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VASCULAR AND ENDOTHELIAL FUNCTION IN HUMAN HYPERTENSION, AND THE IMPORTANCE OF THE RENIN-ANGIOTENSIN-ALDOSTERONE SYSTEM

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Stockholm 2018

Cover illustration: *Marey's Sphygmograph, 1885. A portable specialized instrument to be placed above the radial artery to magnify pulse waves and records them on paper with an attached pen.*

Reference: Marey EJ. La méthode graphique dans les sciences expérimentales et principalement en physiologie et en médecine. Ed: G Masson. Paris: Lahure, 1885 (2nd Edition).

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VASCULAR AND ENDOTHELIAL FUNCTION IN HUMAN HYPERTENSION, AND THE IMPORTANCE OF THE RENIN-ANGIOTENSIN-ALDOSTERONE SYSTEM

THESIS FOR DOCTORAL DEGREE (Ph.D.)

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In remembrance of my beloved father, Kurt Jekell

“Lernen ist Erfahrung. Alles andere ist einfach nur Information.”

Albert Einstein

ABSTRACT

Background: Hypertension induces structural vascular and cardiac changes with increased arterial stiffness and left ventricular (LV) hypertrophy and is major risk factor for cardio-vascular morbidity and mortality. The renin-angiotensin-aldosterone system (RAAS) and the sympathetic nervous system are important for blood pressure regulation and vascular function. Angiotensin II, the main effector of the RAAS, induces vasoconstriction, inflammation, structural vascular changes, and LV hypertrophy. Thus, treating hypertension with drugs blocking the RAAS might have advantages compared to other drug classes.

The overall objective of this thesis was to increase our knowledge about the evaluation of arterial structure and function in human hypertension. Thus, the effects of treatment on indices of arterial stiffness and endothelial function were studied, and the effects beyond blood pressure reduction by blocking the RAAS were evaluated by comparison to drugs acting on the sympathetic nervous system.

Material and methods: This work is based on two clinical studies. In the "Swedish irbesartan left ventricular hypertrophy versus atenolol project" (SILVHIA), 115 patients with hypertension and LV hypertrophy were randomized to treatment based on the AT1-receptor blocker irbesartan or the beta-adrenoceptor blocker atenolol for 48 weeks. Two matched control groups consisting of hypertensive patients with no LV hypertrophy and normotensive control subjects were also investigated. We studied arterial stiffness (by pulse pressure, total vascular compliance, and ambulatory arterial stiffness index) and circulating markers of inflammation and of endothelial activation. In the "Doxazosin-ramipril study" (DoRa), 71 hypertensive patients were randomized to treatment with the angiotensin-converting enzyme inhibitor ramipril or the alpha 1-adrenoceptor blocker doxazosin for 12 weeks. The effects of treatment on arterial stiffness (by pulse wave analysis with applanation tonometry and by an oscillometric single-arm cuff method) and on endothelial function were evaluated simultaneously in different vascular beds (by forearm flow-mediated vasodilatation, pulse wave analysis and beta 2-adrenoceptor agonist stimulation, skin microcirculation by laser Doppler fluxmetry and iontophoresis, and myocardial microcirculation by the subendocardial viability ratio).

Results and conclusions: Antihypertensive treatment improved indices of arterial stiffness, and blocking the RAAS had additional effects on arterial stiffness beyond blood pressure reduction. There were no effects on endothelial function from the treatment. The oscillometric single cuff method was a simple and useful method to assess arterial function and to evaluate drug-induced treatment effects. Endothelial functions in different vascular beds were all related to future cardiovascular mortality risk (according to the "Systematic coronary risk evaluation", SCORE), but not to hypertension-induced heart disease. However, the studied methods to evaluate endothelial function were poorly interrelated. Thus, drugs blocking the RAAS may offer an advantage in the treatment of hypertension beyond the effects on blood pressure reduction, as compared to other drug classes.

LIST OF SCIENTIFIC PAPERS

I. **Jekell A**, Malmqvist K, Wallén NH, Mörtsell D, Kahan T. Markers of inflammation, endothelial activation, and arterial stiffness in hypertensive heart disease and the effects of treatment: Results from the SILVHIA study. *J Cardiovasc Pharmacol* 2013; 62:559-566.

II. **Jekell A**, Kalani M, Kahan T. The effects of alpha 1-adrenoceptor blockade and angiotensin converting enzyme inhibition on central and brachial blood pressure and vascular reactivity: the doxazosin-ramipril study. *Heart Vessels* 2017; 32:674-684.

III. **Jekell A**, Kahan T. The usefulness of a single arm oscillometric method (Arteriograph) to assess changes in central aortic blood pressure and arterial stiffness by antihypertensive treatment: results from the Doxazosin-Ramipril Study. *Blood Press* 2018; 27:88-98.

IV. **Jekell A**, Kalani M, Kahan T. Endothelial function and microvascular reactivity in relation to cardiovascular risk in hypertension: Results from the Doxazosin-ramipril study. *Manuscript.*

CONTENTS

LIST OF ABBREVIATIONS

AASI	Ambulatory arterial stiffness index
ACE	Angiotensin converting enzyme
Ach	Acetylcholine
AIx	Augmentation index
ANG	Angiotensin
AT1	Angiotensin II type 1-receptor
AT2	Angiotensin II type 2-receptor
BP	Blood pressure
CV	Cardiovascular
DoRa	Doxazosin-ramipril study
EDV	Endothelium dependent vasodilatation
EIDV	Endothelium independent vasodilatation
EFI	Endothelial functional index
FMD	Flow mediated vasodilatation
GTN	Glyceryl trinitrate
hsCRP	High-sensitivity C-reactive protein
ICAM	Inctracellular adhesion molecule
IL	Interleukin
LDF	Laser Doppler fluxmetry
LV	Left ventricular
NO	Nitric oxide
PWA	Pulse wave analysis
PWV	Pulse wave velocity
RAAS	Renin-angiotensin-aldosterone system
RI	Reflection index
SCORE	Systematic coronary risk evaluation
SILVHIA	Swedish left ventricular hypertrophy investigation versus atenolol
SEVR	Subendocardial viability ratio
SNP	Sodium nitroprusside
VCAM	Vascular adhesion molecule

1 INTRODUCTION

1.1 BLOOD PRESSURE REGULATION AND THE ROLE OF NEUROHORMONAL ACTIVATION

Essential hypertension is characterized by a disturbance in the central sympathetic nervous outflow with increased activation to several organs and a subsequent increase in vascular tone. Enhanced noradrenaline release from sympathetic vasoconstrictor nerve endings and reduced neuronal reuptake induces vasoconstriction by stimulation of postjunctional alpha 1-adrenoceptors on vascular smooth muscle cells in the resistance arteries. Impaired vasodilation and mechanical shear stress in the vessel walls also contribute to vascular remodeling, with further elevation of peripheral vascular resistance (1-3). In addition, sympatho-adrenal activation increases cardiac contractility, heart rate, and pulse wave amplitude, with an increased pressure workload on the vasculature, thus causing myocardial remodeling and impaired cardiac function.

There are different drugs to reduce theses effects of sympathetic overactivation in hypertension. Alpha-adrenoceptor antagonists act on the vasculature to reduce vasoconstriction. Also, centrally acting alpha-adrenoceptor agonists acting on inhibitory pathways in the control of blood pressure are used to achieve blood pressure reduction. The exact mechanism of action through which beta-adrenoceptor antagonists reduce blood pressure remains to be established. However, the mechanism is likely to include effects on the central nervous system to reduce central sympathetic nervous outflow, and these antagonists interact with the renin-angiotensin-aldosterone system (RAAS) by inhibiting beta 1-adrenoceptor-mediated renin release from the kidneys. In addition, they inhibit beta-adrenoceptor-mediated chronotropic, inotropic, and lusitropic effects on the myocardium.

Renal prorenin and renin are synthesized and stored in the juxtaglomerular cells, which are located in the vascular walls of the afferent arterioles. Increased renin release is mediated by the renal baroreceptor reflex in the afferent arterioles due to a reduction in perfusion pressure. Renin release is also stimulated by the juxtaglomerular apparatus due to decreases in tubular salt delivery. The juxtaglomerular cells are directly innervated by efferent renal sympathetic nerves and postjunctional beta 1-adrenoceptors. In addition, renin release is regulated by intracellular calcium levels in the juxtaglomerular cells (4).

Renin induces formation of angiotensin (ANG) I from circulating angiotensinogen, and ANG I is further converted by angiotensin converting enzyme (ACE) to ANG II. ANG II can act on ANG II type 1 (AT1)-receptors to mediate vasoconstriction in vascular smooth muscle cells and to release vasopressin and aldosterone, leading to retention of water and sodium with further increase in blood pressure (BP). In addition, ANG II has trophic, pro-inflammatory, and pro-thrombotic effects and can thus induce oxidative stress, hypertrophy, fibrosis, and vascular remodeling through AT1-receptor stimulation. ANG II also acts on ANG II type 2 (AT2)-receptors, which have reciprocal effects compared to AT1-receptor stimulation and induce a vascular repair response (Figure 1). Thus, AT2-receptor activation causes

vasodilatation by elevation of nitric oxide (NO) synthesis, inhibits cellular growth, and promotes cellular differentiation. Recent evidence shows that the RAAS is far more complex than this brief description, with different physiologically active peptide fragments of ANG II and converting enzymes having both systemic and local effects in different tissues, as reviewed elsewhere (4,5).

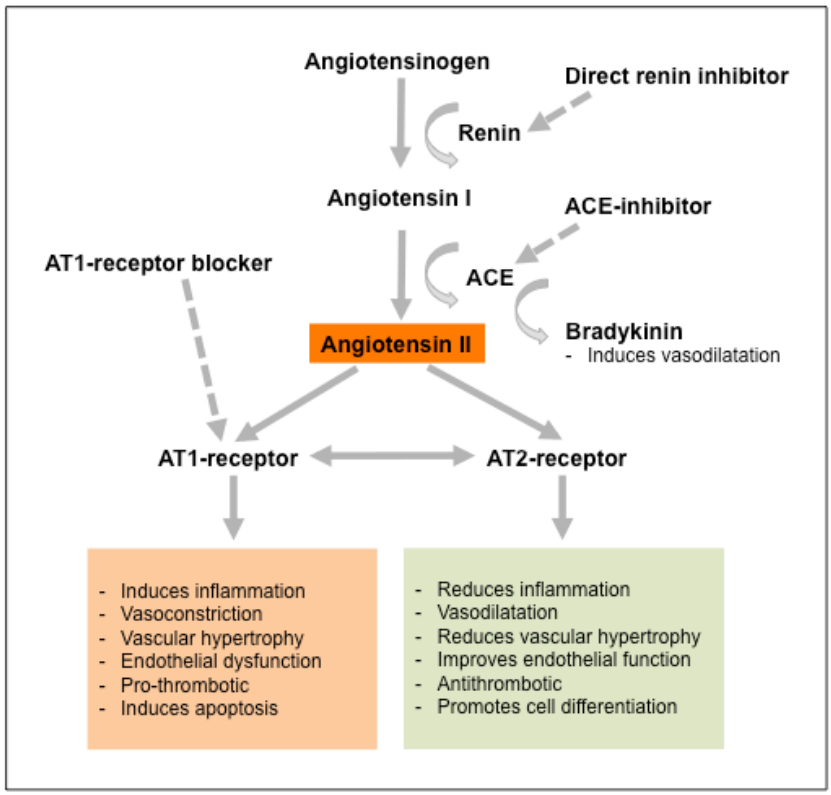

Figure 1. The renin-angiotensin-aldosterone system and the receptor-mediated effects of angiotensin II. ACE, angiotensin converting enzyme, AT1 (angiotensin II type I)-receptor: AT2 (angiotensin II type 2)-receptor.

Both ACE-inhibitors and AT1-receptor blockers induce vasodilatation. While ACE inhibitors prevent formation of ANG II, they also increase levels of bradykinin by inhibiting the breakdown of bradykinin by ACE. Bradykinin increases NO synthesis and induces vasodilatation (Figure 1). However, AT1-receptor blockers inhibit the direct effects of ANG II on AT1-receptors, and this leads to impaired negative feedback and thus increased ANG II formation, which will cause increased stimulation of AT2-receptors and might contribute to vasodilatation. A consequence of long-term RAAS inhibition is a renin escape mechanism with increased synthesis of renin. Direct renin inhibitors can inhibit this compensatory mechanism by blocking the renin/prorenin-receptor. Hence, renin production, which is the rate-limiting step of ANG II formation, is blocked (6). There is evidence that direct renin inhibitors improve vascular structure and function by improving endothelial function and reducing the development of atherosclerosis, especially in combination with an AT1-receptor blocker (7). Thus, blocking the RAAS at several levels should theoretically be beneficial by blocking the compensatory renin increase. However, clinical studies have shown a

disadvantage of combination therapy in patients with diabetes with an increased risk of hyperkalaemia and cardiovascular (CV) events (8) In addition, there are other therapeutic agents under development to modulate the RAAS. In animal models, compound 21, an AT2-receptor agonist, seems to reduce atherosclerosis (4).

1.2 THE ENDOTHELIUM AND THE ROLE OF ANGIOTENSIN II

The endothelium is a single cell layer that acts as a semipermeable membrane adherent to the vascular lumen. The endothelium is involved in the control of vascular tone, and mechanical shear stress and substances stimulating receptors on the endothelial surface can cause vasoconstriction or vasodilation. Endothelium-dependent vasodilatation (EDV) is induced by NO, which is derived from the transformation of L-arginine to citrulline by the activity of the constitutive endothelial enzyme NO synthase. NO causes vasodilatation through relaxation of smooth muscle cells by stimulation of cyclic guanosine monophosphate. An increase in NO synthesis is caused by mechanical forces on the vessel wall through increased shear stress and by endothelial receptor-mediated stimulation by agonists such as acetylcholine (Ach), bradykinin, substance P, and serotonin. Also, endothelium-derived relaxing factors like prostacyclins and endothelium-derived hyperpolarizing factors can induce vasodilatation if less NO is available (9). Vasoconstriction is mediated by ANG II, endothelin 1, thromboxane A2, and prostaglandin H2. Increased levels of reactive oxygen species, mainly superoxide anion, degenerates NO. ANG II increases oxidative stress, and cytokine release promotes up-regulation of adhesion molecules and stimulates uptake of oxidized low-density lipoproteins, thereby causing vascular remodeling and atherosclerosis (10,11).

1.3 METHODS TO EVALUATE ENDOTHELIAL FUNCTION

Endothelial function in humans can be studied in different vascular beds, such as the coronary circulation, skeletal muscle, and skin microcirculation. The gold standard method to evaluate endothelial function in the coronary circulation is to measure changes in coronary blood induced by intra-arterial infusion of Ach, which is a measure of EDV (12). The invasive forearm blood-flow technique with venous occlusion plethysmography is another reference method, where changes in forearm blood flow, which represent modification of the local vascular resistance, are measured before and after intra-arterial infusion with Ach and sodium nitroprusside (SNP) to represent EDV and endothelium-independent vasodilatation (EIDV), respectively (2). Endothelial function in small resistance arteries can be evaluated ex vivo by muscular or subcutaneous fat biopsies to evaluate the effects of Ach or SNP in noradrenaline precontracted vessels (13).

Well-established non-invasive methods include post-ischemic flow-mediated vasodilatation (FMD) in the brachial artery (14,15) and beta 2-adrenoceptor agonist-induced changes in the reflecting pulse wave form as assessed by pulse wave analysis (PWA) with applanation

tonometry for skeletal muscle (16,17). Peripheral artery tonometry, where plethysmographic pressure changes in the fingertip after arterial occlusion of the forearm (reactive hyperemia index) are measured, has been proposed as an alternative method to evaluate endothelial function in the peripheral circulation. Skin microvascular reactivity can be measured by laser Doppler fluxmetry following post-ischemic or heat-induced hyperaemia or by local iontophoretic application of Ach and SNP (18,19). In the myocardium, the subendocardial viability ratio (SEVR) can be calculated from PWA with applanation tonometry and the aortic pulse waveform, where the ratio of the diastolic to the systolic time pressure index gives information about the coronary subendocardial perfusion and microcirculatory function (20,21).

1.4 METHODS TO EVALUATE VASCULAR FUNCTION AND INDICES OF ARTERIAL STIFFNESS

A high pulse pressure (i.e. the differences between systolic and diastolic BP) is a simple but crude reflection of arterial stiffness. A better measure of vascular elasticity is compliance. Arterial compliance is defined as a change in the area, diameter, or volume of an artery or arterial bed for a given change in pressure. Thus, one way to define (total) arterial compliance is to calculate stroke volume divided by pulse pressure (SV/PP). Hemodynamic changes with elevated BP, increased heart rate, and structural vascular changes with loss of elasticity in the aorta and larger conduit arteries increase pulse pressure and thus reduce arterial compliance. An increased pulsatile load derived from the aorta and conduit arteries also influences smaller resistance arteries causing arterial stiffness. Thus, the interaction between the macrocirculation and microcirculation in hypertension is important (22).

In clinical research and in the specialized clinical settings, the use of applanation tonometry for PWA is common. PWA of the radial and aortic waveform by applanation tonometry allows the central BP, the augmentation index (AIx), and the pulse wave velocity (PWV) to be calculated, as presented in Figure 2 (23,24). Interestingly, the Sphygmograph, a mechanical device used to measure BP, was developed already in 1854 by German physiologist K von Vierordt. In 1863, French physiologist EJ Marey, improved the device by making it portable (25). He demonstrated how this device could graphically show variations of the radial pulse waveform using tonometry (see illustration on front page). Today, the evaluation of arterial stiffness and central hemodynamic measures can be achieved by different techniques. Invasive direct measurements during arterial catheterization, e.g. during coronary angiography, is preferable but is more complicated, compared to non-invasive methods. PWV and hence arterial stiffness can, in addition to the use of PWA and applanation tonometry, also be evaluated by the piezoelectric principle, which is based on the time difference of the carotid and femoral pulse wave propagation, or by echo tracking, where the pressure curve and flow curve in the common carotid artery are analyzed.

The estimation of central BP and indices of arterial stiffness can also be achieved by cuff-based methods. One single brachial cuff oscillometric method, the Arteriograph, is a device where brachial BP readings are registered by an automated arm cuff (26). This technique is quick, easy to use, and operator independent, and might thus have important advantages to PWA by applanation tonometry. The Arteriograph device has been well validated against invasive measurements (27) but has not yet been used for studying the effects of antihypertensive treatment on vascular structure and function. Also, comparisons of the two methodologies concerning antihypertensive effects on vascular structure and function are lacking. This added knowledge about the ability to detect effects of treatment is therefore important.

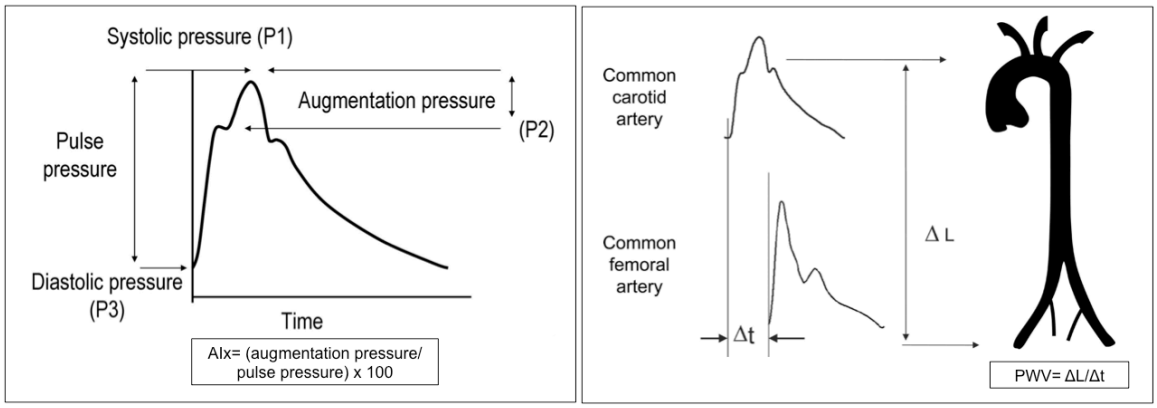

Figure 2. Augmentation Index (AIx) and carotid-femoral pulse wave velocity (PWV). Adapted from (24).

1.5 HYPERTENSION-INDUCED CARDIAC AND VASCULAR REMODELING IS ASSOCIATED WITH INCREASED CV RISK

Hypertension-induced cardiac remodeling and the development of left ventricular (LV) hypertrophy can reflect LV dysfunction and is associated with an increased risk of cardiovascular (CV) morbidity and mortality and all cause death (28,29). Also, vascular remodeling, including increased peripheral resistance, increased arterial stiffness with elevated pulse wave velocity, and augmented central pulse pressure is all associated with increased CV risk (Table 1). In addition, different CV risk factors like hyperglycemia and diabetes type 2 accelerate this development and induce early structural vascular changes (30).

In the microcirculation, hypertension induced remodelling of small resistance arteries has prognostic importance, where a greater media-to-lumen ratio is associated with increased risk (31). Furthermore, there is an association of structural vascular changes in smaller resistance arteries and coronary flow reserve (32). Impaired endothelial function in the forearm measured invasively or non-invasively, and in the coronary circulation, measured invasively or with ultrasound and Doppler flow as the coronary flow reserve, is related to cardiovascular risk according to the Framingham risk score, and is of prognostic importance (12,33,34).

There is also a relation between impaired endothelial function in the conduit arteries and increased arterial stiffness, augmentation index and central pulse pressure (35,36).

Table 1. Measurements to evaluate vascular structure and function and indices of arterial stiffness and their relation to cardiovascular risk.

Method	Measurement	Prognosis
Brachial pulse pressure	Systolic BP - diastolic BP (Papers I-III).	Increased pulse pressure increases the risk of coronary heart disease (37).
Stroke volume/ pulse pressure (SV/PP)	Arterial compliance (Paper I).	Reduced SV/PP increases the risk of CV events independently of LV mass index and age (38).
Ambulatory arterial stiffness index (AASI)	Derived from ambulatory BP measurements (Paper I).	Increased AASI is associated with CV events and predicts CV mortality risk (39,40).
Pulse wave velocity (PWV)	Measurement of arterial stiffness. Applanation tonometry and the SphygmoCor device (Paper II) and the oscillometric single- cuff method by Arteriograph (Paper III).	Elevated PWV increases the risk of CHD and stroke and improves risk prediction of future CV events (41,42).
Augmentation index (AIx) and aortic BP	Aortic pulse wave analysis and augmented central pulse pressure. Applanation tonometry and the SphygmoCor device (Paper II), and the oscillometric single-cuff method by Arteriograph (Paper III).	Increased AIx predicts risk of CV events independently of peripheral BP (43). Central aortic BP is better related to future CV events compared to brachial BP (44).
Carotid-femoral PWV / carotid-radial PWV	PWV velocity ratio by applanation tonometry and the SphygmoCor device (Papers II,IV).	Aortic-brachial stiffness mismatch is associated with CV mortality in high-risk patients (45).

Furthermore, impaired skin microvascular reactivity is related to the risk of developing coronary heart disease, and is associated with diabetes and with an increased risk in patients with an acute coronary syndrome (46,47).

Circulating biomarkers of vascular function such as the intracellular adhesion molecule (ICAM)-1, vascular adhesion molecule (VCAM)-1, and E-selectin reflect endothelial activation and the risk of atherosclerotic disease (48,49). In hypertension, there are signs of systemic inflammation with elevation of circulating markers of inflammation and endothelial activation like interleukin (IL)-6 and ICAM-1 (50). Low-grade systemic inflammation with increased high-sensitivity C-reactive protein (hsCRP) seems to promote vascular fibrosis with remodeling of the arterial wall causing arterial stiffness (51). Also, cardiac remodeling is associated with early signs of fibrosis and cytokine activation, suggesting the importance of inflammation in hypertension with LV hypertrophy (52,53). However, the influence of inflammation, vascular stiffness, and activation of the vascular endothelium in relation to LV hypertrophy and BP is not well studied.

1.6 HYPERTENSION AND ESTIMATION OF CARDIOVASCULAR RISK

Hypertension is one of several major risk factors for CV morbidity and mortality. Thus, hypertension is a major threat to public health and remains the most important risk factor for premature mortality worldwide (54). There has been a transition during the last 40 years from the highest prevalence of hypertension being seen in high-income countries to the highest prevalence being seen in low-income and middle-income countries due to population growth and increased population age (55). Improvement of BP control to prevent CV disease is therefore warranted.

Treatment of hypertension is based on lifestyle interventions such as reduced salt intake, increased exercise, weight reduction, moderation of alcohol intake, and smoking cessation. Most often, however, this is not enough to reach the target BP. Antihypertensive treatment is effective, well documented, and reduces CV morbidity and mortality (56). It is of great importance to assess global CV risk in each individual in order to determine which actions are best suited in order to prevent future CV events. Furthermore, stratification of the individual CV risk can be assessed by different risk models – like the European SCORE (Systematic coronary risk evaluation) (57) or the American Framingham risk score (58) – in order to improve prevention strategies.

One important component in risk factor assessment is to identify early and often asymptomatic signs of subclinical target organ damage such as LV hypertrophy, microalbuminuria, and white matter lesions in the brain, because these findings motivate intensive treatment.

1.7 THE EFFECTS OF ANTIHYPERTENSIVE TREATMENT ON BP AND CARDIAC AND VASCULAR STRUCTURE AND FUNCTION

Central aortic BP provides better risk prediction than peripheral brachial BP (43,44). Some data suggest that ACE inhibitors lower aortic BP more than the brachial BP and reduce PWV (i.e. aortic stiffness) to a greater extent than other antihypertensive drugs (59,60), but results are not consistent (61). Such effects of ACE inhibitors have been proposed to improve CV outcome in high-risk patients beyond the effects of BP reduction (62,63). Furthermore, there is evidence that antihypertensive treatment with ACE inhibitors or AT1-receptor blockers has additional effects beyond BP reduction through the reduction of cardiac fibrosis and improvement of LV diastolic function compared to other drug classes (64,65). Taken together, both the sympathetic nervous system and the RAAS are central for BP regulation and vascular control, and there might be beneficial effects on vascular function beyond BP reduction by treatment with drugs that inhibit the RAAS. However, the effects of treatment on endothelial function in patients with mild to moderate primary hypertension are unclear and the results are often conflicting (66-68). Thus, comparison of treatment with drugs blocking the RAAS and other BP-lowering drug classes are warranted. Furthermore, the effects of alpha 1-adrenoceptor blockers on endothelial function and indices of aortic stiffness are not well studied.

Endothelial dysfunction is related to CV risk, and endothelial function can be evaluated in different vascular beds with different techniques. However, it is still unclear how endothelial function in larger conduit arteries is related to findings in smaller resistance arteries and to skin microvascular reactivity and coronary microcirculatory function. This interesting question needs to be further addressed.

2 AIMS

The overall objective of this project was to obtain greater knowledge about the evaluation of arterial structure and function in hypertensive patients. Furthermore, we wished to study the effects of antihypertensive treatment on vascular structure and function because this might help to individualize antihypertensive treatment in order to reduce future risk of CV disease.

More specifically, we had the following aims:

1. To study the effects of antihypertensive treatment on arterial stiffness and endothelial function.

2. To study whether blocking the RAAS has effects on arterial stiffness and endothelial function beyond BP reduction alone.

3. To evaluate an oscillometric single cuff-based method to assess central arterial function compared to established methods using PWA with applanation tonometry.

4. To compare several non-invasive physiological methods in the evaluation of endothelial function in different vascular beds in humans and their relation to the risk of future CV disease.

3 MATERIALS AND METHODS

3.1 DESIGN AND STUDY POPULATION

This thesis is based on two clinical studies – the "Swedish irbesartan left ventricular hypertrophy versus atenolol project", SILVHIA (Paper I), and the "Doxazosin-ramipril study", DoRa (Papers II–IV). Baseline characteristics of the study populations are presented in table 2.

Table 2. Baseline characteristics of study populations.

	SILVHIA			**DoRa**
	NT control	HT no LVH	HT and LVH	
n	38	38	114	71
Male/female, %	74/26	74/26	67/33	63/37
Age, yrs. (range)	54 ± 9 (37-75)	54 ± 9 (36-74)	54 ± 9 (31-74)	55 ± 13 (23-76)
Body mass index (kg/m^2)	24.8 ± 2.3	26.6 ± 3.4	27.1 ± 3.4	26.8 ± 4.7
LV mass index (g/m^2)	88	99	149	103
Systolic BP (mm Hg)	121 ± 10	148 ± 14	162 ± 19	154 ± 10
Diastolic BP (mm Hg)	78 ± 7	97 ± 6	104 ± 8	93 ± 9
Heart rate (bpm)	61 ± 7	68 ± 8	68 ± 10	61 ± 8
Smoking (%)	19	16	24	6
Cholesterol (mmol/L)	5.8 ± 1.2	5.9 ± 1.0	6.0 ± 1.0	5.4 ± 1.1
Glucose (mmol/L)	5.1 ± 0.6	5.3 ± 0.5	5.4 ± 2.6	5.4 ± 0.6
eGFR ($ml/min/1.73m^2$)	87.2 ± 18.0	97 ± 26.5	95 ± 24.8	90.4 ± 14.5

Mean values ± SD. NT, normotensive; HT, hypertension; LVH, left ventricular hypertrophy; LV, left ventricular; BP, blood pressure; eGFR, estimated glomerular filtration rate.

3.1.1 Paper I

The aim of the SILVHIA project was to study the treatment effects of the AT1-receptor blocker irbesartan compared to the beta-adrenoceptor blocker atenolol in terms of BP reduction, LV geometry, and LV systolic function in patients with hypertension and LV hypertrophy (69,70).

The primary aim in paper I was to study indices of arterial stiffness and markers of endothelial function and inflammation in patients with hypertensive heart disease compared to hypertensive patients with no LV hypertrophy and compared to normotensive subjects. The secondary aim was to compare the effects beyond BP reduction on vascular structure and function by blocking the RAAS as compared to blocking the sympathetic nervous system.

The SILVHIA study included 115 patients with hypertension (diastolic BP 90–115 mm Hg) and LV hypertrophy, 38 hypertensive patients with no LV hypertrophy, and 38 normotensive controls (diastolic BP <90 mm Hg and a normal LV mass) matched for both age and sex (69,70). Exclusion criteria were an ejection fraction <45%, secondary hypertension, renal failure, congestive heart failure, and coronary and/or valvular heart disease. All drugs were withdrawn and followed by a 4–6 week period of single-blind placebo treatment. Patients with hypertension and LV hypertrophy were randomized to double-blind treatment with irbesartan (150 mg o.d.) or atenolol (50 mg o.d.). If diastolic BP after 6 weeks was ≥90 mm Hg, the study drug dose was doubled. Open-label hydrochlorothiazide and felodipine were added if required to achieve a diastolic BP <90 mm Hg. The duration of the study was 48 weeks. Evaluation of vascular structure and function and biochemical markers of endothelial activation and inflammation was performed at baseline and at weeks 12 and 48. The study design is presented in figure 3.

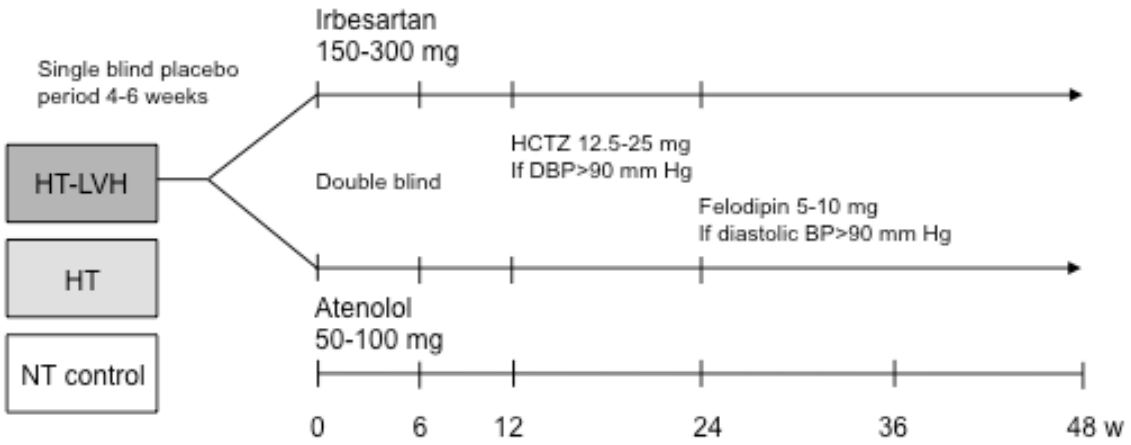

Figure 3. The SILVHIA study design. HT, hypertension, LVH, Left ventricular hypertrophy; NT, normotensive.

3.1.2 Papers II–IV

The primary aim in paper II was to study the effect of antihypertensive therapy on arterial stiffness and on endothelial function in patients with mild to moderate primary hypertension. The secondary aim was to evaluate the potential effects on vascular structure and function beyond BP reduction by blocking the RAAS as compared to blocking the sympathetic nervous system.

The DoRa study was a randomized double-blind parallel group study in 71 patients above 18 years of age with mild to moderate hypertension. Included patients were previously untreated or were randomized after an additional wash-out period of 4 weeks. BP criteria for inclusion was an office systolic BP >140 mm Hg and/or diastolic BP >90 mm Hg. Secondary hypertension was ruled out by clinical examination and routine screening of blood tests. Exclusion criteria were severe hypertension (BP >180/110 mm Hg), structural heart disease, congestive heart failure, arrhythmias, diabetes, and hyperlipidaemia. Patients were randomized to the alpha 1-adrenergic blocker doxazosin 4 mg or the ACE inhibitor ramipril 5 mg, with doubling of the dose after two weeks for additional 10 weeks of treatment. A total of 61 patients completed the study. The study design is presented in Figure 4. BP measurements, study blood samples, vascular investigations of arterial stiffness (applanation tonometry and the oscillometric single-cuff method), and assessment of endothelial function (FMD, PWA with beta 2-adrenoceptor agonist stimulation, and LDF and iontophoresis) was performed at baseline and at 12 weeks, see paper II for details. Vascular examinations were performed on two consecutive days to avoid pharmacological interaction of the vascular study protocols. Study medication was taken routinely at the same hour by the patients, and at week 12 the BP and vascular investigations were performed 2 hours after intake of the study medication in order to achieve the maximum pharmacological effects of treatment.

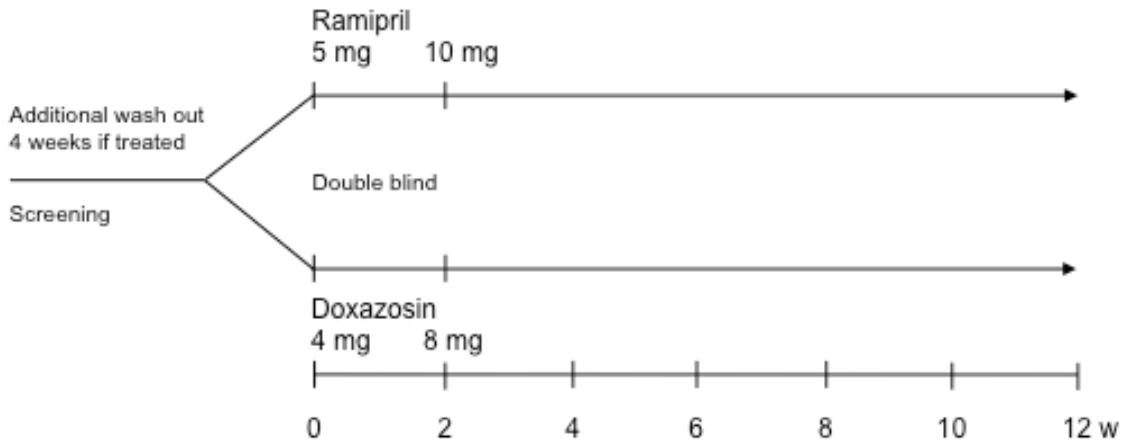

Figure 4. The DoRa study design.

Paper III was a methodological study comparing applanation tonometry and the SphygmoCor device to the oscillometric single-cuff technique by Arteriograph. Simultaneous baseline measurements from 71 patients from paper II were compared. Indices of arterial stiffness (aortic BP, AIx, and PWV) by both techniques were evaluated. The oscillometric single-cuff method was also used to evaluate the effects of treatment on BP reduction and on indices of arterial stiffness.

In paper IV, four different methods (FMD, PWA with beta 2-adrenoceptor agonist stimulation, LDF and iontophoresis in skin microcirculation, and coronary microcirculation as measured by the SEVR) were used to evaluate the interrelationship of endothelial function in different vascular beds. We also studied how endothelial functions in different vascular beds were related to CV risk, assessed by SCORE, and to signs of hypertensive heart disease.

3.2 METHODS

3.2.1 Measurements of BP, vascular compliance, and arterial stiffness

In paper I, seated systolic and diastolic BP was determined as the average of three consecutive measurements using a mercury sphygmomanometer after resting for at least 10 minutes. In paper II, the systolic and diastolic BP was determined as the average of three consecutive measurements taken 1 minute apart after a 10 min rest in supine position using an oscillometric device (OMRON 705IT, OMRON Healthcare Co., Ltd. Kyoto Japan). Pulse pressure was calculated as the systolic minus diastolic BP (papers I–IV), mean arterial pressure was calculated as the diastolic BP + 1/3 × pulse pressure (papers II–III), and arterial compliance was measured as the stroke volume divided by pulse pressure (SV/PP) (paper I). Ambulatory BP recordings were performed in the SILVHIA project (71), and we used these values to calculate the ambulatory arterial stiffness index (AASI) as a measure of arterial stiffness. The AASI was obtained by plotting diastolic BP against systolic BP, where AASI is defined as 1 minus the regression slope of the diastolic BP to the systolic BP (72).

Two different methods for evaluation of central aortic BP and indices and arterial stiffness were used simultaneously in DoRa – PWA by applanation tonometry and the SphygmoCor software and equipment in papers II and III, and the oscillometric single-cuff technique with the Arteriograph in paper III.

PWA was assessed by applanation tonometry (Millar Instruments, Houston, TX, US), and the SphygmoCor device (AtCor Pty Ltd, West Ryde, NSW, Australia). The central aortic waveform was calculated from radial applanation tonometry using a general transfer function by the device software, and central BP was derived. The carotid-femoral PWV was calculated as the carotid-to-femoral distance divided by the transit time difference of the carotid and femoral pulse wave propagation (24). The carotid-radial PWV was calculated similarly in order to obtain the carotid-femoral/carotid-radial PWV ratio, which is a measure of the aortic-brachial mismatch (45).

The oscillometric cuff-based method (Arteriograph, TensioMed Kft, Budapest, Hungary) uses a single-arm cuff device, and the brachial systolic and diastolic BP is measured by automatic inflation of the cuff. The pressure change during suprasystolic BP (>35 mm Hg above peak systolic value) is driven to the device by the hose from the cuff and is subsequently recorded by a piezoelectric sensor. The approximate time difference of the first systolic peak (the ejection of blood from the left ventricle to the aorta) and the second peak (the retrograde reflected pulse wave from the iliac bifurcation) represents the return time. PWV was calculated by dividing the traveled distance (the tapered jugulum to the symphysis distance) by the return time / 2. AIx was calculated as 100 × (second – first systolic wave) / pulse pressure. Aortic BP was obtained by an algorithm in the software based on invasively measured aortic BP (27).

3.2.2 Functional methods to evaluate endothelial function

A summary of methods to evaluate endothelial function in different vascular beds is presented in Table 3.

FMD was measured by post-ischemic hyperaemia in the non-dominant arm. The resting basal diameter of the brachial artery was measured for 1 minute by a Vivid 7 Dimension ultrasound device with a 9 MHz linear transducer (GE Medical System, Horten, Norway). Thereafter, an inflated pneumatic tourniquet placed around the forearm to a pressure of 250 mm Hg for 5 minutes induced occlusion of the brachial artery. After cuff deflation, the maximum change in diameter was achieved by repeated measurements (30, 60, and 90 s). The relative change from baseline diameter was taken as a measure of FMD. Finally, after 10 min of additional rest, EIDV was induced by 0.4 mg sublingual glyceryl trinitrate (GTN) (Nitrolingual, G Pohl-Boskamp GmbH & Co KG, Hohenlockstedt, Germany). Relative changes in artery diameter were calculated from rest to 4 min following GTN administration (15). In addition, the endothelial function index (EFI), i.e. FMD/GTN ratio, was used to improve the validation of the EDV in relation to the EIDV, as reported elsewhere (73).

EDV was also evaluated by applanation tonometry and PWA with beta 2-adrenoceptor agonist stimulation. PWA was performed before and 15 and 20 min after 0.25 mg sc terbutaline (Bricanyl, AstraZeneca, Mölndal, Sweden) to evaluate the maximum effect of beta 2-adrenoceptor agonist stimulation. Aortic waveforms were generated by the SphygmoCor software from radial artery applanation tonometry. This receptor-mediated mechanism represents EDV in the resistance arteries. The reflection index (RI) is defined as the relative height of the first diastolic reflective pulse wave in relation to the first systolic peak, as presented in Figure 5 (17). A smaller relative change after beta 2-adrenoceptor agonist stimulation is associated with impaired endothelial function. Also, the relative change in radial AIx and aortic AIx can be an alternative way to measure wave reflection by this technique (16). Another method is photoplethysmography and digital volume pulse, as described by others (74).

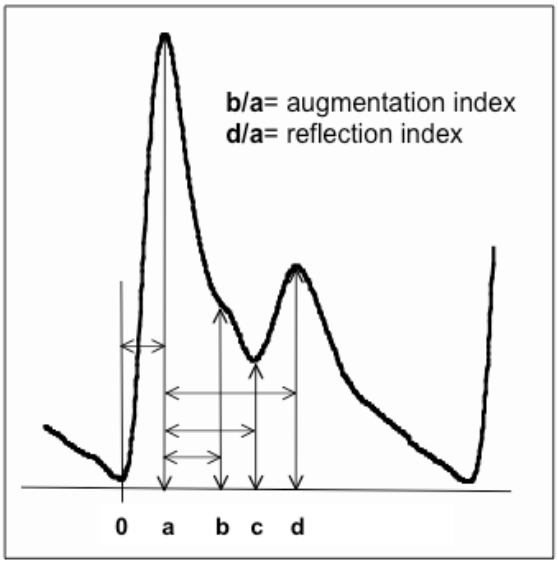

Figure 5. Pulse wave analysis with applanation tonometry of the radial artery. 0,onset of systole; a, peak systole; b,reflected wave in systole; c, dicrotic notch; d, reflected wave in diastole. Modified from (17).

3.2.3 Evaluation of skin microcirculation

Forearm skin microvascular reactivity (vasodilatation) was assessed by LDF and 60 s transcutaneous iontophoretic administration (Periflux system 5000, PF 5010 LDPM Unit, PF5010 Temp Unit, and 481-1 Single Probe, Perimed, Järfälla, Sweden) of small amounts of Ach (Sigma-Aldrich AB, Stockholm, Sweden) and SNP (Hospira, Inc., Lake Forest, IL, USA) to represent EDV and EIDV, respectively (18). Skin microvascular peak flux was recorded continuously up to 16 min after iontophoresis and was expressed in arbitrary units. To determine maximum skin microvascular hyperaemia, peak flux was evaluated after local heating of the forearm skin to 44°C for 6 min. Heat-induced maximum hyperaemia after local heating gives information about the total microvascular reactivity, and the maximum skin hyperaemia seems to be enhanced by NO (19). Reduced maximum hyperaemia is an indicator of increased CV risk in diabetic patients with acute coronary syndrome (47), and statin treatment improves maximum hyperemia in patients with coronary artery disease and hyperglycaemia (75).

3.2.4 Evaluation of coronary microcirculation

The SEVR was calculated by PWA and the aortic pulse waveform using the SphygmoCor device in paper IV. The SEVR is an indirect measure of subendocardial perfusion capacity and is an index of oxygen supply and demand of the myocardium (21). It was calculated as the systolic to diastolic pressure time integral. The SEVR is associated with coronary flow reserve, which is a measure of coronary microcirculation (20), and it is associated with CV risk and can be used to improve risk prediction in high-risk patients (76,77).

Table 3. Methods to measure macrovascular endothelial function and microvascular reactivity in different vascular beds.

Method	Mechanism	Vascular bed
FMD	Post-ischemic reactive hyperemia. Shear stress. EDV (Papers II, IV)	Conduit arteries (15)
GTN-mediated vasodilation	NO donor mediates relaxation of smooth muscle cells. EIDV (papers II, IV)	Conduit arteries (15)
PWA and beta 2-adrenoceptor agonist stimulation	Receptor-mediated stimulation. EDV. Change in the reflective pulse wave form (papers II, IV)	Resistance arteries (16)
Iontophoresis and LDF	Acetylcholine-mediated EDV and sodium nitroprusside-mediated EIDV vasodilatation (papers II, IV)	Forearm skin microcirculation (18)
Local heating	Maximum reactive hyperaemia (papers II, IV).	Forearm skin microcirculation (19)
SEVR	Indirect measurement of coronary microcirculation, calculated from PWA and the SphygmoCor (paper IV)	Coronary microcirculation (20)

FMD, flow mediated vasodilation; GTN, glyceryl trinitrate; PWA, pulse wave analysis; LDF, laser Doppler fluxmetry; SEVR, subendocardial viability ratio; EDV, endothelium-dependent vasodilation; EIDV, endothelium-independent vasodilation

3.2.5 Echocardiography

Echocardiography was performed by standard procedures in the supine position. Measurements of LV dimensions and wall thickness were made using the M-mode technique. The Penn convention was used for calculation of LV mass (78), which was corrected for body surface area, i.e. the LV mass index. LV hypertrophy was defined as a LV mass index >131 g/m^2 and >100 g/m^2 (paper I) and >115 g/m^2 and >95 g/m^2 (paper IV) for men and women, respectively, reflecting changes in guideline recommendations over time. Relative wall thickness was calculated as (interventricular septum thickness + posterior wall thickness) / LV end diastolic diameter, and it was considered increased if >0.45 (paper I) or >0.42 (paper IV). LV volumes in systole and diastole were calculated from area tracings using the disc summation method (modified Simpson's rule) to calculate stroke volume (paper I). Evaluation of diastolic function was made by pulsed Doppler registrations. The mitral peak flow velocities of the early (E) and late (A) waves were used for the E/A ratio calculations.

Tissue Doppler echocardiography was performed in the apical four-chamber view, and pulsed wave Doppler was used to register the mitral annular systolic (s´) and early diastolic (e´) velocities. Calculation of the e´ mean (mean of the e´ septal and e´ lateral registrations) was used to assess the LV diastolic filling pressure, E/é. The left atrial volume was also measured as a diastolic parameter. For methodological details and reproducibility, see elsewhere (69,79).

3.2.6 Assessment of global cardiovascular risk by SCORE

The SCORE algorithm (57) was used to predict CV risk in patients (paper IV). SCORE is calculated by adding information on age, sex, smoking, systolic BP, and total cholesterol to predict the 10-year risk for CV mortality. (57). For better risk prediction, a web-based calculator was used with high-density lipoprotein included in the model as recommended in current guidelines (80).

3.2.7 Circulating biomarkers of endothelial activation and inflammation

Blood samples were obtained under fasting conditions from an antecubital vein after 20 minutes of supine rest by blood collection needles into Vacutainer tubes on ice. Following centrifugation (+4°C, 1500 × *g* for 15 min), plasma and serum were frozen immediately at −70°C. Routine biochemistry was performed following standard procedures.

In paper I, serum concentrations of hsCRP were immunologically measured in plasma (Dade Behring, Marburg, Germany), and serum concentrations of IL-6 were measured with the enzyme-linked immunosorbent assay technique (Novakemi AB, Stockholm, Sweden). E-selectin, ICAM-1 and VCAM-1 were measured by enzyme immunoassays (R&D systems, Abingdon, UK). In paper II, ICAM-3, IL-6, IL-8, hsCRP, tumor necrosis factor-α, and monocyte chemoattractant protein-1 were measured by assays from MesoScale Diagnostics (Human Cytokine Assay, Ultra-Sensitive Kit, MSD, Bethesda, USA).

3.3 STATISTICAL ANALYSIS

Data are presented as mean values ± SD (or SEM for calculated differences) or as medians and interquartile ranges. Skewed variables were log transformed. Group comparisons were made by the analysis of variance or by multivariate analysis of variance. Multiple linear regression analysis was used to assess the effects of treatment in papers I–IV. Pearson's correlation coefficient (r) was used to study markers of inflammation, endothelial activation and arterial function in relation to BP and LV hypertrophy (paper I), to study the relationship between the oscillometric single-cuff method by Arteriograph and the applanation tonometry method by SphygmoCor (paper III) and to study the relationship between endothelial

functional measurements and CV risk assessed by SCORE (paper IV). In addition to the linear regression model, Bland-Altman agreement analysis was used in paper III. In paper IV, associations with CV risk, as assessed by SCORE, were also assessed in a multivariable logistic regression model, including PWV and microalbuminuria, to improve CV risk prediction, as compared to SCORE alone (81,82).

The primary outcome in the SILVHIA study was to evaluate the reduction of LV mass by treatment with irbesartan and atenolol. A sample size of 48 patients per treatment group was selected to detect a difference of LV mass index from week 0 to 24 of 7.9 g/m^2 between groups, with 2 alpha 0.05 and beta 0.90. To allow for a dropout rate of 15%, 115 subjects were included (69).

The two co-primary outcomes in paper II were change in endothelial function, as assessed by flow-mediated vasodilatation, and change in hemostatic function, as measured by the generation of thrombin–antithrombin complex. To determine the size of the study population, assuming 2 alpha 0.05 and beta 0.80, we a priori calculated 2 × 24 subjects as sufficient to detect a 0.6% difference between the two study groups in flow-mediated vasodilatation by treatment, and 2 × 26 subjects as sufficient to detect a 0.4 μg/L difference in thrombin–antithrombin complex by treatment between the two groups (paper II). All statistical tests were 2-sided and carried out to a significance level *P* of <0.05.

3.4 ETHICAL CONSIDERATIONS

All studies were conducted in accordance with the Declaration of Helsinki and were approved by the Regional Ethics Committee in Stockholm. All participants gave their oral and written informed consent.

4 RESULTS

4.1 ARTERIAL STIFFNESS AND INFLAMMATION IN RELATION TO HYPERTENSION-INDUCED HEART DISEASE AND BP (PAPER I)

Patients with hypertension and LV hypertrophy had higher baseline BP compared with hypertensive patients with no LV hypertrophy (Table 2). The measures of metabolic status and kidney function were similar between the groups. Hypertensive subjects showed increased arterial stiffness compared to controls, and patients with hypertension and LV hypertrophy showed signs of increased arterial stiffness compared to hypertensive patients without LV hypertrophy and compared to controls. Furthermore, they also showed increased levels of inflammatory markers (hsCRP and IL-6), as presented in Figure 6. Levels of endothelial markers and leukocyte counts were similar in the normotensive controls and the hypertensive groups. Arterial stiffness was independently related to mean arterial BP and LV mass, and the AASI was independently related to mean arterial BP and hsCRP but not to

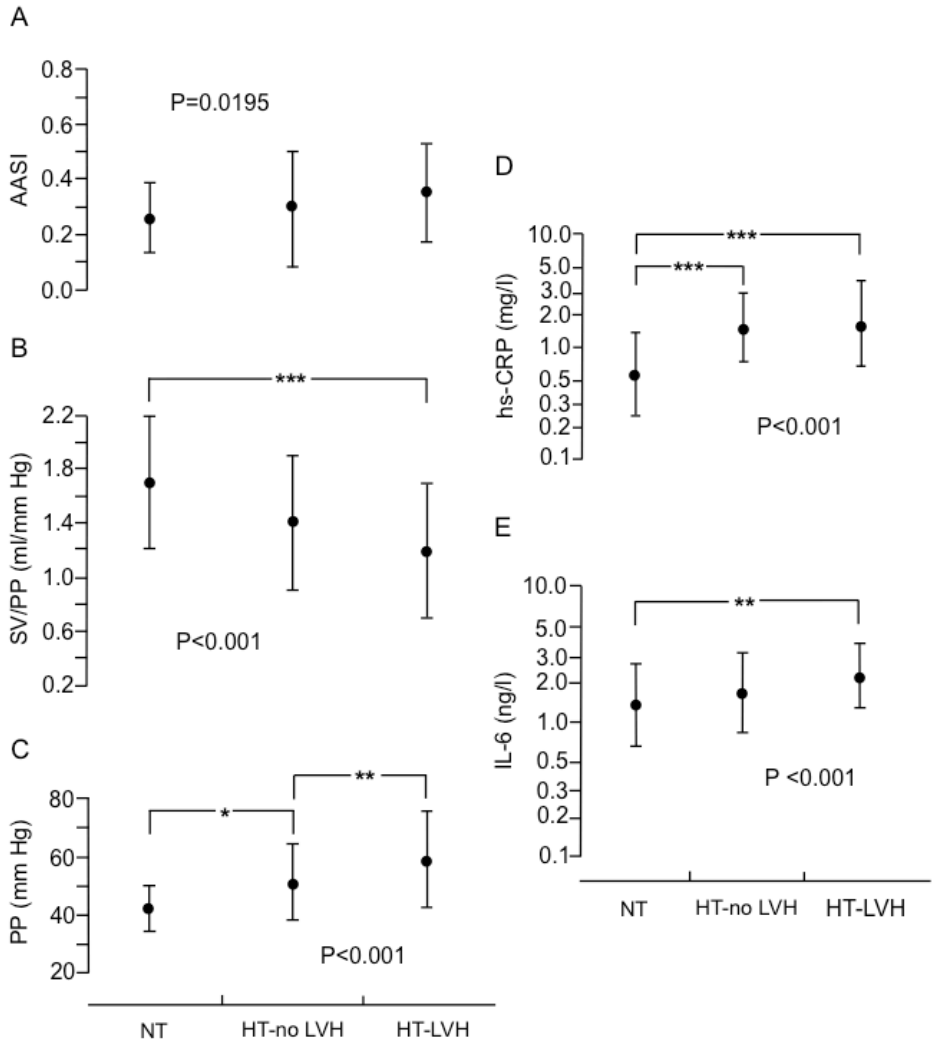

Figure 6. All data are presented as mean values ± SD. * p < 0.05; ** p < 0.01; *** p < 0.001. NT, normotensive controls; HT, hypertension; LVH, left ventricular hypertrophy; AASI, ambulatory arterial stiffness index, SV/PP, stroke volume/pulse pressure; PP, pulse pressure; hs-CRP, high-sensitivity C-reactive protein; IL-6, interleukin-6. From paper I.

endothelial adhesion molecules. Thus, hypertensive subjects also had signs of subclinical inflammation that were elevated with more pronounced hypertension and increased LV mass. Endothelial functional markers were similar comparing groups, and there were no relations between endothelial markers and BP or LV mass.

4.2 THE EFFECTS OF TREATMENT ON INDICES OF ARTERIAL STIFFNESS AND ENDOTHELIAL FUNCTION (PAPERS I AND II)

In paper I, BP reduction was similar in the irbesartan and atenolol groups, but reduction of LV mass was greater in the irbesartan group, as previously reported (69). Treatment had no effect on the AASI, and there were no differences when comparing treatment groups (Figure 7 a). Arterial compliance (SV/PP) was improved in both treatments groups, and SV/PP was

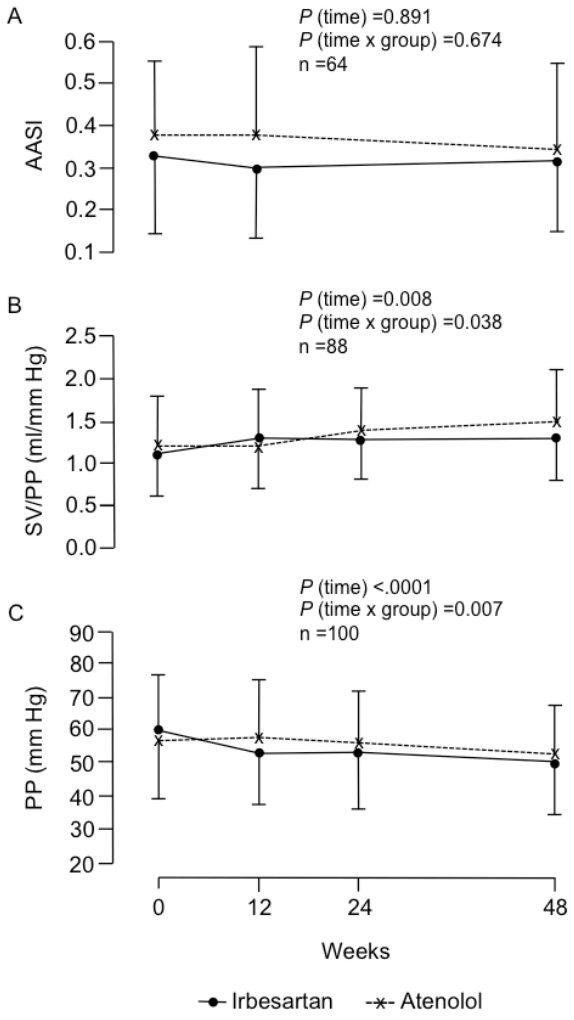

Figure 7. The effects of treatment with irbesartan (n = 35–54) and atenolol (n = 42–57) Mean values and SD. Multivariate analysis of variance, adjusted for treatment group. AASI, ambulatory arterial stiffness index, SV/PP, stroke volume/pulse pressure; PP, pulse pressure. From paper I

improved to a greater extent by atenolol, as compared to irbesartan. The greater reduction in heart rate (increasing stroke volume) by atenolol might be an explaining factor to this finding (Figure 7 b). Pulse pressure was reduced by both irbesartan and atenolol, with a larger reduction by the AT1-receptor blocker than by the beta adrenoceptor-blocker (Figure 7 c).

In paper II, the brachial and aortic BP was reduced by both treatments, with a larger reduction for ramipril, as compared to doxazosin. Central aortic BP was reduced more than brachial BP, with no differences between treatment groups. The carotid-femoral PWV and carotid-radial PWV was reduced by ramipril, as compared to doxazosin. AIx was reduced by both treatments, but there were no significant differences between treatments.

Relative changes comparing treatments are presented in Figure 8, which corresponds to the absolute values presented in Table 4.

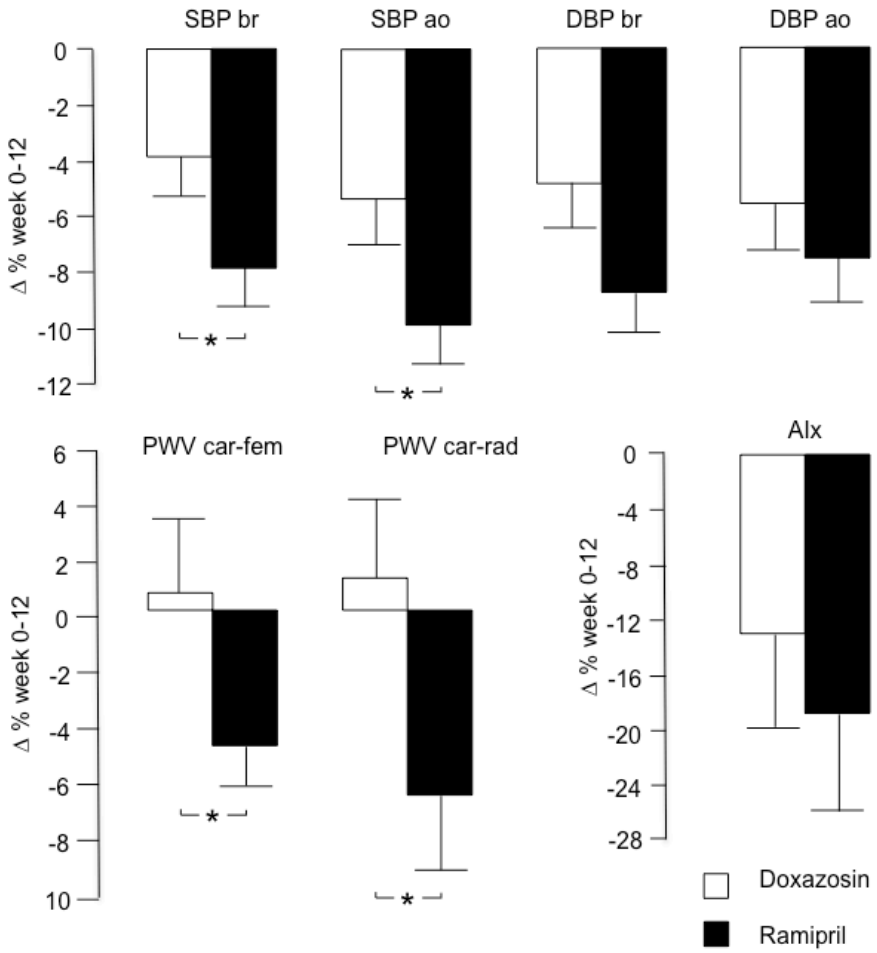

Figure 8. Relative changes (mean values ± SEM) in BP and vascular function by treatment. Significant treatment-induced changes between groups are shown as *$P < 0.05$. SBP, systolic blood pressure; br, brachial; ao, aortic; DBP, diastolic blood pressure; PWV, pulse wave velocity; car-fem, carotid-femoral; car-rad, carotid-radial; AIx, augmentation index. From paper II.

Table 4. Treatment effects on BP and indices of arterial stiffness (paper II)

	Week	Doxazosin	Ramipril	p-value by repeated measures MANOVA	
SBP br (mm Hg)	0	148.0 ± 11.0	148.3 ± 16.3	time	<0.001
	12	142.3 ± 12.1	136.2 ± 11.6	group	0.27
				time x group	0.030
SBP ao (mm Hg)	0	140.3 ± 12.9	139.2 ± 15.8	time	<0.001
	12	131.7 ± 14.8	124.7 ± 13.3	group	0.19
				time x group	0.039
DBP br (mm Hg)	0	89.0 ± 10.3	88.0 ± 8.1	time	<0.001
	12	84.6 ± 10.3	80.1 ± 8.7	group	0.21
				time x group	0.073
DBP ao (mm Hg)	0	90.9 ± 10.0	87.8 ± 7.5	time	<0.001
	12	85.2 ± 10.5	80.8 ± 7.1	group	0.058
				time x group	0.35
Heart rate (bpm)	0	58.9 ± 7.6	61.9 ± 8.1	time	0.79
	12	58.8 ± 9.7	61.3 ± 7.4	group	0.14
				time x group	0.90
PWV car-fem (m/s)	0	8.5 ± 1.5	8.9 ± 2.0	time	0.070
	12	8.3 ± 1.7	8.4 ± 1.9	group	0.42
				time x group	0.037
PWV car-rad (m/s)	0	8.7 ± 1.5	9.1 ± 1.0	time	0.38
	12	8.7 ± 1.2	8.4 ± 1.1	group	0.43
				time x group	0.034
AIx (%)	0	29.3 ± 10.4	30.7 ± 13.6	time	0.37
	12	27.1 ± 11.4	26.8 ± 12.1	group	0.78
				time x group	0.37

Mean values ± SD at week 0 and 12 for 27–33 subjects in each treatment group, including all subjects with valid measurements at week 0 or 12. *P* denotes significant changes by repeated measured MANOVA. For SBP, systolic blood pressure; br, brachial; ao, aortic; DBP, diastolic blood pressure; PWV, pulse wave velocity; car-fem, carotid-femoral; car-rad, carotid-radial; AIx, augmentation index.

In paper II, there were small effects of treatment on endothelial function as evaluated with FMD, GTN-mediated vasodilation and PWA, and change of RI, but no difference was seen between treatment groups as presented in Table 5. In addition, skin microcirculation – as assessed by LDF and iontophoresis – and heat-induced maximum hyperaemia remained unchanged by treatment.

Table 5. Assessment of endothelial function by treatment (Paper II).

	Week	**Doxazosin**	**Ramipril**	***p-value* by repeated measures MANOVA**	
FMD (%)	0	6.3 ± 4.4	5.3 ± 4.2	time	0.34
	12	5.5 ± 3.1	4.5 ± 4.3	group	0.75
	Δ 0-12	−0.3 ± 1.0	−1.1 ± 1.0	time x group	0.57
GTN (%)	0	15.5 ± 6.8	14.4 ± 7.0	time	0.92
	12	14.4 ± 7.0	14.4 ± 6.9	group	0.97
	Δ 0-12	−0.5 ± 1.3	0.3 ± 1.3	time x group	0.67
EFI	0	0.47 ± 0.38	0.49 ± 0.56	time	0.98
	12	0.51 ± 0.41	0.44 ± 0.64	group	0.90
	Δ 0-12	0.07 ± 0.12	0.07 ± 0.12	time x group	0.42
RI (%)	0	−7.3 ± 2.8	−6.8 ± 3.2	time	0.68
	12	−6.6 ± 3.1	−7.7 ± 3.8	group	0.54
	Δ 0-12	0.3 ± 0.9	−0.8 ± 1.0	time x group	0.43

Mean values ± SD for relative changes before and following drug treatment, and absolute changes by treatment (Δ, mean values ± SEM); 23-32 subjects in each treatment group. *P*-values for the differences calculated by repeated measures MANOVA, adjusted for age. FMD, flow mediated vasodilation; GTN, glyceryl trinitrate; EFI, endothelial functional index; RI, reflection index.

4.3 THE EFFECTS OF TREATMENT ON CIRCULATING ENDOTHELIAL AND INFLAMMATORY MARKERS (PAPERS I AND II)

In SILVHIA, there were small effects of treatment on circulating endothelial and inflammatory markers (paper I). Table 6 presents the results of treatment on circulating endothelial and inflammatory markers from SILVHIA during the initial 12 weeks with single drug therapy with irbesartan and atenolol (paper I) and from DoRa with ramipril or doxazosin under similar conditions (83). Taken together, there were no clear effects from blocking the RAAS on circulating endothelial and inflammatory markers compared to treatment with atenolol or doxazosin. It is important to note, however, that the biomarkers that were assessed and the analytical procedures that were used were somewhat different between the two studies.

4.4 EVALUATION OF A SINGLE-ARM OSCILLOMETRIC CUFF METHOD IN COMPARISON TO PWA WITH APPLANATION TONOMETRY (PAPER III)

First, we compared measures of indices of arterial stiffness by the oscillometric single-cuff method with the Arteriograph device to PWA by applanation tonometry by the SphygmoCor device. Baseline measurements of BP and indices of arterial stiffness are presented in Figure 9. As shown, the methods were closely related. However, the oscillometric cuff-based method recorded higher values as shown in the corresponding Bland-Altman analysis of mean differences (Figure 9). The relation for PWV values was weaker, but significant. This suggests that PWV measurements estimated with the oscillometric cuff method and those estimated by PWA with applanation tonometry are related but might not be interchangeable.

Second, the effects of treatment on central aortic BP and indices of arterial stiffness were evaluated in 58 patients at baseline who completed the study period of 12 weeks. At baseline, office BP values were, 151 ± 8/93 ± 10 mm Hg and 155 ± 9/93 ± 7 mm Hg for the doxazosin and ramipril groups, respectively. Treatment reduced brachial and aortic BP as assessed by the oscillometric cuff method, and reductions in both brachial and aortic BP were greater for ramipril compared to doxazosin. Treatment reduced the aortic systolic BP more than the brachial systolic BP, and this effect was greater in the ramipril group compared to the doxazosin group. Treatment improved the AIx in both treatments groups. By univariate analysis, this change in AIx was shown to be greater in the ramipril group compared to the doxazosin group. However, when adjusting for potential confounders this difference was weakened. Both doxazosin and ramipril reduced PWV and increased the transit time, with no significant differences between treatments. To conclude, the oscillometric single-cuff method was suitable for detecting treatment-induced changes in BP values and indices of arterial stiffness.

Table 6. Circulating endothelial markers and inflammatory markers in papers I and II

SILVHIA	**Irbesartan**		**Atenolol**			
Week	**0**	**12**	**0**	**12**	***P* (time)**	***P* (time x group)**
E-selectin (ng/ml)	48 [37;59]	48 [40;62]	42 [34;57]	39 [31;55]	0.029	0.031
ICAM-1 (ng/ml)	227 [191;256]	228 [200;259]	229 [194;272]	216 [191;272]	0.79	0.047
VCAM-1 (ng/ml)	613 [522;841]	603 [473;728]	613 [528;744]	654 [532;745]	0.81	0.08
hsCRP (mg/l)	1.7 [1.0;2.4]	1.2 [0.8;2.6]	1.3 [0.9;4.1]	1.4 [0.7;2.9]	0.03	0.08
IL-6 (ng/l)	2.2 [1.7;3.2]	2.4 [1.5;3.1]	1.9 [1.4;2.8]	1.9 [1.5;3.1]	0.64	0.24
DoRa	**Ramipril**		**Doxazosin**			
Week	**0**	**12**	**0**	**12**	***P* (time)**	***P* (time x group)**
E-selectin (ng/ml)	2.7 [1.5;4.0]	2.6 [1.7;5.1]	2.9 [1.8;4.0]	2.5 [1.7;3.7]	0.92	0.14
P-selectin (ng/ml)	14.1 [10.8;18;.9]	14.9 [10.8;22.0]	16.6 [12.1;20.9]	14.2 [10.9;18.1]	0.71	0.15
ICAM-3 (ng/ml)	0.4 [0.3;0.6]	0.5 [0.2;0.6]	0.5 [0.3;0.6]	0.4 [0.3;0.5]	0.60	0.56
hsCRP (mg/l)	1.3 [0.7;2.1]	1.4 [0.7;2.7]	1.8 [1.2;3.0]	1.4 [0.9;2.3]	0.52	0.11
IL-6 (pg/ml)	0.3 [0.2;0.4]	0.4 [0.2;0.5]	0.3 [0.2;0.4]	0.3 [0.2;0.4]	0.78	0.012
IL-8 (pg/ml)	1.9 [1.3;0.4]	1.7 [1.3;2.0]	1.8 [1.5;2.2]	1.5 [1.2;1.8]	0.13	0.69
TNF-α (pg/ml)	1.0 [0.8;1.1]	1.3 [0.8;1.2]	1.0 [0.8;1.2]	0.9 [0.8;1.1]	0.41	0.81
MCP-1 (pg/ml)	42 [37;48]	41 [35;43]	42 [34;51]	42 [36;52]	0.14	0.90

Median and interquartile range. Multivariate analysis of variance adjusted for study drug treatment and smoking. From (83) and unpublished data. ICAM-1, intracellular adhesion molecule-1; VCAM-1, vascular adhesion molecule-1; hsCRP, high sensitive C-reactive protein, IL-6, interleukin 6; IL-8, interleukin 8, TNF- α, tumor necrosis factor-α, MCP-1, monocyte chemoattractant protein-1.

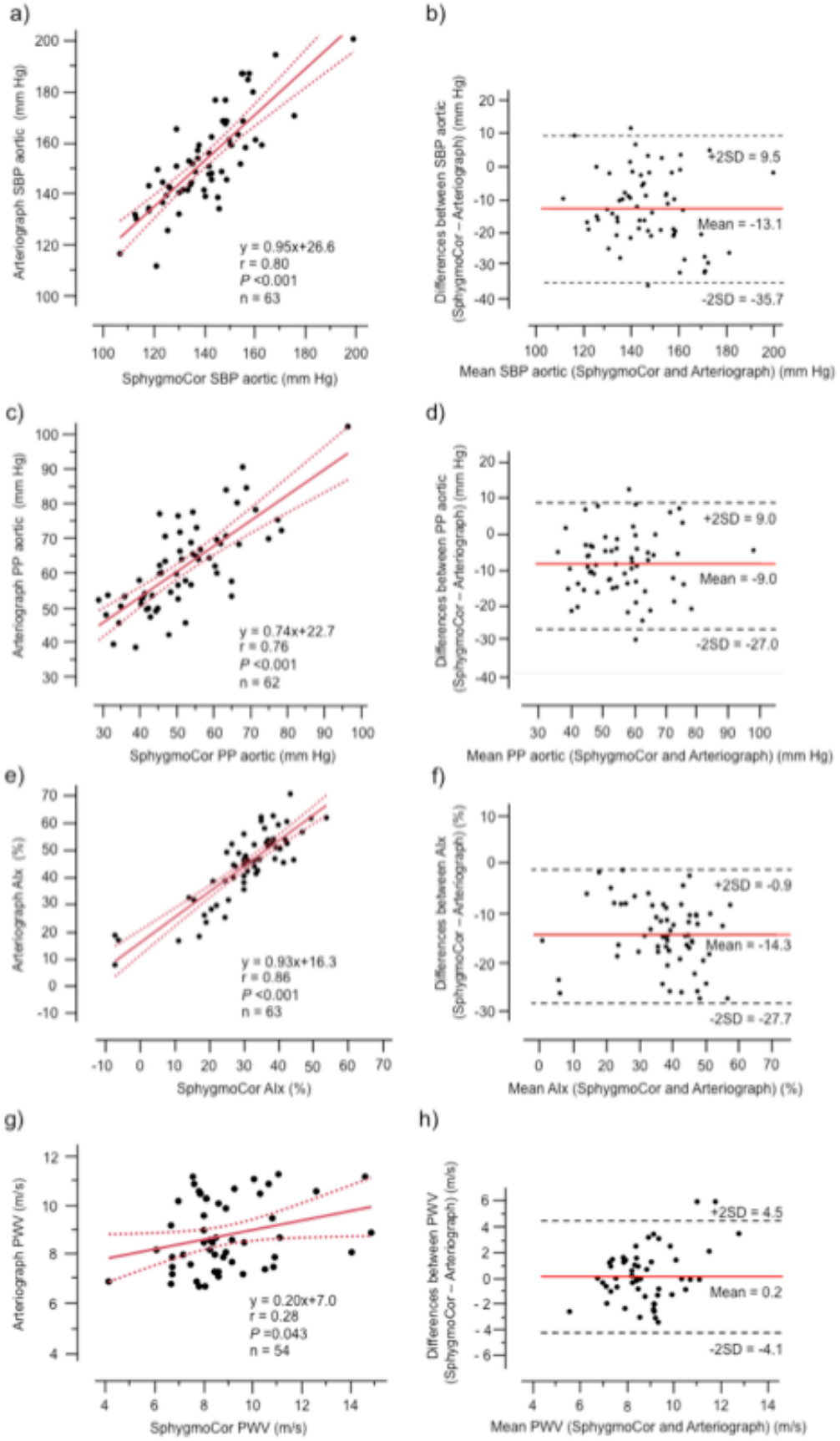

Figure 9. Comparison of the oscillometric single-cuff method and PWA with applanation tonometry. Pearson´s correlation coefficients and Bland-Altman agreement analysis of the difference in mean values for (a-b) systolic aortic BP (SBP), (c-d) aortic pulse pressure (PP), (e-f) augmentation index (AIx), and (g-h) pulse wave velocity (PWV). From paper III.

4.5 ENDOTHELIAL FUNCTION AND MICROVASCULAR REACTIVITY IN RELATION TO CV RISK, AS ASSESSED BY SCORE, AND TO HYPERTENSION-INDUCED HEART DISEASE (PAPER IV)

Our results showed that endothelial function in conduit arteries, as assessed by FMD, and the EFI were inversely related to SCORE (Figure 10). EIDV was not related to SCORE, and the change in RI was not related to SCORE. SEVR was not related to SCORE (Figure 11). Skin microcirculation was assessed by LDF and iontophoresis, and maximum skin-reactive hyperemia was evaluated by local heating. The relative change in peak flux after Ach iontophoresis was not related to SCORE, but both the EIDV relative change in peak flux and the heat-induced maximal hyperemia were inversely related to SCORE (figure 11).

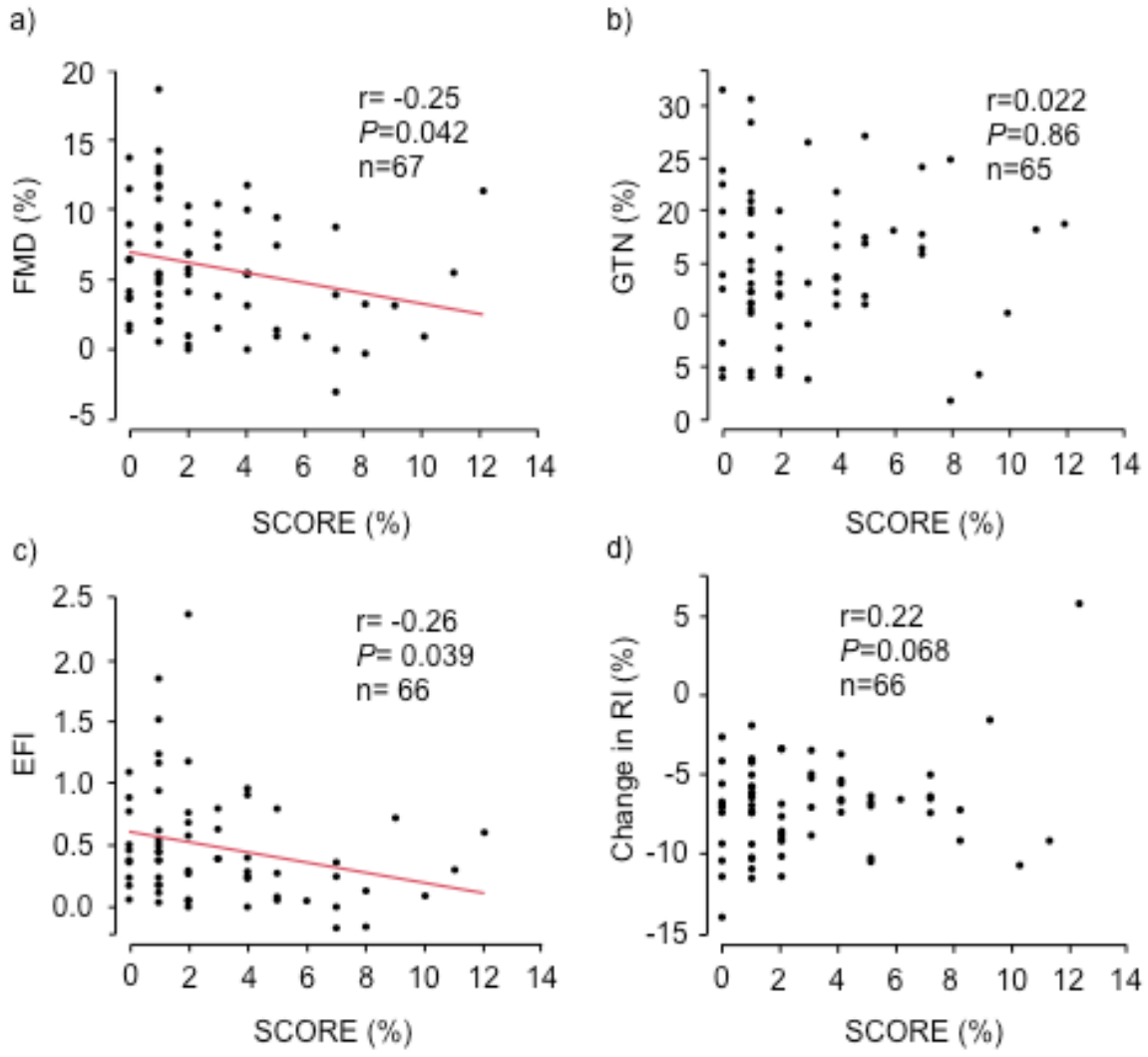

Figure 10. The relations between (a) flow-mediated vasodilatation (FMD), (b) glyceryl trinitrate (GTN)-mediated vasodilation, (c) endothelial functional index (EFI), and (d) relative change in reflection index (RI) before and after beta 2-adrenoceptor agonist stimulation, and the 10-year risk for a fatal cardio-vascular event as assessed by the systematic coronary risk evaluation (SCORE). From paper IV.

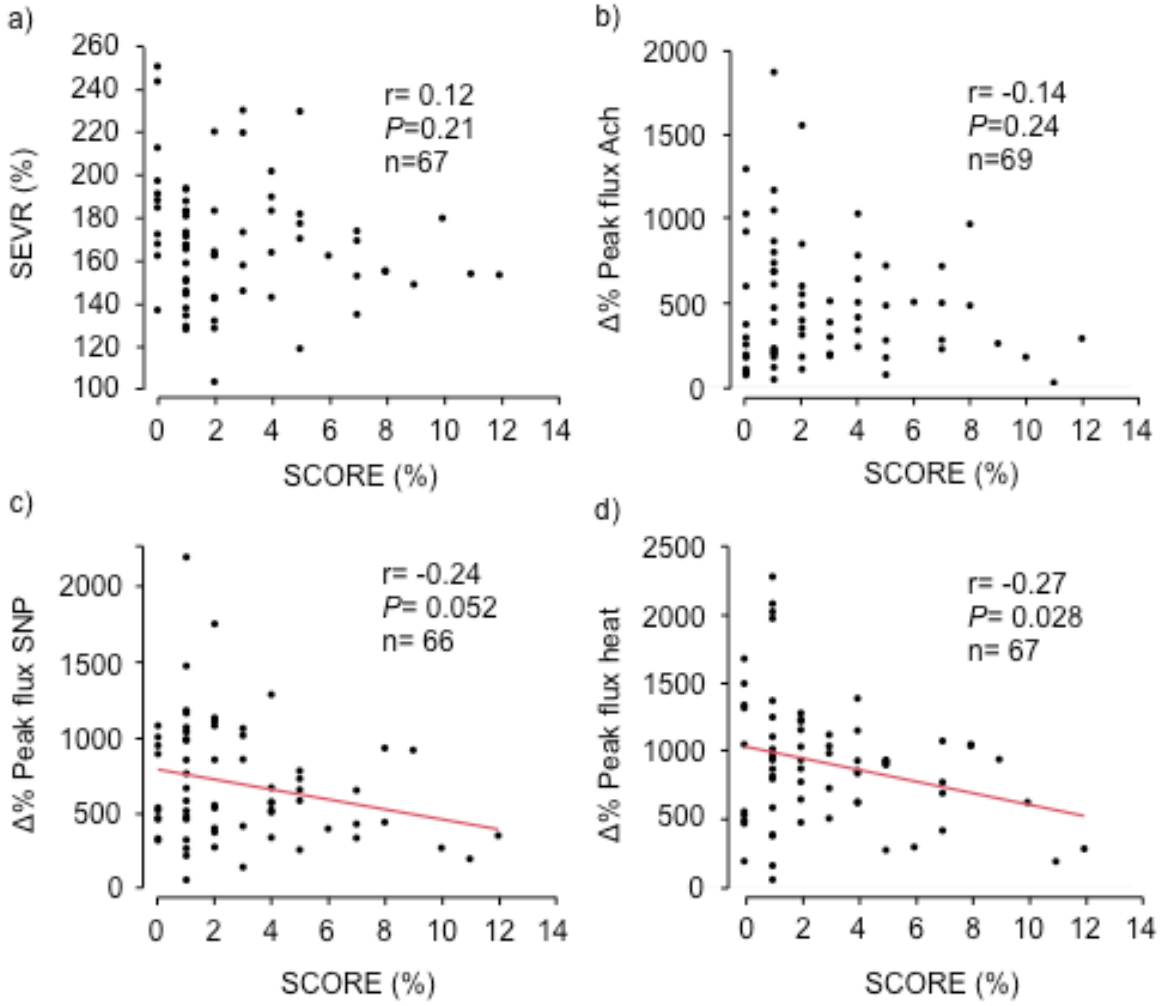

Figure 11. The relations between (a) the subendocardial viability ratio (SEVR), (b) the relative change in endothelial-dependent peak flux (Δ % Peak flux Ach), (c) the relative change in endothelial-independent peak flux (Δ % Peak flux SNP), and (d) the relative change in the peak flux after heat-induced maximal hyperemia (Δ % Peak flux heat) and the 10-year risk for a fatal cardiovascular event as assessed by the systematic coronary risk evaluation (SCORE). From paper IV.

FMD was not related to LV mass, and there were no relations between FMD and diastolic parameters (i.e. relative wall thickness, E/A, E/e´, or left arterial size). The EFI tended to be inversely related to the left arterial volume, but no other diastolic measures approached significance. The SEVR tended to be improved by reduced filling pressures, E/é, but the change in RI and the skin microcirculation were not related to LV mass index or diastolic function. FMD tended to be related to the aortic pulse pressure, and the SEVR was inversely related to aortic pulse pressure (Figure 12 a-b). Both FMD and the SEVR were inversely related to PWV (Figure 12 c-d). GTN-mediated vasodilation, EFI, and the change in RI were not related to PWV or aortic pulse pressure. In skin microcirculation, both the Ach peak flux and the peak flux after heat-induced maximal hyperemia were related to the carotid-femoral PWV / carotid-radial PWV ratio (Figure 12 e-f). The SNP-induced peak flux was not related to the PWV ratio.

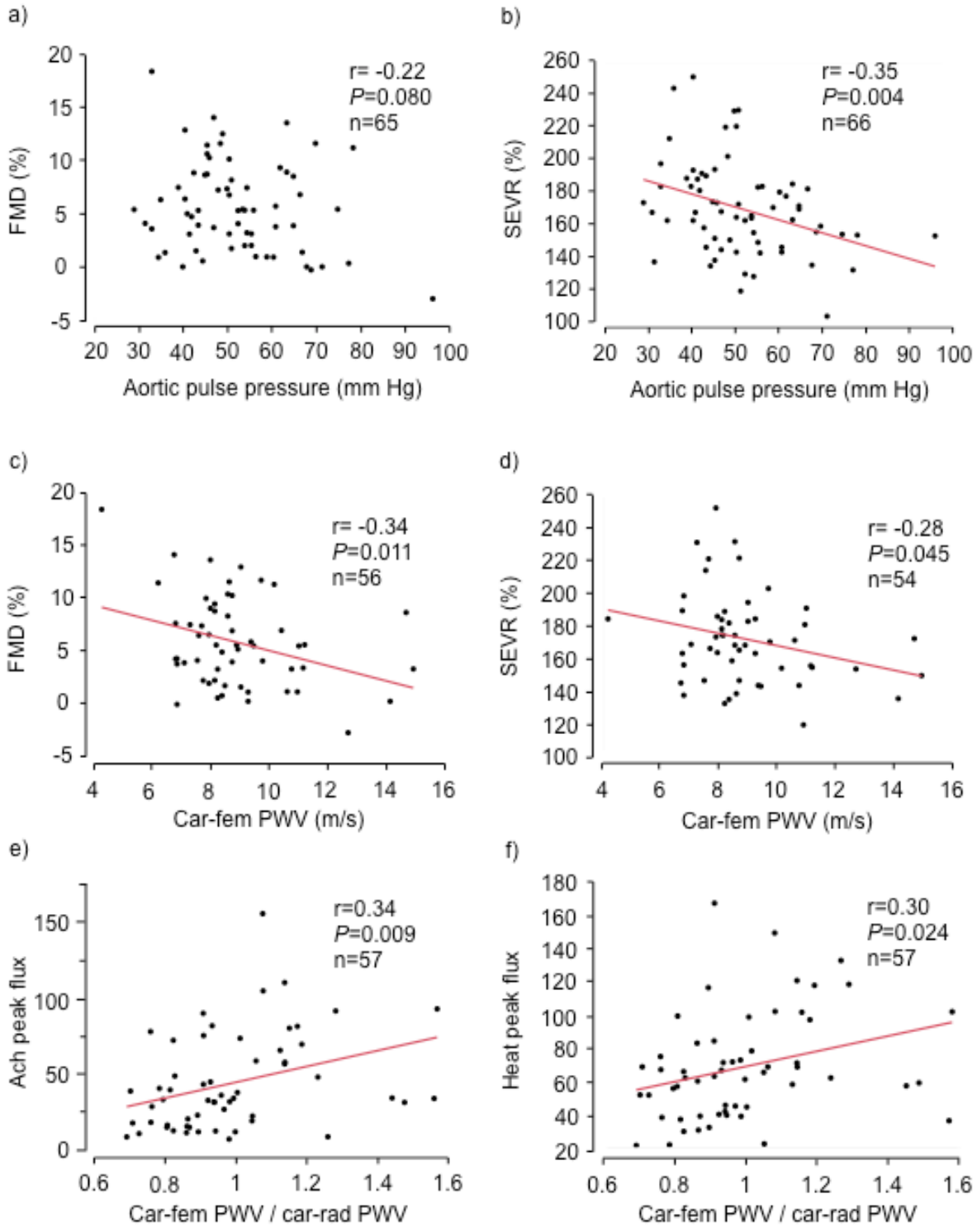

Figure 12. The relations between (a) flow mediated vasodilataion (FMD), (b) the subendocardial viability ratio (SEVR), and aortic pulse pressure, (c) FMD, (d) SEVR, and carotid femoral pulse wave velocity (car-fem PWV), (e) Acetylcholine mediated peak flux (Ach peak flux), (f) heat induced peak flux (Heat peak flux), and the carotid-femoral to carotid-radial pulse wave velocity ratio (car-fem PWV / car-rad PWV). From paper IV.

4.6 COMPARISON OF METHODS TO EVALUATE INDICES OF ENDOTHELIAL FUNCTION IN DIFFERENT VASCULAR BEDS (PAPER IV)

Methods representing different vascular beds were poorly interrelated. Thus, endothelial function in the conduit arteries as assessed by FMD did not relate to the RI change, the SEVR, or the skin microcirculation. GTN-mediated vasodilation tended to be related to the RI change and tended to be inversely related to the skin microcirculatory response to the Ach peak flux. The EFI tended to be related to the SEVR. There was a trend for the RI change to be inversely related to the skin microcirculatory response to the Ach peak flux, and the RI change was inversely related to the Ach mediated relative peak flux change. This means that a larger reduction of RI after beta 2-adrenoceptor agonist stimulation – which indicates improved endothelial function in the resistance arteries – is related to skin microvascular endothelial function. Interestingly, there were no relations between coronary microcirculation and skin microcirculation.

5 GENERAL DISCUSSION

5.1 THE RELATIONS BETWEEN BP, ARTERIAL STIFFNESS, INFLAMMATION, AND HYPERTENSION-INDUCED HEART DISEASE

Patients with hypertension show signs of increased systemic inflammation (84,85) and increased arterial stiffness (51), and our results showed that both were independently related to BP (paper I). In patients with hypertension-induced LV hypertrophy, these signs of inflammation and increased arterial stiffness are more pronounced. These results suggest that arterial stiffness contributes to an increase in LV mass beyond the effects of elevated BP. In contrast, circulating markers of endothelial activation were not related to BP or LV mass. Thus, endothelial activation appears to be less prominent in patients with uncomplicated hypertension, as compared to subjects with established atherosclerotic disease.

5.2 THE EFFECT OF ANTIHYPERTENSIVE TREAMENT ON VASCULAR STUCTURE AND FUNCTION

Our results in papers I–III showed that antihypertensive treatment with different drug classes to lower BP also reduces arterial stiffness. The effects of treatment on BP and the indices of arterial stiffness in the SILVHIA and DoRa trials are summarized in Table 7. Furthermore, the prevailing evidence that inhibition of the RAAS has additional effects on vascular structure and function beyond BP reduction is supported by the current results. Thus, there was a greater reduction in pulse pressure by an AT1-receptor blocker, as compared to a beta-adrenoceptor blocker, and the effects on blood pressure and arterial stiffness were related to the degree of activation of the RAAS (paper I). Also, the reductions in central aortic BP, PWV, and AIx were greater with an ACE inhibitor, as compared to an alpha-adrenoceptor blocker (papers II and III), suggesting that both aortic stiffness and resistance vessels were affected by the treatment.

The effects of AT1-receptor blockers are mediated by blocking ANG II-induced stimulation of AT1-receptors. The interrupted negative feedback by AT1-receptor blockers on ANG II formation will increase the concentrations of ANG II, and this will stimulate AT2- receptors and reduce vasoconstriction, hypertrophy, and vascular inflammation. AT1-receptor blockers thus seem to have additional effects on vascular structure and function beyond their BP-lowering effect (86,87). However, the relative contribution of AT1- and AT2-receptor-mediated effects to this reduction in BP cannot be evaluated with the present results. Furthermore, ACE inhibitors also seem to have beneficial effects beyond BP reduction (60,61), and they have been shown to reduce aortic stiffness and wave reflection more than beta-adrenoceptor blockers, and to reduce central BP more than other drug classes (59,88). This remodeling process by ACE inhibitors to reduce arterial stiffness and to increase NO availability appears to be dose-dependent (89,90). ACE inhibitors also block bradykinin inactivation, and bradykinin is a strong stimulus for NO release that induces NO-mediated

Table 7. Effects of antihypertensive treatment by blocking the RAAS versus blocking the sympathetic nervous system on blood pressure and indices of arterial stiffness

	SILVHIA (paper I)		DoRa (paper II)	
	AT1-receptor blocker	**Beta-adrenoceptor blocker**	**ACE inhibitor**	**Alpha-adrenoceptor blocker**
SBP brach	↓↓	↓↓	↓↓↓	↓↓
DBP brach	↓↓	↓↓	↓↓	↓↓
SBP aortic			↓↓↓	↓↓
DBP aortic			↓↓	↓↓
HR	↓	↓↓↓	→	→
PP brach	↓↓↓	↓↓	↓↓↓	↓
PP aortic			↓↓↓	↓
SV/PP	↑↑	↑↑↑		
AASI	↓	↓		
PWV			↓↓↓	↓↓
AIx			↓↓	↓↓

SBP, systolic blood pressure; brach, brachial; DBP, diastolic blood pressure, HR, heart rate; PP, pulse pressure; SV, stroke volume; AASI, ambulatory arterial stiffness index; PWV, pulse wave velocity; AIx, augmentation index.

vasodilation in muscular arteries. These effects through bradykinin might also contribute to the beneficial effects of ACE inhibitors in preventing CV events in high-risk patients (62,63).

Endothelial function was evaluated by different techniques in several different vascular beds, and no effects of antihypertensive treatment were seen on endothelial function (paper II). Also, there were no differences between treatments with ramipril and doxazosin. Our results

are in agreement with others showing only small effects of ACE inhibition on endothelial function, as evaluated by FMD (91). Further circumstantial support for these findings was provided by the results of circulating markers of endothelial activation that showed only minor effects on these markers by treatment (paper I, Table 6). Our results are in agreement with others showing small effects on circulating endothelial and inflammatory markers in hypertensive patients (92,93). Thus, blocking the RAAS has no significant effect on circulating endothelial biomarkers in patients with mild-to-moderate hypertension and no signs of atherosclerotic disease.

In conclusion, antihypertensive treatment improves vascular structure and function. This suggests that antihypertensive treatment to achieve target BP is important in order to reduce vascular abnormalities and to maintain normal vascular function. Blocking the RAAS has additional effects beyond BP reduction on indices of arterial stiffness. Treatment might have little effect on systemic inflammation or endothelial activation in hypertensive subjects with no overt atherosclerotic disease, although beneficial effects might be observed in patients with established atherosclerotic disease.

5.3 THE EVALUATION OF AN OSCILLOMETRIC SINGLE-CUFF METHOD COMPARED TO PWA WITH APPLANATION TONOMETRY

An oscillometric single-arm cuff method (Arteriograph) was compared to PWA with applanation tonometry (SphygmoCor) (paper III). Simultaneously measured values of aortic BP, aortic pulse pressure, and AIx were closely related with the two devices, while values of estimated PWV were more weakly related, but still significant. These two methods are based on different technologies to measure central BP and indices of arterial stiffness. PWA by radial applanation tonometry with the SphygmoCor uses a general transfer function in the software of the device to recalibrate the radial pulse waveform to the aortic waveform. Furthermore, BP from the brachial artery is used for this recalculation of aortic BP, but this causes a systematic error with an underestimation of the aortic systolic BP and an overestimation of the aortic diastolic BP compared to invasive BP measurements (94). This error can be corrected with calibrations, as discussed by others (95-97). Also, the estimated AIx seems to be underestimated due to underestimated measurements of aortic BP. In contrast, the oscillometric cuff method measures brachial BP, and it detects pulse wave propagation in the aortic arch and large arteries. The calculation of aortic BP is based on the reflected late systolic wave, and it shows agreement with invasive aortic systolic BP recordings (98). PWV is also obtained differently with the two methods. PWV by applanation tonometry is based on several pulse wave registrations, where the variation of the isovolumetric contraction time over time can influences the results. The oscillometric cuff method uses a single pulse wave registration and the transit time of the returning pulse wave from the aortic bifurcation. It has been argued that PWV estimated by the oscillometric single-cuff device might be dependent on stiffness from the brachial artery and the fact that the reflecting pulse wave is originating from the branching of the subclavian artery from the

aorta (99). However, these assumptions are based on a mathematical model, and there is no in vivo data supporting these results (100).

In addition, the ability of the oscillometric single-cuff method to assess effects of treatment on BP and indices of arterial stiffness was evaluated (paper III). The single-cuff method detected greater reductions in brachial to aortic systolic BP levels and AIx by ramipril than by doxazosin. This interesting finding suggests that this method might be more sensitive for detecting treatment-induced changes on vascular stiffness compared with PWA by applanation tonometry. In addition, the oscillometric single-cuff method might be more sensitive to detecting changes in pulsatile flow that represent early structural changes. This is suggested by the greater reduction in aortic BP and AIx by ramipril, as compared to doxazosin as assessed by PWA with applanation tonometry.

In conclusion, the oscillometric single-cuff appears suited for risk evaluation and for evaluation of the temporal changes of aortic BP and indices of arterial stiffness that are induced by antihypertensive treatment in the clinical setting. This method is easy to use compared to the more complicated and operator-dependent PWA method using applanation tonometry.

5.4 ENDOTHELIAL FUNCTION IN RELATION TO RISK ASSESSMENT BY SCORE AND BY HYPERTENSION–INDUCED HEART DISEASE

A cross sectional analysis evaluated indices of endothelial function and microvascular reactivity simultaneously in different vascular beds in relation to global CV risk, as assessed by SCORE, and by signs of hypertension-induced heart disease (paper IV). Our findings show that endothelial function in different vascular beds are all related to global CV risk, and thus the impaired endothelial function in hypertension is to some degree generalized and provides prognostic information in the hypertensive patient. Furthermore, the inverse relationship between FMD and SCORE was strengthened by adding carotid-femoral PWV into the statistical model, suggesting that PWV might improve CV risk assessment with SCORE (81,82).

However, these different methods were poorly interrelated, suggesting that they represent different aspects of future CV risk. There are several potential reasons as to why the methods were poorly related. The key factor is that these methods measure endothelial function in different vascular beds. FMD represents endothelial function in larger conduit arteries, the PWA method with beta 2-adrenoceptor agonist represents smaller resistance arteries, LDF and iontophoresis the skin microcirculation, and the SEVR represents the coronary microcirculation. Vascular structure and function are different throughout the cardiovascular system. Local NO availability varies in different vascular beds, and larger conduit arteries have higher NO-synthase activity, as compared to smaller vessels (101). In resistance arteries, about one third of the EDV is mediated by NO, and about two thirds of the response is

mediated by endothelium hyperpolarizing factor and influence on potassium channels (13). In hypertensive subjects, there are signs of vascular remodeling with no relation to endothelial dysfunction (102,103). Thus, this might suggest that patients with mild-to-moderate hypertension might undergo remodeling in smaller arteries before the development of endothelial dysfunction. Also the stimulus for EDV is of importance. For FMD, the post-ischemic reactive hyperaemia after cuff release causes local mechanical shear stress, which mediates NO release. In contrast, receptor-mediated stimulation is used for PWA with applanation tonometry and for iontophoresis and skin microcirculation. Also the route of administration, the dose, and the pharmacological agent can influence the vascular response.

There was no relation between endothelial functional measurements and hypertension-induced heart disease. This finding suggests that the risk factors related to endothelial dysfunction and SCORE and the risk factors related to hypertension-induced heart disease are not the same. For endothelial dysfunction, risk factors like smoking, hyperglycemia, and dyslipidemia are important, whereas for the development of LV hypertrophy and diastolic dysfunction, the influence of an activated RAAS and the sympathetic nervous system and BP are more important mediators.

In conclusion, it is important to evaluate endothelial function in hypertensive patients because this might improve risk prediction. Endothelial functional measurements in different vascular beds are associated with global CV risk, as assessed by SCORE, but not to hypertension-induced heart disease. However, these different methods are poorly interrelated. Thus, in a clinical setting the evaluation of arterial stiffness and endothelial function in conduit arteries should be evaluated simultaneously in hypertensive patients with mild or moderate risk, in addition to SCORE values, in order to further improve risk prediction.

5.5 CLINICAL IMPLICATIONS AND FUTURE PERSPECTIVES

Hypertension is a major contributor to CV morbidity and mortality. Thus, BP reduction and improvement of preventive strategies in the clinical settings are of great importance to reduce risk of future CV events. Global risk assessment plays a central role in guiding the doctor in the evaluation of the hypertensive patient to improve decision-making regarding therapy, which is of outmost prognostic importance. In addition, it has an informative and educational value for the individual patient to learn more about the importance of life style changes in combination with the effects of treatment, to achieve an optimal BP.

In paper I, our results showed an independent relation between arterial stiffness and hypertension induced heart disease. In addition, hypertension seems to be associated with vascular inflammation, which contributes to the evolution of arterial stiffness, in addition to BP. Thus, evaluation of vascular stiffness is important. We showed in paper III that the oscillometric single-cuff method is well suited to evaluate indices of arterial stiffness and could detect treatment-induced changes. Arterial stiffness is easy to evaluate by PWV, which

is an independent predictor of future CV events, and improves risk prediction (41,42). Furthermore PWV can be used to reclassify hypertensive patients with intermediate risk, as recommended in current guidelines (104). In addition, evaluation of central BP and wave reflection is important, as the aortic BP levels are different from brachial BP levels. Thus, the effects of treatment on central hemodynamics give additional information to conventional brachial BP measurements in order to improve risk evaluation and the effects of treatment in hypertensive patients.

Our results from paper IV suggested that endothelial function in the macrocirculation and microcirculation is related to increased CV risk, assessed by the SCORE algorithm. Thus, endothelial dysfunction in patients with uncomplicated mild-to-moderate hypertension appears to be associated with increased CV risk and assessment of endothelial function may improve risk prediction. Increased LV mass is also a known CV risk factor, but in contrast, our results showed no relations between endothelial functional measurements and hypertension induced heart disease. Our results suggest that endothelial dysfunction and increased LV mass represents different aspects of CV risk.

An improvement of risk evaluation where the information of vascular structure and function is taken in account in risk prediction is important. In hypertensive patients with mild-to-moderate risk the evaluation of arterial stiffness and endothelial function in different vascular beds could give additional information of future CV risk. It would be interesting to evaluate treatment effects on indices of arterial stiffness, as compared to BP reduction, in a randomized prospective outcome study in hypertensive patients with intermediate risk. Furthermore, it would be interesting to evaluate the contribution of adding PWV and AIx to the SCORE algorithm to improve risk evaluation in hypertensive patients.

6 CONCLUSIONS

1. Hypertension induced heart disease is independently related to both BP and indices of arterial stiffness.

2. Circulating inflammatory markers are elevated in hypertensive patients, and further increased in hypertensive patients who in addition are present with hypertension induced heart disease, with LV hypertrophy.

3. Risk prediction in hypertensive patients may be improved by evaluation of endothelial function in addition to global CV risk, assessed by SCORE.

4. Indices of endothelial function in different vascular beds are not closely interrelated, and are not related to hypertension-induced heart disease, suggesting that endothelial dysfunction and LV hypertrophy represents different aspects of CV risk.

5. An oscillometric single-cuff method (Arteriograph) can be used to evaluate arterial stiffness and central haemodynamics, and this technique is well suited to evaluate the effects of antihypertensive treatment on central BP and indices of arterial stiffness.

6. Endothelial function in macro- and microcirculation is unchanged by antihypertensive treatment in patients with hypertension and no signs of atherosclerotic disease.

7. Circulating endothelial biomarkers and inflammatory markers are not influenced by antihypertensive therapy in patients with mild-to-moderate hypertension. Treatment induced improvement of endothelial function and systemic inflammation with antihypertensive treatment may require the presence of more advanced atherosclerotic disease.

8. Blocking the RAAS by AT1-receptor blockers or ACE inhibitors improves indices of arterial stiffness more than antihypertensive treatment based on drugs blocking the effects of the sympathetic nervous system (alpha 1-adrenoceptor blockers or beta 1-adrenoceptor blockers).

9. Thus, drugs blocking the RAAS may offer an advantage in the treatment of hypertension beyond the effects on blood pressure reduction, as compared to other drug classes.

7 SVENSK SAMMANFATTNING

Bakgrund: Högt blodtryck (hypertoni), leder till förändringar i blodkärlen och hjärtat vilket orsakar en ökad kärlstyvhet och förtjockning av hjärtmuskeln (vänsterkammarhypertrofi). Detta är kända riskfaktorer för framtida kardiovaskulär sjukdom.

Regleringen av blodtrycket styrs bland annat av det sympatiska nervsystemet och renin-angiotensin-aldosteron systemet (RAAS). Ett aktiverat RAAS leder till att angiotensin II bildas, vilket orsakar inflammation och strukturella förändringar i hjärta och kärl. Det finns därför mycket som talar för att blodtrycksmediciner som blockerar RAAS har positiva effekter utöver att sänka blodtrycket.

Det övergripande syftet med detta projekt var att öka kunskapen om strukturella och funktionella kärlförändringar vid hypertoni hos människa. Vidare studerades hur blodtrycks-handling påverkade kärlstyvhet och endotelfunktion. Blodtrycksmediciner som hämmar RAAS jämfördes med mediciner som hämmar det sympatiska nervsystemet för att studera behandlingseffekter utöver blodtryckssänkningen.

Material och metoder: Denna avhandling baseras på två kliniska studier. I SILVHIA (Swedish irbesartan left ventricular hypertrophy investigation versus atenolol; delarbete I) studerades 115 patienter med hypertoni och vänsterkammarhypertrofi, som randomiserades till behandling med en angiotensin II-antagonist (irbesartan) eller en beta-receptorblockerare (atenolol) under 48 veckor. Dessa jämfördes med en grupp som hade hypertoni utan vänster-kammarhypertrofi, och en grupp friska kontroller med normalt blodtryck. Olika mått på kärlstyvhet och cirkulerande biomarkörer för endotelcellsaktivitet och inflammation studerades vid studiestart samt efter 12 och 48 veckors behandling.

I DoRa (Doxazosin-ramipril studien; delabete II-IV) studerades 71 patienter med obehandlad mild till måttlig primär hypertoni. Patienterna randomiserades till blodtryckssänkande behandling med en ACE-hämmare (ramipril) eller en alfa-receptor blockerare (doxazosin) under 12 veckor. Effekten på kärlstyvhet mättes med pulsvågsanalys och applanations-tonometri (SphygmoCor), och med en oscillometrisk metod med blodtrycksmanschett (Arteriograph). Endotelfunktionen mättes samtidigt i flera kärlbäddar med olika icke invasiva metoder: flödesmedierad vasodilatation (större konduktanskärl), pulsvågsanalys med beta 2-agonist stimulering (mindre resistenskärl), laser Doppler fluxmetri med jontofores (hudens mikrocirkulation), och pulsvågsanalys (kranskärlens mikrocirkulation). Undersökningarna genomfördes vid studiestart och efter 12 veckors behandling.

Resultat och slutsatser: Patienter med hypertoni visade tecken på en ökad inflammatorisk aktivitet, mätt med cirkulerande biomarkörer, och en ökad kärlstyvhet. Inflammation var oberoende relaterad till blodtryck, och kärlstyvhet var oberoende relaterad till blodtryck och till vänsterkammarmassa (delarbete I). Behandling sänkte blodtrycket och minskade kärlstyv-heten. Blodtrycksmediciner som hämmade RAAS minskade kärlstyvheten mer jämfört med

mediciner som hämmade det sympatiska nervsystemet (delarbete I och II). Däremot sågs inga effekter av behandling på endotelfunktionen mätt med de olika metoderna (delarbete II). Även cirkulerande biomarkörer för endotelcellsaktivitet och inflammation var oförändrade och behandlingen hade liten effekt (delarbete I och II). Den användarvänliga oscillometriska metoden med en blodtrycksmanschett (Arteriograph) var lämpad för att utvärdera effekter av behandling på kärlfunktion (delarbete III). Endotelfunktion mätt i olika kärlbäddar relaterade till riskdiagrammet SCORE, men det fanns ingen relation mellan endotelfunktion och hypertensiv hjärtsjukdom. Detta talar för att olika riskfaktorer bidrar till utvecklingen av endoteldysfunktion respektive hypertensiv hjärtsjukdom. Endotelfunktionsmätningar i olika kärlbäddar var inte relaterade, vilket talar för att endotelfunktionen i stora och små blodkärl inte är direkt jämförbara (delarbete IV).

Sammanfattningsvis visar våra resultat att blodtrycksbehandling som hämmar RAAS har fördelaktiga tilläggseffekter utöver den blodtryckssänkande effekten hos patienter med primär mild till måttlig hypertoni. Detta talar för att patienter med hypertoni i första hand ska behandlas med blodtrycksmediciner som hämmar RAAS.

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9 REFERENCES

1. Folkow B, Grimby G, Thulesius O. Adaptive structural changes of the vascular walls in hypertension and their relation to the control of the peripheral resistance. Acta Physiol Scand 1958;44:255-72.

2. Panza JA, Quyyumi AA, Brush JE, Jr., Epstein SE. Abnormal endothelium-dependent vascular relaxation in patients with essential hypertension. N Engl J Med 1990;323:22-7.

3. Grassi G, Mark A, Esler M. The sympathetic nervous system alterations in human hypertension. Circ Res 2015;116:976-90.

4. Te Riet L, van Esch JH, Roks AJ, van den Meiracker AH, Danser AH. Hypertension: renin-angiotensin-aldosterone system alterations. Circ Res 2015;116:960-75.

5. Fyhrquist F, Saijonmaa O. Renin-angiotensin system revisited. J Intern Med 2008;264:224-36.

6. Nguyen G, Delarue F, Burckle C, Bouzhir L, Giller T, Sraer JD. Pivotal role of the renin/prorenin receptor in angiotensin II production and cellular responses to renin. J Clin Invest 2002;109:1417-27.

7. Imanishi T, Tsujioka H, Ikejima H et al. Renin inhibitor aliskiren improves impaired nitric oxide bioavailability and protects against atherosclerotic changes. Hypertension 2008;52:563-72.

8. Parving HH, Brenner BM, McMurray JJ et al. Cardiorenal end points in a trial of aliskiren for type 2 diabetes. N Engl J Med 2012;367:2204-13.

9. Ghiadoni L, Taddei S, Virdis A. Hypertension and endothelial dysfunction: therapeutic approach. Curr Vasc Pharmacol 2012;10:42-60.

10. Montezano AC, Nguyen Dinh Cat A, Rios FJ, Touyz RM. Angiotensin II and vascular injury. Curr Hypertens Rep 2014;16:431.

11. Marchesi C, Paradis P, Schiffrin EL. Role of the renin-angiotensin system in vascular inflammation. Trends Pharmacol Sci 2008;29:367-74.

12. Anderson TJ, Uehata A, Gerhard MD et al. Close relation of endothelial function in the human coronary and peripheral circulations. J Am Coll Cardiol 1995;26:1235-41.

13. Deng LY, Li JS, Schiffrin EL. Endothelium-dependent relaxation of small arteries from essential hypertensive patients: mechanisms and comparison with normotensive subjects and with responses of vessels from spontaneously hypertensive rats. Clin Sci (Lond) 1995;88:611-22.

14. Corretti MC, Anderson TJ, Benjamin EJ et al. Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery: a report of the International Brachial Artery Reactivity Task Force. J Am Coll Cardiol 2002;39:257-65.

15. Celermajer DS, Sorensen KE, Gooch VM et al. Non-invasive detection of endothelial dysfunction in children and adults at risk of atherosclerosis. Lancet 1992;340:1111-5.

16. Wilkinson IB, Hall IR, MacCallum H et al. Pulse-wave analysis: clinical evaluation of a noninvasive, widely applicable method for assessing endothelial function. Arterioscler Thromb Vasc Biol 2002;22:147-52.

17. Lind L, Fors N, Hall J, Marttala K, Stenborg A. A comparison of three different methods to evaluate endothelium-dependent vasodilation in the elderly: the Prospective Investigation of the Vasculature in Uppsala Seniors (PIVUS) study. Arterioscler Thromb Vasc Biol 2005;25:2368-75.

18. Morris SJ, Shore AC, Tooke JE. Responses of the skin microcirculation to acetylcholine and sodium nitroprusside in patients with NIDDM. Diabetologia 1995;38:1337-44.

19. Gooding KM, Hannemann MM, Tooke JE, Clough GF, Shore AC. Maximum skin hyperaemia induced by local heating: possible mechanisms. J Vasc Res 2006;43:270-7.

20. Tsiachris D, Tsioufis C, Syrseloudis D et al. Subendocardial viability ratio as an index of impaired coronary flow reserve in hypertensives without significant coronary artery stenoses. J Hum Hypetens 2012;26:64-70.

21. Hoffman JI, Buckberg GD. The myocardial supply:demand ratio--a critical review. Am J Cardiol 1978;41:327-32.

22. Safar ME, Struijker-Boudier HA. Cross-talk between macro- and microcirculation. Acta Physiol (Oxf) 2010;198:417-30.

23. O'Rourke MF, Adji A. An updated clinical primer on large artery mechanics: implications of pulse waveform analysis and arterial tonometry. Curr Opin Cardiol 2005;20:275-81.

24. Laurent S, Cockcroft J, Van Bortel L et al. Expert consensus document on arterial stiffness: methodological issues and clinical applications. Eur Heart J 2006;27:2588-605.

25. Marey EJ. La méthode graphique dans les sciences expérimentales et principalement en physiologie et en médecine. Ed: G Masson. Paris: Lahure, 1885 (2nd Edition).

26. Baulmann J, Schillings U, Rickert S et al. A new oscillometric method for assessment of arterial stiffness: comparison with tonometric and piezo-electronic methods. J Hypertens 2008;26:523-8.

27. Horvath IG, Nemeth A, Lenkey Z et al. Invasive validation of a new oscillometric device (Arteriograph) for measuring augmentation index, central blood pressure and aortic pulse wave velocity. J Hypertens 2010;28:2068-75.

28. Verdecchia P, Schillaci G, Borgioni C et al. Prognostic significance of serial changes in left ventricular mass in essential hypertension. Circulation 1998;97:48-54.

29. Krumholz HM, Larson M, Levy D. Prognosis of left ventricular geometric patterns in the Framingham Heart Study. J Am Coll Cardiol 1995;25:879-84.

30. Nilsson PM, Lurbe E, Laurent S. The early life origins of vascular ageing and cardiovascular risk: the EVA syndrome. J Hypertens 2008;26:1049-57.

31. Rizzoni D, Porteri E, Boari GE et al. Prognostic significance of small-artery structure in hypertension. Circulation 2003;108:2230-5.

32. Rizzoni D, Palombo C, Porteri E et al. Relationships between coronary flow vasodilator capacity and small artery remodelling in hypertensive patients. J Hypertens 2003;21:625-31.

33. Perticone F, Ceravolo R, Pujia A et al. Prognostic significance of endothelial dysfunction in hypertensive patients. Circulation 2001;104:191-6.

34. Campuzano R, Moya JL, Garcia-Lledo A et al. Endothelial dysfunction, intima-media thickness and coronary reserve in relation to risk factors and Framingham score in patients without clinical atherosclerosis. J Hypertens 2006;24:1581-8.

35. Nigam A, Mitchell GF, Lambert J, Tardif J-C. Relation between conduit vessel stiffness (assessed by tonometry) and endothelial function (assessed by flow-mediated dilatation) in patients with and without coronary heart disease. Am J Cardiol 2003;92:395-399.

36. McEniery CM, Wallace S, Mackenzie IS et al. Endothelial function is associated with pulse pressure, pulse wave velocity, and augmentation index in healthy humans. Hypertension 2006;48:602-8.

37. Franklin SS, Khan SA, Wong ND, Larson MG, Levy D. Is pulse pressure useful in predicting risk for coronary heart Disease? The Framingham heart study. Circulation 1999;100:354-60.

38. de Simone G, Roman MJ, Koren MJ, Mensah GA, Ganau A, Devereux RB. Stroke volume/pulse pressure ratio and cardiovascular risk in arterial hypertension. Hypertension 1999;33:800-5.

39. Dolan E, Thijs L, Li Y et al. Ambulatory arterial stiffness index as a predictor of cardiovascular mortality in the Dublin Outcome Study. Hypertension 2006;47:365-70.

40. Hansen TW, Staessen JA, Torp-Pedersen C et al. Ambulatory arterial stiffness index predicts stroke in a general population. J Hypertens 2006;24:2247-53.

41. Mattace-Raso FU, van der Cammen TJ, Hofman A et al. Arterial stiffness and risk of coronary heart disease and stroke: the Rotterdam Study. Circulation 2006;113:657-63.

42. Ben-Shlomo Y, Spears M, Boustred C et al. Aortic pulse wave velocity improves cardiovascular event prediction: an individual participant meta-analysis of prospective observational data from 17,635 subjects. J Am Coll Cardiol 2014;63:636-46.

43. Vlachopoulos C, Aznaouridis K, O'Rourke MF, Safar ME, Baou K, Stefanadis C. Prediction of cardiovascular events and all-cause mortality with central haemodynamics: a systematic review and meta-analysis. Eur Heart J 2010;31:1865-71.

44. McEniery CM, Cockcroft JR, Roman MJ, Franklin SS, Wilkinson IB. Central blood pressure: current evidence and clinical importance. Eur Heart J 2014;35:1719-25.

45. Fortier C, Mac-Way F, Desmeules S et al. Aortic-brachial stiffness mismatch and mortality in dialysis population. Hypertension 2015;65:378-84.

46. RG IJ, de Jongh RT, Beijk MA et al. Individuals at increased coronary heart disease risk are characterized by an impaired microvascular function in skin. Eur J Clin Invest 2003;33:536-42.

47. Östlund Papadogeorgos N, Jörneskog G, Bengtsson M, Kahan T, Kalani M. Severely impaired microvascular reactivity in diabetic patients with an acute coronary syndrome. Cardiovasc Diabetol 2016;15:66.

48. Blann AD, McCollum CN. Circulating endothelial cell/leukocyte adhesion molecules in atherosclerosis. Thromb Haemost 1994;72:151-4.

49. Blankenberg S, Barbaux S, Tiret L. Adhesion molecules and atherosclerosis. Atherosclerosis 2003;170:191-203.

50. Chae CU, Lee RT, Rifai N, Ridker PM. Blood pressure and inflammation in apparently healthy men. Hypertension 2001;38:399-403.

51. Mahmud A, Feely J. Arterial stiffness is related to systemic inflammation in essential hypertension. Hypertension 2005;46:1118-22.

52. Müller-Brunotte R, Kahan T, Lopez B et al. Myocardial fibrosis and diastolic dysfunction in patients with hypertension: results from the Swedish Irbesartan Left Ventricular Hypertrophy Investigation versus Atenolol (SILVHIA). J Hypertens 2007;25:1958-66.

53. Kuznetsova T, Haddad F, Knez J et al. Cytokines profile in hypertensive patients with left ventricular remodeling and dysfunction. J Am Soc Hypertens 2015;9:975-984.

54. Collaborators GBDRF. Global, regional, and national comparative risk assessment of 79 behavioural, environmental and occupational, and metabolic risks or clusters of risks, 1990-2015: a systematic analysis for the Global Burden of Disease Study 2015. Lancet 2016;388:1659-1724.

55. Collaboration NCDRF. Worldwide trends in blood pressure from 1975 to 2015: a pooled analysis of 1479 population-based measurement studies with 19.1 million participants. Lancet 2017;389:37-55.

56. Ettehad D, Emdin CA, Kiran A et al. Blood pressure lowering for prevention of cardiovascular disease and death: a systematic review and meta-analysis. Lancet 2016;387:957-967

57. Conroy RM, Pyorala K, Fitzgerald AP et al. Estimation of ten-year risk of fatal cardiovascular disease in Europe: the SCORE project. Eur Heart J 2003;24:987-1003.

58. D'Agostino RB, Sr., Vasan RS, Pencina MJ et al. General cardiovascular risk profile for use in primary care: the Framingham Heart Study. Circulation 2008;117:743-53.

59. Morgan T, Lauri J, Bertram D, Anderson A. Effect of different antihypertensive drug classes on central aortic pressure. Am J Hypertens 2004;17:118-23.

60. Ong KT, Delerme S, Pannier B et al. Aortic stiffness is reduced beyond blood pressure lowering by short-term and long-term antihypertensive treatment: a meta-analysis of individual data in 294 patients. J Hypertens 2011;29:1034-42.

61. Shahin Y, Khan JA, Chetter I. Angiotensin converting enzyme inhibitors effect on arterial stiffness and wave reflections: a meta-analysis and meta-regression of randomised controlled trials. Atherosclerosis 2012;221:18-33.

62. Yusuf S, Sleight P, Pogue J, Bosch J, Davies R, Dagenais G. Effects of an angiotensin-converting-enzyme inhibitor, ramipril, on cardiovascular events in high-risk patients. The Heart Outcomes Prevention Evaluation Study Investigators. N Engl J Med 2000;342:145-53.

63. Sleight P, Yusuf S, Pogue J et al. Blood-pressure reduction and cardiovascular risk in HOPE study. Lancet 2001;358:2130-1.

64. Brilla CG, Funck RC, Rupp H. Lisinopril-mediated regression of myocardial fibrosis in patients with hypertensive heart disease. Circulation 2000;102:1388-93.

65. Müller-Brunotte R, Edner M, Malmqvist K, Kahan T. Irbesartan and atenolol improve diastolic function in patients with hypertensive left ventricular hypertrophy. J Hypertens 2005;23:633-40.

66. Kiowski W, Linder L, Nuesch R, Martina B. Effects of cilazapril on vascular structure and function in essential hypertension. Hypertension 1996;27:371-6.

67. Ghiadoni L, Magagna A, Versari D et al. Different effect of antihypertensive drugs on conduit artery endothelial function. Hypertension 2003;41:1281-6.

68. Benndorf RA, Appel D, Maas R, Schwedhelm E, Wenzel UO, Boger RH. Telmisartan improves endothelial function in patients with essential hypertension. J Cardiovasc Pharmacol 2007;50:367-71.

69. Malmqvist K, Kahan T, Edner M et al. Regression of left ventricular hypertrophy in human hypertension with irbesartan. J Hypertens 2001;19:1167-76.

70. Malmqvist K, Wallen HN, Held C, Kahan T. Soluble cell adhesion molecules in hypertensive concentric left ventricular hypertrophy. J Hypertens 2002;20:1563-9.

71. Nyström F, Malmqvist K, Lind L, Kahan T. Nurse-recorded clinic and ambulatory blood pressures correlate equally well with left ventricular mass and carotid intima-media thickness. J Intern Med 2005;257:514-22.

72. Li Y, Wang JG, Dolan E et al. Ambulatory arterial stiffness index derived from 24-hour ambulatory blood pressure monitoring. Hypertension 2006;47:359-64.

73. Millgard J, Hagg A, Sarabi M, Lind L. Endothelium-dependent vasodilation in normotensive subjects with a familial history of essential hypertension and in young subjects with borderline hypertension. Blood Press 2002;11:279-84.

74. Chowienczyk PJ, Kelly RP, MacCallum H et al. Photoplethysmographic assessment of pulse wave reflection: blunted response to endothelium-dependent beta2-adrenergic vasodilation in type II diabetes mellitus. J Am Coll Cardiol 1999;34:2007-14.

75. Settergren M, Böhm F, Ryden L, Pernow J, Kalani M. Lipid lowering versus pleiotropic effects of statins on skin microvascular function in patients with dysglycaemia and coronary artery disease. J Intern Med 2009;266:492-8.

76. Laugesen E, Hoyem P, Fleischer J et al. Reduced Subendocardial Viability Ratio Is Associated With Unfavorable Cardiovascular Risk Profile in Women With Short Duration of Type 2 Diabetes. Am J Hypertens 2016;29:1165-72.

77. Di Micco L, Salvi P, Bellasi A, Sirico ML, Di Iorio B. Subendocardial viability ratio predicts cardiovascular mortality in chronic kidney disease patients. Blood Purif 2013;36:26-8.

78. Devereux RB, Reichek N. Echocardiographic determination of left ventricular mass in man. Anatomic validation of the method. Circulation 1977;55:613-8.

79. Iacobaeus C, Kahan T, Jorneskog G, Bremme K, Thorsell M, Andolf E. Fetal growth is associated with first-trimester maternal vascular function. Ultrasound Obstet Gynecol 2016;48:483-490.

80. Piepoli MF, Hoes AW, Agewall S et al. 2016 European Guidelines on cardiovascular disease prevention in clinical practice: The Sixth Joint Task Force of the European Society of Cardiology and Other Societies on Cardiovascular Disease Prevention in Clinical Practice (constituted by representatives of 10 societies and by invited

experts)Developed with the special contribution of the European Association for Cardiovascular Prevention & Rehabilitation (EACPR). Eur Heart J 2016;37:2315-81.

81. Sehestedt T, Jeppesen J, Hansen TW et al. Risk prediction is improved by adding markers of subclinical organ damage to SCORE. Eur Heart J 2010;31:883-91.

82. Sehestedt T, Jeppesen J, Hansen TW et al. Thresholds for pulse wave velocity, urine albumin creatinine ratio and left ventricular mass index using SCORE, Framingham and ESH/ESC risk charts. J Hypertens 2012;30:1928-36.

83. Ekholm M, Jekell A, Wallén NH, Gigante B, Kahan T. The effects of angiotensin converting enzyme inhibition and alpha 1-adrenergic receptor blockade on inflammation and hemostasis in human hypertension. J Cardiovasc Pharmacol 2018 Jan 30 [Epub ahead of print].

84. Harrison DG, Guzik TJ, Lob HE et al. Inflammation, immunity, and hypertension. Hypertension 2011;57:132-40.

85. Schiffrin EL. The immune system: role in hypertension. Can J Cardiol 2013;29:543-8.

86. Schiffrin EL, Park JB, Pu Q. Effect of crossing over hypertensive patients from a beta-blocker to an angiotensin receptor antagonist on resistance artery structure and on endothelial function. J Hypertens 2002;20:71-8.

87. Laurent S, Boutouyrie P, Vascular Mechanism C. Dose-dependent arterial destiffening and inward remodeling after olmesartan in hypertensives with metabolic syndrome. Hypertension 2014;64:709-16.

88. Pannier BM, Guerin AP, Marchais SJ, London GM. Different aortic reflection wave responses following long-term angiotensin-converting enzyme inhibition and beta-blocker in essential hypertension. Clin Exp Pharmacol Physiol 2001;28:1074-7.

89. Ghiadoni L, Versari D, Magagna A et al. Ramipril dose-dependently increases nitric oxide availability in the radial artery of essential hypertension patients. J Hypertens 2007;25:361-6.

90. Tropeano AI, Boutouyrie P, Pannier B et al. Brachial pressure-independent reduction in carotid stiffness after long-term angiotensin-converting enzyme inhibition in diabetic hypertensives. Hypertension 2006;48:80-6.

91. Bots ML, Remme WJ, Luscher TF et al. ACE inhibition and endothelial function: main findings of PERFECT, a sub-study of the EUROPA trial. Cardiovasc Drugs Ther 2007;21:269-79.

92. Hlubocka Z, Umnerova V, Heller S et al. Circulating intercellular cell adhesion molecule-1, endothelin-1 and von Willebrand factor-markers of endothelial dysfunction in uncomplicated essential hypertension: the effect of treatment with ACE inhibitors. J Hum Hypertens 2002;16:557-62.

93. Cesari M, Kritchevsky SB, Atkinson HH et al. Angiotensin-converting enzyme inhibition and novel cardiovascular risk biomarkers: results from the Trial of Angiotensin Converting Enzyme Inhibition and Novel Cardiovascular Risk Factors (TRAIN) study. Am Heart J 2009;157:334 e1-8.

94. Cloud GC, Rajkumar C, Kooner J, Cooke J, Bulpitt CJ. Estimation of central aortic pressure by SphygmoCor requires intra-arterial peripheral pressures. Clin Sci (Lond) 2003;105:219-25.

95. Rezai MR, Goudot G, Winters C, Finn JD, Wu FC, Cruickshank JK. Calibration mode influences central blood pressure differences between SphygmoCor and two newer devices, the Arteriograph and Omron HEM-9000. Hypertens Res 2011;34:1046-51.

96. Jatoi NA, Mahmud A, Bennett K, Feely J. Assessment of arterial stiffness in hypertension: comparison of oscillometric (Arteriograph), piezoelectronic (Complior) and tonometric (SphygmoCor) techniques. J Hypertens 2009;27:2186-91.

97. Ring M, Eriksson MJ, Zierath JR, Caidahl K. Arterial stiffness estimation in healthy subjects: a validation of oscillometric (Arteriograph) and tonometric (SphygmoCor) techniques. Hypertens Res 2014;37:999-1007.

98. Hickson SS, Butlin M, Mir FA et al. The accuracy of central SBP determined from the second systolic peak of the peripheral pressure waveform. J Hypertens 2009;27:1784-8.

99. Trachet B, Reymond P, Kips J et al. Numerical validation of a new method to assess aortic pulse wave velocity from a single recording of a brachial artery waveform with an occluding cuff. Ann Biomed Eng 2010;38:876-88.

100. Parati G, De Buyzere M. Evaluating aortic stiffness through an arm cuff oscillometric device: is validation against invasive measurements enough? J Hypertens 2010;28:2003-6.

101. Boegehold MA. Heterogeneity of endothelial function within the circulation. Curr Opin Nephrol Hypertens 1998;7:71-8.

102. Park JB, Schiffrin EL. Small artery remodeling is the most prevalent (earliest?) form of target organ damage in mild essential hypertension. J Hypertens 2001;19:921-30.

103. Rizzoni D, Porteri E, Castellano M et al. Endothelial dysfunction in hypertension is independent from the etiology and from vascular structure. Hypertension 1998;31:335-41.

104. Mancia G, Fagard R, Narkiewicz K et al. 2013 ESH/ESC Guidelines for the management of arterial hypertension: the Task Force for the management of arterial hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC). J Hypertens 2013;31:1281-357.