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MARTIN SERG

Therapeutic aspects of central haemodynamics, arterial stiffness and oxidative stress in hypertension

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To my beloved parents,
my wife Monika and my children

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LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original publications referred to in the text by their Roman numerals (I–IV):

I Serg M, Kampus P, Kals J, Zagura M, Muda P, Tuomainen TP, Zilmer K, Salum E, Zilmer M, Eha J. Association between asymmetric dimethylarginine and indices of vascular function in patients with essential hypertension. Blood Pressure 2011; 20:111–116.

II Kampus P, Serg M, Kals J, Zagura M, Muda P, Karu K, Zilmer M, Eha J. Differential effects of nebivolol and metoprolol on central aortic pressure and left ventricular wall thickness in hypertensive patients. Hypertension 2011; 57:1122–1128.

III Serg M, Kampus P, Kals J, Zagura M, Zilmer M, Zilmer K, Kullisaar T, Eha J. Nebivolol and metoprolol: long-term effects on inflammation and oxidative stress in essential hypertension. Scandinavian Journal of Clinical and Laboratory Investigation 2012; 72:427–432.

IV Serg M, Graggaber J, Kampus P, Zagura M, Kals J, Mäki-Petäjä K, Cheriyan J, Zilmer M, Eha J, McEniery CM, Wilkinson IB. Baseline augmentation index and pulse pressure amplification determine the response to antihypertensive therapy. (submitted for publication)

Author's contribution:

Papers I, III: Collecting clinical data, data analysis, main writer of the paper

Paper II: Collecting clinical data, participating in data analysis, and writing the paper

Paper IV: Study design, identifying and recruiting patients, collecting clinical data, data analysis, main writer of the paper

ABBREVIATIONS

ACEI	angiotensin converting enzyme inhibitor
ADMA	asymmetric dimethylarginine
ASCOT	Anglo-Scandinavian Cardiac Outcomes Trial
AIx	augmentation index
AIx@75	augmentation index corrected for heart rate 75 beats *per* minute
AP	augmentation pressure
ARB	angiotensin II receptor blocker
BB	beta-blocker
BMI	body mass index
BP	blood pressure
CAFE	Conduit Artery Function Evaluation
CCB	calcium channel blocker
CRP	C-reactive protein
CO	cardiac output
CVD	cardiovascular disease
DDAH	dimethylaminohydrolase
EDV	endothelium-dependent vasodilation
eGFR	estimated glomerular filtration rate
eNOS	endothelial nitric oxide synthase
ECG	electrocardiography
ELISA	Enzyme-Linked Immunosorbent Assay
ESC	European Society of Cardiology
ESH	European Society of Hypertension
GTN	glyceryl trinitrate
HDL	high-density lipoprotein
IL-6	interleukin-6
IMT	intima-media thickness
ISMN	isosorbide mononitrate
LIFE	Losartan Intervention for Endpoint Reduction in Hypertension
LDL	low-density lipoprotein
LVH	left ventricular hypertrophy
MAP	mean arterial pressure
NO	nitric oxide
OxLDL	oxidized low-density lipoprotein

OxS	oxidative stress
PP	pulse pressure
PPA	pulse pressure amplification
PVR	peripheral vascular resistance
PWA	pulse wave analysis
PWV	pulse wave velocity
REASON	Preterax in Regression of Arterial Stiffness in a Controlled Double-Blind
sICAM-1	soluble intercellular adhesion molecule-1
SV	stroke volume
Tr	timing of the reflected waveform

1. INTRODUCTION

Arterial hypertension is a leading cause of death globally, which affects approximately 1 billion people worldwide (World Health Organization 2009). The value of elevated blood pressure (BP) as a predictor of future cardiovascular disease (CVD) is firmly established (Kannel 1996). However, only a small fraction of hypertensive patients have elevated BP levels alone, with the great majority presenting additional risk factors, leading to the development of subclinical target organ damage and increased total cardiovascular risk (Mancia *et al.* 2007). Furthermore, cardiovascular risk cannot be fully explained by traditional risk factors (Cohn *et al.* 2004).

The vascular tree is lined with the endothelium. Endothelial cells produce several vasoactive substances, including nitric oxide (NO), which is the main vasodilating and a principal antiatherogenic biomolecule in the human arteries (Davignon and Ganz 2004). It has been shown that NO-mediated endothelium-dependent vasodilation (EDV), a hallmark of endothelial function, is decreased in hypertension (Panza *et al.* 1990). Endothelial dysfunction is considered as an early process in arterial stiffening and atherosclerotic lesion formation (Wilkinson *et al.* 2002b; Halcox *et al.* 2009). There is evidence that early intervention in hypertensive patients is prognostically important (Julius *et al.* 2004). Consequently, several biochemical and functional markers of endothelial dysfunction have been acknowledged in order to more precisely and timely stratify cardiovascular risk in these patients (Mancia *et al.* 2007). Other independent predictors of cardiovascular risk, oxidative stress (OxS) (Heitzer *et al.* 2001) and inflammation (Ridker *et al.* 2002), are increased in hypertension (Russo *et al.* 1998; Kampus *et al.* 2006). Moreover, reversing non-physiological levels of OxS and inflammation reduces endothelial dysfunction (Taddei *et al.* 1998; Mäki-Petäjä *et al.* 2007) and the progression of atherosclerosis (Nissen *et al.* 2005; Ono *et al.* 2008). Thus, the maintenance of both OxS and inflammation may have an additional merit in antihypertensive therapy.

Large comparative studies and meta-analyses have shown that brachial BP reduction *per se* reduces cardiovascular risk (Staessen *et al.* 2001; ALLHAT Officers and Coordinators 2002; Turnbull *et al.* 2003). The European Society of Hypertension (ESH) and the European Society of Cardiology (ESC) recommend lowering of BP below 140/90 mmHg in essential hypertension (Mancia *et al.* 2007). Although a large range of antihypertensive drugs are available, less than 30% of the treated hypertensive patients are at or below target BP levels (Primatesta *et al.* 2001; Grassi *et al.* 2011). A significant number of these patients remain above target level despite adequate compliance with therapy. Current hypertension guidelines emphasise the need for treatment individualisation based on the level of BP, presence of cardiovascular risk factors, subclinical target organ damage, or concomitant conditions (Mancia *et al.* 2009). Essential hypertension is characterised by an altered haemodynamic profile of the patients (Franklin *et al.* 1997). There is evidence that the assess-

ment of the haemodynamic profile of hypertensive subjects could predict the reduction of BP with antihypertensive therapy (Smith *et al.* 2006; Protogerou *et al.* 2009a). Hence, haemodynamic profiling could lead to improved and more rapid BP control, and a reduction in the number of antihypertensive drugs required in an individual patient.

Traditionally, BP is measured from the brachial artery. However, BP in the brachial (peripheral) artery and in the aorta (central artery) differ significantly due to arterial stiffness and pulse wave reflection phenomena. There is an increasing amount of evidence that central BP is more relevant than brachial BP for the development of subclinical target organ damage (Agabiti-Rosei *et al.* 2007a; Wang *et al.* 2009). Furthermore, antihypertensive drugs exert differential effects on brachial and central BP. Nitrates, calcium channel blockers (CCBs), angiotensin converting enzyme inhibitors (ACEIs), and angiotensin II receptor blockers (ARBs) have been shown to effectively reduce both brachial and central BP (Protogerou *et al.* 2009b). Data about beta-blockers (BBs) are controversial. The most extensively studied conventional BB, atenolol, has been shown to effectively reduce brachial BP, but to have minimal or no effect on central BP (Williams *et al.* 2006; Mackenzie *et al.* 2009). This detrimental effect of atenolol is considered as an explanation for its inferiority to other antihypertensive drugs in reducing cardiovascular events in patients with uncomplicated hypertension (Wilkinson *et al.* 2006). However, BBs are pharmacologically heterogeneous (Panjrath and Messerli 2006) and newer BBs (e.g. nebivolol) may have a greater effect on central BP (Dhakam *et al.* 2008; Mahmud and Feely 2008). There are no data about the effect of metoprolol succinate, the most widely used conventional BB in Northern and Eastern Europe, on central BP.

The main purpose of the present thesis was to assess the biochemical, functional, and structural parameters and properties of the vasculature in hypertension, as well as to investigate the effect of antihypertensive therapy on these parameters.

2. REVIEW OF THE LITERATURE

2.1. Arterial hypertension

Arterial hypertension is an important public health challenge because of its high prevalence and association with CVD (Kearney *et al.* 2005). The perception of elevated BP as a risk factor for CVD is based on the results from the Framingham Heart Study launched in 1948 (Kannel *et al.* 1961). To date, elevated BP has been identified as a major risk factor for coronary artery disease, heart failure, stroke, peripheral artery disease, and renal failure (Mancia *et al.* 2007). Historically, diastolic rather than systolic BP was emphasised as a predictor of cardiovascular events (MacMahon *et al.* 1990). Diastolic BP was regarded as the resistance that the heart had to overcome, while systolic BP was taken as a measure of cardiac strength (Vlachopoulos and O'Rourke 2000). However, the accumulating amount of data confirmed that both systolic and diastolic BP have continuous independent relationship with cardiovascular morbidity and mortality (Kannel *et al.* 1971; Kannel *et al.* 1981; Lewington *et al.* 2002). Arterial hypertension is widely defined as persistent brachial systolic and/or diastolic BP >140/90 mmHg. Current ESH/ESC guidelines state that the classification of hypertension and its risk assessment should be based on systolic and diastolic BP, risk factors, subclinical target organ damage, and concomitant diseases (Mancia *et al.* 2007). Estimation of total cardiovascular risk is important for the choice of primary and secondary cardiovascular prevention in hypertensive patients.

Treating systolic and diastolic BP until they are less than 140/90 mmHg is associated with an improvement in cardiovascular outcome (Mancia *et al.* 2009). While lifestyle changes (e.g. smoking cessation, sodium restriction, weight reduction) may be appropriate, these kinds of interventions should not unnecessarily delay the initiation of pharmacological treatment for hypertension, especially in patients at high level of risk (Mancia *et al.* 2007). Currently, five major classes of antihypertensive drugs – thiazide diuretics, CCBs, ACEIs, ARBs, and BBs – in monotherapy or combined, are suitable for the treatment of hypertension (Mancia *et al.* 2007). Despite the fact that elevated BP has been known to be an important risk factor for CVD for half a century and a variety of antihypertensive drugs are available, the target of 140/90 mmHg has remained to be poorly achieved (Pereira *et al.* 2009). Furthermore, several large-scale comparative studies have shown that, despite similar brachial BP reduction, 'older' antihypertensive drugs are inferior to 'newer' antihypertensive drugs in reducing cardiovascular risk (Dahlöf *et al.* 2002; Dahlöf *et al.* 2005). These data have called for the development of novel methods and markers of cardiovascular risk assessment. Moreover, the effects of antihypertensive drugs on these markers have been shown to better predict cardiovascular outcome than brachial BP (Williams *et al.* 2006).

2.2. Central haemodynamics

The BP is the force that the blood exerts on the vascular wall. Arterial pressure can be divided into the steady [mean arterial pressure (MAP)] and the pulsatile [pulse pressure (PP)] components (Cheriyan *et al.* 2010). The MAP is determined by cardiac output (CO) and vascular resistance, whereas the PP component is influenced by left ventricular ejection, large artery stiffness, pulse wave reflection, and heart rate (Franklin *et al.* 1997). The arterial system receives blood in spurts from the left ventricle of the heart. In the systole, the left ventricle generates a forward moving pulse wave. The increasing impedance in resistance arteries (small muscular arteries and arterioles) and the branching of arteries cause a backward reflecting pulse wave (O'Rourke 1982). Consequently, the pulse waveform at any site of the arterial tree is the sum of the forward traveling wave and the backward traveling wave (Nichols and O'Rourke 2005). In healthy large conduit arteries the reflecting wave arrives to the heart in the diastole, thus increasing diastolic BP and improving coronary artery perfusion. However, in stiff arteries pulse wave velocity (PWV) is increased, i.e. the incident and the reflected pulse waves are accelerated, causing the reflected pulse wave to merge with the incident pulse wave in the systole, augmenting central systolic BP and PP (Williams 2004). This in turn leads to increased left ventricular afterload and oxygen demand and reduced coronary perfusion, resulting in left ventricular hypertrophy (LVH). This pulse wave reflection can be quantified through the measurement of augmentation index (AIx) which can be measured using pulse wave analysis (PWA) (Pauca *et al.* 2001; Van Bortel *et al.* 2001) (Figure 1). Due to the pulse pressure amplification (PPA) phenomenon, systolic BP is amplified when moving from the aorta to the periphery. Typically, MAP and diastolic BP fall by only 1–2 mmHg. This small fall in MAP causes blood to flow forwards, not backwards. Consequently, the pulsatile components (systolic BP and PP) of central and peripheral pressures may vary significantly. In general, brachial systolic BP and PP tend to overestimate central systolic BP and PP. In younger individuals PPA is more pronounced, whereas with vascular ageing amplification is reduced (Agabiti-Rosei *et al.* 2007a).

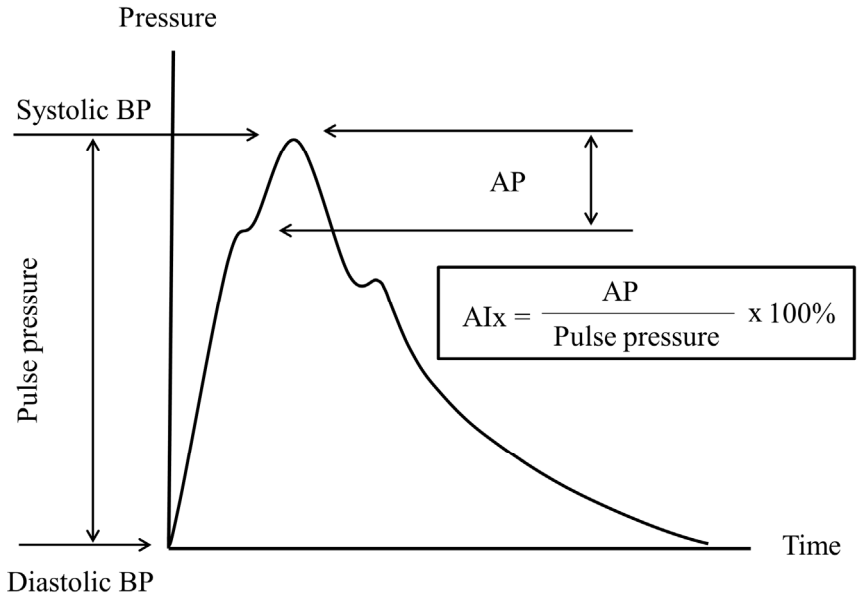

Figure 1. Pulse wave analysis. AIx is the ratio of augmentation pressure (AP) to PP (in percentage). Modified from Serg *et al.* 2010.

Central haemodynamics in arterial hypertension

Arterial hypertension is a physiologically and haemodynamically heterogeneous disease. Altered haemodynamics can play a central role in the development and perpetuation of high BP (Franklin *et al.* 1997). Hypertension is often considered to result from an increase in peripheral vascular resistance (PVR) (Julius 1990). Although this is largely true in middle-aged subjects with elevation of both systolic and diastolic BP (i.e. mixed systolic/diastolic hypertension) (Izzo 2005), it has recently been shown that this is not true in subjects with isolated systolic hypertension (Mitchell *et al.* 2004; Yasmin *et al.* 2005), which is the most common form of hypertension in the elderly (Franklin *et al.* 2001) and prevalent in adolescents (Sorof *et al.* 2002) and in young adults (Mallion *et al.* 2003). In young subjects with isolated systolic hypertension, PVR is normal and hypertension results primarily from either an elevation in CO or increased stiffness of the large arteries (McEniery *et al.* 2005b). The mechanisms underlying increased arterial stiffness in young hypertensives have remained unknown. Long-term follow-up studies have shown that in young hypertensives with primarily increased CO, CO normalises with age and is followed by an increase in PVR, resulting in mixed systolic/diastolic hypertension (Lund-Johansen 1994). In older adults with isolated systolic hypertension the main physiological abnormality is the stiffening of the large arteries (Franklin *et al.* 1997).

Current ESH/ESC guidelines recommend the use of a specific antihypertensive drug based on level of BP, presence of cardiovascular risk factors, subclinical target organ damage, or concomitant conditions (Mancia *et al.* 2009). However, haemodynamic profiling of hypertensive subjects could predict BP reduction with antihypertensive therapy (Smith *et al.* 2006; Protogerou *et al.* 2009a). Hence, haemodynamic profiling could lead to improved and more rapid BP control, and a reduction in the number of antihypertensive drugs required in an individual patient. One could predict that drugs that reduce CO (i.e. BBs) (Conway 1983) would be more efficacious in individuals with high CO. Similarly, vasodilators (i.e. alpha-blockers) could be more effective in individuals with increased PVR (Lund-Johansen and Omvik 1991). There is evidence that ARBs have BP-independent effects on arterial stiffness, thus they could be efficacious in patients with high aortic PWV (Mahmud and Feely 2002). Oral nitrate preparations have been shown to reduce significantly AIx, indicating that they may be useful in patients with increased pulse wave reflection (Stokes *et al.* 2003).

2.2.1. Central blood pressure

2.2.1.1. Assessment of central blood pressure

Brief history of methods for measuring central blood pressure

Classically, central BP has been measured invasively. The first measurement of BP was conducted by a Cambridge University graduate, Stephen Hales (1733), in a series of experiments. In his classical experiment, Hales opened an artery of a mare, inserted a narrow brass tube into the artery and fitted a 9-foot-long vertical glass tube to the pipe. The pressure of the horse's circulation forced the blood up the glass tube (Booth 1977). In the early 19th century, Richard Bright identified increased arterial tension from the 'hardness' of the arterial pulse, and associated LVH and vascular damage to high arterial pressure (O'Rourke and Gallagher 1996). The pioneering studies by Marey in Paris and Mahomed in London (O'Rourke 1992) followed with non-invasive graphic methods to record the radial pulse wave at the end of the 19th century. However, interest in the pulse wave methodologies lapsed with the introduction of the cuff sphygmomanometer by Riva-Rocci in 1896 and the improvement of the method by Korotkov in 1905 (Hirata *et al.* 2006). The introduction of high-fidelity manometers by Murgo and Millar in 1972 and tonometers for invasive and non-invasive pulse wave recording (Drzewiecki *et al.* 1983; Kelly *et al.* 1989) as well as the development of methods to characterise and analyse the arterial pulse (Nichols and O'Rourke 2005) marked the 'renaissance' of PWA. Furthermore, by that time the limitations of the cuff sphygmomanometer (mainly the ability to only measure the extremes of the pulse in the brachial artery) were better recognised (O'Rourke and Gallagher 1996).

Current methods for measuring central blood pressure

Invasive techniques during open chest surgery or cardiac catheterisation remain to be the most accurate methods of central BP measurement (Wilkinson *et al.* 2010). However, these methods are not appropriate in clinical studies and routine practice. Several non-invasive techniques have been developed for the assessment of central BP (Van Bortel *et al.* 2008). The most widely used approach is to perform radial artery tonometry and then apply a radial-to-aorta transfer function (Sphygmocor, Atcor Medical, Australia) to calculate the aortic pulse waveform from the radial waveform (Laurent *et al.* 2006). The radial artery is well supported by bony tissue, making optimal applanation easy to achieve. Arterial tonometry is based on the principle that when the artery is immobilised and the arterial wall is flattened against a pressure sensor, pressure within the lumen is directly transmitted to the sensor (Nichols and O'Rourke 2005). The tonometer generates a radial artery waveform which can then be transformed using a mathematical radial-to-aortic transfer function to derive an aortic waveform. By entering the data from simultaneous, non-invasive, oscillometric measurement of brachial BP, the waveforms and transfer function have been used to derive central haemodynamic indices. It has been shown that applanation tonometry with transfer function is a validated, accurate, and reproducible method for measuring central BP (Smulyan *et al.* 2003; Sharman *et al.* 2006). Particularly, Pauca *et al.* (2001) elegantly showed that the estimated central PP measured with the use of the transfer function calibrated with invasive radial BP differed from actual aortic PP by only 0.7 ± 4.21 mmHg. Meanwhile, the Association for the Advancement of Medical Instrumentation criteria accept BP measurement using oscillometric sphygmomanometers to deviate by 5 ± 8 mmHg from the gold standard (White *et al.* 1993). Hence, the largest drawback for measuring central BP using the transfer function is considered to be the inaccuracy of the oscillometric sphygmomanometer (Wilkinson and Cockcroft 2004).

2.2.1.2. Clinical implications of central blood pressure

The value of brachial BP as a predictor of future CVD is firmly established (Kannel 1996). Recently, greater emphasis has been placed on brachial PP, a surrogate measure of large artery stiffness, especially in older individuals (Franklin *et al.* 1999; Wilkinson *et al.* 2004; Mancia *et al.* 2007). However, there is a gradual widening of PP from the central to the peripheral arteries due to the PPA phenomenon. Central (aortic, carotid) systolic BP and PP are pathophysiologically more relevant than peripheral pressures for the pathogenesis of CVD (Agabiti-Rosei *et al.* 2007a). It is central systolic BP that the left ventricle encounters during the systole (afterload) and aortic diastolic BP which determines coronary perfusion. Furthermore, distending pressure (MAP) in the large elastic-type arteries (aorta and carotid) but not in the peripheral muscular

arteries (brachial, radial) is the key determinant of arterial stiffening that characterises accelerated vascular ageing and hypertension (Vlachopoulos *et al.* 2010a). With ageing and vascular disease the stiffness of the central arteries approaches that of the peripheral arteries, resulting in a relative similarity of the values of brachial and central PP (Kotsis *et al.* 2011; Wojciechowska *et al.* 2012), decreasing thus PPA. Central systolic BP or PP are more closely correlated with measures of subclinical target organ damage, such as left ventricular mass (Covic *et al.* 2000) and carotid intima-media thickness (IMT) (Boutouyrie *et al.* 1999; Roman *et al.* 2007), compared to peripheral (brachial and radial) systolic BP or PP. It has been shown that 70% of individuals with high-normal brachial BP have similar aortic pressures as those with stage 1 hypertension (McEniery *et al.* 2008). Thus, assessment of central BP may improve the identification and management of patients with elevated cardiovascular risk.

Data from the Strong Heart Study showed that wider brachial PP is associated with higher cardiovascular mortality independently of the traditional risk factors, LVH, and reduced ejection fraction (Palmieri *et al.* 2006). Subsequent analysis of the same study revealed that central PP is superior over brachial PP for prediction of cardiovascular events (Roman *et al.* 2007; Roman *et al.* 2009). A community based study from China showed that central systolic BP was more strongly related to target organ indices, such as left ventricular mass, carotid IMT, estimated glomerular filtration rate (eGFR), and cardiovascular mortality than brachial BP (Wang *et al.* 2009). The predictive value of central BP for all-cause and cardiovascular mortality has been shown in patients with end-stage renal disease (Safar *et al.* 2002) and coronary artery disease (Chirinos *et al.* 2005). Furthermore, central systolic BP, but not brachial BP, independently predicts cardiovascular events and cardiovascular mortality in the elderly (Pini *et al.* 2008). The Conduit Artery Function Evaluation (CAFE) study showed that central PP is significantly associated with a post hoc-defined composite outcome of cardiovascular events/procedures and development of renal impairment in hypertensive patients, suggesting that lowering central BP rather than brachial BP may be a more important goal of antihypertensive treatment (Williams *et al.* 2006).

2.2.1.3. Antihypertensive therapy and central blood pressure

The largest hypertension study to date, the Antihypertensive and Lipid-Lowering Treatment to Prevent Heart Attack Trial (ALLHAT Officers and Coordinators 2002), and meta-analyses (Staessen *et al.* 2001; Turnbull *et al.* 2003) have shown that in hypertensive patients brachial BP lowering *per se* improves cardiovascular outcome. However, several large-scale hypertension trials have demonstrated that 'newer' antihypertensive drugs may reduce cardiovascular outcome beyond BP control. Particularly, the Losartan Intervention For Endpoint reduction in hypertension (LIFE) study (Dahlöf *et al.*

2002) and the Anglo-Scandinavian Cardiac Outcomes Trial (ASCOT) (Dahlöf *et al.* 2005) underlined the 'beyond BP reduction' paradox where an ACEI, an ARB, or a CCB were superior to a conventional BB atenolol in reducing cardiovascular events. There is an accumulating amount of evidence that this 'beyond (brachial) BP reduction' paradox can be explained by the effect of antihypertensive drugs on central BP.

It has been shown that the reduction in central BP but not MAP by antihypertensive treatment is involved in the reduction in a major subclinical target organ damage marker, carotid IMT (Boutouyrie *et al.* 2000). The results from the Preterax in Regression of Arterial Stiffness in a Controlled Double-Blind (REASON) study show that the regression of left ventricular mass with long-term antihypertensive therapy is linked to the reduction in central and not brachial BP (de Luca *et al.* 2004).

Although nitrates are not routinely used in the management of essential hypertension, they were the first drug group whose effects on central BP were tested. In one of the first of these studies, Kelly *et al.* (1990) demonstrated that a 0.3 mg sublingual dose of glyceryl trinitrate (GTN) reduced central systolic BP markedly more than brachial systolic BP (22.1 vs. 11.8 mmHg, respectively). However, to date, only acute effects of low- and high-dose nitrates on central BP have been studied (Stokes *et al.* 2003).

'Newer' antihypertensive drugs, such as ACEIs (Jiang *et al.* 2007), ARBs (Dhakam *et al.* 2006) and CCBs (Morgan *et al.* 2004), are powerful vasodilators which effectively reduce central BP. Due to the reduction in PVR, they have been shown to shift the effective reflection distance more distally, thus delaying the arrival of the reflected wave at the central artery (Morgan *et al.* 2004; Dhakam *et al.* 2006). The long-term (one-year) REASON study highlighted the superiority of an ACEI/diuretic (perindopril/indapamide) combination over atenolol in reducing central BP (Asmar *et al.* 2001). The results from the REASON study were taken further in a study by Jiang *et al.* (2007), which allowed to show that while an ACEI enalapril effectively reduced central BP, indapamide had no effect on central BP 'beyond' brachial BP. Hence, the results of the REASON study could be attributed to angiotensin converting enzyme inhibition alone. The LIFE study demonstrated the superiority of an ARB losartan over atenolol in reducing cardiovascular events and LVH (Dahlöf *et al.* 2002). Whether these effects of an ARB could be attributed to its effects on central BP were tested by Dhakam *et al.* (2006). In this study an ARB eprosartan and atenolol reduced brachial BP to a similar degree while eprosartan reduced central BP and plasma brain natriuretic peptide (marker of heart afterload) significantly more than atenolol. The CCBs have been shown to effectively reduce central BP in short- (Morgan *et al.* 2004) and long-term (London *et al.* 1994) studies. Comparisons between combination therapies have also been conducted regarding their effects on central BP. The ASCOT demonstrated superiority in cardiovascular outcomes from primary treatment of hypertension with a CCB/ACEI (amlodipine/perindopril) combination

compared to a BB/thiazide diuretic (atenolol/bendroflumethiazide) combination (Dahlöf *et al.* 2005). The CAFE study, a substudy of ASCOT, including 2199 patients with hypertension, demonstrated that despite similar brachial BP reduction, the CCB treatment arm was substantially superior to the BB treatment arm in lowering central BP, which may explain the outcome data (Williams *et al.* 2006). The superiority of a CCB/ARB combination over a combination including a CCB and a BB or a diuretic have been demonstrated in the EXPLOR study (Boutouyrie *et al.* 2010) and in a study by Matsui *et al.* (2009). Several studies have shown that diuretics have a neutral or negative effect on central BP, i.e. without a favourable effect beyond brachial BP reduction (Protogerou *et al.* 2009b).

The BBs are beta-adrenoceptor antagonists. However, the mechanisms leading to BP reduction with these agents have remained unclear (Cheriyan *et al.* 2010). It has been suggested that the BBs have antihypertensive effects owing to a decrease in CO, inhibition of renin secretion, central sympathetic outflow, and the release of noradrenaline from postganglionic adrenergic nerve endings; and resetting of baroreceptor levels (Panjrath and Messerli 2006). The BBs significantly reduce heart rate and consequently increase the duration of the systole. This causes the reflecting pulse wave to arrive in the heart during the systole, concomitantly increasing the afterload and central systolic BP. Indeed, the most extensively studied conventional BB, atenolol, has been shown to effectively reduce brachial BP but to have a minimal or no effect on central BP (Williams *et al.* 2006; Mackenzie *et al.* 2009). However, the BBs vary considerably in their chemical and pharmacodynamic properties (Panjrath and Messerli 2006). The hypothesis that the inferiority of atenolol in reducing central BP is a class effect of BBs has recently been questioned (Wilkinson *et al.* 2006). Dhakam *et al.* (2008) showed that nebivolol, a novel vasodilating BB, decreases central systolic BP more than atenolol despite their similar reduction in brachial BP. Atenolol is a relatively selective beta-1 adrenoceptor antagonist and has a weak effect on peripheral vascular tone (Burns *et al.* 2004). Newer BBs, e.g. nebivolol, are more beta-1 adrenoceptor selective but have substantial vasodilating properties due to several mechanisms. Firstly, they induce endothelial NO production in the endothelium (Cockcroft *et al.* 1995; McEniery *et al.* 2004), secondly, they stimulate beta-3 adrenoceptors which mediate NO release (Ladage *et al.* 2006), and thirdly, they reduce OxS (Troost *et al.* 2000). Vasodilating BBs have additional beneficial haemodynamic effects: they reduce heart rate to a lesser degree compared to atenolol and have a neutral effect on CO (Kamp *et al.* 2003). However, there are no data about the effect of metoprolol succinate, the most widely used BB in Northern and Eastern European countries, on central BP.

2.2.2. Arterial stiffness

The arterial wall is divided into three concentric layers: the intima, the media, and the adventitia. Functionally, there are two types of arteries. Firstly, there are large elastic arteries (i.e. aorta, common carotid artery), which can distend and accommodate large volumes of blood during the systole because the media is composed of concentric layers of elastic fibres. Secondly, there are intermediate size muscular arteries (i.e. femoral and brachial arteries), with a relatively thick media, composed mainly of smooth muscle fibres that allow them to regulate blood flow by constriction and dilation (Nichols and O'Rourke 2005).

With ageing and CVD, the cushion and conduit functions of the large arteries are impaired (Benetos *et al.* 1993; O'Rourke 1995). In this regard two distinct terms, atherosclerosis and arteriosclerosis, often used interchangeably, can be distinguished. Atherosclerosis (e.g. due to hypercholesterolaemia and smoking) is primarily an intima disease, and is almost exclusively associated with the disturbance of conduit function, resulting in narrowing of arteries. Arteriosclerosis, which is characteristic of hypertension and ageing, results from the degeneration of the arterial media with fractures and fragmentation of elastic lamellae, increased collagen and calcium content, and dilation and hypertrophy of the large arteries and the aorta, hence causing increased arterial stiffness (Avolio *et al.* 1998). Arterial stiffness can be defined as a loss of compliance function of the large arteries (Laurent *et al.* 2006). In addition to structural determinants, arterial stiffness is strongly affected by endothelial cell signalling (Wilkinson *et al.* 2002b) and vascular smooth muscle cell tone (Zieman *et al.* 2005).

2.2.2.1. Clinical implications of arterial stiffness

Measurement of PWV is regarded as the most simple, non-invasive, robust, reproducible, and direct method to determine arterial stiffness (Laurent *et al.* 2006). Aortic PWV or carotid PWV is used for stiffness measurement in the elastic arteries, whereas muscular artery stiffness can be measured using femoro-tibial or brachial PWV. In contrast to the large arteries, the stiffness of the muscular arteries does not markedly change with ageing (Benetos *et al.* 1993; Vermeersch *et al.* 2008; Ruitenbeek *et al.* 2008). Indeed, there is a body of evidence that aortic stiffness but not muscular artery stiffness predicts cardiovascular events (Mitchell *et al.* 2010). Furthermore, aortic stiffness has been shown to have a better predictive value than carotid stiffness in hypertensive patients (Paini *et al.* 2006). Aortic PWV is considered as the "gold standard" measurement of regional arterial stiffness (Laurent *et al.* 2006). Aortic PWV is usually measured using the foot-to-foot velocity method from the common carotid artery and the femoral artery, and the time delay (in seconds) between the feet of the two waveforms. The distance covered by the waves is usually measured using a surface distance between the two recording sites

(metres). The PWV is calculated as the distance travelled by the pulse wave divided by the time taken to travel the distance (Laurent *et al.* 2006). Arterial stiffness is considered a marker of subclinical target organ damage in the latest ESH/ESC hypertension guidelines (Mancia *et al.* 2007). According to these guidelines, the cut-off value of increased aortic PWV, using the direct distance between the carotid and femoral sites, is >12 m/s. Furthermore, reference values of aortic PWV have been developed (Boutouyrie and Vermeersch 2010) for different age groups as arterial stiffness is a cumulative measure of previous cardiovascular risk factors.

Arterial stiffness is considered the main determinant of PP increase in hypertension, thus causing isolated systolic hypertension, especially in the elderly (Franklin *et al.* 1997). Arterial stiffness is associated with a number of cardiovascular risk factors, e.g. ageing, smoking, impaired glucose tolerance, diabetes, hypercholesterolaemia, and obesity (Wilkinson *et al.* 2004; Laurent *et al.* 2006). There is a large amount of evidence that aortic PWV is an independent predictor of coronary and cardiovascular events (Boutouyrie *et al.* 2002; Terai *et al.* 2008), fatal strokes (Laurent *et al.* 2003), and cardiovascular and all-cause mortality (Laurent *et al.* 2001) in the general population and in a range of clinical conditions (Laurent *et al.* 2006). Currently as many as 19 studies, the majority of them included in a recent meta-analysis (Vlachopoulos *et al.* 2010b), have consistently shown the independent predictive value of aortic PWV for fatal and non-fatal cardiovascular events in various populations. Arterial stiffness can thus be considered an intermediate end-point of cardiovascular events. The aforementioned meta-analysis showed that with a 1 m/s increase of aortic PWV the risk of cardiovascular events increases by 14% and cardiovascular and all-cause mortality increases by 15% (Vlachopoulos *et al.* 2010b).

Increased arterial stiffness increases the velocity of the incident and reflected pulse waves, which in turn shifts pulse wave reflections from the diastole to the systole. This phenomenon can be described with AIx, which is defined as the difference between the second and first systolic peaks expressed as a percentage of central PP. The AIx is positively correlated with age and inversely with heart rate and height (Kampus *et al.* 2006). The AIx is an indirect surrogate marker of aortic stiffness (Laurent *et al.* 2006). A recent meta-analysis showed that AIx is an independent predictor of cardiovascular events and all-cause mortality (Vlachopoulos *et al.* 2010a). There is evidence that age-related changes in AIx and aortic PWV are nonlinear, with AIx increasing more in younger individuals, whereas changes in aortic PWV are more prominent in older individuals (McEniery *et al.* 2005a; Kampus *et al.* 2007). These data suggest that AIx might be a more sensitive measure of arterial stiffening in younger individuals while aortic PWV is probably a better marker in older individuals.

2.2.2.2. Antihypertensive therapy and arterial stiffness

Firstly, antihypertensive drugs may have direct, BP-independent effects on arterial stiffness, occurring via a modification of the elastic components of the arterial wall, i.e. vascular smooth muscle cells, elastin, and collagen. Secondly, the effects can be indirect, BP-dependent; in elastic tubes stiffness is reduced with lower distending pressure (Vlachopoulos and Stefanadis 2008). The structural degeneration that underlies arterial stiffness is largely irreversible, which emphasises the importance of early prevention of arterial stiffening with non-pharmacological interventions (Sakuragi *et al.* 2010). This is confirmed by recent data showing that increased arterial stiffness (measured using PP or aortic PWV) predicts the inferior BP lowering effects of antihypertensive drugs (Mackenzie *et al.* 2009; Protogerou *et al.* 2009a). Furthermore, the de-stiffening effect of antihypertensive drugs is time-dependent, emphasising the importance of long-term trials (Mahmud and Feely 2003).

Reduction in arterial stiffness with antihypertensive drugs is generally explained by the reduction in MAP (Payne *et al.* 2010). However, in clinical trials differences in arterial stiffness change with different antihypertensive drugs have emerged despite similar BP reduction. The ACEIs (Tropeano *et al.* 2006; Mahmud and Feely 2008) and ARBs (Mahmud and Feely 2002) seem to have a superior effect on arterial stiffness regardless of BP reduction (Vlachopoulos and Stefanadis 2008). It has been suggested that ACEIs and ARBs may have additional de-stiffening effects due to their anti-inflammatory and anti-fibrotic characteristics and improvement in endothelial function (Safar 2010). The BP-independent effect of another group of vasodilators, the CCBs, on arterial stiffness was first shown by Asmar *et al.* (1993). In that study the CCB felodipine and hydrochlorothiazide reduced similarly brachial BP but only felodipine reduced PWV. More recently, Matsui *et al.* (2009) demonstrated that after 24 weeks of treatment ARB/CCB and ARB/diuretic combinations reduced brachial BP to the same extent. However, the ARB/CCB combination showed a significantly greater reduction in aortic PWV (Matsui *et al.* 2009). Nitrates (Laurent *et al.* 1992), alpha-blockers (Komai *et al.* 2002), diuretics (Girerd *et al.* 1998), and BBs (Asmar *et al.* 2001; Mahmud and Feely 2008) have been shown to reduce arterial stiffness, although these effects have been thought to be largely BP-dependent.

Although AIx is considered a surrogate measure of arterial stiffness, the effect of antihypertensive drugs on AIx and aortic PWV is not always in the same direction (Kelly *et al.* 2001). Reduction in AIx is accomplished by the active effect of drugs on the muscular arteries and the passive effect on the elastic arteries. Vasodilating drugs (ACEIs, ARBs, CCBs, and nitrates) reduce pulse wave reflection and hence AIx, through their action on smooth muscle cells in the arterial wall (Nichols and O'Rourke 2005). Recently, the renin inhibitor aliskiren reduced AIx more than the ACEI ramipril despite similar BP reduction (Virdis *et al.* 2012). Diuretics have a neutral effect, while conventional BBs increase pulse wave reflection (Jiang *et al.* 2007; Protogerou

et al. 2009b). However, vasodilating BBs differ from conventional BBs as they effectively reduce AIx (Mahmud and Feely, 2008). Several haemodynamic factors may contribute to this. In short-term studies nebivolol has been shown to significantly reduce PVR (Kamp *et al.* 2003), which is attributed to its endothelial nitric oxide synthase (eNOS) stimulatory effects (Münzel and Gori 2009). In addition, nebivolol reduces heart rate (Dhakam *et al.* 2008) and increases ejection duration less than atenolol (Mahmud and Feely 2008).

2.2.3. Assessment of endothelial function using pulse wave analysis

The vascular endothelium is the inner layer of blood vessels. The endothelium plays a key role in the biology of the arterial wall through the release of vasoactive and trophic factors (Lekakis *et al.* 2011). Endothelial dysfunction is widely defined as the imbalance between vasodilating and vasoconstricting substances produced by endothelial cells (Deanfield *et al.* 2005). When this balance is disrupted, it predisposes the vasculature to vasoconstriction, leukocyte adherence, platelet activation, mitogenesis, pro-oxidation, thrombosis, impaired coagulation, vascular inflammation, and atherosclerosis (Verma and Anderson 2002). The NO, which is produced in the endothelium from L-arginine by eNOS, is the main vasodilating and a major antiatherogenic biomolecule in the arteries (Davignon and Ganz 2004). The NO-mediated EDV is considered a hallmark of endothelial function. It is well accepted that endothelial dysfunction occurs in response to cardiovascular risk factors and precedes the development of atherosclerosis (Shimokawa 1999; Ross 1999). Endothelial dysfunction contributes to hypertension, atherogenesis, and the progression of CVD (Cockcroft 2005). In hypertensive patients endothelial dysfunction is increased (Panza *et al.* 1990) and associated with cardiovascular events (Perticone *et al.* 2001). Furthermore, a study in postmenopausal hypertensive women has shown that a change in endothelial function with antihypertensive treatment predicts cardiovascular outcome (Modena *et al.* 2002).

A variety of methods have been developed for assessment of endothelial function (Lekakis *et al.* 2011; Lind *et al.* 2011). Originally, endothelial function was assessed as the vasomotor response to acetylcholine (which stimulates NO release) in a coronary artery (Ludmer *et al.* 1986). However, the invasiveness of this method restricts its use in clinical trials. A number of less invasive techniques have since been developed for EDV assessment. Venous occlusion plethysmography evaluates EDV in the the resistance arteries of the forearm. Another widely used method, ultrasound-based flow-mediated dilation, evaluates EDV in the conduit arteries (Lekakis *et al.* 2011).

Recently, PWA with pharmacologic tests has emerged as a simple, noninvasive, and reproducible method for assessment of endothelial function (Wilkinson *et al.* 2002a). The advantage of assessment of endothelial function

with the use of PWA is that it embraces global endothelial function, i.e. both the conduit and resistance vessels (McEniery *et al.* 2006). Changes in small artery tone affect pulse wave reflection: vasodilation reduces AIx, whereas vasoconstriction increases it (Nichols *et al.* 2008; Lekakis *et al.* 2011). The GTN, a NO donor, directly induces vasodilation (Kelly *et al.* 1990), thus reducing pulse wave reflections. The observation that acetylcholine, which stimulates the release of NO by the endothelium, reduces pulse wave reflection in vivo (Klemsdal *et al.* 1994) led to the proposition by Chowienczyk *et al.* (1999) that endothelial function could be measured using PWA with pharmacological tests. Augmentation index corrected for heart rate 75 beats/min (AIx@75) is measured at baseline and after the administration of an inhalable beta-2 agonist (i.e. salbutamol) and a NO donor (i.e. GTN). The effects of these drugs on AIx correspond to endothelium-dependent and -independent vasodilation, respectively.

Endothelial function assessed by using PWA with the administration of salbutamol and GTN correlates well with the response to acetylcholine and sodium nitroprusside in the forearm vascular bed (Wilkinson *et al.* 2002a). This response is assessed using venous occlusion plethysmography, which is currently considered the gold standard for assessment of endothelial function (Lind *et al.* 2011). Given the novelty of the technique, relatively few clinical trials have used PWA with a pharmacological test to assess EDV. However, to date reduced EDV, assessed using PWA with pharmacological tests, has been shown in diabetes (Chowienczyk *et al.* 1999), hypercholesterolaemia (Wilkinson *et al.* 2002a), coronary artery disease (Hayward *et al.* 2002), peripheral artery disease (Kals *et al.* 2006), and rheumatoid arthritis (Wållberg-Jonsson *et al.* 2008).

2.3. Additional subclinical markers for target organ damage

Only a small fraction of hypertensive patients have an elevation of BP alone, with the great majority exhibiting additional cardiovascular risk factors, subclinical target organ damage, or CVD (Mancia *et al.* 2007). Furthermore, when concomitantly present, these factors potentiate each other, leading to a total cardiovascular risk which is greater than the sum of its individual components. Thus, assessment of subclinical target organ damage is considered to be an important component of cardiovascular risk assessment in hypertensive patients (Mancia *et al.* 2009). Currently, organ damage assessment of the heart, the blood vessels, and the kidneys is recommended in hypertensive patients by the ESH/ESC hypertension guidelines (Mancia *et al.* 2007).

2.3.1. Carotid artery intima-media thickness

Pignoli *et al.* (1986) validated the concept that carotid IMT as measured by B-mode ultrasound and applied either *in vitro* or *in situ* at the time of autopsy, reflected direct measurements of aortic and/or carotid specimens. Based on this data, carotid IMT is considered a direct measure of the status of the vascular wall, and abnormalities are not a surrogate but a direct measure of atherosclerotic and arteriosclerotic processes (Mancini *et al.* 2004). Carotid IMT using ultrasound is a quick, non-invasive, relatively inexpensive, and easily available method for subclinical target organ damage assessment (Mancia *et al.* 2007). The ESH and ESC have established carotid IMT >0.9 mm or a plaque as a marker for subclinical target organ damage (Mancia *et al.* 2007). Ultrasound scannings limited to the common carotid arteries are likely to measure vascular hypertrophy only (arteriosclerosis), whereas assessment of atherosclerosis also requires scanning of the bifurcations and/or internal carotids where plaques are more frequent (Mancia *et al.* 2007). Despite the lesser atherosclerotic involvement of the common carotid artery, it has increasingly become the site of choice for measurement of carotid IMT. This is mainly because assessment and quantification of IMT in the internal carotids and carotid bifurcations are far more difficult for various technical and methodological reasons (Espeland *et al.* 1999).

Traditional risk factors such as male sex, ageing, being overweight, elevated BP, diabetes, and smoking are positively associated with carotid IMT in epidemiological and observational studies (O'Leary *et al.* 1999; Chambless *et al.* 2002; Van der Meer *et al.* 2003). Additionally, novel cardiovascular risk factors, such as C-reactive protein (CRP), oxidized low-density lipoprotein (oxLDL), and plasma viscosity have been shown to be associated with carotid IMT (Lee *et al.* 1998; Van der Meer *et al.* 2002; Kampus *et al.* 2007). Increased carotid IMT correlates with calcific coronary disease as detected by computed tomography (Davis *et al.* 1999).

Several studies have confirmed that carotid IMT is associated with cardiovascular events and diseases (Craven *et al.* 1990; Salonen and Salonen 1991; Chambless *et al.* 1997; O'Leary *et al.* 1999; Chambless *et al.* 2000). A recent meta-analysis including 37 197 subjects with a mean follow-up of 5.5 years demonstrated that for an absolute carotid IMT difference of 0.1 mm, the future risk of myocardial infarction increases by 10% to 15%, and the stroke risk increases by 13% to 18% (Lorenz *et al.* 2007). Furthermore, carotid IMT has proved to be a valuable marker for therapeutic benefit in clinical trials. A 4-year study in hypertensive patients showed that despite similar clinic BP reduction, lacidipine was superior to atenolol in slowing down carotid IMT progression (Zanchetti *et al.* 2002). These data were confirmed in a recent meta-analysis which showed that in high risk patients ACEIs, BBs, and CCBs reduce the rate of carotid IMT progression compared to placebo or no-treatment (Wang *et al.* 2006). Meanwhile, in the actively-controlled trials CCBs reduced the progression of carotid IMT more than diuretics, BBs, or ACEIs. However,

whether a decrease of IMT progression is associated with a reduction in cardiovascular events and an improvement in prognosis has not been demonstrated (Zanchetti *et al.* 2009).

2.3.2. Left ventricular thickness

The LVH is initially a compensatory effect in hypertension, which represents adaptation to increased heart afterload. However, this is the first step towards the development of overt clinical disease (Agabiti Rosei and Muiesan 2011). The prevalence of LVH increases with age, which might reflect the influence that additional risk factors exert over time (Agabiti-Rosei *et al.* 2007b). Assessing LVH markedly improves the risk stratification in hypertensive patients without evidence of target organ damage after routine examination (Cuspidi *et al.* 2002). The LVH can be assessed using echocardiography or electrocardiography (ECG). However, left ventricular mass index measured using echocardiography is more sensitive than ECG in diagnosing LVH (Reichek and Devereux 1981) and in predicting cardiovascular risk (Levy *et al.* 1990). According to ESH and ESC recommendations (Mancia *et al.* 2007), the assessment of left ventricular mass should include measurements of the interventricular septum, left ventricular posterior wall thickness, and end diastolic diameter. Using these parameters left ventricular mass is calculated using the formula recommended by the American Society of Echocardiography (Lang *et al.* 2005). Subsequently, left ventricular mass is indexed by body height or body surface area (de Simone *et al.* 1992).

The LVH is an independent cardiovascular risk factor in the general population (Levy *et al.* 1990) and in patients with hypertension (Koren *et al.* 1991), end-stage renal disease (Foley *et al.* 1995), and coronary artery disease (Ghali *et al.* 1992). A number of studies have shown that reducing LVH with antihypertensive therapy improves cardiovascular outcome (Muiesan *et al.* 1995; Verdecchia *et al.* 1998; Koren *et al.* 2002). However, the relationship between echocardiographic left ventricular mass and clinical BP is usually weak (Devereux *et al.* 2004). Moreover, a large body of evidence indicates that there is no significant or weak correlation between reduction in left ventricular mass and decrease in brachial BP (Mancia *et al.* 1997; Devereux *et al.* 2004, Gosse *et al.* 2000). Most recently, the LIFE study demonstrated that atenolol was inferior to losartan in reducing LVH despite the fact that both drugs reduced brachial BP to a similar degree (Devereux *et al.* 2004). Left ventricle afterload, the main determinant of LVH, is affected more by central than peripheral haemodynamics (Hashimoto *et al.* 2007). These changes in central haemodynamics are sensed by the heart as measured with the use of plasma brain natriuretic protein (Deary *et al.* 2002). Indeed, compared with brachial BP, central BP is a stronger determinant of LVH (Roman *et al.* 2007). With conventional BB therapy (e.g. atenolol) pulse wave reflection in the arterial tree increases, thus augmenting left ventricular afterload, and compromising normal ventricular relaxation and

coronary filling (de Luca *et al.* 2004). The results of a recent meta-analysis (Fagard *et al.* 2009) are in accordance with previous evidence (Klingbeil *et al.* 2003) that BBs yield less regression of left ventricular mass compared to other antihypertensive drugs. However, the majority of the 31 reviewed studies where BBs were used involved atenolol. There are scarce and only short-term data (Liu *et al.* 1999; Fountoulaki *et al.* 2005) about vasodilating BBs, e.g. nebivolol, which have a beneficial effect on pulse wave reflection (Mahmud and Feely, 2008).

2.4. Biochemical markers for oxidative stress, inflammation and endothelial function

Although several traditional risk factors are known in hypertension, they do not fully explain cardiovascular risk in these patients. Biomarkers are generally considered to be molecules, proteins, or enzymes in body fluids that provide an independent diagnostic or prognostic value by reflecting an underlying disease state or condition (Tsimikas *et al.* 2006). Although firmly inter-related (Liao *et al.* 1994), biomarkers for inflammation, OxS, and endothelial dysfunction, stand as important novel markers of cardiovascular risk. Hence, general aspects of these three classes of markers are reviewed below.

2.4.1. Oxidative stress

Metabolism of oxygen by cells generates potentially deleterious reactive species, also including reactive oxygen species (Zilmer *et al.* 2010). Living organisms have evolved a network of antioxidant defence mechanisms to maintain their survival against abnormal levels of OxS (Dhalla *et al.* 2000). High-grade OxS results from the imbalance between the generation of reactive oxygen species and the antioxidant defence systems. In experimental studies OxS has been shown to induce hypertension (Touyz 2004). Indeed, OxS is increased in essential hypertension (Russo *et al.* 1998). Many mechanisms may contribute to the generation and/or maintenance of hypertension by high-grade OxS, including the generation of vasoconstrictor lipid peroxidation products, such as isoprostanes, quenching of NO by superoxide, and overproduction of oxLDL (Grossman 2008).

Isoprostanes are stereoisomers of prostaglandins that are formed primarily through the non-enzymatic peroxidation of arachidonic acid by reactive oxygen species (Morrow 2006). Isoprostanes are considered the most sensitive markers for lipid peroxidation and the most reliable markers for systemic OxS in the human body (Moore and Roberts 1998; Morrow 2005). Hence, isoprostanes have become the gold standard in measuring OxS in vivo (Tsimikas *et al.* 2006). There are several mechanisms known to account for the hypertensive

effects of isoprostanes. They have potent vasoconstrictor and antinatriuretic effects; furthermore, isoprostanes are related to the renin-angiotensin system and endothelin formation (Romero *et al.* 1999; Ortiz *et al.* 2001). Elevated isoprostane levels have been documented in patients with hypercholesterolaemia, diabetes mellitus, smoking, and renovascular hypertension (Morrow 2005).

The oxLDL is generated with the peroxidation of lipid and protein content of low-density lipoprotein (LDL). Oxidation of LDL occurs primarily in the vessel wall, which activates several inflammatory and atherogenic pathways (Navab *et al.* 2004). The oxLDL is not present in normal arteries. However, it is present in almost all types of atherosclerotic lesions (Tsimikas *et al.* 2006). It has been shown that oxLDL but not native LDL is recognised by scavenger receptors and taken up by macrophages, a pivotal process in the development of atherosclerosis (Steinberg *et al.* 1989). This suggests that plasma levels of oxLDL are influenced mainly by the degree of local OxS in the arterial wall and susceptibility of LDL to oxidation (Tsimikas *et al.* 2006). The oxLDL increases the synthesis of caveolin-1, which inhibits the production of NO by inactivating eNOS (Kinlay *et al.* 2001). Indeed, coronary and brachial endothelial function is strongly correlated with oxLDL levels (Penny *et al.* 2001; Matsumoto *et al.* 2004). The oxLDL is a valuable marker for subclinical atherosclerosis (Kampus *et al.* 2007; Lind *et al.* 2008) and is related to severity of the acute coronary syndrome (Ehara *et al.* 2001).

The OxS contributes to target organ damage and CVD (Heitzer *et al.* 2001, Ehara *et al.* 2001). Thus, reversal of OxS could represent an adjunctive target for antihypertensive treatment. Although large studies have shown that antioxidant supplementation does not lower BP (Heart Protection Study Collaborative Group 2002; Kim *et al.* 2002), it improves endothelial dysfunction in patients with hypertension (Taddei *et al.* 1998), coronary artery disease (Title *et al.* 2000), and cardiovascular risk factors (Heitzer *et al.* 1996; Ting *et al.* 1997). Several studies have demonstrated that antihypertensive therapy with ACEIs, ARBs, and CCBs reduces OxS (Ghiadoni *et al.* 2003; Taddei *et al.* 2003; Sáez *et al.* 2004; Muda *et al.* 2006). However, the data about the effect of BBs on OxS is controversial. There is emerging evidence that vasodilating BBs (e.g. nebivolol) have superior antioxidative effects compared to conventional BBs (Fratta Pasini *et al.* 2005; Celik *et al.* 2006). The majority of studies investigating the effect of antihypertensive treatment on OxS have been of short-to-mid-term duration. However, the short-term effects of drugs on OxS may not reflect their effect during long-term antihypertensive therapy (Sáez *et al.* 2004).

2.4.2. Endothelial dysfunction

A healthy endothelium is responsible for NO production from its precursor, L-arginine, by eNOS. L-arginine is metabolised in vivo by a class of enzymes known as the protein arginine methyltransferases (Khan *et al.* 2007). The main products of these reactions are symmetric dimethylarginine, asymmetric

dimethylarginine (ADMA), and N^G-monomethyl L-arginine. The latter two have been shown to inhibit eNOS; with ADMA having higher concentrations in the body, it is considered the primary inhibitor of eNOS activity (Vallance *et al.* 1992). The ADMA competitively inhibits NO elaboration by displacing L-arginine from eNOS (Vallance *et al.* 1992). It has been suggested that, in addition to its direct eNOS inhibitory effect, ADMA activates the renin-angiotensin system and released angiotensin II activates nicotinamide adenine dinucleotide phosphate-oxidase. Subsequently, superoxide is produced which reduces the bioavailability of NO (Veresh *et al.* 2008). The ADMA is mainly metabolised by the enzyme dimethylarginine dimethylaminohydrolase (DDAH) into L-citrulline and dimethylamine, and is additionally eliminated by the kidneys (Martens-Lobenhoffer and Bode-Böger 2006). Dysfunction of the L-arginine/NO pathway is a common mechanism by which several cardio-vascular risk factors mediate their deleterious effects on the vascular wall (Böger 2005). Furthermore, recent data suggest that ADMA may act as a link between subclinical inflammation and endothelial dysfunction (Antoniades *et al.* 2011).

The ADMA is closely related to endothelial dysfunction measured by using venous occlusion plethysmography (Perticone *et al.* 2005) and flow-mediated dilation (Juonala *et al.* 2007), as well as by the coronary flow reserve (Takiuchi *et al.* 2004). Hence, numerous studies have used ADMA as a single surrogate for endothelial dysfunction in different conditions (Weber *et al.* 2007). The ADMA has been shown to be increased in the presence of other cardiovascular risk factors and several disease states. These cardiovascular risk factors include hypercholesterolaemia (Böger *et al.* 1998), hypertriglyceridaemia (Lundman *et al.* 2001), previous gestational diabetes (Mittermayer *et al.* 2002), insulin resistance (Stühlinger *et al.* 2002), increased carotid IMT (Miyazaki *et al.* 1999; Zoccali *et al.* 2002; Chirinos *et al.* 2008), and non-traditional risk factors such as CRP (Zoccali *et al.* 2002) and vascular cellular adhesion molecules (Nanayakkara *et al.* 2005). Clinical conditions that are associated with increased ADMA concentrations include hypertension (Perticone *et al.* 2005), syndrome X (angina pectoris with normal coronary arteriograms) (Piatti *et al.* 2003), diabetes mellitus (Lin *et al.* 2002), peripheral artery disease (Böger *et al.* 1997), pulmonary hypertension (Gorenflo *et al.* 2001), preeclampsia (Savvidou *et al.* 2003), chronic heart failure (Kielstein *et al.* 2003), ischaemic stroke (Yoo *et al.* 2001), and cerebral small vessel disease (Khan *et al.* 2007). In patients with chronic kidney disease, plasma ADMA is inversely related to eGFR and represents a strong and independent risk marker for progression to end-stage renal disease and mortality (Ravani *et al.* 2005). Furthermore, ADMA predicts all-cause mortality in healthy subjects (Böger *et al.* 2009) and cardiovascular events in patients with coronary artery disease (Lu *et al.* 2003; Schnabel *et al.* 2005), end-stage renal disease (Zoccali *et al.* 2001), and peripheral artery disease (Mittermayer *et al.* 2006).

Various pharmacological agents such as antidiabetic drugs (Stühlinger *et al.* 2002) and oestrogen replacement therapy (Teerlink *et al.* 2003) have been shown to reduce ADMA levels. Regarding antihypertensive drugs, ARBs (telmisartan, losartan, eprosartan) and ACEIs (perindopril, enalapril) have been shown to effectively reduce ADMA levels (Ito *et al.* 2001; Delles *et al.* 2002; Scalera *et al.* 2008). Data about BBs are intriguing. Conventional BBs such as bisoprolol, atenolol, and metoprolol have no effect on ADMA level or even increase it (Ito *et al.* 2001; Oğuz *et al.* 2007; Pasini *et al.* 2008). The vasodilating BB nebivolol has been shown to stimulate NO release in endothelial cells (Cominacini *et al.* 2003) and upregulate DDAH expression (Pasini *et al.* 2008), consequently improving endothelial dysfunction in hypertensive patients (Cockcroft *et al.* 1995). However, there are controversial data about the effect of nebivolol on plasma ADMA levels (Oğuz *et al.* 2007; Pasini *et al.* 2008; Kandavar *et al.* 2011).

2.4.3. Inflammation

Recent evidence suggests that chronic low-grade inflammation has a key role in the pathogenesis of atherosclerosis (Ross 1999). A number of circulating markers of inflammation have been extensively studied regarding their cardiovascular effects (Verma and Anderson 2002). Amongst them, CRP has emerged as a robust and independent risk marker (Bisoendial *et al.* 2007). The CRP is produced by the liver, by macrophages in the atheroma, and by the smooth muscle cells of the arteries in response to proinflammatory cytokines, including interleukin-6 (IL-6) (Torzewski *et al.* 1998; Calabró *et al.* 2003). *In vitro,* CRP decreases eNOS bioactivity (Venugopal *et al.* 2002; Verma *et al.* 2002), and increases the expression of the endothelial receptor for oxLDL (Li *et al.* 2004), thus eliciting endothelial dysfunction. Soluble cellular adhesion molecules are expressed on the surface of endothelial cells and leukocytes in response to certain cardiovascular risk factors and proinflammatory cytokines (Vita *et al.* 2004). They orchestrate the process of leukocyte rolling, adhesion, and transmigration into the subintimal space (Verma and Anderson 2002).

It has been shown in humans that biomarkers of inflammation, such as CRP, IL-6, and soluble intercellular adhesion molecule-1 (sICAM-1), are associated with endothelial dysfunction and arterial stiffness (Van Bussel *et al.* 2011). Furthermore, CRP levels are associated with the risk of developing hypertension (Sesso *et al.* 2003). Arterial hypertension is associated with increased inflammation (Preston *et al.* 2002; Kampus *et al.* 2006). The CRP has consistently been shown to predict cardiovascular events in high-risk patients (Haverkate *et al.* 1997; Ridker *et al.* 1998) and in the general population (Ridker *et al.* 2002). Furthermore, CRP may have an additional value in estimating cardiovascular risk in patients at intermediate risk (Wilson *et al.* 2008). Although CRP has been the most extensively studied inflammatory marker (Albert and Ridker 2006), other markers such as sICAM-1 and IL-6

have been demonstrated to predict cardiovascular events (Ridker *et al.* 2000a; Ridker *et al.* 2000b). Elevated levels of sICAM-1 have been found in patients with cardiovascular risk factors and may predict the development of CVD (Verma and Anderson 2002).

The CRP has been suggested as an attractive target for prevention and treatment of cardiovascular events. In secondary prevention the reduction in CRP has been shown to result in a decreased atheroma burden and in improved cardiovascular outcome, independently of the LDL lowering effect of statins (Nissen *et al.* 2005; Ridker *et al.* 2005). Antihypertensive drugs such as ARBs, ACEIs, and CCBs have been shown to decrease the level of sICAM-1 and CRP (Agabiti Rosei *et al.* 2005; Derosa *et al.* 2010) while thiazide diuretics and conventional BBs have no or small effect (Touyz *et al.* 2007; Martinez-Martin *et al.* 2011).

3. AIMS OF THE THESIS

The general aim of the current thesis was to assess the biochemical, functional and structural properties of the vasculature in hypertension, as well as to investigate the effect of antihypertensive therapy on these parameters.

The specific aims of the present thesis were the following:

1. To investigate the relationship between asymmetric dimethylarginine, carotid intima-media thickness, and endothelial function in hypertensive patients.
2. To determine the long-term effects of the beta-blockers nebivolol and metoprolol succinate on central blood pressure, arterial stiffness, and left ventricular wall thickness.
3. To investigate the association between reduction in central blood pressure and reduction in left ventricular wall thickness in long-term antihypertensive therapy.
4. To compare the long-term effects of nebivolol and metoprolol succinate on oxidative stress and inflammation in hypertension.
5. To study the importance of the underlying haemodynamic abnormality on the response to antihypertensive treatment with an alpha-blocker (doxazosin), a beta-blocker (bisoprolol), an angiotensin II receptor blocker (candesartan), and a nitrate (isosorbide mononitrate).

4. SUBJECTS AND METHODS

4.1. Study subjects

Two groups of patients with essential hypertension were recruited.

The first group of patients (n=80) (Papers I–III) were recruited from general practitioners' offices in the South-Estonian region. Newly diagnosed treatment-naïve patients with mild-to-moderate essential hypertension, aged 30 to 65 years, were recruited between March 2006 and December 2009. All patients were studied in the Department of Cardiology, University of Tartu. Mild or moderate hypertension was defined as seated systolic BP 140 to 179 mmHg and/or diastolic BP 90 to 109 mmHg on ≥ 2 occasions separated by 1 month. Patients were excluded during the screening period in case they had diabetes mellitus; body mass index (BMI) > 30 kg/m^2; coronary artery disease; clinically relevant heart failure (New York Heart Association class II–IV); renal failure; chronic pulmonary disease; valve disease; arrhythmias; secondary hypertension; clinically relevant atherosclerotic disease of the lower extremities; acute or chronic inflammatory disease; hypercholesterolaemia; known hypersensitivity or allergic reaction to BBs; pregnancy or breastfeeding; history of hepatic, renal, metabolic, or endocrine diseases; heavy smoking (> 10 cigarettes a day); and excessive alcohol consumption.

The second group of patients (Paper IV) consisted of newly diagnosed treatment-naïve hypertensive patients (n=53) of both sexes aged 18–80 years. Forty-one patients were studied in the Department of Cardiology, University of Tartu, Tartu, Estonia. Twelve patients were studied in the Clinical Pharmacology Unit, University of Cambridge, Cambridge, UK. Patients were recruited from local hypertension outpatient clinics and from general practitioners' offices. We excluded patients with secondary hypertension, uncontrolled hypertension (brachial BP > 200/100 mmHg), and pregnant or nursing women and women of childbearing age not taking contraceptives. Additionally, patients with gout, asthma, heart failure (New York Heart Association class III–IV), liver failure (defined as an elevation of alanine aminotransferase four times above upper limit of normal), renal failure (chronic kidney disease stage 3 or 4), and terminal illnesses (e.g. cancer), were excluded.

4.2. Methods

4.2.1. Study protocol

Papers I–III: This was a parallel-group, randomised, double-blind, active controlled, one-centre phase IV study. All patients who met the inclusion and exclusion criteria were assigned a patient number in the ascending order. This number determined whether treatment was to be performed with nebivolol or metoprolol. Randomisation was performed in blocks of four. Patients were

randomly assigned to receive either nebivolol (Nebilet, dL-nebivolol hydrochloride, Berlin-Chemie AG, Germany) or metoprolol (Betaloc Zok, metoprolol succinate, AstraZeneca, UK). The allocation of patients into treatment arms remained unknown for all patients throughout the study. All nebivolol and metoprolol tablets were encapsulated in identical form, size, colour, and shape by an accredited pharmacy manufacturing unit. The nebivolol-treated patients received 5 mg of the drug daily, and the metoprolol-treated patients started with 50 mg of the drug daily with possible up-titration to 100 mg daily 2 weeks after randomisation. If the target BP of < 140/90 mmHg was not achieved, the investigator was free to add 12.5 to 25 mg of hydrochlorothiazide (Hypothiazid; Chinoin Pharmaceuticals and Chemical Works Private Co. Ltd., Hungary) daily 4 weeks after randomisation. The duration of the study was 52 weeks plus the screening period of 2 weeks. The patients attended follow-up visits at weeks 2, 4, 12, 24, 40, and 52.

Paper IV: This was a crossover, randomised, double-blind, placebo-controlled two-centre phase IV study. The study involved 4 different antihypertensive drugs and the drug dosages were gradually force titrated. Standard Latin square randomisation in 1 block was used. All drugs and the placebo were encapsulated to look identical by Ipswich Hospital Pharmacy, Ipswich, UK. We used candesartan (Amias, candesartan cilexetil, Takeda UK Ltd., UK) 8 mg for 1 week and thereafter 16 mg; bisoprolol (Cardicor, bisoprolol fumarate, Merck Pharmaceuticals, UK) 2.5 mg for 1 week and thereafter 5 mg; isosorbide mononitrate (ISMN) (Elantan, isosorbide-5-mononitrate, Schwarz Pharma Ltd., UK) 25 mg for 1 week and thereafter 50 mg; doxazosin (Cardura, doxazosin mesilate, Pfizer Ltd., UK) 1 mg for 1 week, 2 mg for the 2nd week and thereafter 4 mg; and the placebo. All drugs were administered once a day. Each treatment phase lasted for 6 weeks after which the patient was switched to the next treatment phase. There were no wash-in or wash-out periods. Thus, each patient remained in the study for up to 30 weeks.

4.2.2. Brachial blood pressure measurement

In Papers I–III, brachial BP was measured in a sitting position from the non-dominant arm as a mean of three consecutive measurements at five-minute intervals using a validated oscillometric technique (OMRON M4-I, Omron Healthcare Europe BV, the Netherlands). The mean of all (Paper I) or the two closest (Paper II and III) BP readings was used in further analysis. Brachial PP was calculated as the difference between brachial systolic BP and diastolic BP. In Paper IV, triplicate supine BP measurements from the dominant arm were used (OMRON HEM-705CP, Omron Corporation, Japan) with a mean of the two closest BP readings used in the analysis.

4.2.3. Assessment of aortic pulse wave velocity

Aortic PWV was determined by sequential acquisition of pulse waveforms from the carotid and femoral arteries (SphygmoCor Px, version 7.1, AtCor Medical, Australia) using the Millar tonometer (SPT-301B, Millar Instruments, USA). The timing of these waveforms was compared with that of the R wave on a simultaneously recorded ECG. Aortic PWV was determined by calculation of the difference between the carotid and the femoral path lengths divided by the difference in the R wave to waveform foot times. Path length for the determination of aortic PWV was measured as the surface distance between the suprasternal notch and the femoral site minus the distance between the suprasternal notch and the carotid site using a tape measure. In Paper IV, aortic PWV was adjusted to MAP. All aortic PWV measurements were made in duplicate, and their mean values were used in subsequent analysis. Aortic PWV measurements were performed by 2 observers and the intra- and inter-observer coefficents of variation have been published previously (Zagura *et al.* 2012).

4.2.4. Pulse wave analysis

Radial artery waveforms were recorded with a high-fidelity micromanometer (applanation tonometry) from the wrist of the dominant arm, and PWA was performed of the systolic portion (SphygmoCor Px, version 7.1, AtCor Medical, Australia) of the pulse curve in accordance with established guidelines (Laurent *et al.* 2006). The corresponding ascending aortic waveforms were then generated, using a validated transfer function, from which central systolic and diastolic BP, central PP, and AIx were calculated. The AIx was defined as the difference between the second and the first systolic peaks of the central arterial waveform, expressed as a percentage of central PP (Laurent *et al.* 2006). In Paper IV, AIx was adjusted to heart rate and MAP. Timing of the reflected waveform (Tr) was the time from the beginning upstroke of the derived aortic systolic pulse waveform to the inflection point. The MAP was calculated from the integration of the radial artery waveform. The degree of PPA was calculated as brachial PP divided by central PP. In Papers I-III the PWA measurements were made in duplicate, and the mean values were used in subsequent analysis. In Paper IV the PWA measurements were made in triplicate, and the means of the 2 closest readings were used in further analysis. The PWA measurements were performed by 2 observers and the intra- and inter-observer coefficents of variation for AIx@75 have been published previously (Zagura *et al.* 2012).

4.2.5. Assessment of endothelial function

Measurements of PWA were performed at baseline (two recordings), and at every five minutes for twenty five minutes after 400 μg of salbutamol inhalation with a spacer (Ventolin, salbutamol sulphate, GlaxoSmithKline, France). After

that, a 500-μg tablet of GTN (Nitroglycerin Nycomed, GTN, Nycomed, Denmark) was placed under the tongue for three minutes and AIx@75 was measured at every three to five minutes for twenty minutes. The greatest difference from baseline AIx@75 with both pharmacological interventions was used to assess EDV and endothelium-independent vasodilation, respectively (Wilkinson *et al.* 2002a; Kals *et al.* 2006).

4.2.6. Ultrasound examination of carotid artery intima-media thickness

The IMT scans of the common carotid artery were performed by a specialist using an ultrasound device (Sonos 7500, Philips Medical Systems, Inc., USA) with a 12-MHz transducer and videotaped on a super-Video Home System videocassette recorder for further analysis. Longitudinal images of distal one cm of the left and right common carotid arteries at the far wall and near wall were measured in three projections (anterolateral, lateral, and posterolateral). The scans were measured with the Prosound software (Caltech, USA) by an independent technician at the University of Eastern Finland, Kuopio, Finland. The mean of mean carotid IMT was defined as the average of the segmental mean carotid IMT values (Papers I and II). The mean of maximum carotid IMT was defined as the average of the segmental maximum carotid IMT values (Paper II) (Stein *et al.* 2008).

4.2.7. Echocardiography

Echocardiographic examination was performed by two experienced sonographers, who were blinded to patient characteristics, using a commercially available device (Sonos 7500, Philips Medical Systems, Inc., USA) with a 3.5-MHz transducer and digital recording capabilities. The images were stored digitally, coded with a random number, and read by two blinded observers. Using the 2D and the M-mode, long-axis measurements were obtained at the level distal to the mitral valve leaflets. The measurements were made according to the recommendations of the American Society of Echocardiography (Lang *et al.* 2005). Left ventricular mass was calculated using the following formula: 0.8{1.04 [(left ventricular internal dimension at the end of the diastole + posterior wall thickness at the end of the diastole + septal wall thickness at the end of the diastole)^3-(left ventricular internal dimension at the end of the diastole)^3]} + 0.6 g. Left ventricular mass was indexed for the power of its allometric or growth relation to height (height in metres$^{2.7}$) (de Simone *et al.* 1992); this method was used to adjust for differences in body size. Relative wall thickness was calculated using the formula (2 x posterior wall thickness at the end of the diastole)/ left ventricular internal dimension at the end of the diastole (Lang *et al.* 2005).

4.2.8. Inert gas re-breathing method

The CO was assessed non-invasively using the inert gas re-breathing system (Innocor, Innovision, Denmark) (Paper IV). The inert gas re-breathing method is a simple, validated, and reproducible method of CO assessment (Agostoni *et al.* 2005; Gabrielsen *et al.* 2002). The Innocor system uses an oxygen-enriched mixture of an inert soluble gas (0.5% nitrous oxide) and an inert insoluble gas (0.1% sulphur hexafluoride) from a 4-litre anaesthesia bag. Subjects breathed the gas mixture over 20 seconds, with a breathing rate of 15/min. Concurrently, heart rate was continuously measured. Expired gases were sampled continuously and analysed by an infrared photoacoustic gas analyser. The use of sulphur hexafluoride allows the measurement of the volume of the lungs, valve, and rebreathing bag. Nitrous oxide concentration decreases during the rebreathing manoeuvre, with a rate proportional to pulmonary blood flow. Subsequently, CO was determined by the Fick principle, and stroke volume (SV) was calculated. The PVR was calculated as MAP divided by CO (McEniery *et al.* 2005b).

4.2.9. Laboratory analyses

White blood cell and red blood cell counts, haematocrit, haemoglobin, and platelets were estimated with a Sysmex XE 2100 autoanalyser (Sysmex Corp., Japan). Plasma glucose, total cholesterol, LDL, high-density lipoprotein (HDL), triglycerides, creatinine, and urine creatinine were determined by standard laboratory methods, using certified assays, in a local clinical laboratory.

The other blood samples were centrifuged within 15 minutes of collection. Centrifuged blood samples and urine samples were divided to aliquots and stored at –70 ºC until analysis. The oxLDL levels were measured using an Enzyme-Linked Immunosorbent Assay (ELISA) kit (Mercodia AB, Sweden). The urinary content of 8-isoprostanes was analysed by a competitive ELISA (Cayman Chemical Co., USA). The urinary concentrations of 8-isoprostanes were corrected by urinary creatinine concentrations to account for differences in renal function. The plasma level ADMA was determined by a competitive ELISA using a commercially available kit (DLD Diagnostika, Germany). The plasma level of high-sensitivity CRP was determined by using a validated latex particle-enhanced immunoturbidimetric assay (Roche Diagnostics Gmbh, Germany). The sICAM-1 levels were measured by ELISA using a commercially available kit (R&D Systems, USA). The IL-6 was analysed by the quantitative sandwich enzyme immunoassay technique (R&D Systems, USA). Fibrinogen was measured by the clotting method after Clauss using the Stago Compact analyser (Diagnostica Stago, France). Renal function was assessed using eGFR calculated with the abbreviated Modification of Diet in Renal Disease equation (Brosius *et al.* 2006).

4.2.10. Statistical analysis

All data were tested for normality using the Kolmogorov-Smirnov test. Normally distributed data are presented as mean ± standard deviation; non-normally distributed data are presented as the median with the interquartile range. The skewed data were log-transformed for analysis to improve normality.

The EDV was defined as a maximum change in AIx@75 after salbutamol administration (Paper I). Correlations between the variables were examined using univariate linear regression and stepwise multiple regression analyses (Papers I, II, and III). The predictors for stepwise correlation analysis were selected on the basis of simple correlation analysis and from among the variables known or likely to be associated with the dependent variable (Papers I and II).

For categorical variables, contingency tables were composed and the chi-square test or the Fisher exact test was used to compare the distributions for the two randomised groups. For continuous variables, which were not normally distributed for ≥1 group, the Mann-Whitney U-test was used to test the difference between the groups. In other cases, the t-test was used to test for difference.

Changes from the baseline to the endpoint were tested for difference from zero using the paired t-test; for continuous variables, which were not normally distributed ≥1 group, the Wilcoxon rank-sum test was employed (Papers II and III). Additional analyses with MAP adjusted OxS and inflammatory markers were conducted (Paper III). The 2-way ANOVA with repeated measures was used to test the interaction between time and drug, as well as error (drug x time) (Paper II).

One-way repeated measures ANOVA with Bonferroni correction was used to analyse the effects of the drugs on haemodynamic parameters (Paper IV). Patients were divided according to the tertiles of baseline haemodynamic parameters (aortic PWV, AIx, PVR, CO, SV, and PPA), and thereafter patients in the 1st and in the 3rd tertile were compared regarding the reduction in brachial systolic, diastolic, and central systolic BP for each drug (Paper IV). The independent samples t-test was used to test for difference in BP reduction between the tertiles (Paper IV). The drug carry-over effect was assessed with treatment order as the independent variable (Paper IV). $P<0.01$ was considered significant in Bonferroni corrected analysis (Paper IV). Otherwise, significance was defined as two-sided $p<0.05$.

Statistical analysis was performed with the Statistica software (version 8; Statsoft, USA) (Paper I), SAS (version 9.1, SAS Institute Inc., USA) and R (www.r-project.org) software (Papers II and III), and the SPSS software (version 18.0, SPSS Inc., USA) (Paper IV).

5. RESULTS

5.1. Association between asymmetric dimethylarginine, carotid artery intima-media thickness and endothelial function (Paper I)

The clinical characteristics of the study group are summarised in Table 1. Ten (12.5%) study subjects were smokers. Hormone replacement therapy was not used by any female patient. One female patient received oral contraceptives as concomitant therapy.

The ADMA levels were significantly decreased in smokers (0.51 ± 0.12 μmol/L) compared with non-smokers (0.65 ± 0.17 μmol/L) (p=0.01). The ADMA was significantly correlated with EDV (r=–0.26; p=0.02) (Figure 2) and with mean of mean carotid IMT (r=0.32; p=0.007) (Figure 3). No correlation was detected between ADMA and AIx, aortic PWV, central and brachial BP values, or eGFR. Mean of mean carotid IMT was positively correlated with age (r=0.39; p=0.001). There was no correlation between EDV and IMT or EDV and age. A multiple regression model was constructed with ADMA as the dependent variable. Female gender, mean of mean carotid IMT, EDV, total cholesterol levels, and smoking status explained 30% of the variability of ADMA (p<0.001) (Table 2).

Table 1. Baseline characteristics of the study subjects.

Parameter	Hypertension patients (n=80)
Age, y	47 ± 10
Weight, kg	79.5 ± 11.9
Height, cm	172.2 ± 9.4
BMI, kg/m^2	26.7 ± 2.5
Waist-to-hip ratio	0.89 ± 0.08
Smokers, %	12.5
Brachial systolic BP, mmHg	145.4 ± 11.9
Brachial diastolic BP, mmHg	90.3 ± 7.6
Brachial PP, mmHg	55.1 ± 10.7
Central systolic BP, mmHg	131.4 ± 17.9
Central diastolic BP, mmHg	84.8 ± 9
Central PP, mmHg	46.6 ± 13.4
Heart rate, beats/min	69 ± 9.1
AIx, %	23.2 ± 13.4
AIx@75, %	19.2 ± 12.8
Tr, ms	146.8 ± 13.7

Table 1. Continuation

Parameter	Hypertension patients (n=80)
Aortic PWV, m/s	7.4 ± 1.4
Mean of mean carotid IMT, mm	0.8 ± 0.1
Total cholesterol, mmol/L	5.3 ± 0.87
LDL cholesterol, mmol/L	3.4 ± 0.8
HDL cholesterol, mmol/L	1.7 ± 0.6
Triglycerides, mmol/L	1 (0.8; 1.5)
ADMA, μmol/L	0.6 ± 0.2
Glucose, mmol/L	5.2 ± 0.6
Creatinine, μmol/L	68.6 ± 12.9
CRP, mg/L	0.9 (0.5; 2.1)
eGFR, mL/min/1.73m^2	102.8 ± 17.2

Values are the mean ± SD or median with the interquartile range.

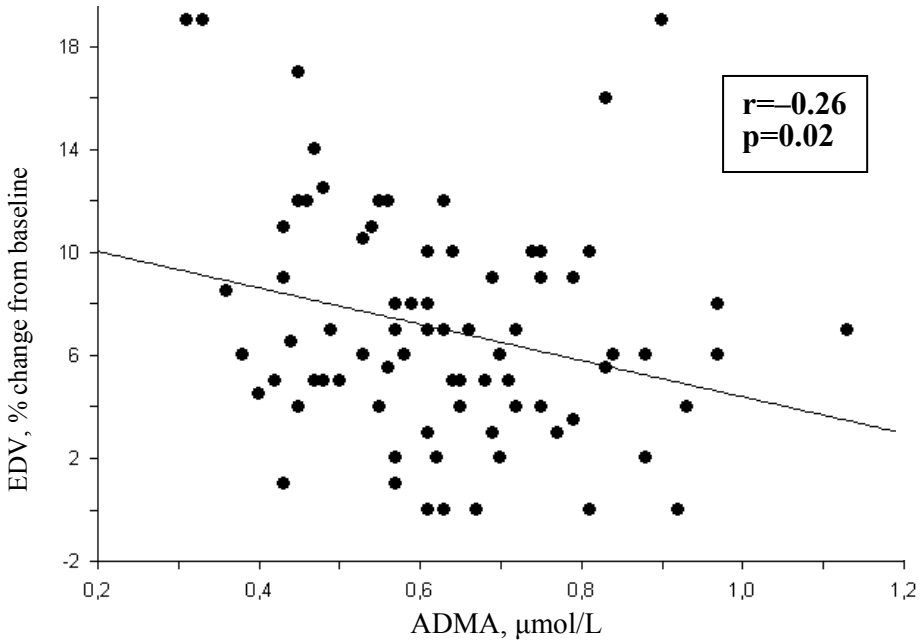

Figure 2. Correlation between ADMA and EDV.

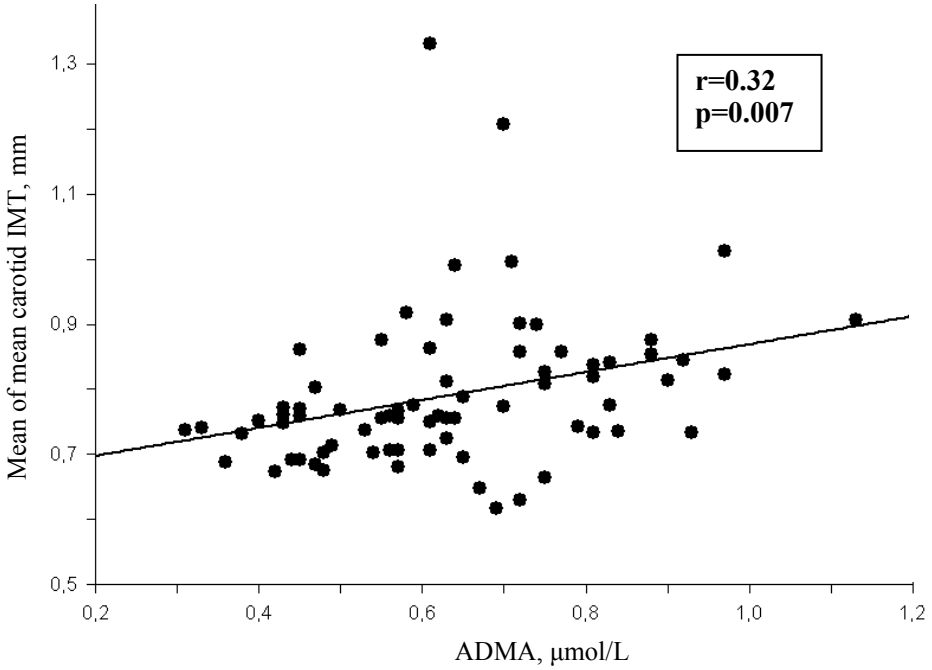

Figure 3. Correlation between ADMA and mean of mean carotid IMT.

Table 2. Results of multiple regression analysis with ADMA as the dependent variable.

Parameter	Regression coefficient	Standard error	p value
Mean of mean carotid IMT, mm	0.53	0.15	0.001
Total cholesterol, mmol/L	–0.06	0.02	0.008
EDV, % change from baseline	–0.01	0.004	0.02
Gender (female)	0.07	0.04	0.05
Smoking status	–0.09	0.05	0.08

R^2=0.3; $p<0.001$.

5.2. The long-term effects of nebivolol and metoprolol succinate on central blood pressure and left ventricular wall thickness (Paper II)

The baseline characteristics of the untreated hypertensive subjects are presented in Table 3. Before randomisation there were no statistically significant differences in the demographic and clinical characteristics between the treatment groups. A total of 40 patients (50%) were enrolled in the nebivolol arm and 40 (50%) were enrolled in the metoprolol arm. Out of the 80 patients enrolled, 63 (79%) completed the study. Seventeen patients were withdrawn from the study for various reasons: seven patients for lack of compliance (four in the nebivolol group and three in the metoprolol group), four patients for dizziness (two patients in each group), two patients for being non-respondent (both in the nebivolol group), two patients for bradycardia (one patient in each group), one patient for hyperglycaemia (in the metoprolol group), and one patient for anxiety (in the nebivolol group).

Up-titration of metoprolol to 100 mg was performed for 13 patients (32%). During the treatment period 30 % of the patients (12 subjects) in the nebivolol group and 22.5 % of the patients (9 subjects) in the metoprolol group (p=0.5) received additionally 12.5 to 25 mg hydrochlorothiazide.

The haemodynamic indices for each treatment group prior to and after one year of therapy are presented in Table 4. Brachial and central systolic or diastolic BP were not different for the groups at baseline. The AIx, AIx@75, and central PP were significantly higher in the nebivolol group at baseline.

Both drugs significantly reduced heart rate, brachial systolic and diastolic BP, and MAP (Table 4), without differences between the groups (Figure 4). However, only the patients of the nebivolol treatment group showed significantly decreased brachial PP (p=0.02). The reduction in central systolic BP, central diastolic BP (Figure 5), and central PP was significant only in the nebivolol group. At the same time, these parameters did not display significant changes in the metoprolol group (Table 4). Mean reduction in central PP was 6.2 mmHg in the nebivolol group and 0.3 mmHg in the metoprolol group (p=0.01). The PPA did not change during the treatment period in either treatment arm.

The AIx@75 (22.9 ± 11.9 % to 18.6 ± 11.3 %; p=0.02) and aortic PWV 7.5 ± 1.5 m/s to 6.8 ± 1.2 m/s; p=0.03) were significantly decreased after 6 months of treatment only in the nebivolol group. However, no significant change was detected in AIx, AIx@75, or aortic PWV after the one-year treatment period for either treatment group (Table 4). There was a trend for correlation, but not significant, between heart rate change and AIx change for the whole study group (r=–0.24; p=0.06). However, the correlation was significant only for the nebivolol treatment arm (r=–0.40; p=0.03).

There occurred a significant reduction in left ventricular posterior wall thickness, left ventricular relative wall thickness as well as a trend for reduction

in left ventricular septal wall thickness and indexed left ventricular mass compared to the baseline values only for the nebivolol group (Table 4). Moreover, changes in septal wall thickness were more significantly correlated with changes in central systolic BP (r=0.41; p=0.001) and with changes in central PP (r=0.32; p=0.01) compared with changes in brachial BP values (r=0.32; p=0.01 and r=0.26; p=0.04, respectively) (Figure 6). Multiple regression analysis showed that only change in central systolic BP (p=0.009) was independently correlated with changes in septal wall thickness as the dependent variable but not with medication used, BMI, changes is MAP, or changes in heart rate (R^2=0.2; p<0.01). Finally, no correlation was revealed between changes in brachial systolic BP and changes in septal wall thickness after adjustment for MAP in multiple regression analysis.

Table 3. Baseline characteristics of the study subjects.

Parameter	Nebivolol (n=40)	Metoprolol (n=40)	p value
Age, y	48.6 ± 10.5	44.4 ± 9.0	0.07
Gender, male/female, n	20/20	21/19	0.8
BMI, kg/m^2	26.6 ± 2.7	26.8 ± 2.4	0.8
Weight, kg	78.3 ± 12.0	80.7 ± 11.8	0.4
Height, m	1.7 ± 0.1	1.7 ± 0.1	0.4
Smokers, n	5	5	1
Glucose, mmol/L	5.2 ± 0.4	5.2 ± 0.8	0.9
Total cholesterol, mmol/L	5.3 ± 0.8	5.2 ± 0.9	0.6
LDL cholesterol, mmol/L	3.4 ± 0.8	3.4 ± 0.9	0.9
Triglycerides, mmol/L	1.2 ± 0.8	1.4 ± 1.1	0.4
HDL cholesterol, mmol/L	1.8 ± 0.7	1.6 ± 0.4	0.3
Creatinine, μmol/L	69.2 ± 13.1	68.0 ± 12.8	0.7
CRP, mg/L	1.7 (0.6;2.3)	1.9 (0.5;2.5)	0.6

Values are the mean ± SD, number, or median with the interquartile range.

Table 4. Haemodynamic, left ventricular wall thickness and carotid IMT indices before and after 1-year treatment.

Parameter	Nebivolol (n=30)		Metoprolol (n=33)		Significance p		
	Baseline	1 year	Baseline	1 year	From baseline		2-Way ANOVA
					NEB	MET	Drug x Time
Brachial systolic BP, mmHg	146.3 ± 12.5	129.3 ± 8.3	144.6 ± 11.4	134.1 ± 4.6	<0.001	<0.001	0.1
Brachial diastolic BP, mmHg	90.0 ± 8.2	78.2 ± 7.6	90.6 ± 7.1	79.7 ± 7.6	<0.001	<0.001	0.5
Brachial PP, mmHg	56.3 ± 11.1	51.1 ± 7.2	54.0 ± 10.3	55.0 ± 11.7	0.02	0.7	0.1
Central systolic BP, mmHg	134.8 ± 19.1	122.4 ± 12.5	128.0 ± 16.2	124.6 ± 16.6	<0.001	0.3	0.07
Central diastolic BP, mmHg	85.6 ± 9.5	79.6 ± 9.1	84.0 ± 8.5	81.7 ± 10.8	0.01	0.1	0.7
Central PP, mmHg	49.2 ± 12.9	42.9 ± 7.2	44.0 ± 13.5	43.1 ± 10.1	0.002	0.7	0.004
PPA	1.18 ± 0.2	1.3 ± 0.3	1.2 ± 0.2	1.31 ± 0.2	0.5	0.9	0.1
PWV, m/s	7.5 ± 1.5	7.1 ± 1.2	7.3 ± 1.3	7.3 ± 1.3	0.3	0.9	0.6
AIx, %	27.2 ± 11.4	28.9 ± 11.3	19.2 ± 14.1	19.1 ± 12.3	0.6	0.4	0.1
AIx@75, %	22.9 ± 11.9	20.8 ± 10.8	14.6 ± 13.1	15.3 ± 11.6	0.1	0.7	0.09
Tr, ms	146.2 ± 14.4	149.8 ± 15.9	147.2 ± 13.7	153.5 ± 13.8	0.1	0.6	0.7
Heart rate, beats/min	67.9 ± 9.1	60.1 ± 7.5	70.2 ± 9.2	64.6 ± 9.8	<0.001	<0.001	0.8
LVED diameter, mm	44.3 ± 5.2	45.5 ± 0.5	45.8 ± 4.7	45.7 ± 4.9	0.1	0.9	–
IVS thickness, mm	10.3 ± 1.6	9.7 ± 1.5	9.9 ± 1.6	9.7 ± 1.6	0.06	0.4	–
LVPW thickness, mm	10.3 ± 1.5	9.3 ± 1.3	9.6 ± 1.5	9.3 ± 1.4	<0.001	0.4	–
LV relative wall thickness	0.5 ± 0.1	0.4 ± 0.1	0.4 ± 0.1	0.4 ± 0.1	<0.001	0.6	–
LV mass, g	159.8 ± 49.7	149.2 ± 42.3	154.6 ± 39.0	148.3 ± 33.7	0.09	0.3	–
LV mass index, $g/m^{2.7}$	37.3 ± 9.9	34.9 ± 8.3	34.4 ± 7.6	33.1 ± 6.9	0.09	0.2	–
Mean of mean carotid IMT, mm	0.8 ± 0.1	0.8 ± 0.1	0.8 ± 0.1	0.8 ± 0.1	0.5	0.2	–
Mean of maximum carotid IMT, mm	1.1 ± 0.2	1.1 ± 0.2	1.0 ± 0.2	1.0 ± 0.2	0.5	0.7	–

IVS indicates interventricular septal wall; LV, left ventricular; LVED, left ventricular end diastolic; LVPW, left ventricular posterior wall; MET, metoprolol; NEB, nebivolol. Values are the mean ± SD.

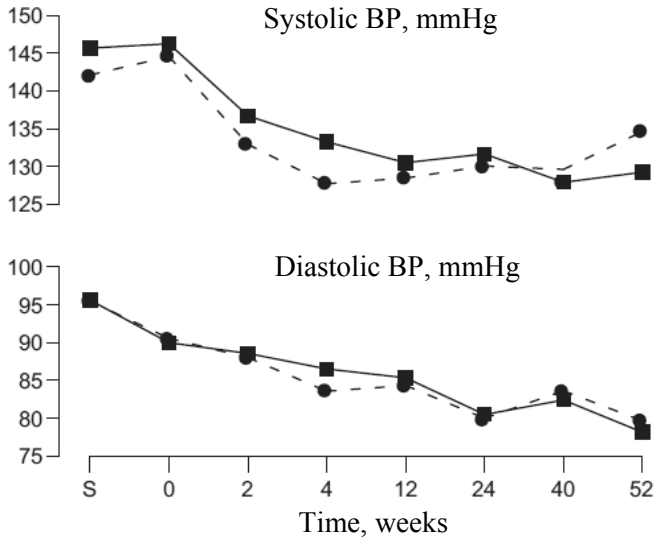

Figure 4. Mean reduction in brachial systolic (top) and diastolic (bottom) BP during the study period. Both treatment groups display significantly reduced brachial systolic and diastolic BPs ($p<0.001$) during 52 weeks of treatment, without difference between the treatment arms. ■ indicates nebivolol; ●, metoprolol; S, screening period.

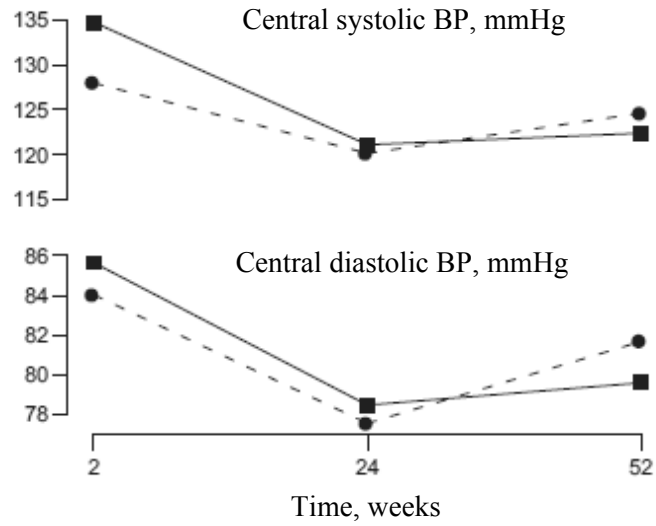

Figure 5. Mean reduction in central systolic (top) and diastolic (bottom) BP measured at baseline, and at weeks 24 and 52. Only the nebivolol group displays significantly reduced central systolic ($p<0.001$) and diastolic ($p=0.01$) BPs after 52 weeks of treatment compared with baseline values. ■ indicates nebivolol; ●, metoprolol.

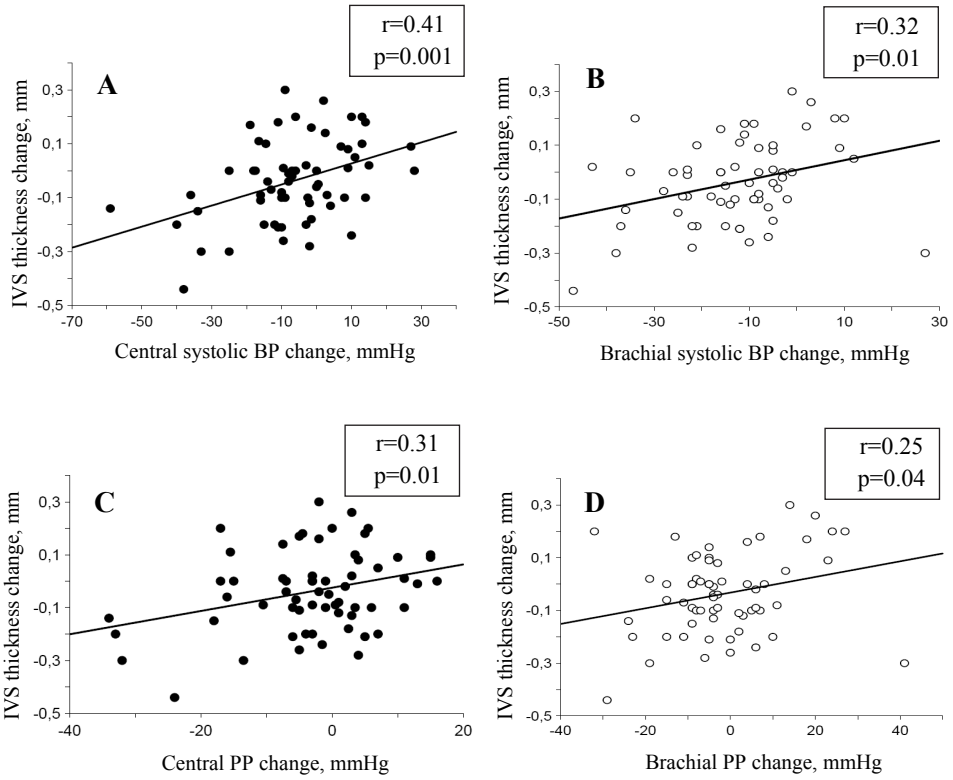

Figure 6. A, Correlation between central systolic BP change and IVS thickness change (r=0.41; p=0.001) for the whole study group. **B**, Correlation between brachial systolic BP change and IVS thickness change (r=0.32; p=0.01) for the whole study group. **C**, Correlation between central PP change and IVS thickness change (r=0.31; p=0.01) for the whole study group. **D,** Correlation between brachial PP change and IVS thickness change (r=0.25; p=0.04) for the whole study group. IVS indicates interventricular septal thickness.

5.3. The long-term effects of nebivolol and metoprolol succinate on oxidative stress and inflammation (Paper III)

The baseline demographic and clinical characteristics of the untreated hypertensive subjects are presented in Table 3. Before randomisation there were no statistically significant differences in the demographic and clinical characteristics between the treatment groups.

The unadjusted and MAP-adjusted data of OxS and inflammatory markers are presented in Table 5. At baseline there were no differences in OxS or

Table 5. Markers for inflammation and OxS at baseline and after 1 year of treatment.

Parameter	Nebivolol	Metoprolol	p
oxLDL, U/L			
Before	85.5 ± 33.8	88.6 ± 31.9	0.7
After	62.0 ± 18.7	65.5 ± 29.1	
p	<0.01	<0.01	
p*	<0.01	<0.01	
8-isoprostanes, ng/mmol creatinine			
Before	43.0 ± 28.4	43.2 ± 34.9	0.7
After	21.8 ± 18.6	34.4 ± 25.7	
p	0.01	0.4	
p*	0.01	0.1	
sICAM-1, ng/mL			
Before	235.7 ± 57.1	234.0 ± 57.2	0.9
After	200.8 ± 55.9	213.1 ± 45.5	
p	<0.01	<0.01	
p*	<0.01	<0.01	
ADMA, μmol/L			
Before	0.6 ± 0.2	0.6 ± 0.2	0.5
After	0.7 ± 0.2	0.6 ± 0.1	
p	0.4	0.3	
p*	0.8	0.3	
IL-6, pg/mL			
Before	1.6 ± 0.7	1.6 ± 0.9	0.9
After	1.7 ± 1	2 ± 1.7	
p	0.8	0.8	
p*	0.7	0.7	
CRP, mg/L			
Before	1.0 (0.4; 1.7)	0.9 (0.5; 2.6)	0.3
After	1.2 (0.7; 2.1)	1 (0.5; 3.3)	
p	1	0.9	
p*	0.8	0.7	
Fibrinogen, g/L			
Before	3.1 ± 0.5	2.9 ± 0.5	0.1
After	3.1 ± 0.7	2.9 ± 0.8	
p	1	0.7	
p*	0.8	0.4	
White blood cell count, x 10^9/L)			
Before	6.1 ± 1.4	5.6 ± 1.6	0.1
After	6.2 ± 1.3	5.9 ± 1.4	
p	0.2	0.2	
p*	0.2	0.2	

p*, p adjusted for treatment MAP. Values are the mean ± SD or median with the interquartile range.

inflammatory markers between the treatment arms. Both drugs reduced significantly oxLDL levels ($p<0.01$ for both drugs). Only nebivolol reduced significantly 8-isoprostane levels ($p=0.01$). Both metoprolol and nebivolol reduced significantly sICAM-1 levels ($p<0.01$ for both drugs). There were no changes in white blood cell count, CRP, IL-6, fibrinogen, or ADMA levels in either group. Adjustment to MAP did not change the statistical significance of the effects of the drugs on OxS and inflammatory markers. In the nebivolol group changes in oxLDL and 8-isoprostane levels were not correlated with change in brachial systolic BP, diastolic BP, or MAP. In the metoprolol group change in oxLDL levels was correlated with systolic BP change ($r=0.45$; $p<0.01$) and PP change ($r=0.47$; $p<0.01$). There were no correlations between change in sICAM-1 level and change in systolic and diastolic BP or MAP in either group.

Total cholesterol, triglycerides, and glucose did not change significantly in either treatment group, but both drugs reduced significantly HDL cholesterol ($p<0.01$ for both drugs) (Table 6). An increase in LDL cholesterol level was observed only in the metoprolol group ($p=0.02$).

Table 6. Comparison of the basic biochemical parameters between the treatment groups.

Parameter	Nebivolol	Metoprolol	p
Total cholesterol, mmol/L			
Before	5.3 ± 0.8	5.2 ± 0.8	0.6
After	5.3 ± 1.0	5.4 ± 1.0	
p	0.9	0.2	
LDL cholesterol, mmol/L			
Before	3.4 ± 0.8	3.5 ± 0.8	0.9
After	3.5 ± 1.0	3.8 ± 1.0	
p	0.9	0.02	
HDL cholesterol, mmol/L			
Before	1.7 ± 0.4	1.6 ± 0.4	0.2
After	1.5 ± 0.4	1.4 ± 0.4	
p	< 0.01	< 0.01	
Triglycerides, mmol/L			
Before	1.2 ± 0.8	1.4 ± 1.1	0.4
After	1.4 ± 1.2	1.3 ± 0.7	
p	0.08	0.4	
Glucose, mmol/L			
Before	5.2 ± 0.5	5.2 ± 0.8	0.9
After	5.3 ± 0.6	5.3 ± 0.8	
p	0.23	0.42	

Values are the mean ± SD.

5.4. Augmentation index as an underlying haemodynamic abnormality and the efficacy of antihypertensive therapy (Paper IV)

The descriptive characteristics of the study subjects are summarised in Table 7. In total, 53 patients (41 patients, Tartu, Estonia; 12 patients, Cambridge, UK) completed the study.

The haemodynamic indices after placebo and all active drugs are shown in Table 8. All drugs significantly reduced brachial, and central BP, and MAP. However, candesartan reduced brachial and central systolic BP and PP the most. All drugs except bisoprolol increased PPA ($p<0.001$). Aortic PWV corrected to MAP did not change with any drug. The AIx corrected to MAP and heart rate was reduced by all drugs except bisoprolol, with ISMN having the relatively largest effect (Δ for ISMN: –5.4%) ($p<0.001$). The CO increased with doxazosin (Δ: +0.4 L/min) and was not affected by the other drugs ($p<0.001$). Bisoprolol (Δ: +14.4 mL) and doxazosin (Δ: +7.3 mL) significantly increased SV, while ISMN (Δ: –5.2 mL) reduced it ($p<0.001$). Doxazosin, candesartan, and bisoprolol significantly decreased PVR ($p<0.001$). There was no drug carry-over effect.

Comparison of the 1st and 3rd tertiles of the baseline haemodynamic parameters revealed baseline AIx and PPA as determinants of BP reduction. The patients in the highest tertile of baseline AIx (AIx value: 36.3–48.2%), comprising mainly women, were significantly older, shorter, and more hypertensive than the patients in the lowest tertile (AIx value: 1.7–28.9%) (Table 9). There was a significant difference in BP change between the tertiles of AIx for all drugs except ISMN (Figure 7). With candesartan, reduction in brachial and central systolic BP was the largest, regardless of the baseline AIx tertile. Bisoprolol was relatively weak at reducing brachial and central BP in the 1st tertile of AIx. However, in the 3rd tertile the BP lowering effect of bisoprolol (Δ brachial systolic BP: –23.4 mmHg) was roughly comparable to that of doxazosin (Δ brachial systolic BP: –22.3 mmHg) and candesartan (Δ brachial systolic BP: –26.4 mmHg).

The patients in the highest tertile of PPA (baseline PPA value: 1.22–1.87) comprising mainly men, were significantly younger, taller, less hypertensive and had lower AIx and heart rate than the patients in the lowest tertile of PPA (baseline PPA value: 1.05–1.11) (Table 10). There was a significant difference in BP change between the tertiles of PPA for all drugs except candesartan (Figure 8). However, candesartan reduced brachial and central BP significantly more than the other drugs, irrespective of the tertile of baseline PPA. With bisoprolol, the reduction in brachial and central systolic BP was more significant in patients with low baseline PPA (Δ brachial systolic BP: –23.1 mmHg and Δ central systolic BP: –21.1 mmHg) compared to patients with high baseline PPA (Δ brachial systolic BP: –11.1 mmHg and Δ central systolic BP: –7.7 mmHg) ($p<0.05$). Comparison of the 1st and 3rd tertiles of baseline SV, CO, aortic PWV, or PVR revealed no significant differences in the BP change with the study drugs.

Table 7. Baseline characteristics of the study subjects.

Parameter	Hypertensive patients (n=53)
Age, y	54.5 ± 12.1
Sex, n	25 M / 28 F
Height, cm	168.3 ± 8.5
Weight, kg	81.7 ± 13.1
BMI, kg/m^2	28.8 ± 4.1
Brachial systolic BP, mmHg	151.3 ± 18.6
Brachial diastolic BP, mmHg	89.7 ± 9.7
Brachial PP, mmHg	61.6 ± 15
Central systolic BP, mmHg	142.8 ± 18.9
Central diastolic BP, mmHg	90.8 ± 9.8
Central PP, mmHg	52 ± 14.7
MAP, mmHg	112.8 ± 12.4
PPA	1.21 ± 0.17
AIx, %	31.5 ± 6.2
Heart rate, beats/min	63.2 ± 9.4
Aortic PWV, m/s	8.9 ± 3
CO, L/min	6.0 ± 1.7
SV, mL	81.2 ± 20.2
PVR, dyne/s	20.1 ± 5.2
Glucose, mmol/L	5.3 ± 0.6
Total cholesterol, mmol/L	5.6 ± 1
HDL cholesterol, mmol/L	1.4 ± 0.5
LDL cholesterol, mmol/L	3.8 ± 1
Triglycerides, mmol/L	1.5 ± 0.8

Values are the mean ± SD or number.

Table 8. Crossover comparison of the haemodynamic variables following treatment with each of the four antihypertensive drugs.

Parameter	Placebo	Doxazosin	Candesartan	Bisoprolol	ISMN	p value
Brachial SBP, mmHg	148.7 ± 16	131.6 ± 15.8 #	127.3 ± 13.4 #	132.5 ± 15.2 #	135.6 ± 16.8 #*	<0.001
Brachial DBP, mmHg	87 ± 8.5	78.8 ± 9.6 #	77.5 ± 7.8 #	78.3 ± 8.6 #	80.5 ± 9 #	<0.001
Brachial PP, mmHg	61.7 ± 13	52.8 ± 12.8 #	49.8 ± 11.2 #	54.2 ± 12.8 #*	55.1 ± 13.6 #	<0.01
Central SBP, mmHg	139.6 ± 16.3	120.9 ± 15.1 #	117.3 ± 14.7 #	125.8 ± 14.3 #*	124.3 ± 17.5 #	<0.001
Central DBP, mmHg	88.1 ± 8.5	79.7 ± 9.4 #	79 ± 8.3 #	79.3 ± 8.7 #	81.4 ± 9.2 #	<0.001
Central PP, mmHg	51.6 ± 12.8	41.2 ± 11.1 #‡	38.3 ± 12.2 #‡	46.5 ± 12.1 #	42.8 ± 14.2 #	<0.001
MAP, mmHg	109.4 ± 10.8	97.2 ± 11 #	95 ± 9.7 #	98.6 ± 10 #	99.1 ± 11.4 #	<0.001
PPA	1.2 ± 0.2	1.3 ± 0.3 #‡	1.4 ± 0.4 #‡	1.2 ± 0.2	1.3 ± 0.2 #‡	<0.001
Aortic PWV, m/s ¶	8.2 ± 1.6	8.5 ± 1.4	8.6 ± 1.7	8.3 ± 1.6	8.5 ± 1.6	0.8
AIx, % §	30.6 ± 11.5	28 ± 10.5 #	27.7 ± 12 #	28.1 ± 9.3	25.2 ± 10.6 #	<0.001
Heart rate , beats/min	63.1 ± 9.4	61.8 ± 9 ‡	63.3 ± 8.6 ‡	54.6 ± 8.3 #	64.2 ± 8.6 ‡	<0.001
CO, L/min	5.9 ± 1.8	6.3 ± 1.8 #†‡	6.1 ± 1.6	5.9 ± 1.5	5.8 ± 1.8	<0.001
SV, mL	81.7 ± 23.3	89 ± 25.2 #†	86.3 ± 22.9	96.1 ± 22 #*†	76.5 ± 23.1 *	<0.001
PVR, dyne/s	20.1 ± 5.9	16.4 ± 4.2 #†‡	16.6 ± 4.8 #†	17.7 ± 4.1 #	18.5 ± 5.1	<0.001

DBP indicates diastolic blood pressure; SBP, systolic blood pressure; #, p<0.01 vs. placebo; *, p<0.01 vs. candesartan; †, p<0.01 vs. ISMN; ‡, p<0.01 vs. bisoprolol; ¶, data corrected for MAP and heart rate; §, data corrected for heart rate. Values are the mean ± SD.

Table 9. Subject characteristics at baseline according to the tertiles of baseline AIx.

	Tertiles of AIx, %		
Parameter	First (1.7–28.9) (n=17)	Third (36.3–48.2) (n=18)	p value
Age, y	49.1 ± 14.1	58.1 ± 9.6	<0.05
Males, %	15 (88.2)	4 (22)	<0.001
Weight, kg	86.9 ± 13.5	78.2 ± 6.9	<0.05
Height, cm	176.5 ± 6.7	163.9 ± 6.1	<0.001
BMI, kg/m^2	27.9 ± 3.6	29.2 ± 2.9	0.3
Aortic PWV, m/s	8.1 ± 1.4	8.7 ± 1.6	0.3
Brachial SBP, mmHg	146.9 ± 17	162.4 ± 20.9	<0.05
Brachial DBP, mmHg	86.9 ± 8.3	95.7 ± 10.0	<0.01
Heart rate, beats/min	62.1 ± 9.7	64.0 ± 10.8	0.6
MAP, mmHg	107.5 ± 10.3	122.9 ± 12.8	<0.001

DBP indicates diastolic blood pressure; SBP, systolic blood pressure. Values are the mean ± SD or number (%).

Table 10. Subject characteristics at baseline according to the tertiles of baseline PPA.

	Tertiles of PPA		
Parameter	First (1.05–1.11) (n=17)	Third (1.22–1.87) (n=18)	p value
Age, y	57.4 ± 9.3	47.5 ± 12.7	0.01
Males, %	4 (24)	15 (83)	0.001
Weight, kg	78.9 ± 11.3	86.1 ± 13.6	0.1
Height, cm	162.7 ± 6.4	174.0 ± 8.1	<0.001
BMI, kg/m^2	29.8 ± 3.9	28.4 ± 3.6	0.3
AIx, %	38.5 ± 3.9	25.4 ± 13.1	0.001
Aortic PWV, m/s	8.9 ± 1.7	8.1 ± 1.4	0.2
Brachial SBP, mmHg	159.9 ± 20.6	145.9 ± 14.5	<0.05
Brachial DBP, mmHg	91.8 ± 10.8	87.8 ± 7.3	0.2
Heart rate, beats/min	57.4 ± 5.7	70.2 ± 10.4	<0.001
MAP, mmHg	118.7 ± 12.8	108.1 ± 7.9	<0.01

DBP indicates diastolic blood pressure; SBP, systolic blood pressure. Values are the mean ± SD or number (%).

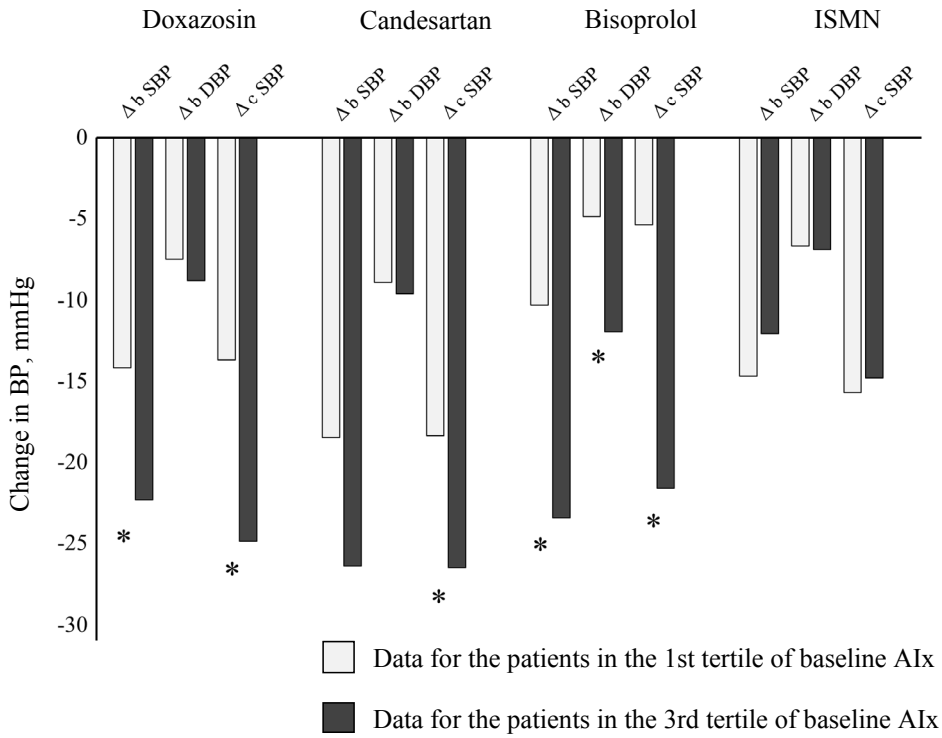

Figure 7. Comparison of BP changes in patients divided according to the tertiles of baseline AIx. * indicates $p<0.05$. b DBP, brachial diastolic blood pressure; b SBP, brachial systolic blood pressure; c SBP, central systolic blood pressure.

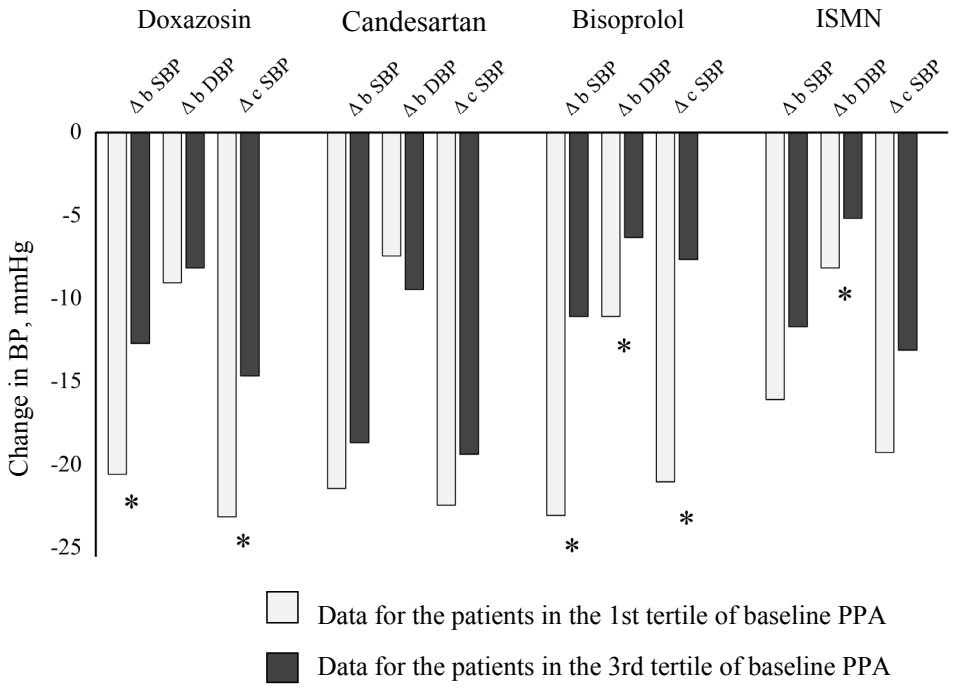

Figure 8. Comparison of BP changes in patients divided according to the tertiles of baseline PPA. * indicates $p<0.05$. b DBP, brachial diastolic blood pressure; b SBP, brachial systolic blood pressure; c SBP, central systolic blood pressure.

6. DISCUSSION

6.1. Asymmetric dimethylarginine as a marker for endothelial dysfunction and carotid artery intima-media thickness (Paper I)

The main finding of Paper I was that in hypertensive patients ADMA is independently associated with endothelial dysfunction measured by using PWA with pharmacological tests, carotid IMT, female gender, and total cholesterol. To our knowledge, this is the first study where PWA with pharmacological tests has been used in hypertensive patients.

The NO is the main vasodilating, antiatherogenic, and antiproliferative molecule produced in the endothelium. The reduction in NO bioavailability is considered a hallmark of endothelial dysfunction. The production of NO by eNOS from L-arginine is regulated by several physiological and pathological pathways (Ignarro 2002). The eNOS inhibitor ADMA competes with L-arginine to bind the active site of eNOS, thus reducing NO availability. Clinically, endothelial dysfunction is evidenced by an impaired vasodilatory response to acetylcholine. The NO also influences wave reflection, and hence the shape of the waveform (Kelly *et al.* 1990). Administration of salbutamol causes the release of NO, resulting in EDV (Wilkinson *et al.* 2002a). It has been shown that administration of an eNOS inhibitor, L^G-monomethyl L-arginine, mitigates the effect of salbutamol on wave reflection (Hayward *et al.* 2002) hence the effect of salbutamol is truly endothelium-dependent. The association between ADMA and EDV in our study is in accordance with the finding of a previous study where venous occlusion plethysmography, currently regarded as the gold standard for endothelial function assessment (Lind *et al.* 2011), was used for EDV assessment (Perticone *et al.* 2005). The independent and inverse association between ADMA and endothelial function assessed by using PWA with pharmacological tests in our study further supports this technique for assessment of endothelial function.

The aortic PWV is considered a gold standard measure of arterial stiffness (Laurent *et al.* 2006), which independently predicts outcome in hypertensive patients (Boutouyrie *et al.* 2002). However, in our study ADMA and EDV were not correlated with arterial stiffness. Our findings are concordant with the results of the PREVENCION study which confirmed that ADMA is associated with carotid IMT and traditional cardiovascular risk factors but not with arterial stiffness (Chirinos *et al.* 2008). To date, the correlation between EDV and aortic PWV has only been shown in middle-aged healthy persons (McEniery *et al.* 2006) and in elderly isolated systolic hypertension patients (Wallace *et al.* 2007). This could be explained by the fact that in elderly patients isolated systolic hypertension results from increased arterial stiffness, i.e. aortic PWV, which differs considerably from aortic PWV in non-isolated systolic hypertension patients. The mean aortic PWV in our study subjects was 7.4 m/sec,

which is considerably lower than it is in isolated systolic hypertension patients (9.65 m/sec) (Wallace *et al.* 2007). Owing to the very strict inclusion criteria of the present study, our patients were at low or moderate total cardiovascular risk, which may also explain the normal range of aortic PWV.

The present study did not reveal significant correlations between the values of central/brachial BP and ADMA. Several experimental studies have shown that the elevated plasma concentration of ADMA increases MAP (Achan *et al.* 2003; Kielstein *et al.* 2004). This controversy can be explained by a lower concentration of plasma ADMA in the clinical setting because ADMA is concentrated intracellularly and is rapidly eliminated by DDAH. Our finding is in accordance with previous observational evidence that ADMA is not correlated with brachial BP (Chirinos *et al.* 2008).

In this study we showed that ADMA is associated with an early marker for subclinical target organ damage, carotid IMT, in hypertension patients. It has been shown previously that ADMA is associated with increased carotid IMT in healthy subjects (Miyazaki *et al.* 1999). In the present study, carotid IMT was positively correlated with age, which is in accordance with the findings of other similar studies (Tanaka *et al.* 2001). We confirmed that EDV and carotid IMT are not correlated, which suggests that they measure different aspects and stages of early atherosclerosis (Yan *et al.* 2005). It is possible that endothelial dysfunction as the earliest event in the process of lesion formation precedes structural changes in the arteries; thus increase in carotid IMT could be a consequence of endothelial dysfunction (Lundman *et al.* 2001).

Interestingly, ADMA was increased in non-smokers compared to smokers in our study. Data about the relationship between ADMA and smoking status are conflicting (Sobczak *et al.* 2009). Smoking has been associated with decreased levels of ADMA in healthy individuals and in high risk patients (Eid *et al.* 2004; Maas *et al.* 2007). In our study, this effect could be due to light smoking (less than 10 cigarettes a day). Furthermore, to minimise the acute effect of smoking on arterial stiffness and several biomarkers, the patients discontinued smoking at least 12 hours before the study. There is evidence that smoking enhances the expression of DDAH which may decrease ADMA levels (Maas *et al.* 2007). It has also been suggested that endothelial dysfunction caused by tobacco smoke is probably not related to ADMA (Sobczak *et al.* 2009).

6.2. Beta-blockers, central haemodynamics and arterial stiffness (Paper II)

The main findings of Paper II were that in long-term antihypertensive therapy both metoprolol and nebivolol reduced similarly brachial BP, however, only the patients receiving nebivolol showed a significant reduction in central BP. Nebivolol but not metoprolol succinate reduced left ventricular posterior wall thickness and left ventricular relative wall thickness. Change in septal wall

thickness correlated more significantly with change in central systolic and PP than with change in brachial systolic and PP. Hence, central BP reduction with antihypertensive treatment is more important than brachial BP reduction in reducing subclinical target organ damage.

For many years, BBs have been advocated as first-line therapy for most patients with hypertension. This class of antihypertensive drugs flourished during the past 50 years as the mainstay for the prevention and treatment of various cardiovascular disorders such as cardiac arrhythmias, hypertension, myocardial infarction, and heart failure (Shah *et al.* 2011). Recently, the role of BBs in uncomplicated hypertension has been questioned. Several meta-analyses suggest that although BBs effectively lower brachial BP they are inferior to other antihypertensive drugs regarding cardiovascular outcome in uncomplicated hypertension (Lindholm *et al.* 2005; Wiysonge *et al.* 2007). However, the majority of studies (70–75%) included in these meta-analyses involved atenolol. The results of these meta-analyses caused the extinction of BBs as an initial drug of choice for hypertension management in the British Hypertension Society guidelines (NICE 2011). Several factors have been ascribed to the inferiority of atenolol (Pollare *et al.* 1989; Dahlöf *et al.* 2002). There is an accumulating amount of evidence that conventional BBs, especially atenolol, despite having similar effects on brachial BP, are inferior to other antihypertensive drugs in lowering central BP (Williams *et al.* 2006; Mackenzie *et al.* 2009; Matsui *et al.* 2009; Boutouyrie *et al.* 2010), explaining the 'beyond (brachial) BP' paradox. However, novel vasodilating BBs (e.g. nebivolol) have been shown to significantly reduce central BP in short-term (4 to 5 weeks) studies (Dhakam *et al.* 2008; Mahmud and Feely 2008). Furthermore, a recent study showed that nebivolol is comparable to the ARB irbesartan regarding its effect on central BP (Vitale *et al.* 2012). There are no data about the effect of metoprolol succinate, the most widely used BB in Northern and Eastern European countries, on central BP.

In the present study the effect of nebivolol and metoprolol was tested during a one-year period. The results of the study confirm that BBs without vasodilating properties have less impact on central BP. Overall central PP reduction was 6 mmHg for the nebivolol group and only 0.3 mmHg for the metoprolol group. Moreover, the results from the CAFE study (Williams *et al.* 2006) indicate that even a 3 mmHg reduction in central PP was associated with better cardiovascular outcome. In the present study both treatment arms reduced similarly heart rate and MAP. It could be suggested that the main mechanism for the reduction in central BP in the nebivolol arm acted through vasodilation and structural remodelling of the small arteries, leading to the reduction in pulse wave reflection site distance and intensity. It has been demonstrated that nebivolol dilates the human forearm vasculature through the L-arginine pathway (Cockcroft *et al.* 1995), improves resistance artery (Tzemos *et al.* 2001) and cutaneous endothelial function and small artery distensibility (Arosio *et al.* 2002). At the same time, conventional BBs do not reduce PVR and sympathetic

activity (Burns *et al.* 2004), which may lead to small artery vasoconstriction and a higher media:lumen ratio (Schiffrin 2004).

The AIx and PPA are related to pulse wave reflection depending on the amplitude and site of wave reflection and on the speed at which the pulse waves travel along the arterial tree. In the present study, AIx and PPA did not change during the one-year treatment period in either treatment arm. Previous data about the effect of nebivolol on AIx and PPA have been conflicting. In patients with isolated systolic hypertension, Dhakam *et al.* (2008) showed a slight increase of AIx and no effect on PPA after five weeks of treatment with nebivolol. On the contrary, Mahmud and Feely (2008) demonstrated, after treatment with nebivolol, a significant reduction in AIx and an increase in PPA in patients with essential hypertension. In the above studies, PPA and AIx were found to be very strongly correlated with heart rate change, which may explain the described controversial results. The present study also revealed a trend for correlation, although not significant, between heart rate change and AIx change for the whole study group. Moreover, the correlation was significant only for the nebivolol treatment arm. No correlation was detected between central PP change and heart rate change. This may suggest that the more pronounced central BP reduction in the nebivolol-treated patients appeared was a concomitant effect of the change in heart rate and AIx and the reduction in PVR rather than the effect of PWV change. It should be noted that there was already a difference in baseline AIx, and the reduction in heart rate was not sufficient to produce significant AIx change after one-year therapy, either.

In the present study there occurred a significant reduction in aortic PWV after 6 months of treatment in the nebivolol group. However, after the one-year treatment period there was no significant difference in the change in aortic PWV, radial-carotid PWV (data not shown), or Tr between the two treatment arms. Previous studies have demonstrated a significant reduction in aortic PWV after treatment with nebivolol (Dhakam *et al.* 2008; Mahmud and Feely 2008). It has been suggested that the effect of BBs on aortic PWV may be related to the concomitant effect of reduction in MAP, sympathetic tone and heart rate. Consequently, one possible explanation for the non-significant change in aortic PWV in the present study is also the significantly smaller reduction in heart rate during the one-year treatment period (approx. 6 beats/min). It should be noted that previous studies were of short duration, and long-term use of BBs may not have such a significant effect on heart rate and sympathetic activity, which are considered important determinants of aortic PWV. The time-dependent effect of BBs on vascular stiffness was also suggested in a recent review by Protogerou *et al.* (2009b). Another possibility is that in the present study baseline mean aortic PWV was in the normal range. Also, owing to the very strict inclusion criteria of the present study, the patients were at low total cardiovascular risk, which may also explain the weak effect of treatment on aortic PWV.

The present study provides the first long-term evidence that the reduction in central systolic BP in the nebivolol group was directly related to the reduction

in left ventricular septal and posterior wall thickness. It has been recently demonstrated that compared to brachial BP central BP is a stronger determinant for LVH (Roman *et al.* 2007; Wohlfahrt *et al.* 2012). Data from the Strong Heart Study also indicate that in terms of reduction in LVH, it is more important to target central systolic than brachial BP (Roman *et al.* 2010). Moreover, the LIFE study demonstrated that atenolol was less effective than losartan at reducing LVH (Devereux *et al.* 2004), despite the fact that both drugs reduced brachial BP to a similar degree. In a small open-study, nebivolol monotherapy for up to 12 months resulted in a reduction in left ventricular wall thickness (Liu *et al.* 1999) and 3-month treatment with nebivolol was as effective as telmisartan in reducing left ventricular mass (Fountoulaki *et al.* 2005). However, a recent meta-analysis provides evidence that BBs show less regression of left ventricular mass compared to other antihypertensive drugs (Fagard *et al.* 2009). Regrettably, the results of 20 of the reviewed 31 studies were obtained with atenolol, the results of three studies, with metoprolol, and the results of only one study, with nebivolol. One plausible explanation for the lesser regression of LVH by BBs in the above meta-analysis is that central BP may not be reduced as effectively as brachial BP, which provides less afterload reduction with conventional BBs without vasodilating properties.

The current study has several limitations. When the trial was designed in 2005, there were no studies of appropriate size providing the data about the effect of antihypertensive drugs on central BP, derived from applanation tonometry, in order to undertake formal power calculation; therefore larger studies are needed to confirm our results. Also, 17 of the 80 study patients were withdrawn, which is quite a high fraction. The main reason for poor treatment compliance was that the study patients were relatively young with newly diagnosed mild-to-moderate hypertension, who did not have any symptoms or complaints attributable to high BP. In the case of mild adverse events after the initiation of treatment, they tended to withdraw their consent. Also, the inclusion criteria of the study were very strict and the patients were at low cardiovascular risk, which may explain the relatively normal mean values of central haemodynamics, as well as may account for the disparity in several parameters studied by us and other researchers. Although both study groups were comparable with regard to brachial BP, there occurred a shift in central haemodynamics (AIx and central PP) at baseline. We cannot exclude an additional effect of the thiazide diuretic on the results. However, it has been proposed that diuretics have a neutral or minimal beneficial effect on central BP, aortic stiffness, and pulse wave reflection (Protogerou *et al.* 2009b; Matsui *et al.* 2009).

6.3. The effect of beta-blocker therapy on oxidative stress and inflammation (Paper III)

The main finding of Paper III was that in long-term antihypertensive therapy both nebivolol and metoprolol reduced oxLDL and sICAM-1 levels in essential hypertension patients while only nebivolol reduced 8-isoprostane levels. Furthermore, the effect of metoprolol on oxLDL was associated with BP change while nebivolol decreased oxLDL and 8-isoprostane levels independently of BP reduction. To our knowledge, this is the first study to assess the long-term effects of nebivolol and metoprolol on OxS and inflammatory markers.

Harmful OxS results from the imbalance between the generation of reactive oxygen species and the antioxidant defence systems (Zilmer *et al.* 2010). It has been shown that such OxS reduces the bioavailability of NO, leading to endothelial dysfunction and atherosclerosis (Taddei *et al.* 1998). Thus, reversal of elevated OxS could represent an adjunctive target for antihypertensive treatment (Ono *et al.* 2008). Sáez *et al.* (2004) have previously shown that antihypertensive treatment reduces OxS markers in a time-dependent manner. This evidence emphasises the need for long-term studies to investigate the antioxidative properties of antihypertensive drugs.

Urine isoprostanes were recently accepted by the European Food Safety Authority as a new biochemical marker for OxS (EFSA 2011). Isoprostanes are stereoisomers of prostaglandins that are formed primarily through the non-enzymatic peroxidation of arachidonic acid by reactive oxygen species (Morrow 2005). To our knowledge, only Napoli *et al.* (2008) have investigated the long-term effect of antihypertensive drugs (ACEIs) on isoprostane levels. However, they measured plasma values of isoprostanes, which may overestimate true isoprostane levels because of the auto-oxidation of lipids (Morrow 2005). Our results suggest that conventional BBs have no significant effect on 8-isoprostane levels, which is in agreement with previous short-term studies where metoprolol (Fahlbusch *et al.* 2004) and atenolol (Flammer *et al.* 2007; Pasini *et al.* 2007) have been used. Furthermore, in a recent study metoprolol increased urine isoprostane levels in patients with metabolic syndrome (Ayers *et al.* 2012). Our study showed that nebivolol decreased urinary 8-isoprostane levels independently of BP reduction in two respects. Firstly, reduction in 8-isoprostane levels was not correlated with reduction in BP. Secondly, nebivolol reduced 8-isoprostane levels even when 8-isoprostane levels were adjusted for BP. Our results suggest that in long-term antihypertensive therapy nebivolol possesses a BP-independent effect on systemic OxS.

Plasma levels of oxLDL are mainly influenced by degree of local OxS in arterial wall and by susceptibility of LDL to oxidation (Sjogren *et al.* 2005). The patients included into our study had been recently diagnosed with hypertension; they were relatively young and were at low cardiovascular risk, which suggests low probability of the presence of plaques in the vasculature as a

source of oxLDL. It is plausible that increased shear stress induces oxidation of LDL cholesterol. Thus, reducing BP *per se* might reduce oxLDL.

In hypertension several pro-inflammatory factors activate endothelial cells to express adhesion molecules, such as sICAM-1, which initiates recruitment of circulating lymphocytes to blood vessel wall. Several studies have shown that renin-angiotensin system inhibitors and CCBs decrease effectively sICAM-1 levels in hypertension (Derosa *et al.* 2010; Martinez-Martin *et al.* 2011). Our study is the first one to assess the effect of metoprolol or nebivolol on sICAM-1 levels in hypertensive patients. Our study suggests that both drugs potentially inhibit endothelial activation in vessel wall.

There was no effect of metoprolol or nebivolol on ADMA levels in long-term antihypertensive therapy. In addition to beta 1-adrenoceptor antagonist characteristics, nebivolol has beta 3-adrenoceptor agonist properties. There is evidence that beta 3-adrenoceptor agonist properties contribute to NO release while beta 1-adrenoceptor antagonism has converse effects (Evangelista *et al.* 2007). These two pharmacological effects of nebivolol may account for the finding that in some studies nebivolol decreases ADMA levels, while in our and other studies ADMA concentration did not change (Oğuz *et al.* 2007; Kandavar *et al.* 2011; Ayers *et al.* 2012). Neither drug had an effect on inflammatory markers (CRP, white blood cell count, fibrinogen, and IL-6). It could be speculated that the studied patients were indeed at a relatively low cardiovascular risk, according to the CRP (0.85 – 0.95 mg/L) risk categories (Pearson *et al.* 2003). Furthermore, CRP values were somewhat higher in a similar study population (1.94 mg/L) (Cottone *et al.* 2007).

There is evidence that BBs have a detrimental effect on lipoprotein and glucose metabolism (Pollare *et al.* 1989; Bakris *et al.* 2004). This effect involves an increase in triglyceride levels and a decrease in HDL cholesterol levels. In our study only metoprolol increased LDL cholesterol levels. The HDL cholesterol was decreased by both drugs. Triglyceride levels did not change significantly in either group. It has been shown that metoprolol and atenolol have a similar effect on glucose, triglyceride, and HDL metabolism, which has been thought to account for their inferior reduction in the incidence of coronary artery disease in several large-scale hypertension studies (Pollare *et al.* 1989). However, there is no evidence that the change in the lipoprotein profile associated with BBs could negatively affect cardiovascular outcome.

6.4. The underlying haemodynamic profile and the efficacy of antihypertensive treatment (Paper IV)

We studied whether the haemodynamic profile of patients with essential hypertension determines the efficacy of an alpha-blocker, a BB, an ARB, or a nitrate. To our knowledge, this is the first study to systematically investigate a complex of haemodynamic parameters (i.e. SV, CO, aortic PWV, AIx, PPA, and PVR) which could influence the efficacy of antihypertensive drugs. We found that

baseline AIx and PPA determined brachial or central BP reduction. Haemodynamic profiling by baseline AIx determined BP reduction with doxazosin, bisoprolol, and candesartan. Haemodynamic profiling by PPA determined BP reduction with doxazosin, bisoprolol, and ISMN. The largest effect of haemodynamic profiling by AIx and PPA which determined BP reduction was demonstrated with bisoprolol. Baseline aortic PWV, SV, CO, and PVR did not determine the response to the drugs used in our study. Candesartan had the most impressive brachial and central BP reducing properties, regardless of the baseline haemodynamic profile.

Augmentation of the pulse wave is dependent on the speed of pulse wave travel, the amplitude of the reflected wave, the reflectance sites, and the duration and pattern of ventricular ejection, including heart rate and ventricular contractility (Nichols and O'Rourke 2005). In contrast to other drugs, only bisoprolol did not change AIx in our study. It is well known that BBs have a negative or neutral effect on AIx. Due to heart rate reduction, they favour the arrival of the reflected wave in the relatively earlier phase in the systole, instead of the diastole (Protogerou *et al.* 2009b; Avolio *et al.* 2009). As expected, only bisoprolol reduced heart rate significantly, which may explain our result. Nitrates can be regarded as the most potent drug to reduce pulse wave reflection in isolated systolic hypertension patients (Stokes *et al.* 2003). In our study, although ISMN reduced AIx the most, the change in AIx was smaller than expected from previous studies (Stokes *et al.* 2003). The modest effect of ISMN on AIx may explain the relatively small reduction in BP with this drug. Among the patients in the 3rd tertile of AIx, the alpha-blocker, the BB, and the ARB effectively reduced BP. Among these drugs, alpha-blockers and ARBs are known to cause vasodilation in the peripheral arteries (Lund-Johansen and Omvik 1991; Protogerou *et al.* 2009b). However, the results concerning BBs are intriguing. Our results suggest that in patients with low AIx, BBs are comparatively less effective at reducing BP. In the 3rd tertile, however, BP reduction with the alpha-blocker was comparable to BP reduction with the ARB. These results can be explained by the baseline characteristics of these patients. Patients with higher AIx had substantially higher baseline BP. There is evidence that higher baseline BP is associated with a larger extent of decrease in BP (Gill *et al.* 1985).

Brachial BP differs from central BP owing to the phenomenon of PPA (Agabiti-Rosei *et al.* 2007a). There is evidence that central BP predicts cardiovascular risk better than brachial BP does (Roman *et al.* 2009). Moreover, different antihypertensive drugs reduce central BP differentially (Agabiti-Rosei *et al.* 2007a; Mackenzie *et al.* 2009), which can be a better determinant of subclinical organ damage and clinical outcome (Williams *et al.* 2006; Roman *et al.* 2010). In our study, candesartan reduced both brachial and central BP the most. Hence, direct comparison of on-treatment central BP may underestimate the differential effect of study drugs on central BP because the drug classes had a dissimilar effect on brachial BP. Therefore, PPA could be a better measure of inter-drug comparison in our study because it takes into account both brachial

and central BP. All drugs except bisoprolol increased PPA, which is in accordance with previous evidence (Protogerou *et al.* 2009b). Baseline PPA predicted the BP lowering effects of doxazosin, bisoprolol, and ISMN. However, the results regarding PPA as an underlying haemodynamic alteration cannot be considered independently. This is because it is determined by pulse wave reflections within the arterial tree and by arterial stiffness (Avolio *et al.* 2009).

There is long-term evidence from the REASON study where atenolol and perindopril/indapamide reduced aortic PWV to a similar degree (Asmar *et al.* 2001). The post hoc analysis of the REASON study showed that aortic PWV predicts BP reduction in hypertension (Protogerou *et al.* 2009a). However, in our study aortic PWV corrected for MAP was not changed by any drug and baseline aortic PWV did not predict the reduction of BP by the studied drugs. There may be several possible explanations for this controversy. The REASON study comprised mainly patients with diastolic and systolic hypertension while the patients included into our study can be classified as isolated systolic hypertension patients considering their mean BP levels. It is known that arterial stiffness is a major contributor to elevated BP in patients with isolated systolic hypertension (Franklin *et al.* 1997). Recent short-term data about isolated systolic hypertension patients show that antihypertensive drugs have no effect on aortic PWV and are less effective in reducing BP in patients with increased aortic PWV (Mackenzie *et al.* 2009). It is possible that in our study the period for detection of complete BP reduction and "destiffening" (6 weeks) was inadequate (McEniery *et al.* 2009).

In our study, CO did not predict BP reduction with antihypertensive treatment, which would have been expected with bisoprolol. The failure to show CO as a predictor of BP reduction with the BB in our study may be due to the neutral effect of bisoprolol on CO. The mean baseline CO in our study patients was 6.0 L/min, which is smaller than it was in a similar group of patients (6.4 L/min) (Kim *et al.* 2011) and even in young normotensive subjects (6.9 L/min) (McEniery *et al.* 2005b). It is possible that the compensatory mechanism, i.e. increase in stroke volume (Δ for bisoprolol: + 14.4 mL) mitigated the decrease of CO with bisoprolol. Furthermore, it is possible that in young patients with isolated systolic hypertension, in whom CO is significantly higher (8.1 ± 1.9 L/min) (McEniery *et al.* 2005b), bisoprolol could be more effective in reducing CO; hence CO could determine BP reduction in these patients. The PVR is positively related to MAP and inversely related to CO. While doxazosin reduced MAP similarly to the other drugs it significantly increased CO, which might have contributed to its larger effect on PVR in our study. However, haemodynamic profiling with the use of PVR failed to predict BP reduction with doxazosin.

7. CONCLUSIONS

1. Asymmetric dimethylarginine was independently associated with endothelial function measured by using pulse wave analysis with pharmacological tests and carotid artery intima-media thickness. Our results suggest that asymmetric dimethylarginine is a marker for endothelial dysfunction and intima-media thickening in hypertensive patients.
2. In long-term (one-year) antihypertensive therapy metoprolol succinate and nebivolol had similar effects on brachial blood pressure and arterial stiffness. Only nebivolol reduced significantly central systolic, diastolic, and pulse pressure. In addition, long-term antihypertensive therapy with nebivolol, but not metoprolol succinate, reduced left ventricular posterior wall thickness and left ventricular relative wall thickness. Our results demonstrate that the vasodilating beta-blocker nebivolol is superior to the conventional beta-blocker metoprolol succinate regarding their effects on central blood pressure and reduction of target organ damage.
3. Change in interventricular septal wall thickness correlated more significantly with change in central systolic and pulse pressure than with change in brachial systolic and pulse pressure. Our results indicate that central blood pressure reduction with antihypertensive treatment is more important than brachial blood pressure reduction in reducing target organ damage.
4. In long-term (one-year) antihypertensive treatment both nebivolol and metoprolol succinate reduced the levels of oxidized low-density lipoprotein and soluble intercellular adhesion molecule-1. However, only nebivolol reduced urinary 8-isoprostane levels. Nebivolol reduced oxidized low-density lipoprotein and 8-isoprostanes independently of blood pressure reduction, while metoprolol succinate had blood pressure-dependent effects on oxidized low-density lipoprotein. This finding demonstrates that in the long-term treatment of essential hypertension nebivolol is superior to metoprolol succinate regarding their systemic antioxidant influences.
5. Augmentation index and pulse pressure amplification as underlying haemodynamic alterations determined the reduction of brachial systolic, diastolic, and central systolic blood pressure with antihypertensive treatment. Baseline augmentation index predicted blood pressure reduction for doxazosin and candesartan, and particularly for bisoprolol. Baseline pulse pressure amplification predicted the reduction in blood pressure for doxazosin, bisoprolol, and isosorbide mononitrate. These results suggest that haemodynamic profiling with the use of augmentation index may be especially beneficial in case beta-blockers are considered for treatment of essential hypertension.

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9. SUMMARY IN ESTONIAN

Tsentraalse hemodünaamika, arterite jäikuse ja oksüdatiivse stressi raviaspektid hüpertensiooniga patsientidel

Kõrgenenud arteriaalne vererõhk on oluliseks kardiovaskulaarseks riskiteguriks. Arteriaalne hüpertensioon on püsiv vererõhu tõus ≥140/90 mmHg. Lisaks kõrgenenud vererõhuväärtustele on hüpertensiooniga haigetel oluline hinnata ka teisi kardiovaskulaarseid riskitegureid ning subkliinilist organkahjustust (näit. unearteri sise- ja keskkesta ning vasaku vatsakese seina paksenemist), mis summaarselt tõstavad individuaalse patsiendi kardiovaskulaarset riski ja määravad edasise ravistrateegia. Samas ei selgita mitmed traditsioonilised riskitegurid (suitsetamine, hüperkolesteroleemia jne.) täielikult patsiendi kardiovaskulaarset riski.

Veresoonte sisemine kiht, endoteel, toodab lämmastikoksiidi, mis on organismi võimsaimaks veresooni lõõgastavaks ja antiaterogeenseks molekuliks. Mitmete riskitegurite, hüpertensiooni ja südame-veresoonkonna haiguste puhul on endoteeli funktsioon häirunud. Endoteeli funktsiooni häirumine on veresooneseina kahjustuse varaseim etapp, mis põhjustab ka arterite jäikuse suurenemist ja subkliinilise organkahjustuse teket, mis on oluliselt seotud hüpertensioonihaigete kardiovaskulaarse suremusega. Seega on varajane endoteeli funktsiooni hindamine kliiniliselt olulise tähtsusega. Uudseks endoteeli funktsiooni peegeldavaks biomarkeriks on asümmeetriline dimetüülarginiin, mis on lämmastikoksiidi sünteesi inhibiitor. Lisaks on hüpertensiooni puhul suurenenud oksüdatiivse stressi (sh. oksüdeeritud LDL-kolesterooli ja 8-isoprostaanide) ning subkliinilise põletiku (sh. C-reaktiivse valgu ja rakuvahelise adhesiooni molekul-1) tase, mis põhjustavad veresoonte kahjustust ning on iseseisvalt seotud kardiovaskulaarsete tüsistustega. Seega oksüdatiivse stressi ja subkliinilise põletiku vähendamine omavad hüpertensiooniga patsientidel soodsat toimet.

Mitmed meta-analüüsid on näidanud, et vererõhu langetamine iseenesest vähendab hüpertensiooniga patsiendi kardiovaskulaarset riski. Samas vaatamata heale ravisoostumusele saavutatakse vähem kui 30% ravitavatest hüpertensiooniga patsientidest eesmärkvererõhk (<140/90 mmHg). Hüpertensiooniga patsiente iseloomustab normist kõrvalekalduv hüpertensiooni hemodünaamiline profiil. Mitmed uuringud on näidatud, et patsiendi hüpertensiooni hemodünaamiline profiil määrab vererõhu languse antihüpertensiivse raviga. Seega võib patsientide hüpertensiooni hemodünaamilise profiili määramine viia efektiivsema vererõhu normaliseerumiseni ja vajaolevate antihüpertensiivsete ravimite vähenemiseni.

Tavaliselt mõõdetakse vererõhku õlavarrelt. Samas vererõhk õlavarrearteris (perifeerne arter) erineb oluliselt vererõhust aordis (tsentraalne arter) arterite jäikuse ja pulsilaine peegeldumise tõttu. Tsentraalne vererõhk on perifeersest vererõhust olulisem subkliinilise organkahjustuse tekkes ja kardiovaskulaarse

riski määramisel. On näidatud, et antihüpertensiivsed ravimid eristuvad ka oma toime poolest tsentraalsele vererõhule. Peamised antihüpertensiivsed ravimiklassid langetavad efektiivselt tsentraalset vererõhku. Samas beeta-blokaatorite kohta on vastukäiväid andmeid. Klassikalised beeta-blokaatorid (näiteks atenolool) omavad neutraalset või minimaalset toimet tsentraalsele vererõhule. Atenolooli nõrka toimet tsentraalsele vererõhule seostatakse uuringutega, kus atenolool on tüsistumata essentsiaalse hüpertensiooniga patsientidel vähendanud kardiovaskulaarseid tüsistusi vähem võrreldes teiste antihüpertensiivsete ravimitega. Siiski on lühiaegsetes uuringutes näidatud, et uuemad vasodilateerivad beeta-blokaatorid (näiteks nebivolool) langetavad efektiivselt tsentraalset vererõhku. Puuduvad andmed metoprolool suktsinaadi kohta, mis on Ida- ja Põhja-Euroopas enim kasutatav klassikaline beeta-blokaator.

Uurimistöö eesmärgid

1. Uurida asümmeetrilise dimetüülarginiini, unearteri sise- ja keskkesta paksuse ning endoteeli funktsiooni vahelisi seoseid hüpertensiooniga patsientidel.
2. Hinnata nebivolooli ja metoprolooli pikaaegset toimet tsentraalsele vererõhule, arterite jäikusele ja südame vasaku vatsakese seina paksusele.
3. Hinnata seost tsentraalse vererõhu languse ning südame vasaku vatsakese seina paksuse vähenemise vahel pikaaegse antihüpertensiivse ravi käigus.
4. Võrrelda nebivolooli ja metoprolooli toimet oksüdatiivsele stressile ja põletikule pikaaegsel kasutusel hüpertensiooniga patsientidel.
5. Testida, kas hüpertensiooniga patsiendi algne hüpertensiooni hemodünaamiline profiil määrab alfa-blokaatori (doksasosiin), beeta-blokaatori (bisoprolool), angiotensiin II retseptori blokaatori (kandesartaan) ning nitraadi (isosorbiid mononitraat) antihüpertensiivset efektiivsust.

Patsiendid ja meetodid

Uuringus osales 133 hüpertensiooniga patsienti, keda uuriti 2 etapis. Esimeses etapis viidi läbi paralleelgruppidega topeltpime randomiseeritud aktiivselt kontrollitud ühe keskuse IV faasi uuring. Uuringusse kaasati 80 mees- ning naissoost ravimata kerge kuni mõõduka astmega hüpertensiooniga patsienti vanuses 30–65 aastat. Uuringu peamisteks väljaarvamiskriteerimiteks olid sekundaarne hüpertensioon, diabeet, ülekaal, südame isheemiatõbi, südamepuudulikkus, astma, hüperkolesteroleemia ning teised olulised kaasuvad haigused. Uuritavad randomiseeriti saama nebivolooli 5 mg üks kord päevas või metoproloolsuktsinaati 50 mg üks kord päevas 1 aasta jooksul. Juhul kui õlavarre vererõhu tase püsis uuringu käigus >140/90 mmHg, sai ravimidoosi suurendada (metoprolooli grupis *ad* 100 mg üks kord päevas) ning lisada hüdrokloortiasiidi 12.5–25 mg üks kord päevas (mõlemas grupis). Õlavarre vererõhu, pulsilaine kiiruse, pulsilaine analüüsi ning ehhokardiograafia mõõtmised toimusid vahetult enne randomiseerimist ning poole ja ühe aasta möödudes randomiseerimisest. Oksüdatiivse stressi ja põletiku markerite ning unearterite sise- ja keskkesta paksuse mõõtmised toimusid randomiseerimisel ja pärast 1-aastast ravimikasutust.

Randomiseerimiseelselt teostati uuritavatel lisaks endoteeli funktsiooni mõõtmised. Teises etapis viidi läbi ristuva disainiga topeltpime randomiseeritud platseebo-kontrollitud kahe keskuse IV faasi uuring. Uuriti 53 ravimata hüpertensiooniga patsienti vanuses 18–80 aastat. Uuringu väljalülitamiskriteerimiteks olid õlavarre vererõhk >200/110 mmHg, sekundaarne hüpertensioon, diabeet, astma, südamepuudulikkus ning teised olulised kaasuvad haigused. Patsiendid randomiseeriti saama doksasosiini 4 mg, bisoprololi 5 mg, kandesartaani 16 mg, isosorbiidmononitraati 50 mg ja platseebot üks kord päevas. Iga ravimit ja platseebot tarvitati 6 nädalat, kuni kõik ravimifaasid olid läbitud (kokku 30 nädalat). Uuringu alguses ja pärast iga ravimifaasi teostati uuritavatele õlavarre vererõhu, pulsilaine analüüsi, pulsilaine kiiruse ning mitteinvasiivsed südame minutimahu, löögimahu ning perifeerse vaskulaarse resistentsuse mõõtmised.

Kõik uuringud viidi läbi kella 8–11 vahelisel ajal, pärast üleööpaastu. Õlavarre vererõhk mõõdeti pärast 15-minutilist rahuolekut. Pulsilaine analüüs teostati Sphygmocor Px (Atcor Medical, Austraalia) aparatuuriga. Aplanatsiooni tonomeetriga registreeriti pulsilaine radiaalarterilt ning, kasutades valideeritud ülekandefunktsiooni, tuletati tsentraalse pulsilaine parameetrid (mh. augmentatsiooni indeks, mis iseloomustab pulsilainete tagasipeegeldumist) ning tsentraalse vererõhu väärtused. Pulsirõhu amplifikatsioon, mis iseloomustab arterite jäikust ja pulsilainete tagasipeegeldumist, arvutati õlavarre pulsirõhu jagamisel tsentraalse pulsirõhuga. Endoteeli funktsiooni uurimiseks hinnati 400 μg salbutamooli inhalatsiooni (stimuleerib lämmastikoksiidi vabanemist endoteelist) toimet augmentatsiooni indeksile. Sama aparatuuri kasutati ka pulsilaine leviku kiiruse hindamiseks aordis. Selle mõõtmiseks registreeriti aplanatsioonitonomeetriga pulsilained ühisunearterilt ja -reiearterilt samaaegse elektrokardiogrammi registreerimisega. Pulsilaine tekke ajastuse ja pulsilaine poolt läbitud vahemaa andmete põhjal arvutatati pulsilaine leviku kiirust aordis. Valideeritud Innocor aparatuuri (Innovision A/S, Taani) kasutati mitteinvasiivseks südame minutimahu, löögimahu ning perifeerse vaskulaarse resistentsuse hindamiseks. Ehhokardiograafilised mõõtmised viidi läbi 2 spetsialisti poolt kasutades 3.5-MHz andurit ning ultraheli aparatuuri (Sonos 7500, Philips Medical Systems, Inc., USA). Unearteri sise- ja keskkesta paksuse mõõtmiseks salvestati 12-MHz anduriga sama ultraheli aparaatuuri kasutades mõlemas ühisunearteris eesmised, külgmised ja tagakülgmised projektsioonid. Seejärel toimus hilisem digitaalse videosalvestuse analüüs Ida-Soome Ülikoolis (Kuopio, Soome) Prosound tarkvaraga (Caltech, USA).

Uurimistöö tulemused ja järeldused

1. Asümmetriline dimetüülarginiin oli sõltumatult seotud endoteeli funktsiooniga ning unearteri sise- ja keskkesta paksusega. Antud tulemused näitavad, et asümmeetriline dimetüülarginiin on endoteeli funktsiooni ja unearteri sise- ja keskkesta paksuse marker hüpertensiooniga patsientidel.
2. Metoprolool ja nebivolool langetasid võrdselt õlavarrelt mõõdetud vererõhku ja arterite jäikust pikaaegse antihüpertensiivse ravi käigus. Vaid nebivolool

langetas tsentraalset süstoolset, diastoolset ja pulsirõhku. Nebivolool, aga mitte metoprolool vähendas vasaku vatsakese tagumise seina paksust ja vasaku vatsakese suhtelist seina paksust. Seetõttu on tüsistumata arteriaalse hüpertensiooni ravis otstarbekas eelistada vasodilateerivaid, mitte klassikalisi beeta-blokaatoreid.

3. Vatsakestevahelise seina paksuse vähenemine oli oluliselt tugevamini seotud tsentraalse süstoolse ja pulsirõhu vähenemisega võrreldes õlavarrelt mõõdetud vererõhu vähenemisega pikaajalisel antihüpertensiivsel ravil. Antud tulemus kinnitab, et tsentraalse vererõhu langetamine antihüpertensiivse raviga on olulisem subkliinilise organkahjustuse vähendamisel võrreldes õlavarrelt mõõdetud vererõhuga.
4. Pikaajalisel nebivolooli ja metoprolooli kasutamisel langetasid mõlemad ravimid plasma oksüdeeritud LDL-kolesterooli ning rakuvahelise adhesiooni molekul-1 taset. Samas vaid nebivolool langetas 8-isoprostaanide taset uriinis. Metoprolool langetas oksüdeeritud LDL-kolesterooli sõltuvalt vererõhu langusest. Nebivolooli toime oksüdeeritud LDL-kolesterooli ja 8-isoprostaanide tasemele oli vererõhu langusest sõltumatu. Seega vähendavad vasodilateerivad beeta-blokaatorid lokaalset ja süsteemset oksüdatiivset stressi sõltumatult vererõhu langusest.
5. Hüpertensiooniga patsiendi arterialse süsteemi hemodünaamilistest parameetritest määrasid augmentatsiooni indeks ja pulsirõhu amplifikatsioon õlavarrelt mõõdetud ning tsentraalse vererõhu langust antihüpertensiivse raviga. Augmentatsiooni indeks ennustas vererõhu langust doksasosiini, kandesartaani ja eriti bisoprolooli puhul. Pulsirõhu amplifikatsioon ennustas vererõhu langust doksasosiini, bisoprolooli ja isosorbiid mononitraadi puhul. Seega võib hüpertensiooni hemodünaamilise profiili hindamisest olla enim kasu beeta-blokaatori määramisel hüpertensiooniga patsiendile.

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11. PUBLICATIONS

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Teadustegevus:

Teadustöö põhisuundadeks on hemodünaamika, oksüdatiivse stressi, subkliinilise põletiku ning endoteeli funktsiooni uurimine arteriaalse hüpertensiooni patsientidel ning erinevate antihüpertensiivsete ravimite mõju nendele faktoritele.

Ilmunud on 9 teaduslikku artiklit rahvusvahelistes eelretsenseeritavates ajakirjades. 13 ettekannet rahvusvahelistel teaduslikel konverentsidel.

Euroopa Hüpertensiooni Ühingu liige, Artery Ühingu liige, Eesti Hüpertensiooni Ühingu liige, Eesti Kardioloogide Seltsi liige, Eesti Nooremarstide Ühenduse liige, Eesti Farmakoloogia Seltsi liige.

Artiklid rahvusvahelistes eelretsenseeritavates ajakirjades:

1. Serg M, Kampus P, Kals J, Zagura M, Zilmer M, Zilmer K, Kullisaar T, Eha J. Nebivolol and metoprolol: long-term effects on inflammation and oxidative stress in essential hypertension. Scandinavian Journal of Clinical and Laboratory Investigation 2012;72:427-432.
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