in neuroendocrine cells NE is stored in neurosecretory granules in sympathetic nerve endings,(6) allowing ^{123}I-*m*IBG to be employed to study disorders of sympathetic innervation. In patients with heart failure ^{123}I-*m*IBG uptake and washout is associated with increased cardiac events and mortality.(7) In addition, drugs that modify sympathetic activity such as beta-blockers and ACE inhibitors are associated with changes in cardiac sympathetic activity as assessed with ^{123}I-*m*IBG.(8,9) Therefore we hypothesized, based on its sympatholytic target, that cardiac ^{123}I-*m*IBG scintigraphy might be an indicator of RSD treatment success by detecting changes in ^{123}I-*m*IBG cardiac uptake. This pilot study was designed to investigate the influence of RSD on cardiac sympathetic tone as measured by changes in cardiac ^{123}I-*m*IBG washout.

METHODS

Patients

In this study, a group of 21 consecutive patients aged 40-70 years with a clinical indication for RSD for therapy resistant hypertension, defined as a mean daytime BP ≥150/100 mmHg despite the use of 3 or more anti-hypertensive drugs including or with intolerance to a diuretic were included. Secondary causes of hypertension (e.g., renal artery stenosis, pheochromocytoma, primary aldosteronism and hyper- or hypothyroidism) and abnormal renal artery anatomy, including the presence of accessory renal arteries, were ruled out prior to the intervention. Patients with renal insufficiency (estimated glomerular filtration rate (eGFR) <45 mL/min/1.73m^2) or proteinuria (>1 g/24 h) or having a pacemaker, implantable cardioverter-defibrillator (ICD), atrial fibrillation or type 1 diabetes mellitus were excluded. Six weeks prior to the first measurements patients were screened to assess eligibility for study participation. Patients were deemed eligible for study participation if they were at least 3 weeks on stable BP lowering medication prior to the first study visit. BP lowering medication was kept unchanged throughout the study until the final visit 6 weeks after RSD.

When fully informed and willing to participate, patients were asked to provide written informed consent. Six weeks hereafter, office BP was measured and cardiac hemodynamics were assessed by use of a continuous non-invasive BP and cardiac output monitor (Nexfin, Edwards Life Sciences, Irvine, CA, USA). Patients were required to maintain the same anti-hypertensive drug regimen throughout study participation.

All patients provided informed consent before inclusion in the study. The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki and has been approved by the local ethics committee prior to the start of the study and was registered under Dutch national registration number: NL.36755.018.11.

Renal sympathetic denervation

RSD was performed by use of radiofrequency energy delivered by the Symplicity renal-denervation catheter (Medtronic Inc., Santa Rosa, California, USA). Prior to the procedure, midazolam 1.0 mg and metoclopramide 10.0 mg was given intravenously. After inserting an 6F introducer in the right femoral artery, the guiding catheter was introduced in the aorta and an aortagram was made by use of a pigtail catheter. The guiding catheter was advanced in the right and left renal artery in no pre-specified order. The denervation catheter was introduced in the renal artery via the delivery catheter. After nitroglycerine 0.2 mg and fentanyl 0.02 mg intravenously, catheter ablations were performed in a helical pattern with the goal of at least 4 ablations

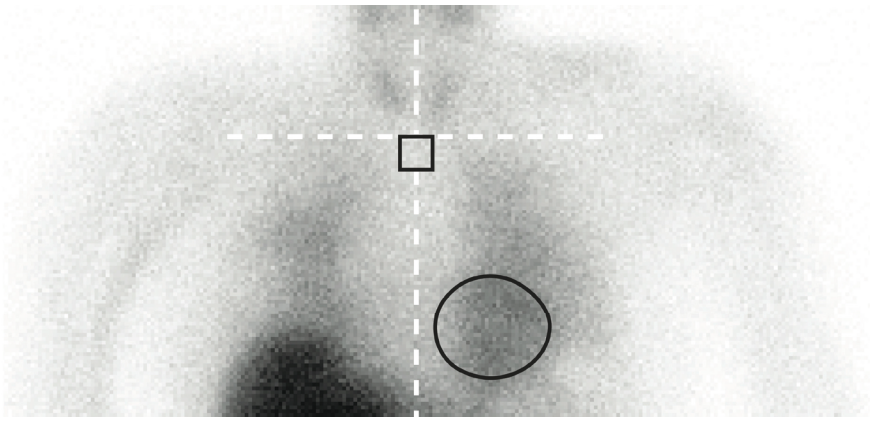
per renal artery to cover each short axis transaxial quadrant, according to the user's instruction of the device.

Cardiac ^{123}I-*m*IBG

Cardiac scintigraphy by use of ^{123}I-*m*IBG was performed at baseline and 6 weeks after RSD. To block thyroid uptake of unlabeled ^{123}I subjects received potassium iodine prior to the intravenous administration of 185 MBq ^{123}I-*m*IBG. To promote ^{123}I-*m*IBG renal clearance a single oral dose of furosemide 60 mg retard was given thirty minutes prior to injection. At 15 minutes (early) and 4 hours (late) post injection 10 min planar anterior acquisitions of the thorax were made (medium energy collimators and 128*128 matrix). On both the early and late images a region of interest (ROI) was drawn over the heart and the upper mediastinum. Obtained values were expressed as mean counts per pixel, i.e. count density (Figure 1). During evaluation of the cardiac scintigraphies, involved investigators (PMvB and HJV) were blinded for subject's identity and the scan's timepoint (i.e. prior to or after RSD). The mean counts per pixel in the two ROI's were used to calculate the early and late heart to mediastinal ratio (early H/M and late H/M). Myocardial ^{123}I-*m*IBG washout between the early and late images was calculated, corrected for aspecific uptake in the mediastinum, using the formula(6):

$$\text{WO (background corrected)} = \frac{(\{H\}e - \{M\}e) - (\{H\}l - \{M\}l) \times 1.21}{(\{H\}e - \{M\}e)} \times 100$$

FIGURE 1 - Semiquantification of ^{123}I-*m*IBG



Semiquantification of cardiac ^{123}I-*m*IBG uptake on a planar acquisition of the thorax. The heart-to-mediastinum ratio (H/M) and myocardial washout (WO) were calculated by drawing a region of interest (ROI) over the heart and the upper mediastinum, on the early (+15min) and late (+4h) images.

Non-invasive peripheral and cardiac hemodynamics

Office BP was measured using an Omron SEM-2 Automatic BP Monitor (Omron Corp., Kyoto, Japan). Ambulatory BP was measured using Spacelabs 90217 Ambulatory BP Monitors (OSI Systems, Inc., Snoqualmie, Washington, USA). Systemic hemodynamics were measured with the Nexfin device (Edwards Lifesciences Corp., Irvine, California, USA), which uses the volume-clamp method to non-invasively measure continuous finger arterial BP.(10) The finger cuff was applied to the third finger of the dominant arm, while brachial BP was reconstructed from the finger arterial pressure.(11) Mean arterial pressure (MAP) was calculated by taking the true integral of the arterial pressure over 1 beat divided by the corresponding beat interval. Stroke volume (SV) was determined by a pulse contour method (Nexfin CO-trek).(12) Cardiac output (CO) was defined as SV divided by the interbeat interval. Systemic vascular resistance (SVR) was calculated as MAP divided by CO. Left-ventricular contractility was estimated as dP/dt max of the pressure pulse. dP/dt max has shown to be significantly increased when cardiac sympathetic nerves are stimulated.(13) Analyses were performed on frames consisting of 30 heart beats after at least 5 minutes of supine rest. To test the autonomic response to BP changes prior to and after intervention, patients were asked to stand-up and stand still for 5 minutes after which the measurements were repeated. Baroreflex sensitivity (xBRS) is the amount of response in heart beat interval to a change in BP, expressed in ms/mmHg. xBRS was obtained in supine position and after standing from a 4 minute beat-to-beat period of systolic BP and inter-beat interval data following 1 minute of calibration.

Laboratory analysis

A venous blood sample was taken in fasting condition at baseline and 6 weeks after RSD. Prior to the visit patients were instructed to collect 24 h urine. Plasma aldosterone, plasma renin activity (PRA) and urinary epinephrine, norepinephrine and normetanephrine were measured by radioimmunoassay. Other measurements were performed using standard laboratory techniques by the Laboratory of Clinical Chemistry, of the Academic Medical Center.

Ambulatory bp monitoring

24-hour ABPM was performed at baseline and at 6 weeks follow-up. Mean systolic, diastolic, MAP and nocturnal dipping patterns were recorded. Recordings were included when at least 70% of the measurements were successful.

Primary and secondary endpoints

The primary endpoint was defined as the change in cardiac sympathetic activity one week prior to and 6 weeks after RSD as measured by a change in uptake and washout of ^{123}I-*m*IBG on cardiac scintigraphy. Secondary end points were the change in office and ambulatory BP, change in catecholamine excretion in 24 h urine, and change in the alteration in cardiac hemodynamics (heart rate (HR), SV, CO and dP/dt max), SVR and xBRS in supine position and after standing.

Statistical analysis

Parametric testing was used for the principal analysis of the exploratory cardiac washout and hemodynamic endpoints. As cardiac ^{123}I-*m*IBG uptake and washout before and after RSD followed a parametric distribution continuous descriptive variables are presented as mean and standard deviation, and are compared by Student's t-test. Levene's test was performed to verify the equality of variances in the samples. All statistical tests were 2-sided, and a p-value <0.05 was considered significant. The calculation of the sample size was based on the findings on mean cardiac ^{123}I-*m*IBG washouts of 37.0 in heart failure patients and the reported drop of 47% in renal NE spillover after RSD .(7)(14) For our calculations, we considered a more conservative change of 20% to be clinical relevant. Thus, a sample size of 18 will have 80% power to detect a difference in means of 7.4 (e.g. a baseline cardiac washout, μ_1, of 37.0 and a follow-up cardiac washout, μ_2, of 29.6 assuming a standard deviation of differences of 12.0, using a paired t-test with a 0.05 one-sided significance level. Because of potential drop-outs, we have finally included a total of 21 patients. All statistical analyses were performed using SPSS v.21.0 (IBM Corp., Armonk, NY, USA).

RESULTS

Patient characteristics

Between July 2011 and December 2013, a total of 21 patients with therapy resistant hypertension underwent RSD. All procedures were performed via the femoral artery by a single, highly experienced intervention radiologist (JAR), especially with regard to renovascular interventions**.** The operator had performed five RSD procedures prior to study enrollment. Baseline characteristics are shown in table 1. Fifteen subjects (71%) were male and sixteen subjects (76%) were of Western-European descent. Mean age was 58.7±8.8 years and body mass index (BMI) was 27.9±3.3 kg/m^2. Baseline office BP was 174.2±17.0 / 99.7±17.4 mmHg. Patients were receiving an average of 4.7±1.3 antihypertensive drugs and their drug regimen remained similar throughout study participation. Mean eGFR was 56.6±8.6 ml/min/1.73m^2. A mean of 4.3±0.6 denervations was performed in the left renal artery wall and 4.2±0.5 denervations were performed in the right renal artery wall. No peri-procedural complications occurred.

TABLE 1 - Baseline characteristics

RSD (n=21)	
Males (%)	15 (71)
Caucasians (%)	16 (76)
Age (years) (SD)	58.7 (8.8)
Body Mass Index (SD)	27.9 (3.3)
Office Systolic Blood Pressure (SD)	174.2 (17.0)
Office Diastolic Blood Pressure (SD)	99.7 (17.4)
Heart Rate (SD)	78.6 (11.2)
Mean Systolic 24 hr Ambulatory Blood Pressure (SD)	161.9 (14.5)
Mean Diastolic 24 hr Ambulatory Blood Pressure (SD)	93.2 (12.7)
Left Ventricular Hypertrophy (%)	6 (29)
Cardiovascular Disease (%)	10 (48)
eGFR (SD)	73.0 (38.6)
Chronic Kidney Disease* (%)	14 (67)
Diabetes (%)	7 (33)
- of which Insulin Dependent Diabetes (%)	5 (24)
Statin Use and/or Dyslipidemia (%)	11 (52)
Number of Anti-hypertensive Drugs (SD)	4.7 (1.3)

SD = Standard Deviation. Chronic Kidney Disease was defined as an estimated glomerular filtration (eGFR) rate of <60 mL per minute per 1.73 m^2 of body-surface area and/or macroalbuminuria (>300mg/L).

Influence of rsd on cardiac sympathetic outflow

The results with regard to the primary efficacy endpoint (change in cardiac sympathetic output by means of uptake and washout of ^{123}I-*m*IBG on cardiac scintigraphy) are shown in table 2. There was no difference in cardiac ^{123}I-*m*IBG uptake between baseline and follow-up (1.7±0.4 vs. 1.7±0.5 at 15 minutes and 1.4±0.4 vs. 1.5±0.5 at 4 hours, respectively) Cardiac washout of ^{123}I-*m*IBG was 34.0±11.2% at baseline vs. 30.9±12.4% at 6 weeks follow-up, and showed a non-significant decrease (delta -3.0% ±, p=0.27, figure 2, upper panel). When the subject with the largest response is not included in the analysis, the small difference in ^{123}I-*m*IBG washout before and after RSD further diminishes: delta -1.2±9.2%, p=0.56).

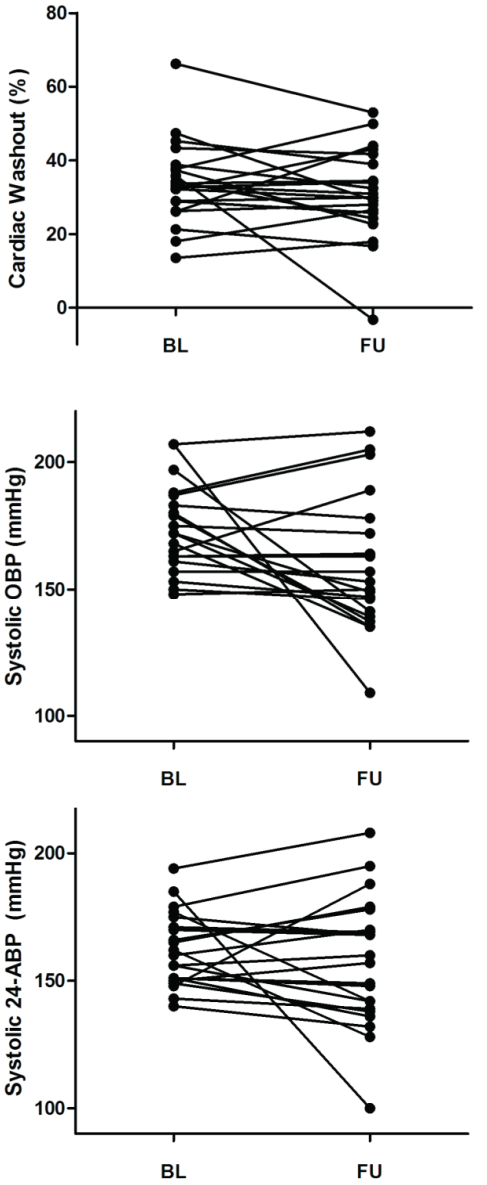
TABLE 2 - Cardiac uptake and washout

CARDIAC ^{123}I-*m*IBG	**Baseline**	**Follow-up**	**p**
Early H/M (15 min) (SD)	1.7 (0.4)	1.7 (0.5)	0.59
Late H/M (4 h) (SD)	1.4 (0.4)	1.5 (0.5)	0.29
Cardiac Washout (SD)	34.0 (11.2)	30.9 (12.4)	0.27

Effects of rsd on bp and cardiac hemodynamics

Changes in office and ambulatory BP, cardiac hemodynamics in supine position and after standing are presented in table 3. Office systolic BP significantly decreased by 12.2 mmHg 6 weeks after RSD (p=0.04, figure 2, middle panel), while diastolic BP and HR did not significantly change. Daytime, nighttime and 24 h ABPM did not show any change in systolic and diastolic BP or dipping pattern (figure 2, lower panel). During the orthostatic challenge, the difference in cardiac hemodynamics (HR, SV, CO and dP/dt max), SVR and xBRS in supine position and after standing did not change after RSD. When the subjects were divided in a responder group (i.e. ≥10.0mmHg drop in systolic BP at follow-up) and non-responder group (i.e. <10.0mmHg drop in systolic BP follow-up), there was no difference in change in ^{123}I-*m*IBG uptake and washout nor in cardiac hemodynamics.

FIGURE 2 - Changes in cardiac washout and blood pressure



^{123}I-mIBG washout rate ('Cardiac Washout', upper panel), systolic office blood pressure (Systolic OBP, middle panel), Systolic 24 h Ambulatory Blood Pressure (Systolic 24-ABP, lower panel) before (BL) and after (FU) renal sympathetic denervation. No significant differences were detected and changes appear randomly. The most prominent decrease in the different panels belongs to separate subjects.

TABLE 3 - Blood pressure, hemodynamics and orthostatic trigger

RSD (n=18)*	BASELINE	FOLLOW-UP	Δ	p
Blood pressure**				
ABPM Systolic BP 24h (mmHg) (SD)	161.9 (14.5)	156.9 (25.4)	-5.0	0.37
ABPM Diastolic BP 24h (mmHg) (SD)	93.2 (12.7)	90.4 (15.5)	-2.8	0.36
Dipping Percentage*(SD)	11.1 (7.7)	10.9 (8.7)	-0.2	0.91
Office Systolic BP (mmHg) (SD)	174.2 (17.0)	160.1 (26.5)	-14.1	0.04
Office Diastolic BP (mmHg) (SD)	99.7 (17.4)	93.8 (19.2)	-5.9	0.16
Office HR (SD)	78.6 (11.2)	77.6 (12.4)	-1.0	0.68
Hemodynamics ***				
Syst Blood Pressure (mmHg) (SD)	170.8 (22.4)	172.6(23.3)	1.8	0.77
Diast Blood Pressure (mmHg) (SD)	88.1(14.4)	89.4(14.9)	1.4	0.68
Mean Arterial Pressure (mmHg) (SD)	120.1(17.6)	121.9(17.7)	1.8	0.69
Pulse Pressure (mmHg) (SD)	82.4(14.4)	82.8(16.2)	0.3	0.92
Cardiac Output (l/min) (SD)	6.3(1.8)	6.1(1.9)	-0.2	0.37
Heart Rate (b/min) (SD)	68.3(12.7)	67.3(10.9)	-1.1	0.50
Stroke Volume (ml) (SD)	91.8(22.4)	90.0(23.7)	-1.8	0.47
Systemic Vascular Resistance (mmHg·min/l)(SD)	1713.8(756.7)	1789.9(701.3)	76.1	0.56
dP/dt max (mmHg/sec) (SD)	1403.7(522.8)	1367.5(417.2)	-36.2	0.63
xBRS (ms/mmHg) (SD)	7.7(5.0)	6.7(4.7)	-0.9	0.32
Orthostatic trigger***	Δ supine-standing	Δ supine-standing		
Syst Blood Pressure (mmHg) (SD)	-3.6 (24.2)	-7.1 (30.7)	-3.5	0.60
Diast Blood Pressure (mmHg) (SD)	5.4 (10.2)	2.6 (14.5)	-2.8	0.32
Mean Arterial Pressure (mmHg) (SD)	1.2 (13.6)	-2.4 (20.1)	-3.6	0.37
Pulse Pressure (mmHg) (SD)	-8.7 (18.2)	-9.7 (19.4)	-1.0	0.83
Cardiac Output (l/min) (SD)	-0.7 (1.1)	-0.4 (1.1)	0.4	0.19
Heart Rate (b/min) (SD)	6.6 (4.9)	6.9 (8.5)	0.2	0.90
Stroke Volume (ml) (SD)	-17.2 (14.1)	-13.0 (14.6)	4.2	0.15
Systemic Vascular Resistance (mmHg·min/l) (SD)	197.9 (333.3)	142.4 (659.3)	-55.5	0.66
dP/dt max (mmHg/sec) (SD)	-154.7 (499.1)	-97.4 (373.2)	57.3	0.68

* Three hemodynamic recordings were of insufficient quality and were therefore not included in the analysis.
** As measured by use of oscillometric office and ambulatory blood pressure monitors
*** As measured by use of a continuous non-invasive blood pressure and cardiac output monitor

Effects of rsd on blood parameters and urinalysis

Results of the effect of RSD on PRA, aldosterone and urinary catecholamine excretion is shown in table 4. There were no significant changes in plasma PRA or aldosterone following RSD and there was no significant change in urinary catecholamine excretion, although urinary epinephrine excretion tended to decrease (delta -6.8 , p=0.16). Kidney function and urinary sodium excretion remained unchanged.

TABLE 4 - Blood and urine analysis

RSD (n=21)	Baseline	FU	p
Blood analysis			
creatinine (µmol/l) (SD)	98.6 (35.5)	94.9 (31.5)	0.27
eGFR (ml/min) (SD)	73.0 (38.5)	76.0 (40.8)	0.12
Aldosteron (nmol/l) (SD)	0.47 (0.30)	0.42 (0.24)	0.37
Plasma Renin Activity (µgA1/l/U) (SD)	2.3 (1.9)	2.2 (3.6)	0.92
Urine analysis			
sodium (mmol/24h) (SD)	169.0 (87.8)	139.7 (60.1)	0.16
Epinephrine (nmol/24h) (SD)	38.7 (35.3)	31.9 (23.6)	0.16
Norepinephrine (nmol/L/24h) (SD)	344.8 (216.8)	334.9 (176.4)	0.75
Normetanefrine (nmol/L/24h) (SD)	2.4 (1.3)	2.3 (1.1)	0.60

DISCUSSION

This study found no influence of RSD on cardiac sympathetic tone as measured by changes in cardiac ^{123}I-*m*IBG washout or uptake. Like previous studies we found a significant reduction in office BP without marked changes in ambulatory BP. No changes were found in cardiac hemodynamics or indices of sympathetic activity. Cardiac ^{123}I-*m*IBG uptake and washout have earlier proven to representatively map cardiac sympathetic innervation and are considered to be a reliable read-out method in stratifying survival risk in heart failure patients.(15,16) In addition, there is a strong association between sympathicomodulatory treatments, including renin-angiotensin system (RAS) inhibitors and cardiac sympathetic activity as measured by ^{123}I-*m*IBG.(15,17) Given its broad dispersion, it could be argued that the sample size was underpowered to demonstrate relevant differences. However, the difference in cardiac ^{123}I-*m*IBG washout in our study was small and appeared to be determined by only one subject with the most pronounced change in cardiac ^{123}I-*m*IBG washout. For comparison, an earlier study showed that low dose of the angiotensin-converting enzyme inhibitor enalapril 10 mg decreased cardiac washout by 8%.(17) The effect of

RSD on ^{123}I-*m*IBG washout as measure of cardiac sympathetic activity seems therefore to be marginal at least.

In line with earlier RSD studies,(18–20) we observed a change in office BP, but no alterations in diastolic office and ambulatory BP. Most probably, the reduction in office systolic BP may be caused by another mechanism than a modulation in autonomic tone alone. After the publication of the sham controlled SYMPLICITY HTN-3 trial results, the value of office BP as a valid endpoint in non-placebo controlled studies has been questioned. Besides placebo effects, increased adherence to antihypertensive drug therapy and other non-procedure related effects may have influenced the decrease in office BP. In theory, these effects may also have contributed to small decreases in cardiac ^{123}I-*m*IBG washout as placebo effects may result in changes in sympathetic outflow.(8,21) In addition, changes in adherence to BP lowering therapy and sodium intake may also have contributed to the decrease in cardiac ^{123}I-*m*IBG, although PRA and 24-hr urinary sodium excretion did not significantly differ following RSD.

There may be different explanations for why RSD failed to show a marked alteration in cardiac sympathetic nerve activity and its related hemodynamic indices. Currently, there is much debate on to what extent ablation is effective, both in a quantitative and a qualitative way. Given the paucity of afferent compared to efferent sympathetic nerve fibers in the renal arterial wall it is conceivable that RSD only has a minor influence on central sympathetic tone.(22) However, in a subanalysis performed by the SYMPLICITY HTN-3 group, a higher drop in BP was found in patients who were ablated more intensively in terms of the number of ablations performed and total energy delivered. (1) This suggests that the inefficacy of the current RSD technique probably lies in a quantitative property rather than in a qualitative one. This is further supported by a case report of a post-mortem examination of a RSD patient, which showed incomplete denervation in the renal arterial wall.(23) Differences in procedural effectiveness may help explain the inconsistent BP lowering and sympatholytic effects across different trials with some studies demonstrating a reduction in sympathetic activity,(2,24) and others showing no difference before and after RSD.(25,26) However, all studies - including our own- used a similar technique with a comparable number of ablations. Nevertheless, baroreflex sensitivity and urinary catecholamine excretion did not show any difference before and after RSD despite a significant decrease in office BP. Since RSD is supposed to have a sympatholytic substrate, a change in hemodynamic compensatory function would be expected. However, the orthostatic trigger in our study did not show any change in cardiovascular responses, which is in line with data from Lenski et al. showing that the hemodynamic response during orthostatic stress is unaltered after RSD.(27) However the absence of orthostatic dysfunction does

reflect a favorable safety profile, as sympatholytic therapy would be expected to affect orthostatic function in a subtle manner.

Finally, sympathetic tone may have been lower in our study population than in patients with cardiac diseases that are characterized by an overshooting sympathetic nervous system. More than half of the study population had a history of cardiovascular disease and, like previous studies, all patients had resistant hypertension which is characterized by increased sympathetic activity. (28) In addition, one third had diabetes and two thirds had chronic kidney disease, both of which are associated with an increase in sympathetic tone.(29,30) One explanation for the lack of effects is that the treatment method is insufficient. The delicate distribution of sympathetic nerves in the renal artery walls and the possible inaccuracy of the present-day ablation techniques make it likely that RSD is inaccurate in its present form. Another explanation to be considered is that the contribution of renal sympathetic activity to the BP increase in patients with therapy resistant hypertension is limited. We believe that intervening in the intricately designed sympathetic matrix is absolutely justifiable, but desirable treatment effects will surface only if the denervation techniques are optimized. Therefore, in line with recommendations made in a recent statement by White, et al., preclinical models should be well characterized and validated prior to initiating the research in human models.(31)

Study limitations

There are some limitations to this study. First, this study has no control group. However, the absence of detection of any significant alterations in cardiac sympathetic outflow make the necessity for a control arm less outspoken. Furthermore, patients characteristics were similar to those in the SYMPLICITY HTN studies, making the study results representative. Secondly, read-out parameters may be overestimated in their significance since the sample size was relatively low. For the same reason the absence of changes in washout, ambulatory BP, serum and urine analyses and hemodynamic changes during the sympathetic stimulus by giving an orthostatic challenge might be underestimated. Thirdly, the use of RAS inhibitors at baseline may have decreased the potency for a treatment effect of RSD. Since both treatment modalities have an sympathomodulating substrate, it is possible that treatment with RAS blockers may have attenuated the effect of RSD on sympathetic tone.

CONCLUSIONS

In this study, we found no signs that RSD alters cardiac sympathetic activity and function. Although past studies have demonstrated that a decrease in sympathetic activity is accompanied by a change in ^{123}I-*m*IBG washout, an alteration of cardiac hemodynamic parameters and changes in ambulatory BP, none of these modalities seem to be affected by RSD. This raises the question whether the current RSD techniques or even the proposed pathophysiological mechanism behind RSD bears sufficient therapeutic potential for cardiac arrhythmias and heart failure in humans. Further pre-clinical work is therefore warranted.

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Chapter 10

Renal 123I-MIBG scintigraphy before and after kidney autotransplantation

Linn C. Dobrowolski
Daan W. Eeftinck Schattenkerk
Mirza M. Idu
Bert-Jan H. van den Born
Hein J. Verberne

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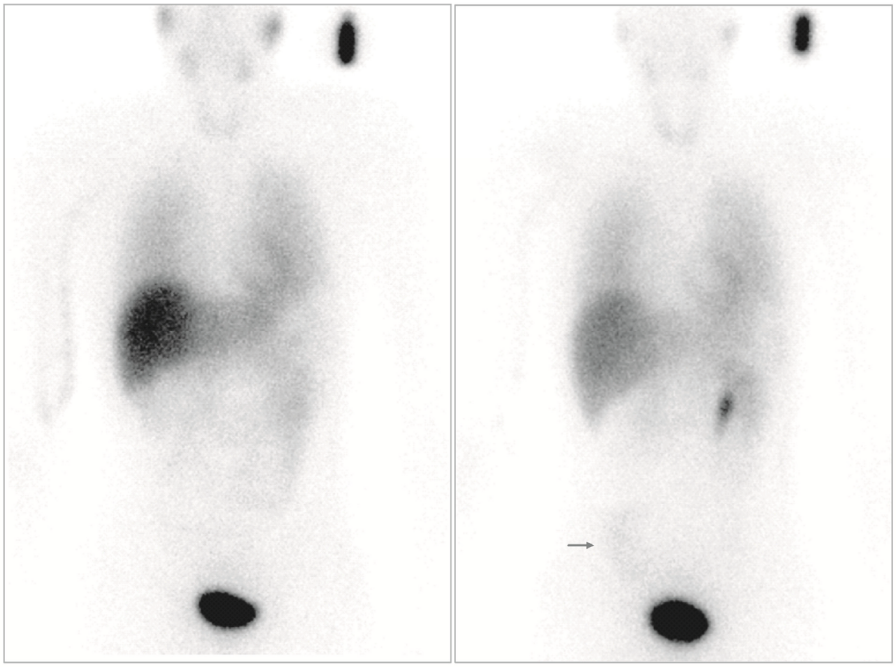
ABSTRACT

A 25-year old man underwent an autotransplantation of his right kidney because of fibromuscular dysplasia induced renal artery stenosis and subsequent hypertension. Since transplantation results in complete kidney denervation, it enabled assessment of renal sympathetic nerve activity changes using renal ^{123}I-*m*IBG scintigraphy. Before and two weeks after transplantation ^{123}I-*m*IBG scintigraphy was performed. Uptake of ^{123}I-*m*IBG in the left (control) kidney increased after transplantation with 4% at 15min and 5% at the 4h post-injection images, whereas ^{123}I-mIBG uptake in the right transplanted kidney decreased with 21% at 15min and with 29% at 4h, demonstrating renal ^{123}I-*m*IBG changes following denervation.

FIGURE 1



A 25-year old patient underwent an autotransplantation of his right kidney for recurrent hypertension caused by fibromuscular dysplasia of the renal artery. Multiple balloon- angioplasties, including stent-placement had been unsuccessful in controlling hypertension (ambulatory BP measurement (ABPM) 150/85 mmHg). Estimated GFR was 96 mL/min/1.73m^2 (CKD-EPI), with 38% contribution of the right kidney to overall kidney function as assessed with ^{T99m}TC-MAG3 renography. Because of his age and recurrent uncontrolled hypertension despite 3 antihypertensive agents, a renal autotransplantation was performed. This normalized ABPM to 119/74 mmHg without the use of antihypertensive drugs and increased kidney function to 118 mL/min/1.73m^2. After transplantation, renal function of the autograft was unchanged with a relative contribution of 46% to overall kidney function on renography. Since autotransplantation assured complete denervation of the kidney, it provided the opportunity to test the feasibility of renal ^{123}I–*meta*-iodobenzylguanidine (^{123}I-*m*IBG, a radiolabeled norepinephrine analogue) scintigraphy for assessing renal sympathetic innervation. Before and 2 weeks after the autotransplantation, we obtained planar images 15 min and 4 h after intravenous administration of ^{123}I-*m*IBG (185 MBq). A vial with a reference amount of ^{123}I was placed next to the head of the patient. Figure 1

shows the planar images 15 min after injection of ^{123}I-*m*IBG before (left panel) and after the transplantation (right panel, the autograft in the right ileac fossa is indicated with the arrow). Subsequently we calculated renal ^{123}I-*m*IBG uptake as a measure of sympathetic innervation and renal ^{123}I-*m*IBG washout as a measure of sympathetic drive by using counts obtained by manually drawn regions of interest. Skeletal muscle served as reference tissue. Excretion of ^{123}I-*m*IBG is visible via the collecting system and native left kidney to the bladder. The accumulation of urinary ^{123}I-*m*IBG in the pyelum of the left kidney was no longer visible 4 h after the administration of ^{123}I-*m*IBG (image not shown).

Mean counts of geometric mean images derived regions of interest were used to calculate relative uptake of ^{123}I-*m*IBG:

$$\frac{(kidney\ uptake\ at\ 15\ min - muscle\ uptake\ at\ 15\ min)}{kidney\ uptake\ at\ 15\ min}$$

Relative uptake at 15 min in the left (control) kidney was 0.81 pre-transplantation vs. 0.85 post-transplantation (+5%) and at 4 h 0.69 vs. 0.72 (+4%) following injection. Relative uptake at 15 min in the right (transplanted) kidney decreased from 0.78 pre to 0.62 (-21%) post-transplantation and at 4 h from 0.66 to 0.47 (-29%). Renal ^{123}I-*m*IBG washout between 15 min and 4 h was relatively unchanged in the left kidney (40% vs. 44%) and decreased in the right (transplanted) kidney (36% vs. 29%).

This case illustrates that after surgical denervation ^{123}I-*m*IBG uptake and washout decrease, but not completely. This may relate to aspecific uptake or change in background signal/noise. Renal ^{123}I-*m*IBG scintigraphy as a measure of renal sympathetic innervation may be an attractive imaging modality to study the effects of interventions that affect renal sympathetic activity.

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Chapter 11

Summary and general discussion

Summary and general discussion

In this thesis we employed novel techniques to gain a better understanding of hypertensive disorders and improve antihypertensive treatment strategies. We discussed novel aspects in phenotyping and treatment modalities. We first addressed central hemodynamics and the (patho)physiological mechanisms involved (part I). Second, we studied individual differences in central hemodynamics and effects of pharmacotherapy (part II). Third, we looked into renal sympathetic denervation, studying its effects on renal and cardiac sympathetic nervous activity by use of 123I-mIBG scintigraphy and illustrated this by a case report of a subject undergoing renal auto transplantation (part III).

Chapter 1 provides a general introduction and outline of this thesis. In short, cardiovascular disease (CVD) is a major global health threat and hypertension is one of its prime risk factors. To improve cardiovascular healthcare, a better understanding and treatment of hypertensive disorders is warranted. This calls for novel diagnostic and therapeutic approaches. Assessment of central hemodynamics may improve hypertensive phenotyping beyond conventional blood pressure (BP) measurement and help to understand differences in the efficacy of BP lowering medication. Further insight in the (patho)physiology of central hemodynamics, including individual differences and impact of antihypertensive medication is therefore of utmost interest (**part I and II**). Renal sympathetic denervation is a novel treatment modality for refractory hypertension with large variations in therapeutic response which are potentially related to individual differences in sympathetic activity and/or effectiveness of ablation (**part III**). The third part of this thesis focuses on modalities to assess the effectiveness of renal denervation.

Part I. Assessment and (patho)physiology of central hemodynamics.

Continuous non-Invasive assessment of blood pressure and hemodynamics

While the gold standard for non-invasive measurement of BP according to Riva-Rocci Korotkoff requires the combined use of a sphygmomanometer and a stethoscope, technological progress has enabled more advanced measurement techniques. The Nexfin® device applies volume-clamp plethysmography according to the Finapres method[1] to non-invasively and continuously assess BP and hemodynamics (including stroke volume, cardiac output, total peripheral resistance, left ventricular contractility and baroreflex sensitivity).[2] Non-invasive, continuous and simultaneous measurement of BP and hemodynamics is valuable to clinical practice and hypertension research. In clinical practice continuous non-invasive measurement of BP and hemodynamics

is often employed in autonomic function testing in for example syncope or volatile hypertension. In research it provides readily applicable hemodynamic assessments in large population studies and facilitates experiments specifically designed to induce rapid changes in BP and hemodynamics: such as head-up-tilt, valsalva manoeuvre or acute-acting pharmaceutics.

In **Chapter 2** we validated the accuracy of Nexfin® continuous non-invasive BP as compared to Riva-Rocci Korotkoff in a random population sample.[3] We demonstrated that the Nexfin® device estimates brachial BP accurately, according to the Association for the Advancement of Medical Instrumentation guidelines, when compared to Riva-Rocci Korotkoff. The beat-to-beat measurements provided by the Nexfin® also enable the tracking of quickly occurring changes in hemodynamics that are under control of the arterial baroreflex. The arterial baroreflex comprises a cardiac arm and a vasomotor arm. Commonly, baroreflex sensitivity addresses the rapid alterations in heart rate in response to variations in BP as sensed by carotid baroreceptors. The slower vasomotor arm, that enacts on arterial smooth muscle cell tone to alter peripheral resistance, is however often neglected. As BP, heart rate and systemic vascular resistance are determined continuously with the Nexfin® device it is possible to study both arms of the arterial baroreflex. In **chapter 3** we explored the determinants of vascular and cardiac baroreflex sensitivity in the same, random population as in chapter 2. We demonstrated that determinants of cardiac and vascular baroreflex are different, supporting the view that they address different modalities of cardiovascular autonomic function.

(Patho)physiology of central hemodynamics

Although BP is usually measured at the level of the upper arm, large differences in BP exist along the major arteries. While diastolic BP is relatively constant, the systolic peak of the BP waveform may be up to 40 mmHg higher in the brachial artery than in the aorta.[4] Differences between central and peripheral systolic BP are a consequence of arterial wave reflection and pressure augmentation.[5-7] In short, the cardiac cycle generates forward pressure waves that travel from the heart down the arterial tree towards the periphery. Impedance mismatch, e.g. in high resistance arteries and arterioles and at bifurcations, results in reflection of these forward waves, giving rise to backward (i.e. reflected) waves that travel in the opposite direction. Depending on their magnitude, morphology and timing, the colliding of forward and reflected pressure waves results in an augmentation of systolic BP, which is often quantified as the augmentation index.[5-7]

Several factors have been identified that influence the process of arterial wave reflection and pressure augmentation and hence differences between central and

peripheral BP, including large artery stiffness, systemic vascular resistance, heart rate and body height.[8-12] Still much remains unknown regarding the (patho)physiological mechanisms involved. A better understanding of wave reflection has important implications. First, as wave reflection potentially raises (central) BP it may indirectly raise CVD risk. Second, indices of wave reflection have also been associated with CVD in numerous populations independent of BP,[13, 14] possibly from detrimental effects on myocardial ejection and perfusion mechanics. Although, conventionally the process of arterial wave reflection and central pressure augmentation is considered to depend heavily on impedance mismatching in high resistance arteries and arterioles, aspects of myocardial function beyond heart rate are often neglected. In **chapter 4** we focused on the heart and studied the effects of changes in cardiac properties on arterial wave reflection and central BP augmentation. We demonstrated that alterations in left ventricular contractility and stroke volume have major effects on arterial wave reflection and central BP augmentation, by inducing changes in forward pressure wave morphology. The effects of nitroglycerin (NTG) on central pressure augmentation have been previously been attributed to reduced wave reflection by arterial smooth muscle cell relaxation and attenuation of total peripheral resistance (TPR).[15] However, NTG in anti-anginal doses has important effects on cardiac preload and myocardial contractility,[16-20] rather than afterload. We demonstrate that low-dose NTG reduces central pressure augmentation by changes in forward wave morphology that are a result of increased left ventricular contractility, in absence of changes in TPR. In addition, during postural stress, AIx decreases despite an increase in TPR and large artery stiffness.[21-24] Our data indicate that that these opposite changes in AIx during postural stress result from changes cardiac preload and ultimately stroke volume, caused by the gravitational translocation of thoracic blood to the legs.[25] This suggests that changes in myocardial performance have important effects on wave reflection and central pressure augmentation. Since indices of wave reflection have been shown to independently predict cardiovascular events, these results may be relevant for interventions targeting left ventricular contractility and cardiac preload.

Part II. Central hemodynamics, individual differences and effects of pharmacotherapy

Individual differences in central hemodynamics

Central BP and arterial wave reflection may improve hypertensive phenotyping. From a pathophysiological point of view central BP is likely to improve cardiovascular risk estimation, since it more accurately reflects the hemodynamic stress imposed on the organs at risk (i.e. the heart, brain and kidneys) than brachial BP. Indeed, data from a variety of cohorts confirms this hypothesis.[26-29] Aortic BP values are more closely associated with generally accepted markers of preclinical organ damage such as

carotid intima media thickness and left ventricular mass.[30] Furthermore, central BP readings have been shown to better predict cardiovascular morbidity and mortality than brachial BP in various populations.[14, 28, 29, 31]. Arterial wave reflection is the main determinant of differences between central and peripheral BP[5-7] and has also independently been associated with CVD risk in various populations.[13, 14]

Differences in central BP and wave reflection may aid in understanding ethnic differences in cardiovascular disease that cannot be explained by conventional risk factors.[32-35] In addition, evaluation of central BP may be important in the assessment of isolated systolic hypertension of the young[36]. In **Chapter 5** we investigated differences in wave reflection and central BP among subjects from diverse ethnic backgrounds, using baseline data of the HELIUS study: a large scale multi-ethnic cohort study, conducted in Amsterdam the Netherlands. We found that African Surinamese and Ghanaians had substantially higher central BP and South-Asians Surinamese had particularly higher augmentation index and closer central to brachial BP, compared to native Dutch, also following adjustment for conventional CVD risk factors. This provides a potential explanation for the well-known ethnic differences in CVD, beyond traditional risk assessment.

Central hemodynamics are also of particular interest in isolated systolic hypertension (ISH) of the young. ISH of the young typically affects tall adolescent males and is characterized by brachial systolic hypertension, but much lower (or "normal") central BP. Whether ISH in the young is a innocuous phenomenon caused by elastic large arteries in tall healthy subjects, with increased brachial but normal aortic systolic BP (i.e. spurious hypertension)[37-39], or is a potentially harmful condition resulting from increased arterial stiffness and larger stroke volume[36, 40, 41], is subject to debate. HELIUS provided us with a unique opportunity to study ISH of the young, since - in contrast to previous studies - HELIUS included concomitant assessment of all known determinants. In **chapter 6** we studied ISH of the young in HELIUS. We found that ISH of the young was more prevalent in males than females and in most instances was associated with larger stroke volume, more elastic arteries, lower wave reflection and lower central BP compared to other hypertensive phenotypes. Furthermore, we found that males with ISH of the young had lower central BP, AIx and large artery stiffness than their female counterparts. This may in part explain the discrepancy in CVD risk associated with ISH between genders as was found in the Chicago Heart Association Study, showing increased risk in females compared to males.[42] Besides gender differences in prevalence and (patho)physiology we also identified ethnic discrepancies in ISH, demonstrating a higher prevalence and less favourable hemodynamics in Ghanaians

compared to the other ethnic groups. The assessment of central hemodynamics in young subjects with ISH may help to distinguish ISH with low from high CVD risk.

Effects of pharmacotherapy

BP lowering drugs may affect wave reflection and have differential effects on aortic versus brachial BP. The mechanisms behind these effects are often incompletely understood,[43, 44] but may still be clinically relevant. The ASCOT trial[45] showed that an atenolol-based regime was inferior compared to an amlodipine based regime in preventing cardiovascular disease, despite an identical effect on brachial BP. A potential explanation was provided by a sub-study of ASCOT: the CAFE study,[44] demonstrating that subjects randomized to amlodipine had lower central BP compared to atenolol despite identical brachial BP. The inferior effect of atenolol on central BP was attributed to the inverse association of heart rate with wave reflection and central BP,[12] likely from an effect on ejection duration. Furthermore, vasodilating antihypertensive drugs such as calcium channel blockers or ACE inhibitors are considered to reduce wave reflection by lowering vascular resistance and have an additional beneficial effect on central BP. By extrapolation, it was postulated that newer beta-blockers such as nebivolol could circumvent the negative impact of heart rate reduction on central BP by reducing wave reflection due to their vasodilating properties. In **chapter 7** we studied the effects of the third generation (vasodilating) beta blocker nebivolol compared to metoprolol on central BP and wave reflection in a double blind-randomized cross-over study. Despite the supposed vasodilating properties, we neither found a differential effect of nebivolol versus metoprolol on central BP nor on wave reflection. Furthermore, despite previously reported changes in forearm blood flow and flow mediated dilatation induced by nebivolol we did not find a differential effect on TPR. Nonetheless, previous studies also suggest that nebivolol may improve endothelial function and elicit anti-proliferative effects. Whether nebivolol has superior efficacy on outcome compared to other beta-blockers, and how this compares to other BP lowering drugs remains to be determined in large prospective trials.

Part III. Renal Sympathetic Denervation

While central hemodynamics may provide an important contribution to cardiovascular risk assessment and potentially guide therapy, current BP-lowering treatment options fall short as many subjects do not reach their desired BP goal. In recent years, catheter-based renal sympathetic denervation (RSD) has emerged as a promising new treatment modality for refractory hypertension.[46, 47] The renal sympathetic nervous system is an important player in the complex pathophysiology of hypertension, as demonstrated in both experimental as well as human studies.[48-54] Theoretically, denervation of

efferent renal sympathetic nerves reduces renin secretion, salt and water retention and enhances renal blood flow. Disruption of afferent renal sympathetic signalling may lower overall central sympathetic outflow, resulting in lower BP by attenuation of peripheral resistance, heart rate, as well as reduced sympathetic drive to kidneys and adrenal glands.

The concept of lowering BP by interrupting the nerves between kidneys and the central sympathetic nervous system is not new. Historically, non-selective surgical sympathectomy, or 'splanchnicectomy', was performed in patients with severe or malignant hypertension in the era where effective antihypertensive therapy was lacking.[55] In these patients, it was demonstrated that surgery reduced sympathetic outflow to the kidneys, increased natriuresis and diuresis, and decreased renin release, without adversely affecting other functions of the kidney. Thanks to technological advancements, selective, minimally-invasive, catheter based RSD was introduced by Medtronic Ardian, using a single point radiofrequency catheter. Following little preclinical studies, the non-sham-controlled Symplicity-HTN 1 and 2 trials, that showed major reductions in office BP in subjects with refractory hypertension, were received with great enthusiam.[46, 47] However in contrast to surgical renal denervation, evidence for ablation effectiveness -i.e. reductions in renal sympathetic activation and renin release or increased natriuresis- following catheter based RSD was lacking.

The initial enthusiasm following Symplicity-HTN 1 and 2 was abolished by the negative results of the Simplicity-HTN 3 trial, that failed to show a beneficial effect on BP of RSD versus sham treatment.[56] The cause for the failure of RSD in Simplicity-HTN 3 is subject to debate. Several non-procedure related effects, including a placebo effect, better medication adherence and regression to the mean may have influenced the outcome of Symplicity-HTN 3. In addition, individual differences in overall and renal sympathetic nerve activity, as well as the contribution of the kidneys to central sympathetic drive, may have affected outcome. Furthermore, although apparently effective in a pre-clinical setting, firm evidence that catheter-based RSD actually does what it's supposed to do in humans –i.e. sympathetically denervate the kidneys- is lacking.

In **chapter 8** we therefore studied the effects of catheter based RSD on renal sympathetic activity. Previous data regarding effectiveness of the ablation of efferent renal sympathetic nerves is scarce. Pre-clinical studies in pigs showed that RSD resulted in a reduction of renal noradrenalin spill-over and content of 80-90%.[46] Yet in humans, proof of efferent sympathetic denervation is less compelling. Symplicity-HTN 1 investigated nor-adrenalin spill-over in ten patients undergoing RSD and found an average reduction of 47% with a large variation (95% CI 28-65%). These

results have not been reproduced, nor has any other convincing proof of efferent denervation been published. The noradrenalin spill-over results of Simplicity-HTN 1 imply at least that efferent denervation is incomplete. A limiting factor lies within the method of assessment as noradrenalin spill-over studies are cumbersome (involving catheterisation of renal artery and vein) and a lack of radioisotopes makes it impossible in many countries. In **chapters 8** and **10**, we therefore set out to use a readily applicable non-invasive alternative to assess the effectiveness of ablation by using 123I-mIBG scintigraphy. This nuclear imaging technique uses a labelled 'false' noradrenalin analogue to visualize organ specific sympathetic activity. **Chapter 10** is a case report of a subject undergoing renal autotransplantation for fibromuscular dysplasia. We observed a reduction in the autotransplanted kidney's 123I-mIBG uptake and washout, indicating the feasibility of this imaging technique. Conversely, in **chapter 8** we performed 123I-mIBG imaging prior to and following RSD, yet we found no change in 123I-mIBG uptake, nor washout following renal denervation. In addition, no change in plasma renin activity was observed after RSD.

Part of the supposed BP lowering effects of renal denervation is attributed to a reduction in afferent renal sympathetic nerve signalling, leading to reduced central sympathetic outflow. Besides lowering BP, this could theoretically give rise to other pleotropic effects, for example on insulin resistance, cardiac arrhythmias and heart failure. Data regarding the effects of RSD on sympathetic nervous activity are conflicting. While some studies showed lower muscle sympathetic nervous activity (i.e. the gold standard for overall sympathetic outflow) following RSD,[57] other studies found it unchanged.[58, 59] In **chapter 9** we studied the effects of RSD on cardiac 123I-mIBG uptake and washout to assess the effect on cardiac sympathetic activity and found it unaffected by RSD. In addition baroreflex sensitivity and urine cathecholamin excretion also remained unchanged. These results are in support of an at best marginal effect of single point, catheter based radiofrequency ablation of renal sympathetic nerves on central sympathetic nervous system activity.

Future perspectives

Central hemodynamics

A growing body of evidence favours the assessment of central hemodynamics in cardiovascular risk prediction. While the added value may be modest in the general population, improved phenotyping by means of central hemodynamics may have a major impact on certain hypertensive subgroups. In this thesis we demonstrated substantial differences in central hemodynamics among subjects of various ethnic backgrounds, providing a potential explanation for the as yet unexplained ethnic

differences in cardiovascular disease. In addition, we found that South-Asian subjects had a much higher central to brachial BP, when compared to other ethnic groups. This implies that South-Asian subjects in particular may be at increased risk of being undertreated if treatment is based on brachial rather than central BP. Vice versa, overtreatment of BP may occur if antihypertensive therapy is initiated in subjects with ISH of the young . It is plausible that subjects with ISH of the young who have raised peripheral, but low central BP, will not benefit from antihypertensive drugs that are frequently prescribed based on brachial BP readings. Despite a great potential for central hemodynamic assessments in antihypertensive treatment strategies, several issues have to be addressed. First, much of the pathophysiological mechanisms involved in central hemodynamics are unclear. We provide evidence for an important role for the heart. Second, several devices are currently available to non-invasively assess central hemodynamics, including piezoelectric, tonometric and newer, more operator friendly, oscillometric devices. These new techniques all have their pros and cons with a tendency to under- (peizoelectronic and tonometric) or overestimation (oscillometric) of true central BP values. The widely accepted and easily implementable non-invasive standard for central BP estimation remains to be chosen. Beyond methodological issues, reference values for central BP are difficult to assess. This is complicated by the fact that BP is strongly dependent on age and gender. Recently typical values for central BP, stratified by age and gender, in a disease free population, have been published.[60] Still the question remains what values should be considered as 'desirable' or 'raised' central BP. Last but not least, whether lowering one's central BP or wave reflection actually lowers cardiovascular risk is -although plausible- formally still unclear, as interventional trials guided by central hemodynamics are lacking. Some evidence is provided by the BP GUIDE study,[61] that demonstrates that the use of central BP guided therapeutic algorithms did not result in increased target organ damage, while using less antihypertensive drugs. However, a true beneficial effect of central BP guided or targeted therapy remains to be shown in a general population or in particular subgroup of interest.

Renal sympathetic denervation

While there is a great demand for better antihypertensive treatment, renal sympathetic denervation did not deliver. However, whether or not catheter-based RSD should be considered as a definite failure remains to be established. Based on the anatomy of the renal nerves, it seems clear that by using current techniques, complete ablation of the renal nerves will not be possible. The extent of renal denervation that is required to elicit a relevant effect on BP is unknown. Post-mortem examination of a patient that underwent RSD, revealed that ablation was far from complete.[62] In a sub-analysis

of Simplicity-HTN 3, a larger fall in BP was observed in subjects that received more extensive renal ablation.[56] While multiple biotech companies were eager to develop their own renal denervation catheters after Simplicity-HTN 1 and 2 and found similar results in their proof of principle and safety studies, research was discontinued following Simplicity-HTN 3. This seems unfortunate as these newer devices employed multiple ablation points, possibly resulting in a more effective ablation. Since ineffectiveness of denervation is considered an important cause of the absence of a significant BP lowering effect following catheter based RSD, data regarding the effectiveness of multi-tip devices could be valuable. With renal 123-IMBG we provided a potential technique to non-invasively assess the effectiveness of ablation. The concept of intervening with the intricately designed sympathetic crosstalk between brain and kidneys is interesting and with ample circumstantial evidence justifying it as a treatment target. However, the expeditious introduction of RSD from hardly any pre-clinical data to man is erroneous. A solid pre-clinical basis should be laid prior to initiating human studies. Once the step from mouse to man is made, at least a properly designed and well controlled trial is paramount to assess the antihypertensive potential of new treatment strategies.

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Chapter 12

Addenda

Nederlandse samenvatting
Authors & Affiliations
Portfolio
Dankwoord

Samenvatting en discussie

In dit proefschrift maken wij gebruik van nieuwe technieken om onze kennis op het gebied van de pathofysiologie en behandeling van hypertensie te vergroten. Hierbij besteden wij onder andere aandacht aan vernieuwingen in de karakterisering (c.q. fenotypering) van hypertensie en antihypertensieve behandelstrategieën. In het eerste deel van het proefschrift (**deel I**) staan wij stil bij de centrale hemodynamiek en de pathofysiologie ervan. Het tweede deel (**deel II**) van dit proefschrift richt zich op individuele verschillende in centrale hemodynamiek en de effecten van farmacotherapie. Tot slot onderzoeken wij in het derde deel van dit proefschrift (**deel III**) de effecten van renale sympathicus denervatie (RSD) op sympathische zenuwactiviteit, waarbij wij gebruik maken van 123I-mIBG scintigrafie scans.

Hoofdstuk 1 is een algemene inleiding en overzicht van dit proefschrift. Hart- en vaatziekten zijn een grote bedreiging voor de mondiale volksgezondheid en hypertensie is de belangrijkste risicofactor. Om de gezondheidszorg op het gebied van hart- en vaatziekten te verbeteren is een beter begrip van hypertensie en haar behandeling cruciaal. Hiervoor zijn nieuwe benaderingen op het gebied van diagnostiek en therapie nodig. Het beoordelen van de centrale hemodynamiek kan leiden tot een betere karakterisering van patiënten met hypertensie dan conventionele spreekkamermetingen en kan verschillen in de effectiviteit van antihypertensiva verklaren. Een bredere kennis van de pathofysiologie van de centrale hemodynamiek, individuele variaties en effecten van farmacotherapie, is daarom van groot belang (**deel I en II**). Renale sympathicus denervatie (RSD) is een nieuwe behandelmethode voor patiënten met therapieresistente hypertensie. RSD kent echter een zeer wisselend therapeutisch effect, hetgeen mogelijk verklaard kan worden door individuele verschillen in sympathische zenuwactiviteit en/of effectiviteit van de denervatie (**deel 3**). In het derde deel van dit proefschrift maken we gebruik van 123I-mIBG scintigrafie om op niet invasieve wijze de effectiviteit van renale denervatie te onderzoeken.

Deel 1. Meting en pathofysiologie van centrale hemodynamiek

Continue en niet-invasief meten van de bloeddruk en hemodynamiek.

Al meer dan een eeuw is de gouden standaard voor het niet-invasief meten van de bloeddruk de methode van Riva-Rocci Korotkoff, waarbij gebruik wordt gemaakt een van sphygmomanometer en stethoscoop. Met de vooruitgang der techniek is het heden mogelijk om op meer geavanceerde wijze de bloeddruk te meten. De Nexfin® bijvoorbeeld, maakt gebruikt van volume-klem plethysmografie volgens de Finapres methode[1] waarbij met een bandje om een vinger continue de bloeddruk

en hemodynamische waarden (oa. slagvolume, systemische vaatweerstand en linker ventrikel contractiliteit) bepaald kunnen worden.[2] Het op deze manier, niet invasief en continue meten van de bloeddruk en hemodynamiek is van grote waarde in de dagelijkse praktijk en voor wetenschappelijk onderzoek. In de reguliere zorg wordt van deze techniek gebruik gemaakt in het kader van autonome functietesten bij personen met bijvoorbeeld syncope. Op het gebied van research biedt het een makkelijk toepasbare methode om bijvoorbeeld hemodynamische metingen te doen in grote populaties of bij experimenten die gepaard gaan met snelle veranderingen in bloeddruk en hemodynamiek zoals bijvoorbeeld de kiepproef, valsalva maneuver of acuut werkende farmaca.

In **hoofdstuk 2** van dit proefschrift hebben wij de nauwkeurigheid van de niet invasieve, continue bloeddrukmeting van de Nexfin® gevalideerd, vergeleken met Riva-Rocci Korotkoff, in een willekeurige onderzoekspopulatie.[3] Wij laten zien dat de Nexfin® de brachiale bloeddruk, volgens de richtlijnen van de Association for the Advancement of Medical Instrumentation, nauwkeurig bepaalt. Dankzij de continue, slag-op-slag metingen van de Nexfin® is het tevens mogelijk om snel optredende veranderingen in hemodynamiek, onder invloed van de arteriële baroreflex, te bestuderen. De arteriële baroreflex bestaat uit een cardiale en een vasomotorische arm. In het algemeen wordt bij baroreflexgevoeligheid gekeken naar de cardiale baroreflex: de snelle veranderingen in hartfrequentie in reactie op schommelingen in bloeddruk die worden waargenomen door de baroreceptoren in de halsslagaderen. De tragere vasomotorische arm van de baroreflex -die verantwoordelijk is voor veranderingen in de vaatwandtonus en daarmee de perifere vaatweerstand reguleert- wordt echter vaak over het hoofd gezien. Doordat de Nexfin® gelijktijdig de bloeddruk, hartfrequentie en systemische vaatweerstand registreert, is het hiermee mogelijk om beide armen van de arteriële baroreflex te bestuderen. In **hoofdstuk 3** hebben wij de determinanten van cardiale en vasculaire baroreflexgevoeligheid bestudeerd in dezelfde populatie als in hoofdstuk 2. Wij laten zien dat deze determinanten verschillend zijn en ondersteunen hiermee de aanname dat zij inderdaad verschillende modaliteiten reflecteren van cardiovasculair autonoom functioneren.

(Patho)fysiologie van centrale hemodynamiek

In de dagelijkse praktijk wordt de bloeddruk met behulp van een stuwband om de bovenarm gemeten. Het is echter bekend dat er grote verschillen in bloeddruk bestaan tussen de grotere slagaderen in het lichaam. Terwijl de diastolische bloeddruk relatief constant is, kan de systolische bloeddruk tot wel 40 mmHg hoger zijn in de bovenarm (a. brachialis) dan in de grote lichaamsslagader (aorta).[4] Verschillen tussen

centrale (aorta) en perifere (a. brachialis) systolische bloeddruk zijn een gevolg van polsgolfreflectie en bloeddrukaugmentatie.[5-7] Kort toegelicht betref dit proces het volgende. De pompende werking van hart genereert drukgolven die zich van het hart door de arteriële vaatboom naar de periferie verplaatsen. Als gevolg van impedantieverschillen onderweg, bijvoorbeeld ter hoogte van weerstandsvaten of ter plaatse van aftakkingen, vindt weerkaatsing van deze voorwaartse drukgolven plaats. Hierbij ontstaan er gereflecteerde, ofwel achterwaartse drukgolven die zich in de tegengestelde richting van de voorwaartse golven verplaatsen (c.q. terug richting het hart). Het 'botsen' van voorwaartse en achterwaartse drukgolven kan, afhankelijk van hun onderlinge timing, grootte en vorm, leiden tot verhoging van de systolische bloeddruk. Deze systolische bloeddrukaugmentatie wordt in het algemeen gekwantificeerd als de augmentatieindex.[5-7] Verschillende factoren kunnen het proces van polsgolfreflectie en bloeddrukaugmentatie, en dus ook het verschil tussen de perifere en centrale bloeddruk, beïnvloeden. Het is onder andere bekend dat de arteriële vaatstijfheid, systemische vaatweerstand, hartfrequentie en lichaamslengte hierbij van belang zijn.[8-12] Het (patho)fysiologisch proces is echter nog onvoldoende onbegrepen, maar is om verscheidene redenen van belang. Allereerst kan polsgolfreflectie leiden tot een verhoging van de centrale bloeddruk en daarmee het cardiovasculair risico verhogen. Ten tweede zijn indices van polsgolfreflectie, onafhankelijk van de hoogte van de bloeddruk, zelf ook geassocieerd met hart- en vaatziekten in verscheidene populaties[13, 14]. Deze associatie is mogelijk o.a. een gevolg van de ongunstige effecten van de weerkaatste bloeddrukgolven op de ejectiefase en diastolische doorbloeding van het hart. Ofschoon men er in het algemeen vanuit gaat dat het proces van bloeddrukgolfreflectie en centrale bloeddrukaugmentatie vrijwel geheel afhankelijk is van het proces van impedantieverschillen in de arteriële vaatboom, wordt de rol van het hart -behoudens frequentie- vaak over het hoofd gezien. In **hoofdstuk 4** hebben wij ons gericht op het hart. We hebben de effecten van veranderingen in cardiale eigenschappen op polsgolfreflectie en centrale bloeddrukaugmentatie bestudeerd. We laten zien dat veranderingen in contractiliteit en slagvolume hier grote effecten op hebben door een effect op de morfologie van de voorwaartse drukgolven. De effecten van nitroglycerine (NTG) op centrale bloeddrukaugmentatie zijn in het verleden steeds toegeschreven aan vermindering van polsgolfreflectie ten gevolge van relaxatie van gladspierweefsel in weerstandsvaten en dientengevolge een verlaging van de systemische vaatweerstand.[15-19] In antiangineuze doseringen heeft NTG juist belangrijke effecten op met name de cardiale preload en contractiliteit, niet zozeer op de afterload. Wij laten zien dat een lage dosis NTG de centrale bloeddrukaugmentatie verminderd door veranderingen in de vorm van voorwaartse bloeddrukgolven, in afwezigheid van veranderingen in systemische vaatweerstand, mogelijk door een effect

op contractiliteit. Voorts is het bekend dat de ten tijde van de houdingsverandering van een liggen naar staan de augmentatieindex daalt, ondanks een stijging in systemische vaatwaarstand een arteriële vaatstijfheid.[20-23] Uit onze data blijkt dat deze tegengestelde veranderingen in AIx ten tijde van orthostatische stress het meest waarschijnlijk een gevolg zijn van veranderingen in preload en uiteindelijk slagvolume doordat de zwaartekracht bloed naar de benen verplaatst.[24] Via verschillende wegen suggereert ons onderzoek dat verscheidene eigenschappen van het hart belangrijk zijn in het proces van polsgolfreflectie en centrale bloeddrukaugmentatie. Aangezien polsgolfreflectie onafhankelijk is geassocieerd met het optreden van cardiovasculaire events en tevens de centrale bloeddruk kan verhogen, kunnen onze resultaten relevant zijn voor interventies die effect hebben op contractiliteit en preload.

Deel II. Centrale hemodynamiek, individuele verschillen en effecten van farmacotherapie

Individuele verschillen in centrale hemodynamiek

Metingen van centrale bloeddruk en polsgolfreflectie kunnen mogelijk leiden tot een betere fenotypering van patiënten met hypertensie. Vanuit een pathofysiologisch oogpunt is het aannemelijk dat de centrale bloeddruk de cardiovasculaire risico-inschatting verbetert, doordat de daadwerkelijke hemodynamische stress, die op de organen van interesse (hart, hersenen en nieren) wordt uitgeoefend, beter wordt weerspiegelt dan de bloeddruk in de bovenarm. Deze hypothese wordt bevestigd door data uit verscheidene cohorten.[25-28] Centrale bloeddrukwaarden zijn nauwer geassocieerd met markers van preklinische orgaanschade zoals a. carotis intima-media-dikte en linker ventrikel massa dan brachiale bloeddrukwaarden.[29] Daarnaast is het in verschillende populaties aangetoond dat de centrale bloeddruk cardiovasculaire morbiditeit en mortaliteit beter voorspelt dan de brachiale bloeddruk.[14, 27, 28, 30] Tot slot is, zoals reeds besproken, polsgolfreflectie -de belangrijkste determinant van verschillen tussen centrale en perifere bloeddruk-[5-7] zelf ook onafhankelijk geassocieerd met hart- en vaatziekten in verschillende studies.[13, 14]

Verschillen in centrale bloeddruk en polsgolfreflectie kunnen helpen in het beter begrijpen van etnische verschillende in hart- en vaatziekten die niet verklaard kunnen worden op basis van de conventionele risicofactoren.[31-34] Daarnaast kan het meten van centrale bloeddruk van belang zijn bij het beoordelen van geïsoleerde systolische hypertensie onder adolescenten en jongvolwassenen.[35] In **hoofdstuk 5** hebben wij verschillen in polsgolfreflectie en centrale bloeddruk onderzocht in personen van diverse etniciteiten. Wij hebben hierbij dankbaar gebruik gemaakt van baseline data van de HEalthy Life In an Urban Setting (HELIUS) studie: een grootschalige multi-etnische cohort studie, uitgevoerd in Amsterdam. Uit onze data bleek dat Afrikaans Surinaamse

en Ghanese deelnemers een aanzienlijk hogere centrale bloeddruk hadden, en met name de Hindoestaans Surinaamse deelnemers een hogere augmentatie index en kleiner verschil tussen centrale en perifere bloeddruk hadden, in vergelijking met de autochtone Nederlanders. Deze verschillen tussen de etnische groepen bleven bestaan na correctie voor conventionele cardiovasculaire risicofactoren. Hiermee geven we een mogelijke verklaring voor de algemeen bekende etnische verschillen in hart- en vaatziekten die niet verklaard kunnen worden met de conventionele risicoschatting.

Centrale hemodynamica zijn in het bijzonder relevant bij adolescenten en jongvolwassenen met geïsoleerde systolische hypertensie (ISH). Het betreft hier veelal jonge, slanke mannen en wordt gekarakteriseerd door brachiale systolische hypertensie, met een vaak veel lagere (of "normale") centrale bloeddruk. Er is echter nog veel discussie of ISH bij jongere personen daadwerkelijk een onschuldig fysiologisch fenomeen is dat wordt veroorzaakt door elastische bloedvaten bij lange mensen met een verhoogde brachiale, maar normale centrale bloeddruk (ook wel spurious hypertension),[36-38] of dat er sprake is van een potentieel schadelijke aandoening die een gevolg is van toegenomen vaatstijfheid en een groter slagvolume.[35, 39, 40] HELIUS gaf ons een unieke kans om ISH in jongvolwassen te onderzoeken, omdat bij HELIUS -in tegenstelling tot eerdere studies- alle eerder veronderstelde determinanten gemeten zijn. In **hoofdstuk 6** hebben wij ISH in jongvolwassen deelnemers van HELIUS onderzocht. Personen met ISH hadden in het algemeen een groter slagvolume, een lagere vaatstijfheid, polsgolfreflectie en centrale bloeddruk en waren langer dan personen met andere vormen van hypertensie. Wij vonden een hogere prevalentie van ISH in mannen dan in vrouwen <40 jaar. Mannen met ISH hadden een lagere centrale bloeddruk, AIx en vaatstijfheid dan vrouwen met ISH. Dit biedt een mogelijk verklaring voor het hogere cardiovasculaire risico van jonge vrouwen met ISH vergeleken met jonge mannen zoals is geobserveerd in de Chicago Heart Association Study.[41] Naast sekse verschillen in prevalentie en (patho)fysiologie vonden wij ook etnische verschillen bij personen met ISH. Onder Ghanese deelnemers was er sprake van een hogere prevalentie van ISH, maar een ongunstiger hemodynamisch profiel vergeleken met de andere etnische groepen. Het beoordelen van de centrale hemodynamiek bij jonge personen met ISH kan mogelijk helpen bij het onderscheiden van ISH dat gepaard gaat met een laag of verhoogd cardiovasculair risico.

Effecten van farmacotherapie

Bloeddrukverlagende medicijnen kunnen van invloed zijn op polsgolfreflectie en een differentieel effect uitoefenen op de aortale versus brachiale bloeddruk. De hiervoor verantwoordelijke mechanismen zijn vaak onvolledig begrepen,[42, 43] maar

desalniettemin mogelijk wel klinisch relevant. In de ASCOT studie werd aangetoond dat een op atenolol gebaseerde antihypertensieve behandeling inferieur was vergeleken met amlodipine in het voorkomen van hart- en vaatziekten, ondanks dat zij een gelijk effect hadden op de brachiale bloeddruk.[44] Een mogelijke verklaring hiervoor werd geboden door een sub-studie van ASCOT: de CAFE studie.[43] Hierin werd aangetoond dat ondanks de gelijke brachiale bloeddruk, personen die met amlodipine behandeld werden een lagere centrale bloeddruk hadden dan personen die naar atenolol waren gerandomiseerd. Het inferieure effect van atenolol werd toegeschreven aan de omgekeerd evenredige associatie van hartfrequentie met polsgolfreflectie en centrale bloeddruk, waarschijnlijk door een effect op ejectieduur.[12] Daarnaast verlagen vaatverwijdende antihypertensiva -zoals calciumkanaal blokkers of ACE-remmers- polsgolfreflectie (mogelijk door verlaging van de systemische vaatweerstand) en hebben ze een additioneel gunstig effect op de centrale bloeddruk. Het werd derhalve gepostuleerd dat derde generatie beta-blockers -zoals nebivolol en carvedilol- het negatieve effect op polsgolfreflectie en centrale bloeddruk door hartfrequentieverlaging, dankzij hun vaatverwijdende eigenschappen, zouden kunnen verminderen. In **hoofdstuk 7** onderzochten wij de effecten van de derde-generatie (vaatverwijdende) beta-blocker nebivolol vergeleken met metoprolol op centrale bloeddruk en polsgolfreflectie in een dubbelblinde, gerandomiseerde cross-over studie. Ondanks de toegedichte vaatverwijdende eigenschappen, vonden wij geen verschil in effect tussen nebivolol en metoprolol op centrale bloeddruk, noch op polsgolfreflectie. Voorts vonden wij, ondanks eerder gerapporteerde vaatverwijdende effecten van nebivolol op onderarms doorbloeding en stroom-gemedieerde vasodilatie, geen differentieel effect tussen beide beta-blockers op systemische vaatweerstand. Nochtans suggereren eerdere studies dat nebivolol de endotheelfunctie kan verbeteren en antiproliferatieve effecten heeft. Of nebivolol een superieur effect heeft op cardiovasculaire uitkomst vergeleken met andere beta-blockers, en hoe zich dit verhoudt met andere bloeddrukverlagende medicatie, valt te bezien in grote prospectieve studies.

Deel III. Renale sympathicus denervatie

Terwijl centrale hemodynamica van belangrijke toegevoegde waarde kunnen zijn bij het bepalen van het cardiovasculair risico en het zo mogelijk ook sturen van therapie, schieten de huidige behandelmogelijkheden voor patiënten met hypertensie te kort. Ondanks een scala aan beschikbare antihypertensieve medicatie wordt in vele gevallen de beoogde bloeddrukverlaging niet behaald. In de afgelopen jaren is renale sympathicus denervatie (RSD) ten tonele verschenen als een veelbelovende nieuwe behandeloptie voor patiënten met therapieresistente hypertensie.[45, 46] Het renale

sympathische zenuwstelsel is een belangrijk speler is in de complexe pathofysiologie van hypertensie, zo blijkt uit experimentele alsook uit humane studies.[47-53] In theorie leidt de denervatie van efferente renale sympathische zenuwvezels tot een verminderde renine afgifte, een afname van water- en zoutretentie en een toegenomen doorbloeding van de nier. Het onderbreken van de afferente sympathische signalen van nier naar het centrale zenuwstelsel kan een lagere centrale sympathische zenuwactiviteit bewerkstelligen, hetgeen op zijn beurt direct in een verlaging van de bloeddruk kan resulteren door afname van de systemische vaatweerstand, verlaging van de hartfrequentie alsmede indirect door afname van de sympathische activiteit naar de nieren en bijnieren.

Het concept van het verlagen van de bloeddruk door de zenuwbanen tussen de nieren en het centrale sympathische zenuwstelsel te onderbreken is niet nieuw. Vanuit een historisch perspectief, werd in het tijdperk voor de introductie van effectieve bloeddrukverlagende medicatie, een niet-selectieve chirurgische sympathectomie of 'splanginectomie' uitgevoerd bij patiënten met ernstige of maligne hypertensie.[54] Bij patiënten die deze ingreep ondergingen werd ook aangetoond dat na de procedure de sympathische zenuwactiviteit naar de nieren en renine afgifte afnamen en dat de natriumexcretie en urineproductie toenamen, zonder dat de nierfunctie negatief beïnvloed werd. Ondanks dat de procedure effectief was in het verlagen van de bloeddruk, had deze grote chirurgische ingreep aanzienlijke risico's en vervelende bijwerkingen. Met dank aan technologische ontwikkelingen werd selectieve, minimaal invasieve RSD met behulp van een radiofrequentieablatie katheter door Medtronic Ardian geïntroduceerd. Na weinig preklinisch onderzoek werden al spoedig de resultaten van de niet-gecontroleerde Symplicity-HTN 1 en 2 trials gepresenteerd. Deze studies lieten indrukwekkende bloeddrukverlagende effecten lieten zien bij patiënten met therapieresistente hypertensie en deze resultaten werden dan ook met veel enthousiasme ontvangen.[45, 46] In tegenstelling tot de chirurgische denervatie werd echter geen overtuigend bewijs geleverd dat RSD met behulp van een radiofrequentieablatie katheter ook daadwerkelijk de sympathische zenuwactiviteit naar de nieren verlaagde.

Het aanvankelijke enthousiasme, waarmee de resultaten van de niet-gecontroleerde Symplicity-HTN 1 en 2 studies waren ontvangen, was echter spoedig over na de negatieve uitkomst van de Symplicity-HTN 3 studie. Uit deze gecontroleerde studie bleek namelijk geen enkel gunstig effect van RSD op de bloeddruk vergeleken met een schijnbehandeling.[55] De oorzaak voor het falen van RSD in de Symplicity-HTN 3 studie is tot op heden nog onderwerp van discussie. Verscheidene, niet aan de behandeling gerelateerde effecten, waaronder een placebo effect, betere therapietrouw en

regressie naar het gemiddelde, kunnen van invloed zijn geweest op de uitkomst van Symplicity-HTN 3. Daarnaast is het mogelijk dat ook individuele verschillen in centrale en renale sympathische zenuwactiviteit, evenals de bijdrage van de nieren aan de centrale activiteit, een rol hebben gespeeld. Tot slot ontbreekt, in tegenstelling tot de operatieve denervatie, sluitend bewijs dat de procedure bij mensen ook daadwerkelijk doet wat het zou moeten doen: de sympathische zenuwvezels van en naar de nieren onderbreken.

In **hoofdstuk 8** onderzochten wij de effecten van RSD met een radiofrequentie-ablatie katheter op renale sympathische zenuwactiviteit. Zoals eerder gezegd, ontbreekt hard bewijs dat de ablatie volgens deze methode effectief is. In beperkt, preklinisch onderzoek werd aangetoond dat RSD bij varkens resulteerde in een afname van renale noradrenaline concentratie en spill-over van 80 tot 90%.[45] In mensen is het bewijs van efferente sympathische denervatie van de nieren echter niet zo overtuigend. In Symplicity HTN-1 werd, in een subset van tien personen die RSD ondergingen, de renale noradrenaline spillover onderzocht. Hierbij vond men na RSD een afname van gemiddeld 47% met een grote variatie in effect(95% CI 25-65%). Deze resultaten zijn niet geproduceerd, noch is er ander overtuigend bewijs, zoals bijvoorbeeld effecten op renine afgifte of natriumexcretie, gepubliceerd van efferente renale denervatie. De noradrenaline spillover resultaten van Symplicity-HTN 1 suggereren ten minste dat de efferente denervatie incompleet is. Een belangrijke beperkende factor is de complexiteit en belasting van noradrenaline spill-over onderzoek, waarbij zowel de arterie als de vene van de nier(en) gekatheteriseerd moeten worden. Daarnaast zijn de benodigde radio-isotopen veelal niet verkrijgbaar, hetgeen een dergelijk onderzoek in veel landen simpelweg onmogelijk maakt. In **hoofdstuk 8** en **10** hebben we door gebruik te maken van 123I-mIBG scintigrafie naar een algemeen beschikbaar, niet-invasief alternatief gezocht om de effectiviteit van de ablatie bij RSD te onderzoeken. Deze nucleair geneeskundige, beeldvormende techniek maakt gebruik van een 'vals' noradrenaline analoog om orgaan specifieke sympathische zenuwactiviteit te visualiseren. **Hoofdstuk 10** betreft een casus van een patiënt die een transplantatie van zijn eigen nier ondergaat in verband met fibromusculaire dysplasie. Wij vonden een afname van 123-I mIBG opname en uitwassing in de getransplanteerde nier, als indicatie voor de potentie van deze beeldvormende techniek. In het onderzoek beschreven in **hoofdstuk 8** hebben wij 123-I mIBG scans verricht vóór en na RSD met radiofrequentie ablatie katheter. Wij vonden hierbij echter geen effect van RSD op de renale 123-I mIBG opname, noch op de uitwas. Daarnaast werden er ook geen effecten gezien op plasma renine activiteit na RSD.

Een deel van het veronderstelde bloeddrukverlagende effect van RSD werd toegeschreven aan een afname in afferente renale sympathische zenuwsignalen, met verlaging van de centrale sympathische zenuwactiviteit tot gevolg. Naast een mogelijk effect op de bloeddruk, zou dit in theorie ook andere gunstige neveneffecten kunnen hebben op bijvoorbeeld insulinegevoeligheid, hartfalen en hartritmestoornissen. De data met betrekking tot de effecten van RSD op sympathische zenuwactiviteit zijn echter niet eenduidig. In sommige studies werd na RSD een afname van sympathische zenuwactiviteit in spieren (de gouden standard voor het meten van algehele sympathische activiteit) geobserveerd,[56] terwijl deze in andere onderzoeken onveranderd bleef.[57, 58] In **hoofdstuk 9** onderzochten wij de effecten van RSD op cardiale 123I-mIBG opname en uitwassing. RSD bleek hierbij geen effect te hebben op cardiale sympathische zenuwactiviteit. Daarnaast bleven ook baroreflexgevoeligheid en urine cathecholamine excretie onveranderd. Onze resultaten ondersteunen de gedachte dat RSD met behulp van een enkelpunts radiofrequentie ablatie katheter op zijn best niet meer dan een marginaal effect heeft op renale en centrale sympathische zenuwactiviteit.

Perspectieven

Central hemodynamiek

Er is een toenemend bewijs ten faveure van de beoordeling van de centrale hemodynamiek bij het opstellen van een cardiovasculair risicoprofiel. Alhoewel de toegevoegde waarde hiervan in de algemene populatie waarschijnlijk beperkt is, kan een betere fenotypering van patiënten met hypertensie door middel van metingen van de centrale hemodynamiek van grote waarde zijn in bepaalde subgroepen. In dit proefschrift laten wij zien dat er aanzienlijke verschillen bestaan in de centrale hemodynamiek tussen personen van verschillende etnische komaf. Hiermee bieden wij een mogelijke verklaring voor de tot op heden onbegrepen etnische verschillen in cardiovasculaire ziekten. Daarnaast vonden wij dat bij Hindoestaans Surinaamse personen vergeleken met andere etnische groepen de centrale bloeddruk veel dichter bij de brachiale bloeddruk ligt. Dit impliceert dat deze Hindoestaans Surinaamse mensen het risico lopen om onderbehandeld te worden wanneer de brachiale bloeddruk als leidraad wordt gebruikt. Vice versa bestaat er in jongvolwassenen met ISH het risico op overbehandeling wanneer de bloeddruk in de bovenarm wordt gemeten. Het is aannemelijk dat de jongvolwassenen met ISH, die weliswaar een verhoogde brachiale maar normale of lage centrale bloeddruk hebben, geen baat hebben -en misschien juist wel schade ondervinden- bij een bloeddrukverlagende behandeling die dikwijls wél wordt voorgeschreven op basis van de brachiale bloeddrukmetingen. Ondanks een groot potentieel van centrale hemodynamische

metingen in de behandeling van hypertensie, dienen verschillende vraagstukken nog te worden opgelost. In de eerste plaats is nog veel van de pathofysiologie van de centrale hemodynamiek, onduidelijk. Wij leveren bewijs voor een belangrijke rol van het hart. Ten tweede zijn er verschillende apparaten beschikbaar om op niet-invasieve wijze centrale hemodynamische metingen te doen. Zo zijn er onder andere piezoelektronisch, tonometrisch en nieuwere, meer gebruiksvriendelijke, oscillometrische apparaten. Al deze technieken hebben hun eigen voor- en nadelen, waarbij de piezoelektronische en tonometrische apparaten de neiging hebben de centrale bloeddruk te onderschatten en de oscillometrische deze kunnen overschatten. Een algemeen geaccepteerde en makkelijke toepasbare standaard voor het niet invasief meten van de centrale bloeddruk dient nog gekozen te worden. Naast methodologische problemen zijn referentiewaarden voor de centrale bloeddruk lastig vast te stellen. Dit wordt verder bemoeilijkt door het feit dat de centrale bloeddruk onder andere afhankelijk is van de leeftijd en het geslacht. Recent zijn er gemiddelde centrale bloeddrukwaarden gepubliceerd, gestratificeerd naar leeftijd en geslacht, in een populatie zonder hart- en vaatziekten.[59] Desalniettemin resteert de vraag wat men als een 'wenselijke' of 'te hoge' centrale bloeddruk zou moeten beoordelen. Als laatste is het –alhoewel plausibel- formeel onbekend of het verlagen van de centrale bloeddruk of polsgolfreflectie ook daadwerkelijk het cardiovasculaire risico verlaagt, omdat interventie studies die sturen op de centrale hemodynamiek schaars zijn. Enig bewijs wordt geleverd door de BP GUIDE studie,[60] waarin men laat zien dat het gebruik van centrale bloeddrukmetingen als behandelstrategie leidt tot het gebruik van minder antihypertensiva zonder een nadelig effect te hebben op eindorgaanschade. Een werkelijk voordelig effect van centrale bloeddruk gestuurde of gerichte therapie in de algemene populatie of een bepaalde deelgroep dient nog te worden aangetoond in groter prospectief onderzoek.

Renale sympathicus denervatie

Ondanks een grote vraag naar betere bloeddrukverlagende behandelingen, slaagde renale sympathisch denervatie er helaas niet in om aan de verwachtingen te voldoen. Of RSD door middel van katheter ablatie volledig moet worden afgeschreven valt nog te bezien. Op basis van de anatomie van de renale zenuwbanen is het duidelijk dat met de huidige technieken complete ablatie van de zenuwen naar de nieren een utopie is. De mate van renale denervatie die nodig is om een relevant effect op de bloeddruk te bewerkstelligen is onbekend. Een post-mortem onderzoek van een patiënt die RSD had ondergaan, toonde inderdaad aan dat de ablatie verre van compleet was.[61] In een sub-analyse van de Symplicity-HTN 3 vond men bij patiënten die een meer uitgebreide denervatie procedure ondergingen een grotere daling in

de bloeddruk.[55] Na de initiële bemoedigende resultaten van de Symplicity HTN-1 en 2 studies waren verschillende biotech bedrijven erop gebrand hun eigen katheters te ontwikkelen. In hun proof-of principle en veiligheidsstudies vonden ook zij grote bloeddrukverlagende effecten, maar staakten na de publicatie van Symplicity HTN-3 hun verdere onderzoek. Dit is begrijpelijk, doch onfortuinlijk, omdat deze nieuwere katheters gebruik maakten van meerdere ablatiepunten, hetgeen mogelijk tot een effectievere denervatie zou kunnen leiden. Aangezien een onvoldoende effectieve ablatie wordt gezien als één van de mogelijke verklaringen van het uitblijven van een significant bloeddrukverlagend effect van RSD, zou data met betrekkingen tot de effectiviteit van deze nieuwere katheters waardevol kunnen zijn. Wij bieden met renale 123-I mIBG een potentiële techniek om op een niet-invasieve wijze de effectiviteit van ablatie bij RSD vast te stellen. Het concept van interveniëren in het complexe sympathische tweerichtingsverkeer tussen de hersenen en de nieren is interessant en door voldoende indirect bewijs ondersteund als behandeldoel. De voortvarende introductie van RSD, van amper enig preklinisch bewijs naar onderzoek in mensen, is onjuist. Een solide preklinische basis dient te worden gelegd alvorens humaan onderzoek te startten. Wanneer de stap van muis naar mens daadwerkelijk wordt gemaakt, is op zijn minst een zorgvuldig ontworpen en gecontroleerde studie van het grootste belang om het bloeddrukverlagende potentieel van nieuwe behandelstrategieën te onderzoeken.

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Authors and Affiliations

Charles O. Agyemang
Department of Public Health, Academic Medical Center, Amsterdam, the Netherlands

Anneke Alkemade
Department of Endocrinology & Metabolism, Academic Medical Center, Amsterdam, the Netherlands

Frederike J. Bemelman
Department of Internal Medicine – Nephrology and Kidney Transplantation, Academic Medical Center, Amsterdam, the Netherlands

Hendrik J. Best
Department of Vascular Medicine, Academic Medical Center, Amsterdam, the Netherlands

Peter H. Bisschop
Department of Endocrinology & Metabolism, Academic Medical Center, Amsterdam, the Netherlands

Stéphane Blanc
BMEYE B.V., Amsterdam, the Netherlands

Bas van den Bogaard
Department of Vascular Medicine, Academic Medical Center, Amsterdam, the Netherlands

Anke J. Borgers
Department of Endocrinology & Metabolism, Academic Medical Center, Amsterdam, the Netherlands

Bert-Jan H. van den Born
Department of Vascular Medicine, Academic Medical Center, Amsterdam, the Netherlands

Peter M. van Brussel
Department of Cardiology, Academic Medical Center, Amsterdam, the Netherlands

Marianne Cammenga
Department of Vascular Medicine, Academic Medical Center, Amsterdam, the Netherlands

Linn C. Dobrowolski
Department of Internal Medicine – Nephrology and Kidney Transplantation, Academic Medical Center, Amsterdam, the Netherlands

Jacqueline van Gorp
Department of Vascular Medicine, Academic Medical Center, Amsterdam, the Netherlands

Mirza M. Idu
Department of Vascular and Transplant Surgery, Academic Medical Center, Amsterdam, the Netherlands

C.T. Paul Krediet
Department of Internal Medicine – Nephrology and Kidney Transplantation, Academic Medical Center, Amsterdam, the Netherlands

Johannes J. van Lieshout
Department of Internal Medicine and Laboratory for Clinical Cardiovascular Physiology Center for Heart Failure Research, Academic Medical Center, Amsterdam, the Netherlands and School of Biomedical Sciences, University of Nottingham Medical School, Queen's Medical Centre, United Kingdom

Anton H. van den Meiracker
Divison of Pharmacology and Vascular and Metabolic Diseases, Department of Internal Medicine, Erasmus Medical Center, Rotterdam, the Netherlands

Gert A. van Montfrans
Department of Vascular Medicine, Academic Medical Center, Amsterdam, the Netherlands

Ron J.G. Peters
Department of Cardiology, Academic Medical Center, Amsterdam, the Netherlands

Jim A. Reekers
Department of Radiology, Academic Medical Center, Amsterdam, the Netherlands

Jos J. Settels
BMEYE B.V., Amsterdam, the Netherlands

Marieke B. Snijder
Department of Public Health, Academic Medical Center, Amsterdam, the Netherlands

Wim J. Stok
Department of Physiology, Academic Medical Center, Amsterdam, the Netherlands

Erik S.G. Stroes
Department of Vascular Medicine, Academic Medical Center, Amsterdam, the Netherlands

Hein J. Verberne
Department of Nuclear Medicine, Academic Medical Center, Amsterdam, the Netherlands

Liffert Vogt
Department of Internal Medicine – Nephrology and Kidney Transplantation, Academic Medical Center, Amsterdam, the Netherlands

Karel H. Wesseling (†)
BMEYE B.V., Amsterdam, the Netherlands

Karel R. Wesseling
BMEYE B.V., Amsterdam, the Netherlands

Berend E. Westerhof
BMEYE B.V., Amsterdam, the Netherlands and department of Lung Diseases, VU Medical Center, Amsterdam , the Netherlands

Wouter Wieling
Department of Internal Medicine, Academic Medical Center, Amsterdam, the Netherlands

Robbert J. de Winter
Department of Cardiology, Academic Medical Center, Amsterdam, the Netherlands

Aeilko H. Zwinderman
Department of Clinical Epidemiology, Biostatistics & Bioinformatics, Academic Medical Center, Amsterdam, the Netherlands

Portfolio

Name PhD student:	Daan W. Eeftinck Schattenkerk
PhD period:	March 2011 – April 2014
Name PhD supervisor:	Prof. Dr. E.S.G. Stroes

1. PHD TRAINING	YEAR	ECTS
General courses		
Good Clinical Practice	2011	0.5
Basiscursus Regelgeving en Organisatie voor Klinisch Onderzoekers (BROK)	2012	0.5
Practical Biostatistics	2012	1.1
Clinical Epidemiology	2013	0.6
Crash Course Chemistry, biochemistry and molecular biology for MD's (re)entering scientific research	2013	0.2
Specific courses		
Laboratory Animal Science (University of Utrecht)	2011	3.9
Seminars, workshops and masterclasses		
Weekly journal club, dept. of Vascular Medicine, AMC	2011-2014	3.0
Weekly clinical education, dept. of Vascular Medicine, AMC	2011-2014	3.0
Weekly hypertension meeting, dept. of Vascular Medicine, AMC	2011-2014	3.0
Presentations		
Determinants of vascular and cardiac baroreflex sensitivity in a random population sample. Poster presentation, European Society of Hypertension	2012	0.5
Renal 123I-MIBG Scintigraphy, a Novel Technique To Assess Efficacy of Renal Sympathetic Denervation: A First Impression. Poster presentation, American Society of Nephrology	2012	0.5
Renal 123I-MIBG scintigraphy, a novel technique to assess efficacy of renal sympathetic denervation: a first impression. Oral presentation, 3e Cardiovasculaire Conferentie	2013	0.5
Renal 123I-MIBG scintigraphy, a novel technique to assess efficacy of renal sympathetic denervation: a first impression. Oral presentation, European Society of Hypertension	2013	0.5

Lack of difference between Nebivolol/hydrochlorothiazide and Metoprolol/hydrochloro-thiazide on central wave augmentation and blood pressure. Oral presentation, European Society of Hypertension	2013	0.5
The decrease in arterial wave reflection upon head-up-tilt is attenuated by unilateral thigh cuff compression. Oral presentation, European Society of Hypertension	2013	0.5
Isolated Systolic Hypertension in HELIUS. Oral presentation, 4e Cardiovasculaire Conferentie	2014	0.5
The effects of Nebivolol/HCTZ vs. Metoprolol/HCTZ on wave reflection and central blood pressure. Oral presentation, Nationaal Hypertensie Congres	2014	0.5

Conferences

XVI International Symposium on Atherosclerosis, Sydney, Australia	2012	1.0
European Society of Hypertension, London, United Kingdom	2012	1.0
3e Cardiovasculaire Conferentie, Noordwijkerhout, the Netherlands	2013	0.5
European Society of Hypertension, Milan, Italy	2013	1.0
4e Cardiovasculaire Conferentie, Ermelo, the Netherlands	2014	0.5
Nationaal Hypertensie Congres, Zeist, Netherlands	2014	0.25

2. TEACHING

		YEAR	ECTS
Supervising			
Jacqueline van Gorp	Bachelor thesis	2012-2013	1.0
Pascale Venema	Bachelor thesis	2013-2014	1.0
Hendrik Best	Bachelor thesis	2013	1.0

3. PARAMETERS OF ESTEEM

	YEAR
Grants, awards and prizes	
European Society of Hypertension – accommodation and registration grant	2012
European Society of Hypertension – accommodation and registration grant	2013
Best Oral Presentation in parallel session, 4e Cardiovasculaire Conferentie	2014

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