Arterial remodeling and hypertensive damage

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Arterial remodeling and hypertensive damage

Clinical studies in patients with essential hypertension

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Arterial remodeling and hypertensive damage

Clinical studies in patients with essential hypertension

PROEFSCHRIFT

Ter verkrijging van de graad van doctor aan de Universiteit Maastricht,
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Abbreviations and definitions used in this thesis

ANOVA	Analysis of variance
APRC	Activated renin concentration
ARB	Angiotensin receptor blocker
BMI	Body mass index
BSA	Body surface area
CFR	Coronary flow reserve
CI	Cardiac index
CKD	Chronic kidney disease
CO	Cardiac output
CSA	Cross-sectional wall area
CVD	Cardiovascular disease
CWS	Circumferential wall stress
CWT	Circumferential wall tension
DBP	Diastolic blood pressure
DM	Diabetes mellitus
eGFR	estimated glomerular filtration rate
ESRD	end-stage renal disease
FF	Filtration fraction
GEE	Global Estimating Equations
GFR	Glomerular filtration rate
HDL	High-density lipoprotein
HR	Heart rate
ICC	Intra-class coefficient
IMT	Intima media thickness
JNC-7	7th Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure
LD	Lumen diameter
LDL	Low-density lipoprotein
MAP	Mean arterial pressure
PAH	Para-immunohippuric acid
PP	Pulse pressure
PP/SV	Pulse-pressure to stroke volume
PV	Plasma volume

PWV	Pulse-wave velocity
RAAS	Renin-angiotensin-aldosterone system
RF	Renal fraction
RPF	Renal plasma flow
RVR	Renal vascular resistance
SBP	Systolic blood pressure
SNS	Sympathetic nervous system
TG	Triglycerides
TPR	Total peripheral resistance

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Chapter 1

Introduction and outline of this thesis

Hypertension: a pressing health problem

Hypertension is the most important risk factor for cardiovascular diseases (CVD) such as stroke, myocardial infarction, and peripheral artery disease. Combined, these diseases are the leading cause of mortality.[1] In 2010, hypertension was responsible for approximately 9.4 million deaths worldwide (approximately 16.7% of the total of all deaths).[2] In addition to cardiovascular morbidity and mortality, hypertension is also a major independent predictor for the development and progression of chronic kidney disease and is the second most common cause of end-stage renal disease requiring dialysis.[3,4] Worldwide, more than 1 billion people are affected by hypertension, which amounts to a prevalence of 22% globally, 34% in the United States, but even up to 46% in Africa for both sexes combined.[1,5] In the Netherlands, the prevalence of hypertension in individuals aged between 30 and 70 years is 37% for men and 26% for women.[6] Both in the Netherlands and globally, the prevalence of hypertension is rising significantly.[1,6] It is estimated that by 2030, approximately 41.4% of US adults will have hypertension.[1] Considering these numbers, the incidence of cardiovascular disease is expected to rise even more over the coming years, and is therefore becoming an increasingly important public health problem. [7]

Pre-hypertension: a distinct entity or transitional phenotype?

Over the past few decades, hypertension in adults has been defined as a systolic blood pressure (SBP) ≥ 140 mmHg and/or a diastolic blood pressure (DBP) of ≥ 90 mmHg, whereas an optimal blood pressure was defined as a SBP <120 mmHg and DBP < 80 mmHg.[8] However, these cut-off values are fairly arbitrary since starting from a blood pressure of 115/75 mmHg, the risk of death by coronary heart disease and stroke nearly doubles with every 20 mmHg in SBP or 10 mmHg of DBP.[9] Several studies have shown that patients with normal or 'high normal' blood pressure (e.g. a SBP 120 – 139 mmHg and/or a DBP of 80 – 89 mmHg) but without overt hypertension have a higher cardiovascular risk and greater risk of developing sustained hypertension over time than normotensive individuals.[10-12] This blood pressure range was termed pre-hypertension in 2003 by the Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure (JNC7). [13] It has been proposed that pre-hypertension can be regarded as a transitional phenotype between normal blood pressure and hypertension with specific risk factors, hemodynamic and pathophysiological characteristics.[14] However, it remains uncertain whether essential hypertension always follows a gradual trajectory from normal blood pressure via prehypertension to overt hypertension, and ultimately to the development of target organ damage. For instance, studies investigating the progression from pre-hypertension to hypertension report rates varying between 7%

to 38%, depending on the studied population.[12,15-17] This shows that a significant part of pre-hypertensives will not develop overt hypertension. For example, while some people will remain pre-hypertensive for many years, others progress rapidly to advanced stages of hypertension. It has been proposed that these different trajectories can be regarded as distinct hypertension phenotypes that are each associated with a different risk of progression to overt hypertension and CVD.[18,19] There is relatively few data about the pathophysiological mechanisms involved in these different stages or phenotypes of essential hypertension and whether these mechanisms differ from those acting in established hypertension. Therefore, research on the pathophysiological mechanisms underlying the development of hypertension and hypertensive target organ damage remains of great importance, in order to develop novel targets for treatment or preventive strategies.

Arterial remodeling and systemic hemodynamics

Among the numerous pathophysiological pathways involved in the etiology of hypertension, arterial remodeling plays a central role. Arterial remodeling involves both functional and structural changes of large and small arteries as well as changes in luminal or overall vessel diameter. These changes are related to alterations in the composition and thickness of the arterial wall, vascular rarefaction, as well as impaired endothelial function, myogenic tone and vasodilatory responses.[20,21] Overall, it is thought that arterial remodeling in hypertension leads to increased stiffness of conductive and resistance arteries, and combined with an increased peripheral vascular resistance, contributes to amplification of systolic blood pressure and augmentation of the pulse pressure.[22] The ensuing increase in blood pressure pulsatility is detrimental for the structure and function of both the macro- and microvasculature and leads to a vicious cycle, which further contributes to arterial damage and compensatory remodeling.[22] Although many studies have identified several mechanisms regarding arterial remodeling in association with hypertension, less is known about the role of these mechanisms in various phases in the development of hypertension over time. Also, it is not fully clear to what extent vascular changes occur in response to an elevated blood pressure and even whether remodeling is a causal factor in the rise of blood pressure. Longitudinal studies in selected patient populations are therefore of paramount importance.

Renal hemodynamics in hypertension

It is well-known that the kidneys play a crucial role in the pathogenesis of essential hypertension. On the one hand, they are important for the regulation of the extracellular fluid volume by adjusting the excretion of salt and water, and for the regulation of the

renin-angiotensin aldosterone system (RAAS). Moreover, the kidney is an important regulator of several metabolic and endocrine processes and these indirectly also affect blood pressure. On the other hand, the kidneys are very susceptible to the damaging effects of high blood pressure. Hypertensive target organ damage occurs frequently and is clinically characterized by (micro-)albuminuria and, in a later stage, a decline in glomerular filtration rate.[23]

The kidneys, like the brain, are subjected to a constant high-flow perfusion and have a relatively low vascular resistance.[24] As a consequence, the blood pressure induced pulsations of the aorta are transmitted to the level of the renal microcirculation. [24] It has been proposed that the increased blood pressure pulsatility, associated with arterial stiffening, is damaging to the renal arterioles and microcirculation.[24] Extensive research on the systemic and renal hemodynamic aspects of hypertension has been performed in the last decades of the 20th century, using clearance techniques to evaluate renal plasma and blood flow. Generally, these studies have shown that in patients with hypertension, renal perfusion (i.e. renal plasma flow [RPF]) is decreased in comparison to normotensive individuals while glomerular filtration rate (GFR) is maintained.[25,26] This means that the filtration fraction (i.e. the quotient of GFR and RPF) is increased in hypertension, reflecting increased postglomerular vascular resistance. Although these pathophysiological features have been well described for established hypertension, only limited studies are available with respect to the early phases of hypertension such as pre-hypertension.[27,28]

Outline of this thesis

In this thesis we aimed to answer several questions with regard to arterial remodeling, systemic and renal hemodynamics as well as its association with renal target organ damage in patients with established hypertension and participants with prehypertension.

As described previously, the processes involved in arterial remodeling are complex and often share multiple common pathways. Therefore, in **chapter 2** we first aimed to provide an overview of several important pathophysiological mechanisms and processes that are involved in arterial remodeling.

As described in chapter 2, hypertensive remodeling commonly results in changes in arterial wall thickness and arterial diameter of muscular arteries like the carotid artery. These changes are thought to reflect a compensatory mechanism to reduce mechanical stress of the vessel wall, caused by increased blood pressure. When this compensation is successful, arterial remodeling can be termed adaptive. [22] However, maladaptive arterial remodeling can also occur, in which the changes to the vessel wall fail to reduce wall tension and stress.[22] There is limited data on whether carotid remodeling in hypertension is adaptive or maladaptive. Therefore, in **chapter 3** we compared several ultrasonographic indicators of arterial

remodeling of the common carotid artery between normotensives and hypertensives. We hypothesized that hypertension is characterized by outward, hypertrophic maladaptive carotid remodeling as summarized by decreased lumen diameter (LD), increased intima-media thickness (IMT), increased cross-sectional area (CSA) and elevated circumferential wall tension (CWT) whereas in normotensives, we hypothesized that although IMT and CSA may be increased, CWT is relatively low, reflecting a pattern of adaptive arterial remodeling. Furthermore, we evaluated the changes in these markers over time.

Next, we explore the association between hypertensive arterial remodeling and the kidney. In **chapter 4** we aimed to assess whether arterial remodeling mediates the development of renal damage in hypertension by studying whether increased arterial stiffness is associated with changes in glomerular filtration rate over time in patients from a general practice with essential hypertension. We hypothesized that patients with higher levels of carotid-femoral pulse-wave velocity (cfPWV) at baseline have higher rates of annual decline in estimated glomerular filtration rate (eGFR), independent of blood pressure. Furthermore, since age is a common determinant of both cfPWV and eGFR, we also tested whether the effect of cfPWV on eGFR-change is different for patients of different age.

As mentioned previously, the specific systemic and renal characteristics in prehypertension and whether or not they are different from those in hypertension, are not fully understood. Therefore, in **chapter 5** we first review available data on hemodynamics in different stages of the hypertensive spectrum such as borderline hypertension, prehypertension and established hypertension. Since the kidney plays such an important role in hypertension, we specifically aimed to review data on (regional) renal hemodynamics between the various stages of essential hypertension. In **chapter 6** we aimed to assess in a historical cohort whether there are differences in systemic and renal hemodynamics between young participants with either normal blood pressure, prehypertension, or hypertension. We hypothesized that in prehypertensive individuals, total peripheral resistance and arterial stiffness are higher than in normotensives, but still lower than in hypertensives. Additionally we hypothesized that compared to normotensives, prehypertensive individuals have a higher renal filtration fraction (reflecting increased vascular resistance), increased renal vascular resistance, and increased renal perfusion.

Finally, we will discuss the main findings of this thesis in **chapter 7** and provide some perspective on possible future studies in this line of research on the pathophysiology of essential hypertension, arterial remodeling, and hemodynamics.

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Chapter 2

Mechanisms of arterial remodeling: lessons from genetic diseases

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Abstract

Vascular disease is still the leading cause of morbidity and mortality in the Western world, and the primary cause of myocardial infarction, stroke, and ischemia. The biology of vascular disease is complex and still poorly understood in terms of causes and consequences. Vascular function is determined by structural and functional properties of the arterial vascular wall. Arterial stiffness, that is a pathological alteration of the vascular wall, ultimately results in target-organ damage and increased mortality. Arterial remodeling is accelerated under conditions that adversely affect the balance between arterial function and structure such as hypertension, atherosclerosis, diabetes mellitus, chronic kidney disease, inflammatory disease, lifestyle aspects (smoking), drugs (vitamin K antagonists), and genetic abnormalities [e.g., pseudoxanthoma elasticum (PXE), Marfan's disease]. The aim of this review is to provide an overview of the complex mechanisms and different factors that underlie arterial remodeling, learning from single gene defect diseases like PXE, and PXE-like, Marfan's disease and Keutel syndrome in vascular remodeling.

Introduction

Arterial remodeling refers to the myriad of structural and functional changes of the vascular wall that occur in response to disease, injury, or aging. Although arterial remodeling can be regarded as a mechanism that naturally occurs with aging, early arterial remodeling is associated with significant hemodynamic changes and cardiovascular morbidity and mortality. Arterial remodeling is set into motion by a variety of complex pathophysiological mechanisms that are closely interrelated, and that influence both the cellular and non-cellular components of the vascular wall. Mechanisms involved in arterial remodeling include fibrosis, hyperplasia of the arterial intima and media, changes in vascular collagen and elastin, endothelial dysfunction, and arterial calcification. Migration and proliferation of vascular smooth muscle cells (VSMCs) contribute to thickening of the arterial intima. Differentiation of VSMCs from their contractile to a secretory or osteogenic phenotype may lead to increased vascular tone, and promotes extracellular matrix (ECM) calcification. Additionally, alterations in the activity of vitamin K-dependent proteins may affect the progression of vascular remodeling, including the induction of calcification. Because of this complexity, it is difficult to study to what extent a single mechanism contributes to arterial remodeling. Monogenetic diseases such as pseudoxanthoma elasticum (PXE), PXE-like syndrome, Marfan's syndrome or Keutel syndrome are characterized by a clinical phenotype that is similar to that of arterial remodeling, but are caused by a specific defect that affects only one or several pathophysiological mechanisms of arterial remodeling. Lessons learned from these relatively rare diseases may therefore ultimately provide insight in more common, multifactorial cardiovascular diseases such as hypertension, diabetes mellitus, and chronic kidney disease as well as in normal vascular aging.

General features of arterial remodeling

Arterial remodeling is thought to reflect adaptation of the vessel wall to mechanical and hemodynamic stimuli (Nichols and O'Rourke, 2005). Arterial remodeling is characterized by alterations in the structure and function of the vascular wall and can be divided into atherosclerosis and arteriosclerosis. Whereas atherosclerosis is characterized by a focal inflammatory process in the intima initiated by accumulation of lipids in plaques, arteriosclerosis is a more diffusely localized alteration of the medial arterial vascular wall (Libby, 2002). Arteriosclerosis is associated with aging and generalized cardiovascular, metabolic, or inflammatory disease. Macroscopically, different types of arterial remodeling can be distinguished, depending on the type and localization of the vessel (**Figure 2.1**) (Mulvany et al., 1996). Arterial remodeling can be either inward or outward and can be hypertrophic (thickening of the vascular wall), eutrophic (constant wall thickness), or hypotrophic (thinning of the vascular

Figure 2.1 Types of vascular remodeling

wall) (Mulvany et al., 1996).

Changes observed in arterial remodeling are mainly seen in large central elastic arteries. They are characterized by increased vessel diameter and thickened intimal and medial layers of the vascular wall (outward hypertrophic remodeling) (O'Rourke and Hashimoto, 2007). On the other hand, remodeling of muscular peripheral vessels is more often inwardly eutrophic or hypertrophic, probably reflecting sustained vasoconstriction of vessels (Mulvany, 2008).

Thickening of the arterial wall is caused by intimal hyperplasia, medial hypertrophy and hyperplasia of VSMCs, and deposition of ECM material including minerals (Virmani et al., 1991; Safar et al., 1998; Schwartz et al., 2000). The normal

composition and lay-out of ECM of the vascular wall is disrupted in arterial remodeling. In the media of the normal arterial wall, elastic fibers are arranged in parallel, concentric, fenestrated layers, alternating with layers of VSMCs anchored to the elastic fibers and structural fibers by glycoproteins and integrins (Dingemans et al., 2000; Nichols and O'Rourke, 2005). These structures, termed elastic lamellae, enable the vessel to expand and buffer the systolic blood pressure pulse, while simultaneously maintaining structural stability. Elastic fibers provide passive elastic buffering, whereas VSMCs dynamically redistribute tensile stress across fibers due to their ability to contract and relax (Rachev and Hayashi, 1999). With arterial remodeling the layered architecture of elastic lamellae is lost as they become progressively fragmented and fibrotic (Farand et al., 2007). At higher levels of blood pressure, vessels dilate which results in increased tensile stress on the vascular wall, in accordance with LaPlace's Law of circumferential wall tension (Nichols and O'Rourke, 2005). Thickening of the arterial wall occurring with arterial remodeling reduces tensile stress. VSMCs of adults do not synthesize new elastin but mainly non-elastic collagen resulting in stiffening of the vascular wall (Greenwald, 2007). Closely related to the degradation of ECM, the deposition of calcium minerals further contributes to stiffening and remodeling of vascular tissue (Blaha et al., 2009; Sekikawa et al., 2012).

In addition to structural changes, endothelial function plays an important role in arterial remodeling. Blood flow and shear stress stimulate endothelial cells to produce nitric oxide (NO), which in turn influences contraction and relaxation of VSMCs. Endothelial function decreases with age and endothelial dysfunction is common in many cardiovascular diseases. Moreover, in response to pathological conditions, such as altered shear stress or inflammation, endothelial cells produce cytokines and growth factors that influence the homeostasis of the vascular wall (Csiszar et al., 2009; Urschel et al., 2012). Endothelial cells produce transforming growth factor-beta (TGF- β) and bone morphogenetic proteins (BMPs) which stimulate VSMCs and vascular pericytes to proliferate, to differentiate and to deposit ECM matrix (discussed in more detail below) (Simionescu et al., 2005; Boström et al., 2011).

Pathogenesis of arterial remodeling

Arterial remodeling is driven by numerous, highly regulated and interrelated processes. Processes that are of particular importance as they are central in arterial remodeling include: (1) VSMC proliferation and differentiation, (2) degradation and fracture of elastin fibers, and (3) calcification and deposition of ECM material (**Figure 2.2**). Genetic diseases with a phenotype resembling vascular disease all affect one or several of these key processes and may thus provide more insight in the mechanisms of vascular disease (**Figure 2.3**).

Figure 2.2 Pathophysiological mechanisms of arterial remodeling.

Pathophysiological mechanisms of arterial remodeling. Cross sectional schematic view of the arterial wall. **(A)** Normal situation. **(B)** Arterial remodeling. Arterial remodeling is characterized by thickening of the wall. Elastic fiber degradation, extracellular matrix calcification and collagen deposition lead to adaptation of the vascular wall. Abbreviations: TGF-β, transforming growth factor-beta; IL-1, interleukin 1; MMP, matrix metalloproteinases; VSMC, vascular smooth muscle cell.

Vascular smooth muscle cell proliferation and differentiation

VSMCs are key regulators of vascular tone and health and insight into their function is of utmost importance for our understanding of the causes of arterial remodeling. In normal arteries, VSMCs in the tunica media regulate vessel tone and diameter in order to maintain hemodynamic balance (Alexander and Owens, 2012).

To fulfill this regulatory function, VSMCs need to have a contractile phenotype. Contractile VSMCs are characterized by a number of phenotype-specific marker proteins such as smooth muscle 22-alpha (SM22 α), alpha-smooth muscle actin (α SMA), and smoothelin (Iyemere et al., 2006; Eys et al., 2007). Although the majority of VSMCs in the vascular wall display a contractile phenotype, studies have shown that a specific subset of medial VSMCs has the ability to differentiate into a synthetic phenotype which can be further subdivided into a migratory-proliferative phenotype, a secretory phenotype or an osteogenic phenotype (Gerthoffer, 2007). Phenotypic flexibility of VSMCs is necessary to deal with the varying conditions of vascular tissue. Stress signals switch gene expression that will modulate VSMC phenotype to adapt. This process of differentiation is termed phenotype switching and is considered to be a key mechanism in arterial remodeling (Iyemere et al., 2006; Alexander and Owens, 2012). Phenotype switching occurs in response to vascular injury or stress and is characterized by reduced expression of genes which are specific for contractile VSMCs and cellular morphology (Alexander and Owens, 2012).

Although the precise mechanisms are still not fully understood, many different stimuli have been identified, some of which are summarized in Table 2.1 (Alexander and Owens, 2012). Migratory stimuli, for instance, alter the cytoskeleton of VSMCs. As a consequence, cell adhesion molecules are detached from the ECM and surrounding vascular cells. Lamellipodia protrude from the leading edge of the cell due to actin polymerization, enabling it to move through the ECM toward a chemotactic stimulus (Willis et al., 2004). This migration contributes to intimal VSMC proliferation and hyperplasia, which is an important cause of arterial wall thickening. Synthetic VSMCs produce elastolytic enzymes (matrix metalloproteinases; MMPs), which facilitate migration by detaching cells from the basement membrane and ECM. Indeed, upregulation of MMPs coincides with the migration of VSMCs (Willis et al., 2004). A genetic disorder that is associated with VSMC phenotype switching is Marfan's disease. It is characterized by abnormal synthesis and function of elastic fibers (Kielty, 2006). Patients with Marfan's disease suffer from abnormal growth, skeletal disorders, ocular problems and increased tendency to develop aneurysms. The gene defect underlying Marfan's disease is a mutation of the fibrillin-1 (FBN-1) gene, which encodes the glycoprotein FBN-1. FBN-1 is essential for maintaining structural stability of elastic fibers, as well as attaching VSMCs to the elastic fibers (Bunton et al., 2001). Because of defective synthesis, elastic fibers are prone to early mechanical fragmentation and therefore disruption of elastic laminae. However, additional studies on the pathophysiological mechanisms in Marfan's disease showed that, preceding elastic

Figure 2.3 Pathophysiological pathways leading to arterial remodeling in genetic and cardiovascular disease.

Abbreviations: PXE, pseudoxanthoma elasticum; MMP, matrix metalloproteinases; VSMC, vascular smooth muscle cell; GGCK, gamma glutamyl transferase.

fiber degradation, impaired binding of VSMCs-induced differentiation into a synthetic proteolytic phenotype (Galis et al., 1994; Bunton et al., 2001; Galis and Khatri, 2002). The resulting production of MMPs damages the already weakened vascular wall (Pratt and Curci, 2010). These patho-mechanistic changes in Marfan's disease help to understand underlying mechanisms leading to general vascular disease.

Indeed, Goodall et al. showed that VSMCs from inferior mesenteric veins of patients with aortic aneurysms display increased MMP-2 production and an increased number of migratory VSMCs (Goodall et al., 2002). Bendeck et al. demonstrated that inhibition of MMP activity inhibited VSMC migration in rats (Bendeck et al., 1996). Moreover, VSMCs are important for atherosclerotic plaque stability. VSMCs and myofibroblasts in the fibrous cap provide stability to atherosclerotic plaques if they deposit collagen. On the contrary, if a significant part of these VSMCs display a proteolytic phenotype, degradation of fibrous cap material may facilitate plaque rupture (Johnson, 2007). Therefore, the role of VSMCs in maintaining atherosclerotic plaque stability largely depends on VSMC phenotype, stressing out the importance to find therapeutic agents that are able to modify the VSMC phenotype (Orr et al., 2010).

Osteogenic VSMC phenotype

Under specific stimuli such as sustained high extracellular levels of calcium and phosphate or in the absence of inhibitors of calcification, VSMCs can differentiate into an osteogenic phenotype in which VSMCs acquire features usually observed in chondrocytes and osteoblasts (Shanahan et al., 1994; Iyemere et al., 2006). Osteogenic

Table 2.1 Stimuli for vascular smooth muscle phenotype switching

Inflammation
Oxidative stress
Hemodynamic shear stress
Mechanical stretch
Advanced glycation end products (AGE)
Increased calcium-phosphate product
SYSTEMIC HORMONAL
Angiotensin II (Ang II)
Aldosterone
PARACRINE STIMULI
Transforming growth factor- β (TGF- β)
Fibroblast growth factor (FGF)
Endothelial growth factor (EGF)
Platelet derived growth factor (PDGF)
Matrix metalloproteinases (MMP)

VSMCs are characterized by down regulation of mineralization inhibitory proteins, upregulation of alkaline phosphatase and release of matrix vesicles (MVs) (Shanahan et

al., 2011). In vitro, culturing VSMCs with elevated phosphate concentrations results in up-regulation of osteogenic markers (Runx2, osterix, and alkaline phosphatase) and down-regulation of VSMC lineage markers (SMA actin, SM22a) (Shanahan et al., 2011).

Downstream, bone morphogenetic protein-2 (BMP-2) induces an osteogenic differentiation of VSMCs. BMP-2 has been shown to be expressed in human atherosclerotic lesions (Boström et al., 1993). The phenotypic switch of VSMCs to chondrocyte- and osteoblast-like cells by BMP-2 is limited by calcification inhibitory proteins such as matrix Gla-protein (MGP). In MGP knock-out mice, the absence of MGP results in heavily calcified elastic fibers, and loss of VSMCs which are differentiated into chondrocytic VSMCs (Luo et al., 1997). Additionally, MGP deficiency in VSMCs results in decreased smooth muscle markers which is accompanied by an up-regulated expression of the bone-specific transcription factor cbf1a/Runx2 and the osteogenic protein osteopontin (Speer et al., 2002).

The ability of MGP to keep VSMCs in the contractile phenotype may be accomplished by binding BMP-2 (Wallin et al., 2000; Zebboudj et al., 2003). Tanimura and co-workers were the first to report an association between small membrane encapsulated particles, MVs, and vascular calcification (Tanimura et al., 1983). Vesicular structures have been found in both intimal and medial layers and were likely derived from VSMCs (Kim, 1976; Bennett et al., 1995; Hsu and Camacho, 1999). The release of vesicle bodies from VSMCs was first described as a rescue mechanism against calcium overload trying to prevent apoptosis of VSMCs (Fleckenstein-Grün et al., 1992). VSMC-derived MVs have been identified in human arteries in association with atherosclerosis and hypertension (Kim, 1976; Kockx et al., 1998). In vitro, MV from VSMCs form the nidus for calcification (Shanahan et al., 1999).

Degradation and fracture of elastin fibers

Elastic fibers consist of polymers of tropoelastin cross-linked to fibrillin-rich microfibrils. In the vasculature, elastin is mainly produced during the fetal and neonatal period by (secretory) VSMCs. Above we discussed the importance of elastin for maintaining arterial wall stability and VSMC homeostasis in Marfan's Disease. Additionally, elastin is also an important nidus for calcification. This is illustrated in PXE disease and its accompanying clinical features. PXE is characterized by extensive calcification that mainly occurs along elastic fibers. Although cutaneous manifestations are primarily of cosmetic concern, presence of characteristic skin lesions signifies risk for development of vascular calcification with considerable morbidity and occasional early mortality (Uitto et al., 2010).

Even in the absence of diseases which directly affect elastin structure and function, similar processes can be observed in vascular aging and aortic stiffening

(Smith et al., 2012). The question remains, what causes disruption of elastic fibers associated with aging? Initially, it was hypothesized that elastin degradation was predominantly the result of material fatigue caused by cyclic stretching of elastic fibers with every heart beat (O'Rourke, 1976; Nichols and O'Rourke, 2005). Diseases such as (systolic) hypertension would accelerate this process, since increased pulse pressure (PP) exerts greater tensile stress on the vascular wall and increased stretch on fibers. In support of this hypothesis, structural alterations in elastin have been demonstrated to be inversely associated with total number of heart beat cycles in vitro (Avolio et al., 1998). However, there are no in vivo studies supporting mechanical fragmentation of elastin.

Calcification and deposition of ECM material

Both VSMC phenotype switching and ECM degradation result in enhanced and accelerated vascular calcification. Initially, vascular calcification was regarded as passive mineral deposition. However, this view has been abandoned since overwhelming evidence exists that vascular calcification actually is a highly regulated process. Soft tissue calcification is thought to result from an imbalance between calcification-promoting and -inhibiting factors (**Table 2.2**). Calcification is the hallmark of patients with genetic diseases like Keutel's syndrome, PXE, and PXE-like syndrome (Ziereisen et al., 1993; Munroe et al., 1999; Vanakker et al., 2007; Rutsch et al.,

Table 2.2 Calcification regulating factors

FACTORS PROMOTING CALCIFICATION

Bone morphogenetic protein2 (BMP-2)
 ↑ Calcium-phosphate product
 Tumor Necrosis Factor α (TNF- α)
 Interleukin 6 (IL-6)
 Receptor activator of nuclear factor κ B (RANK) ligand (RANKL)
 Insulin-like growth factor I (IGF-I)
 Insulin
 ↑ Glucose
 ↑ Parathyroidhormone
 Matrix metalloproteinases (MMP)
 Elastin degradation
 Hydroxyapatite crystals

FACTORS INHIBITING CALCIFICATION

Fetuin-A
 Matrix gla protein (MGP)
 Osteoprotegerin (OPG)

2011). Keutel's syndrome is caused by a mutation in the gene encoding MGP, which is considered to be the most important inhibitor of vascular calcification. MGP is a 14 kD protein which requires vitamin K-dependent carboxylation to become biologically active. Clinically, lessons learned from the mechanisms underlying Keutel's disease can help understanding vitamin K-antagonist-induced vascular calcifications (discussed below) (Rennenberg et al., 2010; Weijs et al., 2011; Schurgers et al., 2012). In PXE, the underlying genetic defect is a loss-of-function mutation of the *abcc6* gene. This gene encodes a transmembrane transporter protein (Multi Drug Resistant Protein 6; MDRP-6). The substrate of the MDRP-6 is not known, and the exact mechanisms by which this mutation leads to elastin calcification are not yet fully understood. Recent studies have pointed toward calcification being stimulated by phenotype switching of VSMCs, oxidative stress, and interference with carboxylation of MGP (Pasquali-Ronchetti et al., 2006; Garcia-Fernandez et al., 2008; Boraldi et al., 2009; Li et al., 2009b; Rutsch et al., 2011). Similarly, in PXE-like syndrome a mutation in the γ -glutamylcarboxylase (GGCX) gene causes elastic fiber calcification as is observed in vitamin K-antagonist-induced vascular calcification (Gheduzzi et al., 2007; Vanakker et al., 2007; Rennenberg et al., 2010; Weijs et al., 2011; Schurgers et al., 2012). The GGCX mutation is associated with increased bleeding tendency due to impairment of vitamin K-dependent coagulation factors (Vanakker et al., 2007; Li et al., 2009a). This has led to the concept that vitamin K-dependent proteins are of importance in inhibiting vascular elastin calcification. The GGCX mutation results in decreased activity of MGP and subsequently an impaired inhibitory potential for calcification, similar to the situation in eutel's syndrome in which MGP is absent (Schurgers et al., 2008; Vanakker et al., 2010). In a similar manner, treatment with vitamin K-antagonists may also induce an increased tendency for calcification (**Figure 2.2**) (Price et al., 1998; Schurgers et al., 2007; Rennenberg et al., 2010; Chatrou et al., 2012). Since vitamin K-antagonists work by inhibiting the Vitamin K cycle and by reducing carboxylation of MGP, these findings confirm the important central role of MGP in the regulation of calcification. Therefore, it is highly probable that in these diseases, MGP also plays an important regulatory role in calcification (Shanahan et al., 1999; Schurgers et al., 2007).

Clinical aspects of arterial remodeling

Since the normal function of vessels is to maintain adequate perfusion of organs and tissues and to buffer oscillating blood pressures, arterial remodeling results in changes in this function. At first, these are compensatory (i.e., reducing wall tension). However, in later stages these compensatory mechanisms become detrimental and initiate a vicious cycle of pathophysiological aberrations.

Arterial remodeling, arterial stiffness and damaging hemodynamics

Fragmentation of the elastic lamina, hyperplasia and hypertrophy of VSMC, loss of contractility of VSMC, deposition of collagen, and arterial calcification lead to stiffening of arteries. Many studies have shown that arterial stiffness, which is clinically measured as the carotid-femoral pulse wave velocity (cfPWV), is independently associated with cardiovascular risk and mortality (Laurent et al., 2001, 2012; Mitchell et al., 2010; Vlachopoulos et al., 2010). In addition, arterial stiffness is independently associated with, and predictive of target organ damage of the heart, kidneys, and brain (Laurent and Boutouyrie, 2005). Arterial stiffness reflects the degree of remodeling in large arteries and is used as a parameter for cardiovascular risk stratification next to traditional cardiovascular risk factors (Nurnberger et al., 2002). The mechanism linking arterial stiffness to an adverse outcome is thought to involve a pathological hemodynamic profile in large, central arteries such as the aorta (Mitchell, 2009). This pathological hemodynamic pattern consists of an increased systolic blood pressure (SBP; i.e., systolic hypertension) and decreased diastolic blood pressure (DBP) resulting in an increased PP. The pressure waveform in the aorta is composed of a forward traveling wave generated by contraction of the left ventricle of the heart, and a backwards traveling wave generated by reflection from peripheral arteries (**Figure 2.4 A**) This reflected wave is generated at vascular bifurcations and at sites where the elastic conduit arteries transition into muscular resistance arteries (Mitchell, 2004). At this site the difference in impedance of the vascular wall causes the forward traveling

Figure 2.4 Hemodynamic changes in arterial stiffening

(A) Aortic blood pressure waveform of a healthy, normotensive person. The forwards traveling wave precedes the (backwards traveling) reflected wave. (B) Aortic pressure wave form of a person with arterial stiffness. Due to increased pulse wave velocity, the forward traveling wave and reflected wave are summated leading to augmented pulse pressure.

wave to be reflected. The shape of the aortic pressure waveform is largely determined by timing and speed with which the pulse wave propagates through the arteries. With arterial stiffening the speed of both the forward and backward traveling wave is increased. Remodeling of arteries causes an earlier wave reflection. As a result of different timing of both waves, the forward traveling wave and the reflected wave are summated, leading to an augmented systolic peak and a relatively low DBP (**Figure 2.4 B**), generating a highly pulsatile flow in aorta and branching arteries. It is this blood pressure pulsatility that is thought to have damaging effects on sensitive target organs as well as on vascular function, and to contribute to the vicious cycle of arterial remodeling.

High blood pressure pulsatility leads to increased mechanical vascular wall stress. With high central PP, the amplitude in which the arterial wall expands and contracts with each consecutive heartbeat is increased. This leads to higher stretch on elastic and collagen fibers in the arterial wall and this in turn may contribute to material fatigue, fracture, and degradation. Additionally, cyclic stretching of VSMC has been demonstrated to stimulate phenotype switching and arterial remodeling (Williams, 1998). Secondly, pathological blood pressure pulsatility adversely affects endothelial function since structure and function of the endothelium are modulated by hemodynamic forces (Gimbrone and García-Cardena, 2012). In hypertensive patients, a high pulse-pressure is associated with endothelial dysfunction, which can be measured as the vasodilator response to acetylcholine (Ceravolo et al., 2003). In the normal situation, a laminar blood flow pattern and cyclic shear stress maintain proper endothelial function such as: NO-mediated regulation of vascular tone, maintaining a non-thrombotic and non-inflammatory state, preserving ECM metabolism, and regulating vascular permeability (Vita and Mitchell, 2003; Gimbrone and García-Cardena, 2012). In arteries with remodeling, blood flow becomes increasingly oscillatory with peaked systolic flows as well as stasis and even flow reversal during diastole (Domanski et al., 1999; Mitchell, 2004). The ensuing turbulent flow and locally altered shear stress patterns cause endothelial dysfunction, which is characterized by impaired NO synthesis and upregulation of pro-inflammatory and pro-atherogenic factors, increased oxidative stress, as well as vasoconstriction (Keulenaer et al., 1998; Blackman et al., 2002; Gimbrone and García-Cardena, 2012). In addition, altered flow and increased pressure pulsatility have been shown to activate the endothelium and induce production of osteogenic factors such as BMP-2 and BMP-4 (Qiu and Tarbell, 2000; Sorescu et al., 2003; Boström et al., 2011). Indeed, BMP-2 transgenic apoE^{-/-} mice display increased calcification of atheromatous lesions, whereas MGP transgenic apoE^{-/-} mice have less atherosclerotic mineralization, suggesting a key role for MGP in suppressing BMP-2-induced vascular mineralization (Nakagawa et al., 2010; Yao et al., 2010). Arterial stiffness and endothelial function not only stimulate the development of atherosclerotic plaques but also further promotes arterial media remodeling. In

this way, arterial stiffness may explain the interrelationship of arteriosclerosis and atherosclerosis.

Finally, the pathological hemodynamic patterns due to arterial stiffness lead to damage of susceptible organs such as kidneys, brain, and heart. It has been established that arterial stiffness and chronic kidney disease are closely interrelated (Safar et al., 2004). Patients with primary kidney disease have accelerated arterial remodeling and calcification due to altered homeostasis of calcium and phosphate, high degrees of inflammation and oxidative stress, uremia, altered cholesterol metabolism, and an activated renin-angiotensin system (RAAS) (Safar et al., 2004). Conversely, increased arterial stiffness and pressure pulsatility induce renal damage (Verhave et al., 2005; Ford et al., 2010; Briet et al., 2011; Chen et al., 2011). Blood pressure pulsatility has been put forward to be able to cause renal damage. Although kidneys are normally protected against high blood pressure by an effective autoregulation, abnormal blood pressure pulsatility has been shown to blunt the renal myogenic response (Bidani and Griffin, 2004; Bidani et al., 2009; Hultström, 2012), exposing the vulnerable glomerular microcirculation to damaging pressure oscillations (Safar et al., 2012).

Calcification as cardiovascular risk factor and possible therapeutic target

In PXE, PXE-like syndrome as well as in Keutel's syndrome, arterial calcification is an important feature of the clinical phenotype. Besides these, arterial calcification is also observed in more common disorders such as diabetes, hyperparathyroidism, and chronic kidney disease as well as in vascular aging. In addition, vascular calcification may be induced by drugs that adversely affect the regulatory balance between factors inducing or inhibiting calcification. For instance, chronic treatment with vitamin K antagonists (such as warfarin) is associated with peripheral artery calcification (Rennenberg et al., 2010). Calcification occurs in both arteriosclerosis and atherosclerosis. Aortic medial calcification has been demonstrated to contribute to arterial stiffness in different populations (Odink et al., 2008; Cecelja et al., 2011; Sekikawa et al., 2012). Moreover, the presence of aortic calcification is predictive of coronary artery disease (Jang et al., 2012). Calcification of coronary arteries predominantly reflects atherosclerosis and can be measured and quantified by computed tomography (CT) using the calcium-score. The calcium score (expressed as Agatston units) has been used as a sensitive tool for risk stratification and decisionmaking regarding coronary revascularization and diagnostic angiography. A negative calcium score indicates that the presence of atherosclerotic plaque is very unlikely, whereas a high calcium score is associated with significant cardiovascular risk (Budoff et al., 2006). The importance of calcification with respect to cardiovascular outcome is further stressed by the fact that rapid annual progression of the calcium score is independently associated with

outcome (Raggi et al., 2004). For this reason, the calcification process may become an important therapeutic target. The challenge is that an intervention should be aimed at a modifiable factor in the pathophysiological process. As can be learned from PXE, PXE-like syndrome and Keutel's syndrome, MGP and the vitamin K cycle are among the most important known regulators of calcification and VSMC phenotype switching. As described above, MGP requires vitamin K mediated carboxylation to be biologically active. Therefore, treatment with vitamin K would theoretically inhibit or possibly reverse arterial calcification and slow down the development of arterial stiffness. Indeed, our group demonstrated that calcification could be reversed in rats that had extensive calcification due to warfarin treatment, by subsequently administering vitamin K (Schurgers et al., 2007). In humans, the 3-year daily supplementation of 500 mcg vitamin K on top of a multi-vitamin resulted in hold on progression of vascular calcification (Shea et al., 2009). In the observational Rotterdam study, high dietary intake of vitamin K was associated with better cardiovascular outcome and reduced coronary artery calcification (Geleijnse et al., 2004; Gast et al., 2009). Also, in post-menopausal women, treatment with vitamin K resulted in improved markers of vascular stiffness (Braam et al., 2003). Furthermore, a recent study by Westenfeld et al. showed that vitamin K2 supplementation reduced plasma levels of inactive, undercarboxylated MGP (Westenfeld et al., 2012). Since vitamin K has no reported adverse side effects, it might be a promising treatment for calcification. Clinical trials investigating the effects of vitamin K supplementation on calcification and arterial remodeling are currently in progress.

Arterial remodeling as potential therapeutic target

In addition to calcification, other pathophysiological pathways of arterial remodeling such as arterial stiffening, fibrosis, or elastin degradation may also be potential candidates for intervention. However, finding suitable, modifiable candidates has proven to be a challenge. Although most existing antihypertensive drugs may reduce arterial stiffness to some extent, it is difficult to determine whether this effect is mainly due to blood pressure reduction or represents a true effect on ECM remodeling (Boutouyrie et al., 2011). Since the RAAS plays an important pro-fibrotic role in arterial remodeling it has been suggested that beneficial effects of RAAS antagonists are (partly)

due to their anti-fibrotic action, independent of their effects on blood pressure. Indeed, Tropeano et al. showed that treatment with 8 mg perindopril was associated with lower carotid stiffness independently of the effects on blood pressure, whereas a dose of 4 mg did not have such an effect (Tropeano et al., 2006). Similar blood-pressure-independent de-stiffening effects have been reported for selective aldosterone antagonists such as plerenone (White et al., 2003), supporting possible effects of RAAS system inhibition

on ECM remodeling. Especially in diabetes, advanced glycation endproducts (AGE) contribute to arterial stiffness by creating cross-links between elastic and collagen fibers. Therefore, the AGE crosslink-breaker alagebrium has received attention as potential de-stiffening drug (Zieman et al., 2007). This α -Aminoguanidine compound improved aortic stiffness and improved peripheral arterial endothelial function in hypertensive patients, independently of blood pressure (Kass et al., 2001; Zieman et al., 2007). However, further research is required to properly assess the effects and safety of this class of drugs.

Conclusion and future perspectives

Studying genetic diseases such as PXE, PXE-like syndrome, Keutel's syndrome and Marfan's disease increase our knowledge about pathophysiological mechanisms underlying arterial remodeling (summarized in **Figures 2.2 and 2.3**). Single gene defects of these specific diseases affect major regulatory pathways such as VSMC phenotype switching, matrix degradation, and calcification that are also involved in common cardiovascular disease and aging. Lessons learned from PXE, PXE-like syndrome and Keutel's syndrome have given attention to the major calcification regulatory protein MGP and has provided a possible new target for intervention. In this way, the continued study of these relatively rare genetic diseases may ultimately provide us with potential new targets for therapeutic intervention above and beyond traditional cardiovascular riskmanagement and treatment of risk factors. Conceivably, since VSMC phenotype switching has such an important regulatory role in arterial remodeling, specifically targeting the direction of VSMC phenotype switching may prove to be promising. Ultimately, these novel concepts learned from studying specific genetic diseases can be applied to general cardiovascular medicine.

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Chapter 3

Differences in long-term changes in carotid remodeling between normotensive and hypertensive persons in a primary care population

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Abstract

Hypertension is associated with an increased intima-media thickness (IMT), but relatively few studies have evaluated additional aspects of carotid remodeling longitudinally. Therefore, we aimed to prospectively investigate whether there are differences between carotid remodeling between hypertensive and normotensive people over time and whether this remodeling is adaptive or maladaptive. To this end, we measured carotid artery remodeling with B-mode ultrasonography using an automated wall-track system, and assessed markers of arterial remodeling in addition to IMT such as lumen diameter (LD), cross-sectional area (CSA), circumferential wall tension (CWT), and circumferential wall stress (CWS), at baseline and after a mean follow-up of 6.1 years. From a cohort of 174 normotensives and 317 hypertensive participants from a single general practice, a total of 128 normotensives and 94 hypertensives consented to follow-up measurements. Overall, hypertensives had significantly higher IMT, LD, CSA, CWT, and CWS than normotensives at both baseline and follow-up, suggesting that hypertensives had established maladaptive carotid remodeling. In hypertensives, IMT, LD, and CSA remained constant over time but in normotensives these values as well as CWT rose significantly, although CWS did not change significantly. Predictors of carotid remodeling were pulse pressure, male sex, smoking and age. Use of angiotensin-receptor blockers was associated with lower CWS in hypertensives. We conclude that although hypertension is associated with maladaptive remodeling the process seems to commence even before overt hypertension is established.

Introduction

Among several other factors, aging and hypertension strongly contribute to carotid artery remodeling.^{1,2} This process serves to reduce mechanical tension and stress on the vascular wall and is characterized by an increase in intima-media thickness (IMT) and carotid luminal diameter. Previous studies have shown that in healthy people both carotid artery diameter and IMT correlate with age and that the annual change in carotid IMT is more pronounced at higher age.³⁻⁵ Likely, these age-related changes reflect a physiological (adaptive) process. In hypertension, such changes are exaggerated in order to cope with the higher transmural pressure. However, intima-media thickening in hypertensive patients cannot fully compensate for the increased circumferential wall stress and wall thickening of the carotid artery is considered to be maladaptive in these patients.²

Although carotid IMT has been studied extensively in various populations, many of these studies have been cross-sectional in design and far less information is available with respect to long-term longitudinal changes. This is also true for other characteristics of carotid artery remodeling such as luminal diameter and wall stress. Moreover, it is not well known whether differences in the longitudinal development of carotid artery remodeling exist between people with normal or elevated blood pressure and whether antihypertensive treatment has any influence on the remodeling process. Therefore, the aim of the present study was to investigate whether there are longitudinal differences in carotid remodeling between hypertensive patients and normotensive individuals and to determine which factors could play a role in carotid remodeling over time.

Methods

The present analysis is based on data which we obtained as part of the HIPPOCRATES study. Details of this study have been published elsewhere.^{6,7} In brief, normotensive and hypertensive participants aged 40 years or older from a single primary care practice in Kerkrade in the Netherlands are followed for several years to study the development of hypertensive target organ damage and cardiovascular disease. All participants have provided written informed consent and the study has been approved by the Maastricht Medical Ethics Committee. The study is conducted in accordance with the Declaration of Helsinki. During the study, treating physicians were at liberty to initiate or to alter antihypertensive treatment when this was deemed to be clinically indicated.

Clinical measurements

At the first study visit, patients were interviewed regarding their medical history and

lifestyle habits. Data regarding use of antihypertensive medication was collected from the automated prescription system of the general practice. At every follow-up visit, a research nurse measured height, weight, waist, and hip circumference. We calculated body mass index (BMI) as weight/height². Systolic and diastolic blood pressure were measured after 10 minutes of rest in sitting position, using a sphygmomanometer and rounded to the nearest 2 mm Hg. Brachial pulse pressure (PP) was calculated as the difference between systolic blood pressure (SBP) and diastolic blood pressure (DBP). Mean arterial pressure (MAP) was calculated as $(SBP + 2 \cdot DBP)/3$. We defined hypertension according to the guidelines of the European Society of Hypertension as a SBP ≥ 140 mm Hg and/or a DBP of ≥ 90 mm Hg.⁸ We also obtained fasting blood samples for measurement of serum lipid concentrations, glucose, HbA1C, and creatinine. Estimated glomerular filtration rate (eGFR) was calculated using the CKD-EPI formula.⁹

Measurements of arterial remodeling

At baseline and follow-up, we obtained B-mode ultrasonographic images of the common carotid artery at both sides, using an automated wall-track system (ArtLab, Esaote, Maastricht, The Netherlands). Carotid IMT was measured 10 mm proximal to the bifurcation in an area free of plaque. At both sides, four consecutive measurements were performed at an angle of 90, 120, 150, and 180 degrees, respectively, and the average of these measurements was taken as the mean IMT. In addition, the inter-adventitial diameter (IAD) was measured. From the ultrasonographic images we calculated carotid artery properties using the following equations: Lumen Diameter (LD) = $IAD - (2 \cdot IMT)$; cross-sectional wall area (CSA_{IMT}) = $\pi \cdot IMT \cdot (IMT + LD)$; circumferential wall tension (CWT) and circumferential wall stress (CWS) according to Laplace's law as $P \cdot (r/w)$ where P is internal pressure, r is lumen radius, and w is wall thickness. CWT was therefore calculated as $MAP \text{ (kPa)} \cdot (LD/2)$ and CWS was calculated by dividing CWT by IMT.¹⁰ The CWT is an estimate of the amount of internal tensile force acting in the radial axis of the vessel and results from the product of the intraluminal pressure (i.e. mean arterial blood pressure) and the internal diameter of the vessel (i.e. lumen diameter). Analogously, CWS estimates the internal mechanical force that the vessel wall experiences resulting from the CWT and reflects the amount of compensation by accounting for the thickness of the vessel wall (in this case IMT).^{11,12} For each marker of remodeling we also calculated the average annual rate of change by dividing the difference between the second and the first measurement by the number of years of follow-up.

Statistical analysis

For the present analysis, we divided the study cohort in two groups: patients with a history of hypertension and normotensive controls. To assess cross-sectional differences between the groups, we used Student's t-tests for continuous variables and Wilcoxon Signed-Rank tests for non-normally distributed variables. The primary

outcome for the longitudinal analysis was the difference between normotensive participants and hypertensive patients in markers of carotid remodeling as well as differences between their rates of change over time. For analysis of repeated (and therefore, intrinsically correlated) data, we used Global Estimating Equations (GEE). This robust method analyses all longitudinal data simultaneously and allows for correction for unequally spaced follow-up intervals as well as for within-subject correlation.^{10,13} We used an autoregressive correlation-structure and corrected for differences in follow-up interval and possible regression to the mean. Furthermore, we added the interaction-term: “[hypertension (1) * follow-up time]” to test for differences in slope of change between both groups, using normotensives as reference. Predictors for the development of longitudinal carotid remodeling were identified with univariable GEE-analysis using an exchangeable correlation matrix. Then, to test for independency, we added all identified predictors in a multivariable model adjusted for follow-up interval. In these models, we also included the interaction-term with time for each predictor variable (i.e. predictor * follow-up time) to assess its effect on the regression slope. Data from GEE-analysis are expressed as beta-coefficient with their 95% confidence intervals and represent the longitudinal effect relative to the normotensive reference group. For all statistical analyses, we used IBM SPSS Statistics version 24 (IBM, Chicago, United States of America). We accepted a significance level (α) of 0.05 as statistically significant. We set an alpha level of 0.025 for two-sided testing and calculated at 90% power the sample size for each of the markers of arterial remodeling, based on previous studies. Data are expressed as means and standard deviations, unless indicated otherwise.

Figure 3.1 Study flow-chart

Table 3.1 Baseline characteristics of the entire cohort and of the follow-up cohort

Characteristic	Entire group (n=587)	Follow-up cohort (n=222)
Number of males (%)	283 (48)	111 (50)
Age (years)	61 ± 11	58 ± 9
BMI (kg/m ²)	27.9 ± 4.5	27.8 ± 4.7
Number of hypertensives (%)	264 (45)	94 (42)
Number of diabetics (%)	71 (12)	20 (9)
Systolic blood pressure (mmHg)	145 ± 22	143 ± 21
DBP (mmHg)	83 ± 9	84 ± 10
Total cholesterol (mmol/L)	5.6 ± 1.1	5.5 ± 1.1
HDL cholesterol (mmol/L)	1.5 ± 0.5	1.5 ± 0.4
LDL cholesterol (mmol/L)	3.4 ± 1.0	3.3 ± 0.9
Triglycerides (mmol/L)	1.6 ± 0.9	1.5 ± 0.9
Glucose (mmol/L)	5.7 ± 1.5	5.6 ± 1.4
eGFR (ml/min/173m ²)	105 ± 14	91 ± 14
Antihypertensive medication:		
Number of participants with diuretics (%)	97 (16.5)	34 (15.3)
Number of participants with beta blockers (%)	116 (19.8)	45 (20.3)
Number of participants with ACE-inhibitors (%)	88 (15.0)	39 (17.6)
Number of participants with ARB (%)	28 (4.8)	12 (5.4)
Number of participants with CCB (%)	42 (7.2)	17 (7.7)

Abbreviations: BMI: body mass index, SBP: Systolic blood pressure, DBP: Diastolic blood pressure, HDL: High density lipoprotein, LDL: low density lipoprotein, eGFR: estimated glomerular filtration rate, ACE: angiotensin converting enzyme, ARB: angiotensin receptor blocker, CCB: calcium-channel blocker.

Results

Altogether, 805 people were eligible for the study but 218 of them refused ultrasound examinations so that the baseline population consisted of 587 individuals. Of these, 222 participants completed follow-up measurements after a mean period of 6.1 ± 1.1 years. The main reasons for not completing follow-up were death, withdrawal of consent or move to another region (**Figure 3.1**). As shown in **Table 3.1**, baseline characteristics were comparable between the follow-up group and the original one. Compared to their normotensive counterparts, hypertensive individuals in the whole group (N=587) had significantly higher baseline values of IMT, LD, CSA, CWT, and CWS (**Table 3.2**). The same was true when normotensives and hypertensives were compared at baseline in the follow-up group only.

Table 3.2 Carotid remodeling in normotensive and hypertensive participants in the entire cohort (N=587)

	Normotensives (N = 323)	Hypertensives (N = 264)	Sig.
IMT (um)	649 ± 130	733 ± 134	p < 0.001
LD (mm)	6.0 ± 0.8	6.5 ± 0.9	p < 0.001
CSA (mm ²)	13.7 ± 3.7	16.7 ± 4.3	p < 0.001
CWT (kPa)	39.8 ± 7.3	47.2 ± 8.4	p < 0.001
CWS (kPa)	63.8 ± 14.8	67.1 ± 15.26	p = 0.009

Abbreviations: IMT: Intima-media thickness, LD: Lumen diameter, CSA: Cross-sectional wall area, CWS: Circumferential wall stress, CWT: Circumferential wall tension

Longitudinal change in carotid remodeling

Figure 3.2 depicts the changes in markers of carotid remodeling over time, comparing hypertensive patients to normotensive participants, unadjusted for differences in duration of follow-up. In the hypertensive group, IMT, LD and CSA did not significantly change over time, whereas they increased significantly in the normotensives. On the other hand, CWS fell significantly in hypertensives, while remaining constant in normotensives. CWT fell in hypertensives but rose in normotensives. In GEE-analysis, hypertensives had significantly higher levels for all markers of carotid remodeling: IMT (β 71.0 95%CI [36.6; 105.4] p < 0.001), CSA (β 2.86 95%CI [1.79; 3.94] p < 0.001), CWS (β 5.5 95%CI [1.6; 9.4]; p < 0.01) and CWT (β 5.99 SE 0.89 95%CI [4.25; 7.73] p < 0.001). Confirming the unadjusted analysis, normotensives had significantly higher rates of change of IMT (β 9.9 95%CI [3.9; 5.9]; p < 0.001), LD (β 0.03 95% CI [0.00; 0.06]; p = 0.044) and CSA (β 0.3 95%CI [0.1; 0.4]; p < 0.001). However, in the adjusted model there was no difference between the slopes of CWS in hypertensives relative to normotensives (β -0.5 95%CI [-1.2; 0.2]; p=0.183). CWT decreased significantly over time (β -0.69 95%CI [-1.05; -0.34] p < 0.001) in hypertensives.

Effect of antihypertensive treatment on carotid remodeling

We first tested in univariate GEE-analyses using an exchangeable correlation-structure whether the composite predictor use of any antihypertensive medication did influence the rate of progression of the different markers of carotid remodeling. Overall use of antihypertensive medication was neither associated with IMT, LD, CSA, or CWS, nor with changes in these parameters over time (data not shown). However, when individual classes of antihypertensive medication were tested, use of angiotensin receptor blockers (ARBs) was associated with lower CWS (β -4.9 95%CI -9.7; -0.2] p < 0.05).

Predictors of carotid remodeling

In **Table 3.3**, the predictors of carotid remodeling identified in univariate GEE-analysis

Figure 3.2 Development of carotid remodeling over time in normotensive (N=128) and hypertensive (N=94) participants

Abbreviations: IMT: Intima-media Thickness, LD: Lumen diameter, CSA: Cross-sectional wall area, CWS: Circumferential wall stress, CWT: Circumferential wall tension

Table 3.3 Determinants of longitudinal carotid remodeling using normotensive participants as reference group (N=222)

	IMT		LD		CSA		CWS		CWT	
	β	95%-CI	β	95%-CI	β	95%-CI	β	95%-CI	β	95%-CI
Male sex			0.7	0.5; 0.9	1.5	0.6; 2.4	†	5.6	2.8; 8.4	*
Baseline age	7.0	5.6; 8.3	*	0.04	0.02; 0.05	*	0.3	0.2; 0.3	*	
Smoking pack years	0.9	0.2; 1.6	†	0.01	0.00; 0.01	†	0.02	0.00; 0.04	†	
Body mass index	5.1	1.3; 8.9	†		0.13	0.02; 0.24	†		0.23	0.05; 0.41
Systolic blood pressure	2.2	1.3; 2.9	*		0.05	0.03; 0.07	*	0.16	0.06; 0.26	*
Diastolic blood pressure					-			0.71	0.5; 0.9	*
Pulse pressure	3.4	2.7; 4.2	*		0.09	0.06; 0.10	*	-	0.18	0.12; 0.24
Change in pulse pressure					-			0.04	0.01; 0.06	*
Cholesterol/LDL-ratio	20.9	7.8; 34.0	†	0.11	0.01; 0.21	†	0.72	0.3; 1.2	†	
Triglycerides	29.9	11.5; 48.3	†		0.83	0.34; 1.33	†			
Diabetes								2.6	0.1; 5.0	

* $p < 0.001$; † $p < 0.01$; ‡ $p < 0.05$

Every model adjusted for baseline value of dependent variable and follow-up time. Beta-coefficients are relative to reference group (i.e. normotensive participants) Abbreviations: BMI: body mass index, SBP: systolic blood pressure, DBP: diastolic blood pressure, PP: pulse pressure, LDL: low density lipoprotein; 95%-CI: 95% confidence interval.

Table 3.4 Multivariable predictors of carotid remodeling (N = 222)

IMT	LD	CSA	CWT	CWS
	Male sex	Male sex	Male sex	Male sex
Baseline age	Baseline age	Baseline age		
Smoking pack-years	Smoking pack-years	Smoking pack-years		
Pulse pressure		Pulse pressure		Change in pulse pressure
			DBP and SBP	

Model adjusted for follow-up time and relative to normotensives.

Abbreviations: IMT: intima media thickness, LD: lumen diameter, CSA: cross-sectional wall area, CWS: circumferential wall stress, CWT: circumferential wall tension.

are shown. IMT was determined by baseline age, smoking pack years, BMI, SBP, PP, cholesterol/LDL-ratio, and serum triglyceride levels. LD was determined by male sex, baseline age, smoking pack-years, and cholesterol/LDL-ratio. This was also the case for CSA which was additionally determined by BMI, SBP and PP. CWT was determined by male sex, BMI, blood pressure (SBP, DBP, and PP) and the presence of diabetes. CWS was also determined by male sex, SBP, and DBP as well as annual increase in PP. When we tested these variables in a model corrected for follow-up duration and baseline value male sex, baseline age, smoking pack years and pulse pressure were independently predictive of carotid remodeling (**Table 3.4**).

Discussion

Maladaptive carotid remodeling in hypertension

The present study aimed to provide more insight into the remodeling of the carotid artery in hypertensives compared to normotensives. First, our results confirm that hypertensives have higher values of IMT compared to normotensive participants, but, more importantly, also show that hypertensives have higher LD, CSA and elevated mean CWS and CWT. This indicates that carotid remodeling is maladaptive in the hypertensive population. These results are in line with previous studies which established an association between hypertension and maladaptive remodeling and/or increased IMT.^{1-3,14}

Longitudinal change in carotid remodeling and its determinants

Secondly, we demonstrated that longitudinally, normotensives display a significantly greater rate of increase in IMT, LD, and CSA over time than hypertensives, in whom these markers did not change significantly after correction for follow-up time and regression to the mean. This finding may reflect that in the present cohort, carotid

remodeling was already at an advanced stage in hypertensive patients whereas in normotensive participants there was still potential for arterial enlargement. Studies investigating changes in carotid IMT over time have yielded heterogeneous results which can be explained by differences in measurement technique, characteristics of the studied population (i.e. ethnicity, comorbidity, age, sex etc.), and/or duration of follow-up. In addition, collinearity between covariates and repeated measures has not always been compensated for adequately.^{15,16} Although the majority of studies have established a relationship between hypertension and progression of IMT¹⁷⁻²⁰, some did not.²¹

The combination of increasing LD, CSA and higher IMT, reflect outward, hypertrophic remodeling, a process that is considered to be a compensatory mechanism to reduce tensile forces (i.e. CWT) and stress (CWS) acting on the vessel wall.^{2,10,22} Outward, hypertrophic carotid remodeling was also observed in healthy, obese participants in a study by Kozako et al. in which higher quartiles of LD were associated with an increased rate of progression of IMT and a corresponding decrease of CWS over time.²³ In our study, however, although CWS tended to decrease over time in normotensives, this change was not statistically significant. Furthermore, CWT significantly increased over time, suggesting that the remodeling was maladaptive in the normotensive group, although in absolute terms, CWT was still significantly lower than in their hypertensive counterparts. A possible explanation for this finding may be that, although strictly speaking the normotensive group had no (history of) hypertension, they still had a relatively high-normal systolic mean blood pressure. Previous cross-sectional studies have shown a gradual association between markers of maladaptive remodeling and increasing levels of blood pressure.²⁴⁻²⁷ Even in normotensives, higher levels of SBP have been associated with increasing levels of CWS²⁸ or arterial enlargement.²⁹ In addition to having a higher blood pressure, our normotensive group consisted of middle-aged to elderly participants among whom were many smokers. Moreover, the entire study population was recruited from a single general practice from a region with a relatively high cardiovascular risk.³⁰ These factors may have contributed to a more maladaptive pattern of remodeling. Indeed, we found in the present study that, in addition to positive history of hypertension, carotid remodeling over time was independently determined by a higher baseline age, male sex, smoking, and pulse pressure. This supports data from other studies which investigated predictors of IMT-progression.^{19,31,32}

Pulse pressure is an important driving force of carotid remodeling in both hypertensive patients and normotensive controls.^{29,33,34} For instance, Di Bello et al. showed that in asymptomatic elderly patients with hypertension, pulse pressure (or isolated systolic hypertension) were predictive of carotid remodeling.³⁵ Pulse pressure is a marker of arterial stiffness and is an important determinant of arterial function, both in cross-sectional and in longitudinal analyses.^{2,36} Also, pulse pressure has been thought to be a driving force of arterial enlargement.³⁶

Interestingly, we found that in hypertensives, CWS and CWT significantly decreased over time and therefore should be interpreted as adaptive remodeling. This is in contrast to other studies in which hypertension generally leads to maladaptive remodeling, unless a treatment effect exists. Although, there was no general effect of antihypertensive treatment on the development of carotid remodeling, use of ARBs was significantly associated with lower CWS in hypertensive patients. This supports previous studies in which use of ARBs led to a more adaptive form of carotid remodeling.³⁷⁻⁴¹

Limitations and strengths Our study has some limitations. For instance, we did not record distension waveforms. As a consequence, it was not possible to assess Young's Elastic Modulus or local carotid pulse pressure. Therefore, measurements of CWS and CWT are based on brachial artery MAP and not on local carotid blood pressure. Nevertheless, there still was a clear link between carotid artery remodeling and peripheral pulse pressure. Also, measurement error is a known factor for variation in sequential IMT measurements, especially with longer follow-up times.¹⁵ Moreover, we could not assess local carotid compliance and, therefore, were unable to compare differences in carotid stiffness between normotensive and hypertensive participants. On the other hand, our study has several strengths. First, it is a longitudinal study investigating the effects of hypertension per se and of treatment with a median follow-up duration of 6 years. Although several studies have assessed the progression of IMT, many of them were designed to evaluate the effect of an intervention or were performed in patients with additional comorbidity.⁴²⁻⁴⁴ Moreover, there are only few studies that evaluate the longitudinal effect change in additional markers of carotid remodeling and hemodynamic mechanical stress above and beyond IMT in a specific hypertensive population.^{45,46} Secondly, the present study was performed in a primary care population in which patients have been treated according to common clinical practice. Therefore, our data reflect a relatively unselected (real-world) population.

Conclusion

In conclusion, we have shown that, relative to normotensive controls, hypertensive patients, have maladaptive arterial remodeling which remains associated with high wall stress and tension in comparison to normotensive participants. However, the latter have higher rates of arterial remodeling over time, and may reflect an earlier phase in the arterial remodeling process. We have shown that pulse pressure, male sex, smoking and age were also predictive of progression of carotid remodeling, but that use of angiotensin-receptor blockers may attenuate circumferential wall stress in hypertensives. Future longitudinal prospective studies that evaluate carotid artery remodeling and hemodynamics in a cohort consisting of persons without hypertension

as well as participants with high-normal blood pressure and patients with established hypertension are warranted to provide more insight in the pathophysiology of carotid remodeling.

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Chapter 4

Arterial stiffness and decline of renal function in a primary care population

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Abstract

Objective: Arterial stiffness is an important pathophysiological factor linking cardiovascular disease and kidney disease. Controversy exists as to whether arterial stiffness causes renal function decline, or kidney dysfunction leads to stiffening, or whether the association is mutual. We aimed to investigate the longitudinal association between arterial stiffness and annual rate of renal function decline.

Methods: We prospectively investigated in a primary care population whether carotid-femoral pulse wave velocity (PWV) was associated with estimated glomerular filtration rate (eGFR) and annual decline in eGFR in participants aged 40 years and older without overt kidney disease.

Results: Baseline data on PWV and eGFR was available for 587 participants; follow-up measurements with a mean duration of 5.6 years were available for 222 patients. PWV, female gender, and mean arterial pressure were independently associated with eGFR at baseline, although age confounded this association. More importantly, baseline PWV, age, and eGFR were independent predictors of renal function decline. Stratification for age showed that the effect of PWV on rate of eGFR decline was amplified with advancing age. On the other hand, baseline eGFR did not determine annual change in PWV, suggesting a unidirectional association between arterial stiffness and eGFR.

Conclusion: Arterial stiffness amplifies age-related renal function decline suggesting that arterial stiffness plays a causal role in the development of renal damage, at least at later stages of age-related renal function decline, possibly through impaired renal autoregulation and increased arterial blood pressure pulsatility.

Introduction

Patients with cardiovascular diseases such as atherosclerosis and hypertension often develop renal damage, which sometimes progresses to chronic kidney disease. However, even in the absence of cardiovascular disease, kidney function progressively declines with advancing age. In both cardiovascular disease and aging, the stiffness of the arterial wall increases, which leads to a rise in systolic blood pressure (SBP) together with a fall in diastolic blood pressure (DBP) causing pulse pressure (PP) to widen.^{1,2} The question remains whether arterial stiffness is an independent pathophysiological mechanism in age-related renal function decline in addition to advancing age. Studies assessing the association between arterial stiffness and renal function provided conflicting results.³⁻⁵ However, many of these studies have been performed in specific populations such as established kidney disease or renal transplant recipients, did not evaluate arterial stiffness using standard measurement techniques, or have been cross-sectional in design, making it difficult to assess a cause-and-effect relationship. Therefore, we aimed to longitudinally evaluate in a primary care population without overt kidney disease whether arterial stiffness, measured as the carotid-femoral Pulse-Wave Velocity, is associated with annual rate of renal function decline and whether this effect is independent of age.

Methods

The present study was based on 587 participants of the HIPPOCRATES project who consented to vascular stiffness measurements. The project is an ongoing study designed to investigate the role of hypertension, target organ damage, and cardiovascular risk in a single primary care population located in Kerkrade in the south of the Netherlands. Details of this study have been published previously.⁶ The present study was conducted in two phases: a baseline, cross-sectional phase and a follow-up phase that commenced at least three years after the baseline phase was completed. A randomly selected part of eligible participants from the baseline phase were invited for follow-up vascular measurements. Patients were eligible for follow-up measurement if they had not experienced a cardiovascular event such as myocardial infarction or stroke and were willing to undergo vascular measurements. Participants provided written informed consent. The study was conducted in accordance with the Declaration of Helsinki and was approved by the Maastricht Medical Ethics Committee.

Clinical measurements

Clinical baseline and follow-up visits were performed at the general practice. At baseline, we interviewed participants about smoking and alcohol consumption and obtained a complete medical and family history. We collected data on baseline cardiovascular medication use from the computerized information system of the general

practice. Patients were treated by their general practitioner according to standard clinical practice. Physicians were at liberty to change medication when clinically required during the course of the study. We measured height, weight, waist and hip circumference at each study visit. Body mass index (BMI) was calculated as weight divided by squared height. We measured blood pressure and heart rate three times using an aneroid sphygmomanometer after the patient had been seated for at least 10 minutes. Hypertension was defined as systolic blood pressure (SBP) > 140 mmHg and/or diastolic blood pressure (DBP) > 90 mmHg or being on antihypertensive medication, according to the 2013 guidelines of the European Society of Hypertension.⁷ Mean arterial pressure (MAP) was calculated using the formula: $MAP = [(2 \times DBP) + SBP] / 3$. We obtained fasting blood samples to determine serum concentrations of creatinine, glucose, total cholesterol, low-density lipoprotein cholesterol (LDL), high-density lipoprotein cholesterol (HDL), and triglycerides (TG). Diabetes was defined as a fasting serum glucose concentration of ≥ 6.5 mmol/L and/or use of insulin or antidiabetic medication.

Arterial stiffness measurements

As marker for arterial stiffness we measured carotid-femoral Pulse-Wave Velocity (PWV), a method considered to be the gold standard marker of arterial stiffness.⁸ We measured PWV two times: once at baseline and once at follow-up. Measurements were performed using a Complior device (Alam Medical, Vincennes, France) which determines the transit time of the pulse-wave propagation between two sensors placed on the skin over the common carotid artery and common femoral artery.⁸ We measured the direct distance between both sensors using a tape measure and was multiplied by 0.8 as was recommended by van Bortel et al.⁹ Carotid-femoral PWV was then calculated by dividing the adjusted distance by the transit time. We averaged four consecutive measurements to reduce measurement variability. Before investigation, participants had been resting in a supine position for 15 minutes in a quiet room with dimmed lights. Certified operators performed all PWV measurements. In our laboratory, the reproducibility of PWV measurements is excellent and comparable to that in literature.¹⁰ The Intraclass Correlation Coefficient (ICC) for intra observer variability was 0.93 ($p < 0.001$), and for inter observer variability 0.92 ($p < 0.001$). Annual change in PWV was calculated as the absolute difference between PWV at follow-up and baseline, divided by the number of years follow-up time.

Creatinine measurements and estimation of glomerular filtration rate

Until February 2007, baseline serum creatinine levels were determined using the Jaffe method on Roche diagnostic systems. Thereafter, follow-up assays were done with an enzymatic method on a Roche Modular PPE analyzer (Roche Analytics, Almere, The Netherlands). Based on internal laboratory validation measurements, in order to compare baseline (Jaffé-based) creatinine measurements to follow-up enzymatic creatinine measurements, all baseline creatinine values were corrected

by the formula: Corrected baseline creatinine = (baseline creatinine x 1.06)⁻²⁵.¹¹ We estimated Glomerular filtration rate (eGFR) using the CKD-EPI equation.¹² Patients with a baseline eGFR below 30 ml/min/1.73 m² were excluded from analysis, as were patients with primary kidney disease as manifested by a nephritic urinary sediment or nephrotic proteinuria. We calculated annual change in eGFR by subtracting baseline eGFR from the eGFR measured at the time of the follow-up vascular measurement and dividing it by the follow-up duration in years.

Statistical analysis

Statistical analyses were performed using Statistical Package for the Social Sciences (SPSS) statistical software, version 20.0 (SPSS, Inc., Chicago, Illinois, USA). Data were analyzed after examination of distribution and skew using appropriate statistical tests. A p-value < 0.05 was considered statistically significant. Data are expressed as means ± standard deviation, unless indicated otherwise. To compare baseline and follow-up measurements, we used paired sample Student's t-test for continuous data, paired sample Wilcoxon Signed Rank test for non-normally distributed data, and the McNemar test for dichotomous data. The main outcome (annual change in eGFR) was analyzed using multivariable linear regression, correcting for confounders such as age, gender, mean arterial pressure (MAP), BMI, total cholesterol, serum glucose levels, smoking (pack years), use of different groups of antihypertensive medication and use of statins.¹³ We did not adjust for pulse pressure since this is also a marker of arterial stiffness. In addition to baseline values of confounders, we adjusted for annual change in PWV, annual change in blood pressure, and baseline eGFR. Since studies have shown that arterial stiffness may have a different effect on the rate of progression of eGFR with advancing age, we performed a sliding mean analysis to assess whether age affects the correlation between PWV and annual change in eGFR.^{3,5} In addition, we performed a subgroup analysis using 62 years as a cut-off value.

Power calculation

For cross-sectional analysis we calculated that with a power of 90% and alpha 5% the minimal detectable difference in mean eGFR was 2.5 ml/min/1.73 m² with the 587 participants of the baseline cohort. For follow-up measurements, to detect a minimal annual change in eGFR of 0.6 ml/min/1.73 m² with a power of 90% and a two-sided alpha of 5%, at least 154 participants would be needed.

Results

Cross sectional association of baseline PWV and eGFR

The baseline HIPPOCRATES study cohort consisted 587 participants. **Table 4.1** shows the baseline characteristics of the studied population. In linear regression analysis cfPWV was significantly and inversely associated with estimated glomerular filtration

Table 4.1 Baseline characteristics of the cross-sectional and follow-up study cohorts

	Cross-sectional cohort (n=587)	Follow-up cohort (n=222)
Age (years)	62 ± 11	58 ± 9
No. Females (%)	304 (52)	111 (50)
Weight (kg)	78.3 ± 14.3	78.5 ± 14.9
Body Mass Index (kg m ⁻²)	27.9 ± 4.5	27.8 ± 4.7
Smoking (packyears)	14 ± 19	12.7 ± 19
Systolic BP (mmHg)	145 ± 22	143 ± 21
Diastolic BP (mmHg)	83 ± 10	84 ± 10
MAP (mmHg)	104 ± 12	103 ± 12
No. Hypertensives (%)	392 (66.8)	153 (68.9)
Heart Rate (bpm)	65 ± 12	64 ± 11
Total Cholesterol (mmol l ⁻¹)	5.6 ± 1.1	5.5 ± 1.1
LDL-Cholesterol (mmol l ⁻¹)	3.4 ± 1.0	3.3 ± 0.9
HDL-Cholesterol (mmol l ⁻¹)	1.5 ± 0.5	1.5 ± 0.4
Triglycerides (mmol l ⁻¹)	1.6 ± 0.9	31 ± 0.9
No. Patients with hyperlipidemia (%)	121 (20.6)	20 (19.3)
Glucose (mmol l ⁻¹)	5.7 ± 1.5	5.6 ± 1.4
Serum creatinine (μmol l ⁻¹)	54 ± 14	72 ± 16
eGFR* (ml min ⁻¹ 1.73 m ⁻²)	105 ± 14	91 ± 14
Carotid-femoral PWV (m s ⁻¹) ^a	9.1 ± 2.0	13.4 ± 2.4
<i>No. patients using antihypertensive medication</i>		
Diuretics (%)	97 (16.5)	31 (14.4)
β-Blockers (%)	121 (20.6)	40 (19.4)
Calcium channel blockers (%)	45 (7.7)	16 (8.1)
ACE inhibitors (%)	92 (15.7)	38 (18.0)
ARB (%)	30 (5.1)	10 (4.5)
Other class of antihypertensives (%)	4 (0.7)	1 (0.5)

Abbreviations: ACE, angiotensin-converting enzyme; ARB, angiotensin receptor blocker; BP, blood pressure; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; LDL, low-density lipoprotein; MAP, mean arterial pressure; PWV, pulse wave velocity.

^aCalculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula

Figure 4.1 Flow-chart of the study

rate (eGFR). In addition to cfPWV, female gender, and MAP were associated with eGFR. In a model adjusting also for age, however, cfPWV was no longer independently associated with eGFR (β -0.41; 95%CI -0.99; 0.18 $p = 0.173$).

Table 4.2 Determinants of annual change in eGFR after follow-up (N=222)

	β	s.e.	95% CI	p-value
<i>Model A (crude, $R^2 = 0.07$)</i>				
Baseline carotid-femoral PWV	-0.22	0.06	-0.33; -0.11	0.007
<i>Model B ($R^2 = 0.28$)</i>				
Baseline carotid-femoral PWV	-0.31	0.07	-0.43; -0.18	< 0.001
Baseline eGFR	-0.06	0.01	-0.07; -0.04	< 0.001
<i>Model C ($R^2 = 0.35$)</i>				
Baseline carotid-femoral PWV	-0.18	0.06	-0.30; -0.06	0.004
Baseline eGFR	-0.07	0.01	-0.08; -0.05	< 0.001
Baseline age	-0.07	0.02	-0.10; -0.03	< 0.001

Abbreviations: 95% CI, 95% confidence interval; eGFR, estimated glomerular filtration rate; PWV, pulse wave velocity.

Model A: crude model.

Model B: analysis adjusted for carotid-femoral PWV, gender, body mass index (BMI), total serum cholesterol, serum glucose levels, smoking pack years, mean arterial pressure (MAP), annual change in PWV, baseline eGFR, annual change in MAP and use of renin-angiotensin blockers (angiotensin-converting enzyme (ACE) inhibitors or angiotensin receptor blocker (ARB)).

Model C: Model B + age

Longitudinal association between baseline PWV and annual decline of eGFR

In total, 222 participants from the baseline study cohort consented to follow-up vascular measurements (see **Figure 4.1**). This group was comparable to the one studied at baseline (**Table 4.1**). The mean duration of follow-up was 5.6 years (range 3.4 – 7.7 years). During that time, eGFR significantly fell by 11.6 ml/min/1.73 m²; the average annual rate of eGFR change was -2.0 ± 1.9 ml/min/1.73 m². Mean carotid-femoral PWV decreased by 0.6 m/s/year. Baseline carotid-femoral PWV was significantly and independently associated with annual decline in eGFR in both univariable (β -0.22; 95%CI -0.33; -0.11, $p=0.007$) and multivariable adjusted regression models (β -0.18 95%CI -0.30; -0.06, $p=0.004$) (**Table 4.2**). Standardized coefficients indicated that both PWV (β -0.22) and baseline age (β -0.31) were strong determinants of annual change in eGFR, responsible for respectively 8 and 7% of variation in the total model (total adjusted $R^2 = 0.35$). There was no significant collinearity between baseline age and baseline PWV. There was no significant interaction between baseline age and baseline PWV. A three-dimensional plot of the linear regression equation representing the mutual association between baseline PWV, baseline age and annual decline in eGFR is shown in **Figure 4.2**. It demonstrates that with increasing baseline age, the rate of decline in eGFR rises. If in addition to age the baseline PWV increases, the rate of eGFR decline is even greater. To further clarify this association between age, PWV and

Figure 4.2 Age, pulse wave velocity (PWV) and annual decline in estimated glomerular filtration rate (eGFR).

Three dimensional plot of the linear regression equation between baseline PWV, baseline age, and annual decline in eGFR.

Figure 4.3 Correlation between baseline PWV and annual change in eGFR.

Correlation between baseline pulse wave velocity (PWV) and annual change in estimated glomerular filtration rate (eGFR). Plot describing the association between baseline age and Pearson's correlation between baseline PWV and annual change in eGFR. Each data point represents the mean of 50 observations. Every consecutive data point differs from the previous by one observation.

annual decline in eGFR, we performed a sliding mean analysis to investigate whether the correlation between baseline PWV and annual change in eGFR was stronger with advancing age. **Figure 4.3** shows that from the age of approximately 62 years, the correlation between PWV and annual change in eGFR becomes stronger. Since several studies have demonstrated that chronic kidney disease is associated with development of arterial stiffness, we analyzed whether this was also the case in the present study. However, neither baseline nor annual change in eGFR were predictive of annual changes in PWV.

Discussion

In the present study, we show that arterial stiffness is inversely associated with eGFR in cross-sectional analysis, but is confounded by age. Our data confirm in a primary care population previous studies that have shown a similar effect.^{3,14} More importantly, we demonstrated that longitudinally both baseline PWV and baseline age were strong determinants of annual rate of decline in eGFR. Since eGFR was not predictive for changes in PWV, our data support the hypothesis that arterial stiffness is an important mechanism in the development of age related renal function decline. This is a new finding since the association between vascular disease and kidney function has long

been primarily attributed to arterial hypertension and changes in vascular resistance.¹⁵ Interestingly, pulse-wave velocity decreased over time in our data. This may reflect a treatment effect, since it has been demonstrated that several antihypertensive drugs have a lowering effect on aortic stiffness, independent of blood pressure.^{16,17}

Several prospective follow-up studies evaluated the role of arterial stiffness and renal function in various populations, with varying results so that the role of arterial stiffness in the pathophysiology still remains debated.^{18,19} For instance, in a study by Upadhyay et al. in 1675 patients with mild-to-moderate kidney disease, baseline arterial stiffness was not associated with incident CKD (< 60 ml/min/1.73 m²) or eGFR.²⁰ Similarly, in a study by Briet et al., no association was found between carotid-femoral PWV and eGFR or incidence of end-stage renal disease (ESRD).²¹ However, in a study by Chen et al. in patients with CKD stage 3 – 5, brachial-ankle PWV was independently predictive of decline in eGFR, progression to dialysis and mortality.²² These differences in outcome could be explained by differences in the duration of follow-up, the underlying cause of kidney disease, or by the possibility that other mechanisms may be more dominant at relatively late stages of CKD than in earlier stages. In the study performed by Tomiyama et al. in healthy Japanese employees aged 33 – 47 years, a higher baseline brachial-ankle PWV was significantly associated with increased decline in eGFR.²³ A meta-analysis by Sedaghat et al. showed that pulse pressure and PWV were predictive of incident chronic kidney disease, defined as a eGFR < 60 ml/min.²⁴ In a study among HIV-infected patients, the presence of chronic kidney disease was associated with a higher central blood pressure, greater augmentation index as well as higher PWV compared to HIV patients without CKD, supporting the association between central artery stiffness and renal damage.²⁵

Age as confounder

As confirmed by the cross-sectional analysis of our data, age has been known to be a strong confounder of the cross-sectional association between arterial stiffness and renal function. Because of this close interrelationship, it has been proposed that age is the main determinant of eGFR. However, in this way age is used as unmodifiable risk factor, where it also can be regarded as a ‘container’ consisting of multiple different and specific physiological mechanisms. These include stiffening of the arteries, widening of arterial pulse-pressure, endothelial dysfunction and others.²⁶ Therefore, when studying the mechanisms that are responsible for age-related renal function decline, adjusting for age attenuates the effect of individual mechanisms occurring with (vascular) aging. This could explain why some studies failed to demonstrate an independent association.

Nevertheless, in our study age and baseline PWV remained significant predictors even after multivariable adjustment, showing that PWV contributes to rate of renal function decline, in addition to normal aging. Moreover, we found this effect mainly occurs above the age of approximately 62 years suggesting that PWV seems to act as an amplifier of the age-related decline in renal function. This suggests that the renal

microcirculation of elderly people is more vulnerable to the damaging hemodynamic effects of arterial stiffness than in younger people. This could reflect an impaired blood pressure buffering capacity of the vascular wall, caused by arterial stiffening, which occurs mainly with advancing age. Indeed, Mitchell et al. demonstrated that central arterial hemodynamics significantly change from the age of approximately 60 years as a result of arterial stiffening and, thereby, contribute to increased blood pressure pulsatility.^{2,27,28}

Mechanism of arterial stiffness-related renal damage

Increased stiffening of the arterial vasculature reduces the pressure buffering capabilities of the vascular wall.² This results in an increased speed of both the forward travelling pulse-wave as well as in earlier pulse-wave reflection, which augments systolic pressure. Because DBP falls with arterial stiffening, the resulting increased central PP contributes to a highly pulsatile flow in the aorta and its branching arteries, including the renal vessels.^{2,28} Normally, the glomeruli and renal microcirculation are protected against blood pressure variations by autoregulation of the vascular tone in afferent and efferent arterioles. The development of hypertensive renal damage would indicate that this autoregulation is somehow disturbed. Although the precise mechanisms are not fully clear, there is evidence from animal models that sustained exposure to increased blood pressure pulsatility induces microvascular remodeling such as fibrosis, which in turn blunts renal autoregulation.²⁹ Indeed, in vitro the myogenic response is mainly determined by systolic blood pressure.³⁰ In this way, the torrential pulsatile flow is transmitted to the vulnerable renal microvasculature, eventually causing either barotrauma or damage through reduced renal perfusion and oxidative stress.^{27,31} In support of this, Fesler et al. showed in human data that the amplitude of wave reflection was associated with increased glomerular pressure.³² In another study, they showed that baseline PP as a marker of stiffness was predictive of renal function decline in patients treated for essential hypertension, which is in line with our present findings.³³

Study limitations and strengths

This study has some limitations. Firstly, renal function was estimated based on creatinine measurements using the Jaffé method at baseline but using an enzymatic measurement method at follow-up. This was caused by a transition of measurement method in the clinical biochemical laboratory during the course of the study. Since the Jaffé method generally overestimates creatinine values compared to enzymatic assays, we corrected the baseline Jaffé values based on internal validation measurements. This resulted in a mean correction factor of 0.78 ± 0.1 , which corresponds to a previously reported correction factor of 0.8, suggesting that change in eGFR may have been reliably assessed in our study.¹¹ Moreover, with lower levels of serum creatinine and, hence, higher eGFR, the association between arterial stiffness and decline in eGFR may have been even larger without this adjustment.

Secondly, we did not measure urinary albumin excretion (UAE) in a quantitative way. It has been demonstrated that UAE is a sensitive marker of renal microvascular dysfunction.³⁴ We did obtain semi-quantitative information about UAE which provided an estimate of the presence of microalbuminuria. Although greater PWV levels tended to be associated with more microalbuminuria at follow-up, this association was not statistically significant and limited by the test method (urinary test-strip). Therefore, the study aimed to assess change in eGFR since this marker is commonly used in clinical practice as measure for kidney disease. Thirdly, we unfortunately had little influence on the use of cardiovascular medication during the follow-up period. Patients were treated by their physicians according to standard medical practice, adjusting medication as required. Although we evaluated which medication participants were currently using at the follow-up visit, not all changes in medication between baseline and follow-up were adequately recorded. We analyzed whether drugs had any effect, however no conclusive information could be obtained. However, this realistically reflects a primary care situation in which patients were not limited to a specific treatment. Our study also has several strengths. First of all, it is a prospective follow-up study in a primary care population. Therefore, the results of our study closely reflect the situation observed in common clinical practice instead of being focused on a specific patient group. Furthermore, arterial stiffness was assessed using the gold standard of non-invasive arterial stiffness measurement, the carotid-femoral PWV. PWV strongly correlates with cardiovascular outcome, and reflects aortic stiffness more accurately than other markers of arterial stiffness such as PP, that only partly reflects arterial compliance.^{8,35}

Conclusion

We demonstrated that carotid-femoral PWV amplifies age-related decline in eGFR during long-term follow-up. Also, we found this association to be unidirectional, supporting the hypothesis that arterial stiffness is an important factor in the development of renal damage, possibly through deleterious effects of altered hemodynamics. Future research should be aimed at investigating the effects of arterial stiffness on renal microcirculation in more detail. Since arterial stiffness seems to play such a key role in the development of age-related kidney function decline, treating arterial stiffness may become an increasingly important therapeutic goal. In recent years, ambulatory methods of measuring arterial stiffness have been developed that may prove useful in further investigating the interrelationship between arterial stiffness, altered hemodynamics and chronic kidney disease.³⁶

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Chapter 5

Hemodynamics of prehypertension

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Abstract

Hemodynamic investigations in prehypertensive people have revealed that already at this early stage of hypertension, total peripheral resistance is increased. This is true even in situations where cardiac output is elevated. Heart rate and arterial stiffness are clearly higher as well in prehypertensives as compared to normotensives. As far as the peripheral circulation has been studied, this shows abnormalities in prehypertension which are also consistent with an increased resistance to flow. Altogether, there is not enough evidence to conclude to hypertension evolves from a high-output to an high-resistance state. Rather, the increase in resistance is a primary phenomenon.

Introduction

Despite decades of intensive research, the etiology of essential hypertension remains unknown. Once this disorder has reached its established phase, it is characterized hemodynamically by an elevated peripheral vascular resistance and a normal or slightly reduced cardiac output (1). In addition, vascular stiffness is increased which over time will result in a further rise in systolic pressure and vascular resistance. This creates a vicious cycle with, if left untreated, an ever-increasing blood pressure. Other pathophysiological features that characterize the phase of established hypertension are reduced renal blood flow, increased filtration fraction and a tendency towards a lower plasma volume. Both the sympathetic nervous system (SNS) and the renin-angiotensin-aldosterone system (RAAS) have been implicated in these abnormalities but their precise role in the initiation and development of the hypertensive process has still not been fully clarified.

The elucidation of the pathogenetic processes leading to established hypertension requires that the factors responsible for the initiation of the disease be known. The ideal way of investigating these factors would be to follow-up normotensive individuals up to the point where they become hypertensive. For obvious reasons, such studies are not feasible, not the least because one would not know who will become hypertensive and who not. In fact, many if not most of them may never develop hypertension at all. Alternatively, one could study the offspring of hypertensive patients and compare this offspring to that of normotensive parents. In doing so, one enriches the population with people who are likely to develop hypertension at some point in their life. This type of approach has been repeatedly applied but again, it is uncertain whether children from hypertensive parents will, indeed, ever become hypertensive. In addition, one runs the risk of mixing up true genetic influences with familial ones such as environment, diet etcetera.

Finally, one could attempt to investigate individuals who are already somewhere on their way from the normotensive to the hypertensive state. Again, this is not an easy category to study but it comprises the people who could be labeled as being prehypertensive. It seems that this group of patients is not too dissimilar from that which was designated in the past with such terms as 'labile hypertension' or 'borderline hypertension'. The term 'labile hypertension' has been largely abandoned because, in fact, nearly all patients with hypertension have some degree of lability of their blood pressure. Borderline hypertensives are people who sometimes cross the line of normality in terms of blood pressure but who at other times are completely normotensive. According to a much-used definition it is a condition in which blood pressure is sometimes below but more often above the arbitrary 140/90 mmHg cutoff point that separates normotension from hypertension. One would think, therefore, that this is a transitory state in which an individual gradually moves from being truly normotensive to being truly hypertensive. As such, one could label this state also as

prehypertension although it is not entirely the same. Prehypertension was defined in the Seventh Report of the Joint National Committee (JNC-7) as a blood pressure, based on the average of two or more properly measured, seated, readings on each of two or more office visits from 120 to 139 mmHg systolic or from 80 to 89 mmHg diastolic (2). Thus, an evolutionary scheme could be: true normotension-prehypertension-borderline hypertension-true hypertension. Admittedly, we do not know with certainty whether people go, indeed, through these stages of prehypertension and borderline hypertension and in the past borderline hypertension has often been considered as an 'illness' in its own right. Still, data from the Framingham study suggest that a normal or high-normal blood pressure frequently progresses to full hypertension (3) and that this is associated with an increased cardiovascular risk (4). So, until there is firm evidence to the contrary, we do best to consider prehypertension and borderline hypertension as, presumably transient, phases in the hypertensive process.

Systemic hemodynamics in borderline hypertension

Blood pressure (BP), in hemodynamic terms, is determined by cardiac output (CO) and total peripheral resistance (TPR) according to the formula: $BP = CO \times TPR$. Whether the very early phases of hypertension are related to a rise in vascular resistance or in cardiac output or both has for years been a matter of vigorous debate. Initially, the hemodynamic studies focused primarily on young, borderline hypertensives. Most of these studies found that cardiac output, when corrected for body size and expressed as cardiac index (l/m^2), as well as heart rate are increased by about 15% in borderline hypertensives as compared to matched normotensives (5, 6). Since cardiac output is the product of heart rate and stroke volume, in theory both components could be involved. However, it turns out that the rise in cardiac output in borderline hypertensives is mainly due to an elevated heart rate and far less to alterations in stroke volume. In a series of elegant experiments, Julius and coworkers have shown that both enhanced sympathetic and reduced parasympathetic activity can be held accountable for the 'hyperkinetic' heart (7). These investigators found that heart rate became normal after total autonomic blockade with propranolol and atropine combined (but not after any one of these alone) which suggests that the pacemaker by itself acts normally but that it is rendered overactive by neurogenic influences. The same researchers also found stroke volume index to be slightly increased but several other studies failed to find a difference in this variable between normotensives and borderline hypertensives. Overall, therefore, the case for a hyperkinetic heart in borderline hypertension seems to be stronger with respect to frequency than to stroke volume. It must be emphasized, though, that in virtually all publications only mean values are presented for the hemodynamic data. Nevertheless, interindividual variations were substantial and true increases are apparent in only about one-third of the patients (6). Finally, total peripheral resistance was, on average, increased in

Figure 5.1 Systemic hemodynamics in normotension (NT), prehypertension (PHT) and hypertension (HT). Adapted from the Strong Heart Study (8).

the group with borderline hypertension. Even though resistance was numerically normal in those with a hyperkinetic heart, it was inappropriately high for the degree of systemic flow. Therefore, it is safe to conclude that borderline hypertension, if we consider this to be an early phase of hypertension, is characterized by an augmented vascular resistance either with or without a hyperkinetic heart.

Systemic hemodynamics in prehypertension

If we want to try to catch potential hemodynamic abnormalities in even earlier phases of the hypertensive process, it is worthwhile to explore systemic hemodynamics in individuals with prehypertension. This has been done, for instance, in the Strong Heart Study which is a population-based survey of cardiovascular risk factors and cardiovascular disease in several American Indian communities (8). At the fourth follow-up examination of this study, Drukteinis and coworkers recruited 1940 participants below 40 years of age (average age 27 years) of whom 971 were normotensive, 294 were hypertensive and 675 fulfilled the criteria of prehypertension (35%). In all these participants, echocardiographic measurements were obtained to estimate cardiac mass and performance. Compared to normotensives, heart rate and cardiac output were significantly higher in the prehypertensives. However, cardiac index did not

differ between groups and averaged 2.67 and 2.73 ml/min.m² respectively in the normotensives and prehypertensives (**Figure 5.1**). These numbers are notably lower than those registered in earlier studies in borderline hypertension (6). Of note, in the prehypertension group more people were obese and/or had diabetes or impaired glucose tolerance. However, adjustment for these confounders did not change the results. Total peripheral resistance index was higher in prehypertension and so was the pulse pressure/stroke index quotient. The latter can be considered as a proxy for arterial stiffness, which apparently is already increased in prehypertension as well. In addition, the prehypertensive group showed a greater left ventricular mass and more often frank left ventricular hypertrophy. Incidentally, besides a higher systolic pressure, the presence of left ventricular hypertrophy also appeared to be a predictor of further progression from prehypertension to hypertension (9).

Almost at the same time, Zhu and coworkers reported on their findings in an even younger group (average age 17 years) with prehypertension (10). In white prehypertensives, these investigators also found a higher heart rate and total peripheral resistance together with a normal cardiac index (measured with impedance cardiography), which is in line with the data from the Strong Heart Study. However, in blacks they found the opposite hemodynamic pattern, i.e. a higher cardiac index but a normal heart rate and total peripheral resistance. Again, the latter still is inappropriately high in relation to the prevailing level of cardiac output because resistance should have fallen in the face of the high systemic flow (**Figure 2**). Another race-related feature was arterial stiffness which was greater in white prehypertensives compared to white normotensives but not different between the two blood pressure groups in blacks.

A little later, Davis and associates published their results with respect to the autonomic and hemodynamic origins of prehypertension (11). They obtained their data from the UCSD twin/family study and compared 340 prehypertensives with 337 normotensives of comparable age. For the hemodynamic measurements, an oscillometric device was used which collects several cardiac and vascular functional data. Also in this study, mean heart rate and cardiac output were significantly higher in the prehypertensive group and so was stroke volume. Remarkably, when normalized for body surface area the differences persisted. Total peripheral resistance was numerically similar in the two groups but one could argue that this was still inappropriately elevated for the height of cardiac output in the prehypertensives (**Figure 2**). Other striking findings in the prehypertensives included enhanced cardiac contractility, a wider pulse pressure and reduced brachial artery distensibility and systemic vascular compliance, which is indicative for an increased vascular stiffness.

Finally, Pal and colleagues studied a group of 118 normotensives and 58 prehypertensives of approximately 20 years of age and found both cardiac output and total peripheral vascular resistance to be significantly higher in the latter (12). Although body mass index was substantially higher in the prehypertensives, the authors failed

Figure 5.2 Balance between cardiac index (CI) and total peripheral resistance (TPR)

Balance between cardiac index (CI=cardiac output normalized for body surface area) and total peripheral resistance index (TPRI=resistance indexed for body surface area) in normotensives (NT), prehypertensives (PHT) and hypertensives (HT). The 'isobars' indicating the lines for a pressure of 120/80 mmHg and 140/90 mmHg mark the boundaries between prehypertension and normotension and between prehypertension and hypertension respectively. Note that in prehypertensives even a high cardiac output is already associated with an inappropriately elevated vascular resistance. Data derived from Drukteinis et al (8), Zhu et al (10), Davis et al (11) and De Leeuw et al (13)

to normalize their hemodynamic data. Thus, we do not know whether the increase in cardiac output was also elevated in relation to body surface area or not. But regardless of cardiac index, their data also point to at least an increase in vascular resistance.

In our own laboratory, we have studied a small group of young, male medical students who at one time had proven to be hypertensive, but later had blood pressures in the prehypertensive range (13). They were compared to another group of young individuals, who were normotensive all the time. In each one of them we recorded blood pressure and non-invasively determined cardiac output and left ventricular ejection time by means of impedance cardiography. Importantly, all participants were put on a mildly sodium-restricted diet to avoid salt-dependent interindividual variations. In our hands, there were no differences in heart rate, stroke volume, cardiac output and left ventricular ejection time between the two groups. Total peripheral vascular resistance, however, was significantly higher in the prehypertensives. Moreover, the pulse pressure over stroke index ratio as a proxy for systemic arterial stiffness was

increased as well in these prehypertensives.

Taken together, the results of the various studies using different populations and different methodology are rather consistent in the sense that they suggest that even in prehypertension the peripheral vasculature is the main source of the elevated pressure. Moreover, an increase in vascular stiffness is a uniform finding (8, 10-15). Undoubtedly, abnormalities in the microcirculation contribute to enhanced vascular stiffness on the one hand and an increased burden to the heart on the other.

Comparison with established hypertension

As little information, there is concerning hemodynamics in prehypertension, as much is there about hemodynamic patterns in patients with established hypertension (6). There is general agreement that in those in whom hypertension is still uncomplicated, the elevated pressure is maintained by an increased total peripheral resistance. By and large, heart rate remains higher in the hypertensives as well, but cardiac output is either normal or only slightly reduced.

In the Strong Heart Study, prehypertensives were not only compared to normotensives but also to hypertensives with respect to their hemodynamic indices (8). These data also show that heart rate was significantly higher in the hypertensives while cardiac index was similar. Total peripheral vascular resistance, when indexed for body surface area, was significantly greater in the hypertensives as well. The pulse pressure to stroke index ratio, as proxy for vascular stiffness, was clearly greater in the hypertensives compared to the normotensives with the prehypertensives taking an intermediate position.

In their twin study, Davis and coworkers found significant trends across their groups of normotensives, prehypertensives and hypertensives with respect to heart rate, cardiac index, pulse pressure and vascular stiffness (11). These were all lowest in the normotensives and highest in the hypertensives. The opposite trend was seen for brachial artery distensibility which was lowest in the hypertensives. Except for pulse pressure, however, post-hoc analysis failed to find statistical differences in any of these variables between the prehypertensives and the hypertensives. Total peripheral vascular resistance was not different across or between the three groups.

Even though conventional significance levels were not reached in most of the post-hoc analyses, the trends are clearly in agreement with the data from the Strong Heart Study in that the 'transition' from prehypertension to frank hypertension is associated with an invariably increased heart rate, no or only small changes in cardiac output, and a further rise in arterial stiffness. In numerical terms, vascular resistance may remain unaltered but even then, it signifies an inability to vasodilate properly in response to a normal or enhanced systemic flow.

In our own study on the medical students, we also compared the prehypertensives to a group of matched hypertensives (unpublished data). The latter

had a lower cardiac index and a higher vascular resistance and stiffness, without any difference in heart rate. Regarding the vascular abnormalities, therefore, these data also tally well with the previous ones.

Regional hemodynamics

Total peripheral resistance is the sum of the resistances (calculated as for parallel circuits) in the various organs of the body. The magnitude of resistance to blood flow in any single organ determines which fraction of the cardiac output will be directed to it. Thus, if we would be able to simultaneously measure cardiac output and regional flows we could explore whether the rise in total resistance is a generalized phenomenon or preferentially occurs in specific organs. A rise in resistance occurs in all vascular beds that have been studied in hypertensives but it is particularly striking in that of the kidney (16). Renal fraction, which is the proportion of cardiac output that flows through the kidneys, falls with age in hypertensives, indicating that the degree of vasoconstriction in the kidney becomes progressively greater than the rise in resistance elsewhere in the body. However, it is impossible to tell whether this preferential renal vasoconstriction is the cause or the consequence of a higher blood pressure.

Even in this established phase of the hypertensive process glomerular filtration rate is well maintained for a long time so that filtration fraction, which is defined as glomerular filtration rate as a percentage of the renal plasma flow gradually rises with the increase in renal vascular resistance. This suggests that the postglomerular resistance increases faster or more than preglomerular resistance. Only when the delivery of blood to the kidneys becomes severely compromised, filtration will fall. Although these pathophysiological features have been well described for established hypertension, only limited information is available with respect to the early phases of hypertension. If we turn again to borderline hypertension, the data from Messerli and coworkers on the renal and the splanchnic vascular beds are of relevance. These investigators studied 41 patients with borderline hypertension who were subdivided in groups with low, normal, or high cardiac output (17). Except for cardiac output they also measured renal and splanchnic blood flow by means of radio-iodinated PAH and indocyanine green clearance, respectively. Both renal and splanchnic blood flow correlated significantly with cardiac output indicating that, at least in this patient population, the fractional distribution of systemic flow to the kidneys and the splanchnic organs remains unaltered. In other words, the observed increase in vascular resistance at this stage is generalized and not preferential in, for instance, the kidneys. In a later study, Messerli's group explored the relationship of renal blood flow and cardiac output with age in normotensives and in borderline hypertensives (18). In both groups, they found a parallel decline in systemic and renal flow with ageing. In other words, at any age the distribution of cardiac output over the kidneys and probably other organs is comparable in normotensives and borderline hypertensives.

Thus, if there is no sustained hypertension, there is no preferential vasoconstriction in the renal vasculature.

Although a few studies have addressed regional flow patterns in prehypertension, no such data exist in combination with estimations of cardiac output except those from our own study in the medical students. In those, renal fraction was not different either between the normotensives and the prehypertensives and, if anything, even slightly higher in the latter (22 vs 20%). Renal vascular resistance in the prehypertensives was numerically comparable to that in normotensives, but given the slightly higher blood pressure in the former, one could still consider this as being too high.

Despite the increase in renal vascular resistance, perfusion of the kidneys was even somewhat greater in the prehypertensive students than in their normotensive counterparts. Such a pattern of relative 'overperfusion' has been seen in other studies as well and seems to 'affect' about one-third of young people in their early stages of hypertension (19, 20). The reason for the increased flow rate is not clear but may involve a mechanism to protect the glomeruli. Indeed, when glomerular filtration rate remains intact for a very long time despite a progressive decline in renal plasma flow, this will lead to an increased filtration fraction just as in patients with established hypertension. It is thought that a rise in postglomerular resistance is necessary to maintain filtration in the face of an enhanced preglomerular resistance but this may also expose some glomeruli to the detrimental effect of an augmented intraglomerular pressure. If the kidney now recruits dormant nephrons and increases total flow in order to perfuse these recruited nephrons, the filtration process can be divided over a greater surface area without the necessity to raise pressure in these glomeruli. This hypothetical sequence of events, however, needs to be confirmed in proper experiments.

As for other organs, there is a study from Turkey in 40 individuals with prehypertension and 50 healthy volunteers who underwent transthoracic Doppler echocardiography to assess cardiac dimensions and coronary flow reserve (CFR).⁽²¹⁾ The two groups did not differ with respect to left ventricular mass and heart rate but CFR was significantly lower in the prehypertension group. Although these data point towards an increased resistance in the coronary vascular bed of prehypertensives, it is impossible to know whether this increase is proportional to that of systemic vascular resistance.

Finally, Italian investigators have shown that in people with prehypertension frequently abnormalities of the retinal circulation are found, including arteriolar narrowing and, consequently, a reduced arteriolar-to venular ratio (22).

Follow-up studies

All of the data described above have been obtained in cross-sectional studies which have only limited value for our understanding of the natural evolution of the hypertensive process. Thus, longitudinal studies are indispensable to explore how hemodynamics change over time. By far the most informative (and only) long-term study in this regard is that of Lund-Johansson (6). This investigator has followed a group of young hypertensive individuals and age-matched normotensive controls for a period of 20 years with similar invasive hemodynamic measurements after 10 and 20 years. Although the hypertensives had slightly elevated blood pressures which precluded a diagnosis of borderline hypertension, they could be considered to be in a very early phase of hypertension that still did not require treatment. At the start of the study, heart rate and cardiac index were about 15% higher in the hypertensives who were then 17 to 29 years of age. After 10 years, blood pressure had changed remarkably little. Nevertheless, total peripheral resistance had increased significantly, while cardiac index and stroke volume index had fallen. Compared to the normotensives, heart rate remained elevated. During the following 10 years, all these changes progressed so that at the 20-year follow-up evaluation cardiac performance was even lower and vascular resistance higher with only minor changes in heart rate.

In our laboratory, we performed repeat examinations of systemic and renal hemodynamics in the prehypertensive group of medical students as well as in the matched hypertensives after two years of follow-up (13). During this time only the hypertensive participants received antihypertensive medication which was discontinued prior to the measurements. Although cardiac output and stroke volume showed a tendency to fall over the two-year period in the prehypertensives, the differences were not statistically significant. The same was true for total peripheral resistance which tended to rise slightly. Heart rate did not change and arterial stiffness remained invariably increased. In the hypertensives, cardiac output fell to a greater extent, together with a rise in resistance and arterial stiffness. Renal blood flow fell slightly in both the prehypertensives and the hypertensives with a rise in renal vascular resistance that was proportional to that in systemic resistance in both groups.

Pathophysiological considerations

According to the classical concept of whole-body autoregulation an increased cardiac output will elicit a vasoconstrictor response to prevent overperfusion of tissues and a disturbance of homeostasis (23). This, in turn, will bring back cardiac output to its original level but at the expense of a raised vascular resistance and, hence, an increased blood pressure. It has long been thought that this sequence of events, which was based on observations in experimental animals, would be applicable to hypertensive

humans as well. Many of the hemodynamic observations that have been obtained in patients in different stages of their hypertension do, indeed, suggest that also in man hypertension evolves from a high output, normal resistance state into a low output, high resistance state. The high output state at the early phase of hypertension or during the period of prehypertension is often explained by enhanced sympathetic activity or altered volume homeostasis. The increase in resistance over time is then seen as the equivalent of the whole-body autoregulation mechanism. There are, however, several arguments against the hypothesis of this hemodynamic transition. First, a high output state does not necessarily lead to an increased resistance or to hypertension. Clinical examples include severe anemia, hyperthyroidism, arteriovenous anastomoses as in Paget's disease, beri-beri, and Gorlin's syndrome. These are all conditions in which cardiac output may sometimes be extremely high, yet is not followed by a (progressive) rise in vascular resistance. Secondly, an autoregulatory vasoconstrictor response occurs only when tissue perfusion exceeds metabolic demands (so-called luxury perfusion) but this does not occur in humans (6). Indeed, the rise in cardiac output is entirely proportional to oxygen consumption. Thirdly, not all patients with borderline hypertension or prehypertension have an increased cardiac output. Thus, a hyperkinetic circulation is not at all a prerequisite to develop sustained hypertension. Finally, there are patients with high-output borderline hypertension or prehypertension who will never progress to the state of hypertension and sometimes may even 'regress' again to normotension.

As a matter of fact, there is no need to invoke a cardiac driver of hypertension if we focus more on vascular resistance itself. As already outlined above, even a numerically normal vascular resistance is still elevated in the face of the prevailing level of cardiac output, regardless of whether output is increased or not. With a high systemic flow that is appropriate in relation to tissue demands, the normal response would be peripheral vasodilation to prevent a rise in blood pressure. Thus, effectively all hemodynamic studies point to a disturbance of vasoregulation, even in the very early stages of hypertension or prehypertension. If we accept the fact that hypertension always starts as an abnormal vasoconstrictor state (from whatever cause), we could see an increased variability of blood pressure and cardiac output just as secondary phenomena. Whether cardiac output will be normal or high will then depend on what 'force' is needed for adequate tissue perfusion.

Collectively, the available data strongly suggest that in prehypertension and borderline hypertension or, for that matter, the early stages of hypertension there is no preferential increase in vascular resistance in any specific organ and certainly not in the kidney. This renders an initiating role of (relative) renal ischemia as the cause of hypertension less likely. It is beyond the scope of this chapter to elaborate on the possible causes of the abnormal resistance but likely genetic, endothelial, and neurohumoral factors will play an important role. Whatever mechanisms are involved, any theory on the pathogenesis of hypertension must account for this generalized,

hence non-localized, increase in resistance.

Conclusions

If we try to reconcile the findings described above in a hypothetical scheme concerning the development of hypertension, it is likely that the transition from normotension to established hypertension may first go through a phase of prehypertension and then borderline hypertension. Likely, the duration of these phases is variable and unpredictable with some people progressing very fast and others staying in one of these phases for a long time with perhaps even a return to normal pressures. The general increase in resistance as seen in prehypertension causes only a minor rise in blood pressure which is maintained because cardiac output cannot fall because of the metabolic demands. In principle, this phase can last for a long time. On the long run, systemic resistance probably further increases due to (inappropriate) vascular remodeling, i.e. increased vascular stiffness, resulting in propagation to borderline and established hypertension. Perhaps it is only when the renal fraction falls and the kidney gets jeopardized that the transition to full-blown hypertension is set in motion with the kidney now probably taking on a culprit role. While these are hypothetical thoughts, they may form a good starting point for future hemodynamic studies in prehypertension and hypertension.

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Chapter 6

Renal and systemic hemodynamics in prehypertension and hypertension.

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Abstract

Prehypertension (i.e. SBP 120-139 and/or DBP 80-90 mm Hg) is an increasingly important clinical problem due to its increased risk on progression to overt hypertension and its association with cardiovascular morbidity and mortality. It is well established that the kidneys play an important role in the etiology of essential hypertension, however it is still incompletely understood whether the kidneys are victim or culprit in the development of hypertension, especially in the earlier stages of the hypertensive process. Therefore we aimed to assess whether there are any differences in renal and systemic hemodynamics between young prehypertensive males, and age and sex-matched normotensive and hypertensive controls. We measured systemic and renal hemodynamic parameters such as cardiac index (CI), heart rate (HR), total peripheral resistance (TPR), stroke-volume to pulse pressure ratio (SV/PP), plasma volume (PV), glomerular filtration rate (GFR, inulin clearance), renal plasma flow (RPF), renal fraction (RF), filtration fraction (FF), and renal vascular resistance (RVR). Additionally, we measured concentrations of noradrenalin, aldosterone, and active plasma renin. In total, 60 males aged between 18 and 21 years (20 prehypertensives, 20 hypertensives, and 20 normotensive controls) participated in the study. CI was comparable between normotensives and prehypertensives, but was significantly lower in hypertensives ($p < 0.001$). TPR rose across the three groups, but was most pronounced in hypertensives. PP/SV tended to follow a similar pattern, but was not statistically significant. GFR was lower across the three groups, but was at a comparable level between prehypertensives and normotensives. Prehypertensives had higher RPF than both normotensives and hypertensives, although FF and RF remained constant across all three groups. RVR increased progressively across groups. Noradrenaline levels were comparable between all three groups, whereas APRC was lower in both prehypertensives and hypertensives. Aldosterone was subsequently higher in prehypertensives and hypertensives. Prehypertension is characterized by increasing peripheral and renal resistance and arterial stiffness, without indications of preferential renal vasoconstriction. Prehypertensives seemed to have renal hyperperfusion since RPF was increased, although GFR, FF and RF were unaltered, which possibly reflects an autoregulatory mechanism. There was no evidence for sympathetic hyperactivity. We conclude that prehypertension is an intermediate stage between normotension and established hypertension in which the kidney seems to compensate for changing hemodynamics.

Introduction

Despite epidemiological data showing that prehypertension, currently defined as a systolic blood pressure (SBP) between 120 and 139 mmHg and/or a diastolic blood pressure (DBP) between 80 and 90 mmHg, is associated with an increased risk of cardiovascular and renal complications[1,2], relatively little is known about the pathogenesis of this condition. The pathophysiological features that characterize uncomplicated established hypertension include a rise in systemic and renal vascular resistance with a normal or reduced cardiac output, reduced renal blood flow with preserved glomerular filtration rate, increased filtration fraction and a tendency towards a lower plasma volume and lower renin levels.[3,4] However, whether similar characteristics can be found in prehypertension, is largely unknown.

Studies in patients with 'borderline' hypertension (which like prehypertension is considered to be an early stage of hypertension) have repeatedly shown that, on average, their cardiac index and heart rate are increased. [5] Although peripheral vascular resistance in these patients was numerically normal, it was already inappropriately high for the level of cardiac output. This has fueled the hypothesis that hypertension, even in its early stage, is caused by vascular rather than cardiac abnormalities. Additionally, other studies have found that in contrast to established hypertensives, renal fraction was unaltered in borderline hypertensives and that renal and systemic blood flow decreased in parallel with advancing age.[6,7]. Such data argue against preferential vasoconstriction in the kidney.

In all likelihood, patients with borderline hypertension who, by definition, regularly cross the line of normality, are one step ahead of those with prehypertension. Thus, the latter group seems to be more appropriate for studying the very early changes that may herald a development into the direction of permanent hypertension. Therefore, the aim of the present study was to investigate possible differences in the hemodynamic and hormonal profile of volunteers with prehypertension as compared to those of normotensive controls and patients with established hypertension. We hypothesized that even prehypertension is associated with a generalized increase in vascular resistance and a compensatory suppression of other pressor systems.

Methods

Population

We asked young, male medical students aged 18-21 years of age to volunteer for this study. Those who agreed to participate were screened by measuring office blood pressure on three different occasions. Volunteers who at the third visit had a systolic blood pressure (SBP) between 120 and 140 mmHg and/or a diastolic blood pressure (DBP) between 80 and 90 mmHg were labeled as prehypertensive, while those with a normal blood pressure (i.e. < 120/80 mmHg) were considered to be normotensive. We

invited age- and sex-matched patients from the outpatient clinic of internal medicine who had established hypertension (i.e. SBP > 140 mmHg or DBP >90 mmHg) but were still untreated to serve as an established hypertensive control group. Participants were excluded if they had signs of preexisting renal damage as indicated by a history of kidney disease, an estimated glomerular filtration-rate (eGFR) < 90 ml/min/1.73m² or the presence of urinary albumin excretion (UAE). The study was conducted in accordance with the Declaration of Helsinki and all participants provided written informed consent.

Protocol

After screening for eligibility and inclusion in the study, patients were asked to adhere to a fixed dietary sodium intake of 55 mmol/day for a duration of seven days prior to the baseline study visit. Adherence to the diet was tested using 24-hour urine collections. All participants were studied after an overnight fast and they had been asked to refrain from smoking. At the study visit, blood pressure and heart rate (HR) were measured at 10-minute intervals during a period of two hours, using an automated oscillatory blood-pressure measurement device (Dinamap 845, Criticon inc., Tampa, Florida, USA). We measured the height and weight of participants and calculated the body surface area using the Du Bois formula. [8] Before the start of renal function measurements, a peripheral intravenous canula was inserted after which participants remained supine in a quiet room. After 30 minutes, fasting blood samples were obtained for determining biochemical and hematological markers such as serum sodium, potassium, creatinine, and hematocrit, as well as the measurement of plasma noradrenaline, and levels of renin and aldosterone. Then, a second intravenous canula was inserted in the opposite arm for the continuous infusion of tracers required for renal function measurements as detailed below.

Measurement of systemic hemodynamics

Stroke volume (SV) was determined using impedance cardiography and cardiac output (CO) was calculated as $CO = SV \times HR$. We adjusted cardiac output for the BSA and averaged the cardiac index (CI) readings of 16 cardiac cycles. Total peripheral vascular resistance (TPR) was calculated using the formula $TPR = MAP \times 80 / CO$. We calculated the ratio between pulse-pressure to stroke-volume ratio (PP/SV) which is an indicator of total arterial stiffness.[9]

We also estimated plasma volume (PV) using the indicator dilution technique with intravenously administered radio-iodinated human serum albumin (RISA) as the tracer substance. [10]

Renal hemodynamics

We determined glomerular filtration rate (GFR) and effective renal plasma flow (ERPF) from the clearance of continuously infused inulin (Inutest, Boehringer-Mannheim,

Germany) and ^{125}I -Hippuran, respectively. Assuming an extraction ratio (ER) of 75%[11], we calculated total renal plasma flow (RPF) as ERPF/ER . Total renal blood flow (RBF) was calculated as $\text{RBF} = \text{ERPF}/(1-\text{Ht})$, where Ht is the participant's hematocrit. Filtration fraction (FF) was calculated by dividing GFR by RPF. Renal vascular resistance (RVR) was calculated using the equation $\text{RVR} = \text{MAP} \times 80.000/\text{RBF}$. Finally, renal fraction (RF), which is the percentage of CO that is flowing through the kidneys, was calculated as $\text{RF} = \text{RPF}/(\text{CO} \times 100\%)$. All measurements were standardized for body surface area (BSA).

Hormonal measurements

Active plasma renin concentration (APRC) was measured by an immunoradiometric assay. [12] Aldosterone concentrations were determined by radioimmunoassay. Noradrenaline was assayed by an HPLC method.

Statistical analysis

All data are expressed as means plus standard deviations (SD) unless specified otherwise. To test for trends and between-group differences, we used one-way analysis of variance (ANOVA) with planned contrasts for normally distributed data. For non-normally distributed data, we used non-parametric tests (e.g. Kruskal-Wallis-test). For all statistical analyses, we used IBM SPSS Statistics version 24 (IBM, Chicago, United States of America). We accepted a significance level (α) of 0.05 as statistically significant. Based on data from a previous study in a comparable population [13], we calculated that for the main outcome measures (renal hemodynamics) we would

Table 6.1 Characteristics of the study population

	Normotensives (N=20)	Prehypertensives (N = 20)	Hypertensives (N = 20)
Age (years)	21 ± 2	20 ± 2	22 ± 3
Height (cm)	183 ± 4	183 ± 5	165 ± 4
Weight (kg)	69 ± 9	74 ± 7	64 ± 16
Body surface area (m ²)	189 ± 8	196 ± 11	170 ± 23
Body mass index (kg/m ²)	20.6 ± 0.8	22.1 ± 1.1	23.5 ± 2.3
Systolic blood pressure (mmHg)	116 ± 8	136 ± 12	144 ± 12
Diastolic blood pressure (mmHg)	72 ± 5	79 ± 8	83 ± 6
Pulse pressure (mmHg)	44 ± 9	57 ± 14	61 ± 13
Mean arterial pressure (mmHg)	86 ± 5	98 ± 7	104 ± 9
Heart rate (beats /min)	65 ± 11	61 ± 13	63 ± 9
eGFR (ml/min/1.73m ²)	122 ± 14	122 ± 15	108 ± 16

require a sample size of 20 participants per group (statistical power 80%, $\alpha = 0.05$; two-sided testing).

Results

The mean age for the entire cohort of 60 participants was 21 ± 2.5 years. The characteristics of the three separate groups are described in **Table 6.1**. Participants were comparable with regard to age. Hypertensives and prehypertensives had a significantly higher BMI than normotensives (ANOVA $p < 0.001$ for trend). Normotensives had a mean blood pressure of 116/72 mmHg and were therefore truly normotensive, whereas the mean blood pressures of prehypertensives and established hypertensives were 136/79 and 144/83 mmHg respectively (**Table 6.1**). There were no differences in heart rate between the three groups. There was no difference in eGFR between the groups.

Systemic hemodynamics

Figure 6.1 shows the systemic hemodynamics of the three groups. CI was significantly lower in hypertensives (ANOVA $p = 0.003$) but was comparable between

Figure 6.1 Systemic hemodynamic characteristics in normotensives, prehypertensives, and hypertensives

Abbreviations: CI: cardiac index; TPR: total peripheral resistance; PV: plasma volume; SV/PP: stroke volume to pulse pressure ratio. NT: normotensives; PreHT: prehypertensives; HT: hypertensives. Bars represent means + 95% confidence intervals.

Figure 6.2 Renal hemodynamic characteristics in normotensives, prehypertensives, and hypertensives

Abbreviations: GFR: glomerular filtration rate; RPF: renal plasma flow; RVR: renal vascular resistance; FF: filtration fraction; RF: renal fraction; NT: normotensives; PreHT: prehypertensives; HT: hypertensives. Bars represent means + 95% confidence intervals

prehypertensives and normotensives. In comparison to normotensives, TPR tended to be higher in both prehypertensives and established hypertensives (ANOVA $F(2,57) = 8.0$, $p < 0.001$ for trend) with the largest difference between prehypertensives and established hypertensives. Compared to normotensives, PV was significantly lower in prehypertensives and established hypertensives but there was no difference between prehypertensives and hypertensives. Pulse-pressure to stroke volume tended to be higher in both prehypertensives and hypertensives, but this trend did not reach statistical significance.

Renal hemodynamics

The results of the renal hemodynamic measurements are displayed in **Figure 6.2**. GFR, adjusted for BSA, tended to be progressively lower in prehypertensives and hypertensives when compared to normotensives (ANOVA $F(2,57) = 3.5$, $p < 0.05$ for trend). Although RPF tended to be higher in prehypertensives, as compared to both normotensives and established hypertensives (650 ± 170 vs. 609 ± 171 and 617 ± 101 ml/min/1.73 m² respectively), the difference did not reach statistical significance. Renal vascular resistance increased across the three groups (ANOVA $F(2,57) = 13.9$, $p < 0.001$) whereas FF and RF were comparable between groups.

Hormonal aspects

In **Figure 6.3** the endocrinological measurements have been summarized. With regard to renin, levels of APRC showed a significant downward trend across the groups, with the lowest values in established hypertensives (ANOVA $F(2,57) = 8.7$, $p < 0.001$ for APRC for trend). Conversely, aldosterone tended to be higher in prehypertensives and hypertensives (ANOVA $F(2,57) = 8.2$, $p < 0.001$). In contrast, noradrenaline levels were not significantly different between the three groups.

Discussion

The ideal way to study the pathogenetic mechanisms leading to hypertension would be to follow a normotensive population for a prolonged period of time up to the point where individuals become hypertensive. As such an approach is not realistic, the best alternative is to study, in a cross-sectional way, people who are in different stages of their development from a true normotensive to a true hypertensive state. In the past, patients with borderline or, as it was sometimes called, labile hypertension served as a model for this transitional state. Since then, however, it has become increasingly clear that even the borderline hypertensive period should already be considered as a relatively 'late' phase. People with blood pressures in the prehypertensives range who do not yet have such upward swings in pressure above the 140/90 mmHg threshold are, therefore, better representatives of the early stages of hypertension.

Figure 6.3. Hormonal data in normotensives, prehypertensives, and hypertensives.

Abbreviations: APRC: active plasma renin concentration; NT: normotensives; PreHT: prehypertensives; HT: hypertensives. Bars represent means + 95% confidence intervals.

Systemic hemodynamics in prehypertension

In the present study, we found that heart rate and cardiac index were comparable in normotensives and prehypertensives. The difference in blood pressure between these two groups must, therefore, be ascribed to a difference in total peripheral vascular resistance. Given the trend that we observed across the three groups, our data suggest that vasoconstriction already begins in prehypertension and continues to rise when established hypertension develops. This is in line with other studies showing an elevated TPR in prehypertensives. [14,15] For instance, data from the Strong Heart Study showed that both cardiac index and peripheral resistance index were significantly increased in prehypertensives. [9] We also found that PP tended to be higher and the SV/PP-ratio lower in prehypertensives, although our study was of insufficient sample size to reach statistical significance. Nevertheless, this points towards an early rise in arterial stiffness, which is a common finding in other studies as well. For example, in the Strong Heart Study the PP/SV-index also tended to be higher in prehypertensives. [16] In the same study, PWV was significantly higher in prehypertensives, compared to normotensives. [14] Several other studies have also reported that aortic PWV is increased in prehypertensives compared to normotensives. [17,18] Interestingly, increased arterial stiffness in prehypertensives was also shown to be associated with the development of incident hypertension over time. [18] Since the combination of an elevated peripheral resistance and increased arterial stiffness is commonly observed in hypertensive arterial remodeling, these data support the hypothesis that hypertrophic remodeling of both large arteries and resistance arteries is an early process in the development of essential hypertension and that prehypertension indeed may be an intermediary situation between normotension and established hypertension.

Renal hemodynamics in prehypertension

Our results indicate that renal vascular resistance increases *pari passu* with systemic

vascular resistance when going from normotension via prehypertension to established hypertension. Thus, vasoconstriction also occurs in the prehypertensive kidney. Since there was no difference in renal fraction between prehypertensives and normotensive or hypertensive controls, our data suggest that vasoconstriction was not greater in the kidneys than in the systemic vasculature. Therefore, in contrast to later stages of hypertension[4], there seems to be no preferential renal vasoconstriction in prehypertensives. Although overall GFR showed a downward trend across the groups, it was only slightly lower in prehypertensives compared normotensives. Moreover, there was no difference in filtration fraction between the three groups. This suggests that (intra)renal hemodynamics are relatively preserved in prehypertension.

Data on the renal circulation in the early stages of hypertension are scarce and sometimes provided conflicting results. For instance, Schmieder et al. demonstrated in borderline hypertensives that renal blood flow was reduced and filtration fraction increased whereas London et al. showed that renal blood flow and cardiac output were increased in borderline hypertensives, while filtration fraction remained constant. [19,20] Possibly, the participants in the former study may already have been in a more advanced stage in the hypertensive process in which renal perfusion falls and filtration fraction rises.

We also found that in comparison to normotensives, active plasma renin levels were suppressed in prehypertensives although not as much as in hypertensives. It is conceivable that in the face of a rising blood pressure, however small, the body tries to compensate for that abnormality by inhibiting other pressure systems such as renin secretion. In this regard, a likely mechanism would be that the increased renal vascular resistance leads to increased preglomerular pressure, which in turn suppresses renin secretion by the juxtaglomerular cells. Perhaps the slight lower plasma volume that we observed in the prehypertensives should also be interpreted in terms of an adaptive phenomenon. Notwithstanding the lower renin levels, aldosterone levels increased as blood pressure was higher. Although it is not clear why this occurs, an elevated aldosterone levels may be secondary to the reduced intravascular volume. Finally, noradrenaline levels were not different between groups which argues against significant sympathetic activation.

The present study has some limitations. Since it was a relatively small study, some trends did not reach statistical significance which may have been caused by lack of statistical power. Also, prehypertensives and hypertensives had a higher body-mass index than normotensives. Obesity is an established risk factor for the development of hypertension and is commonly present in prehypertensive persons.[21] However, since all measures were adjusted for body surface area, this should not have significantly influenced the interpretation of our data. The strength of present study is that it is one of few studies comparing regional renal hemodynamics between age- and sex-matched prehypertensive participants and both normotensive and hypertensive controls.

Conclusion

We conclude that in comparison to normotensives, prehypertensives have elevated peripheral and renal vascular resistance, and tend to have elevated arterial stiffness although not as pronounced as in established hypertension. Our findings further suggest that systemic and renal hemodynamics as well as plasma volume, renin and aldosterone are already altered before established hypertension develops and that prehypertension can be considered as an intermediary phase between normotension and established hypertension.

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Chapter 7

General Discussion

The natural history of hypertension

Already for more than a century, the etiology of essential hypertension is the subject of extensive research. A common view is that in the natural course of essential hypertension blood pressure evolves from true normotension, via pre-hypertension (i.e. high-normal blood pressure), to borderline hypertension (occasional blood pressure elevations above the threshold of high-normal), to established hypertension that, when left untreated, ultimately develops into malignant hypertension. Although in the past decades cut-off values for blood pressure in clinical guidelines have been defined -and redefined-, it is clear that cardiovascular risk linearly increases with rising blood pressure starting from as low as 115/75 mmHg. [1] In addition, hypertensive organ damage does not only occur in patients with established high-grade hypertension, but also in patients with pre-hypertension. [2] Thus, hypertension and its consequences should be considered as the expression of a continuous process without a specific threshold that separates normal from abnormal. [3]

Although the pathophysiological mechanisms underlying essential hypertension and the development of hypertensive organ damage have been studied in great detail, the precise mechanisms which lead to a rise in the pressure are still poorly understood. Nevertheless, it has become increasingly clear that arterial remodeling plays a central role. In this thesis, we aimed to present clinical studies in people with essential hypertension focusing specifically on the role of arterial remodeling and its systemic and renal hemodynamic consequences.

Arterial remodeling in hypertension

Arterial remodeling involves structural and functional changes in both large and small arteries. [4] It results from many different pathophysiological mechanisms that are regulated via complex and often interrelated pathways. Despite many years of research, these pathways have still not been fully elucidated. In chapter 2, we reviewed several biological and biochemical pathways associated with arterial remodeling. Although atherosclerosis is also a form of arterial remodeling which often occurs in patients with hypertension and cardiovascular disease, we focused in this thesis primarily on arteriosclerotic arterial remodeling. Nevertheless, it is important to keep in mind that both arteriosclerosis and atherosclerosis can occur simultaneously, and some pathophysiological mechanisms are shared by both types of arterial remodeling (Chapter 2).

Arterial remodeling differs not only between different types of cardiovascular disease but is also dependent upon age, gender and even arterial site. Indeed, the arterial system is not homogeneous with regard to structural, mechanical and functional properties. [5] For example, the arterial tree can be divided into a proximal (central) component

Figure 7.1. Arterial segments and corresponding vascular wall characteristics.

consisting of mainly large elastic arteries, a distal (peripheral) compartment with predominantly muscular arteries, and finally a compartment consisting of arterioles and capillaries.[6] The elastic arteries are compliant and mainly have a conductive function. Towards the periphery, arteries gradually transform from a compliant, conductive type into a more resistance one, ultimately becoming a mostly resistance-type artery at the level of the arterioles (**Figure 7.1**).[6] These specific properties are important for the study of arterial remodeling in essential hypertension, since different types of remodeling have been observed in different arterial segments. In the proximal aorta (which is an elastic conduit artery), remodeling is primarily characterized by increased length and vessel diameter, increased wall thickness, increased tortuosity and reduced distensibility.[7-9] In the proximal, mixed-type arteries, outward and hypertrophic remodeling is observed in essential hypertension which involves increased intima-media thickness (IMT), lumen enlargement, and increased vessel wall stiffness.[4,10] In contrast, the remodeling observed in small resistance arteries (i.e. small arteries and arterioles) is predominantly inward and eutrophic of nature and is characterized by medial hypertrophy, reduced lumen and external diameter but with unchanged medial cross-sectional area (CSA).[4] Since the resistance arteries greatly influence resistance to flow, even small alterations in their function or structure have significant hemodynamic consequences. [11] Resistance artery remodeling leads to increased peripheral vascular resistance and reflects a vasoconstrictive state, possibly resulting from an impaired vasodilation reserve, an elevated vasoconstrictive state, and altered vessel-wall distensibility. [4,12] Hypertrophic remodeling of resistance arteries has also been observed in secondary forms of hypertension or in association with diabetes mellitus or obesity. [13,14] It has been proposed that over the course of essential hypertension, transition from eutrophic inward to hypertrophic remodeling in resistance arteries may also occur[4,12]. However, there is little longitudinal clinical data available which have systematically assessed changes of arterial remodeling over

time in different vascular regions in patients with essential hypertension.

Systemic hemodynamic aspects of arterial remodeling

The structural and functional changes observed in arterial remodeling are thought to be a homeostatic process to relieve pulsatile mechanical tension that the blood pressure exerts on the arterial wall. According to Young-Laplace's equation ($\delta_\theta = [p \cdot R]/h$), that estimates mechanical stress acting in the tangential direction of the vessel wall (i.e. hoop stress), and that is created by internal pressure on a thin-walled cylinder[15], circumferential wall stress (CWS; δ_θ) increases when circumferential wall tension (CWT), which is the product of mean blood pressure (MBP; p) and lumen diameter (LD, R) rises, while wall thickness (h) remains constant or decreases. Therefore, in order to normalize CWS with rising blood pressure, either wall thickness needs to increase proportionally (i.e. wall hypertrophy), or the vessel lumen diameter needs to decrease (i.e. inward remodeling). When arterial remodeling fails in normalizing CWS or CWT, it is termed maladaptive arterial remodeling.

A consequence of arterial remodeling is that the elastic properties of the arterial wall are altered. Increased wall thickness, an altered collagen/elastin ratio, medial calcification, and other structural remodeling of the vascular wall all contribute to reduced distensibility of the vascular wall and increased arterial stiffness. [12] As described in more detail in Chapter 2, arterial stiffness increases the speed with which the blood pressure waves travel along the blood vessel (due to impaired buffering capacity) and this in turn leads to augmented systolic blood pressure, decreased diastolic blood pressure and therefore elevated blood pressure pulsatility (**Figure 2.4**). It is this blood pressure pulsatility that is thought to be an important factor in not only the progression of arterial remodeling, but also in the development of hypertensive target-organ damage. Not surprisingly, therefore, it is an independent cardiovascular risk factor. [16,17] The hypothesis that arterial remodeling is an adaptation to rising blood pressure has been generally accepted. However, the question remains whether arterial remodeling in itself may also be a cause of elevated blood pressure. In order to answer this question, longitudinal studies comparing arterial properties of normotensives to those in patients with established hypertension and pre-hypertension are required.

Carotid remodeling in essential hypertension

In Chapter 3 we investigated whether there are differences in the longitudinal development of carotid artery remodeling between normotensives and patients with established hypertension. The common carotid artery is a large elastic conduit artery that has been extensively investigated in clinical studies on arterial

remodeling in cardiovascular disease because of its easy accessibility for non-invasive ultrasonographic imaging. Many of these studies have focused on the presence of atherosclerotic plaques and subclinical atherosclerosis as reflected by increased carotid intima-media thickness (IMT) in populations with different cardiovascular risk. They have consistently shown that hypertension is positively and independently associated with an increased carotid IMT and that this association is enhanced in the presence of other risk factors. [18,19] The association was also evident in hypertensive children and adolescents suggesting that it is not only an age-associated phenomenon. [20] A meta-analysis by Kollias et al. showed that both central and brachial blood pressure were associated with an elevated carotid IMT, but that pulse pressure (PP) exhibited the strongest association. [21] The fact that a higher systolic blood pressure (SBP), a greater PP and an increased pulse wave velocity (PWV) show a relationship with carotid IMT, further supports the hypothesis that increased blood pressure pulsatility and arterial stiffness are driving factors for arterial remodeling. [22-25] This is also true for pre-hypertensive patients. [26] In addition, the presence of hypertension or a higher systolic pressure are predictive of progression of IMT over time in longitudinal studies [27-30], although this has not always been confirmed. [31] In our study, although we found that in hypertensives both baseline and follow-up IMT were significantly higher than in normotensives, IMT did not change over time. In contrast, IMT rose significantly in normotensive controls (Chapter 3). In the study by Rosvall et al. the presence of hypertension was independently associated with progression of common carotid IMT over 16 years of follow-up. [30] Interestingly, in this study, the annual rate of change in IMT was determined by the change in SBP between the baseline and the follow-up investigations. When changes in blood pressure amounted to -6 to +5 mmHg no changes in IMT were observed. [30] In our study, we observed a similar pattern in both hypertensives and normotensives.

In contrast to the large number of studies dedicated to IMT, less data is available regarding other indicators of carotid artery remodeling and whether remodeling is adaptive or maladaptive. Therefore, we measured LD, CSA, CWT and CWT in addition to IMT (Chapter 3). We showed that in comparison to normotensives, hypertensives have outward, hypertrophic remodeling as indicated by higher levels of LD, CSA and IMT. Based on the higher CWT and CWS we can conclude that this remodeling is maladaptive. This is in accordance with other studies that have demonstrated an association between hypertension and maladaptive carotid artery remodeling. [32] For instance, in a cross-sectional study by Jiang et al. carotid circumferential wall tension was associated with cardiovascular risk factors, including hypertension, as well as with increased IMT. [33] In addition, we showed that the carotid diameter and wall thickness did not significantly change over time in hypertensives, but that both CWT and CWS fell. These data suggest that although there is no regression of arterial remodeling in the hypertensives, stress and tension still fell, possibly reflecting the influence of treatment. In normotensives, however, IMT, LD, CSA and CWT rose

significantly, whereas CWS remained constant. This pattern reflects active outward, hypertrophic remodeling that still manages to keep the CWS constant over time despite increasing tension (i.e. adaptive remodeling). It is important to note that although the participants in the control-group of our study were formally normotensive, the mean blood pressure was 134/81 mmHg which means that on the basis of current criteria this group can be classified as being prehypertensive. This suggests that the remodeling process already occurs before the 'hypertensive threshold' is reached. This is supported by studies that show carotid artery remodeling in populations without established hypertension. [34,35] Takase et al showed that increased carotid IMT was associated with new onset hypertension in over 850 normotensive participants who were followed for approximately 3 years. [36] Therefore, it is reasonable to propose that although hypertension induces outward, hypertrophic, maladaptive remodeling in the carotid artery, this remodeling may already occur before established hypertension develops. This, in turn, supports the hypothesis that arterial remodeling may be an etiological/causal factor in the development of essential hypertension and hypertensive vascular damage. However, there are relatively few longitudinal clinical studies that have systematically investigated arterial remodeling in the early stages of hypertension and more research is required to further explore the etiology of arterial remodeling and incidence or progression of hypertension.

Arterial remodeling as mediator of hypertensive renal damage

When it comes to the role of the kidney in the pathogenesis of hypertension, this organ can be both culprit and victim. On the one hand, the kidney is an important regulator of blood pressure, but on the other it is also a target of hypertension-related damage. Indeed, hypertensive nephropathy or glomerulosclerosis is the second most common cause of end-stage renal disease (ESRD), but hypertension per se also plays an important role in the progression of chronic kidney disease (CKD) . [37,38] CKD and especially ESRD are strongly associated with cardiovascular events and mortality, and arterial remodeling has been proposed as a linking mechanism between CKD and cardiovascular disease.

Many studies have addressed the association of arterial remodeling with renal damage. Initially, most of these studies focused on patients with ESRD and hemodialysis and consistently showed that renal failure is strongly associated with arterial remodeling, both arteriosclerotic and atherosclerotic.[39] However, as was mentioned in Chapter 2, ESRD is characterized by specific systemic changes such as increased level of inflammatory mediators, altered calcium and phosphate homeostasis and a disturbed cholesterol metabolism, all of which can stimulate arterial remodeling. Therefore, the conditions in ESRD are significantly different from those in patients with hypertension without apparent kidney disease. Several studies have been

performed in patients who are in earlier stages of CKD.[40-43] For example, Briet et al. showed that in patients with mild-to-moderate CKD, a lower eGFR was associated with arterial stiffness and outward, maladaptive carotid remodeling.[42] These data were corroborated by Bruno et al. who found in a population of 314 patients with essential hypertension that in comparison to healthy individuals, the presence of an elevated carotid-femoral PWV was associated with reduced eGFR, even after adjusting for age, sex, mean blood pressure and the presence of diabetes mellitus).[43] When carotid artery stiffness was also present, the odds-ratio even increased further.[43] However, because these and many of these studies are cross-sectional in design, it is impossible to decide whether arterial remodeling causes kidney damage or that the kidneys promote arterial remodeling. Longitudinal studies are necessary, therefore, to better explore this cause-and-effect problem.

In longitudinal studies of kidney function, it is important to note that in healthy people eGFR declines with advancing age at an average rate of 0.4 to 1.7 ml/min/1.73m² per annum. [44] This age-effect has implications for the interpretation of repeated eGFR-measurement as a marker of kidney damage. We showed in Chapter 4 that an increased pulse wave velocity as a proxy of aortic stiffness accelerates the annual decline in eGFR in primary care patients. More importantly, we showed that the age-related decline was especially accelerated in patients older than 60 years. These results are in accordance with those in other studies. For instance, Judson et al. recently showed in a longitudinal study that increasing SBP and widening pulse pressure over time (which reflects arterial stiffness and increased blood pressure pulsatility) were associated with accelerated decline of kidney function. [45] Of interest, this already occurred at median baseline SBP levels of 110 mmHg that increased to 130 mmHg after five years, which are clearly prehypertensive blood pressures. This supports the hypothesis that renal damage may occur already before overt hypertension has developed.[45] Vaes et al. showed similar results, in patients older than 60 years.[46] Sedaghat et al. showed in data from over 2500 patients of the Rotterdam study who were followed-up for a median of 11 years that carotid-femoral PWV and increased PP were associated with a steeper annual decline in eGFR.[47] In addition, the same authors confirmed in a meta-analysis of several longitudinal population-based studies that each standard deviation increase in PP and PWV was associated with respectively 16%, and 8% increased relative risk of incident CKD, (defined as an eGFR <60 ml/min/1.73m²).[47] These clinical data further support the hypothesis that arterial remodeling is detrimental for the kidney. The question remains, however, whether CKD itself promotes arterial remodeling.

Although we found an association between arterial stiffness and the decline of eGFR, neither a lower eGFR, nor its change over time, were significantly associated with progression in PWV (Chapter 4). This is in contrast with some studies showing that the presence of CKD or elevated creatinine levels are predictive for progression of arterial stiffness.[48,49] However, there are also other studies that could not replicate

these findings. For instance, Briet et al. could not find an association between the presence of CKD and progression of aortic stiffness but they did find an association with carotid maladaptive remodeling. It should be noted that in this study, aortic stiffness was associated with decline in eGFR over time.[50] Interestingly, in patients with CKD large artery stiffness and maladaptive remodeling of the carotid artery were reversed within 12 months after living-donor kidney transplantation, independent from the level of glomerular filtration.[51] These data suggest that the damaged kidney somehow promotes arterial remodeling, possibly through uremic toxins or altered regulation of (bone) mineral metabolism (mechanisms also described in Chapter 2) or it may be that transplantation of a healthy kidney counteracts mechanisms promoting arterial remodeling. We must conclude that the precise role of the kidney in the development and progression of arterial stiffening and remodeling remains enigmatic.

Renal hemodynamics in hypertension and prehypertension

Although eGFR and urinary albumin excretion are well-established markers of renal damage which correlate with an adverse outcome, they do not fully reflect all abnormalities which may occur in the hypertensive kidney. Before eGFR starts to decline or urinary albumin excretion occurs, renal damage has already been

Figure 7.2. Hemodynamic effects of large and small artery remodeling in essential hypertension

accumulating. Generally, it is assumed that (hypertensive) arterial remodeling leads to renal damage through the detrimental effects of increased blood pressure pulsatility, that occurs because of arterial stiffening (also described in Chapter 2). [38] This increased blood pressure pulsatility is thought to be transmitted to the vulnerable renal microcirculation and glomeruli, ultimately resulting in nephrosclerosis (**Figure 7.2**). [38] However, since the kidneys are normally protected against large variations in blood pressure by autoregulation of renal blood flow, elevated blood pressure pulsatility can only be damaging to the renal circulation if these autoregulatory mechanisms are somehow impaired. One possibility is that (long-term) exposure to elevated blood pressure pulsatility in itself may impair renal autoregulation or increase susceptibility to hypertensive damage. Indeed, work from Loutzenhiser et al. has shown that rapidly oscillating (peak) systolic blood pressure is an important determinant of the myogenic response in an animal model of impaired renal autoregulation. [52] Another possibility is that there are primary changes in the regulation of renal perfusion in the hypertensive kidney. Thus, the question still remains whether the kidney is victim or culprit in the pathophysiology of essential hypertension and arterial remodeling. Although changes in renal perfusion and renal autoregulation play an important role in the pathophysiology of essential hypertension and the development of hypertensive renal damage, it is as yet not fully known which changes in hemodynamics are present already before overt hypertension develops and to what extent these changes are different from those observed in established hypertension. Therefore, in Chapter 5 we reviewed available literature investigating the systemic and hemodynamic characteristics of both established essential hypertension and its earlier phases borderline hypertension or prehypertension. By and large, borderline hypertension is characterized hemodynamically by an elevated peripheral vascular resistance, which is sometimes accompanied by a 'hyperkinetic heart' (i.e. increased sympathetic tone with an elevated heart rate and/or increased cardiac output). In prehypertensives a similar pattern of increased resistance has frequently been observed, albeit numerically lower than in borderline hypertensives and to a lesser degree associated with an elevated cardiac output. In both borderline hypertension and prehypertension, increased arterial stiffness is a common observation. The combination of greater arterial stiffness and elevated peripheral resistance points towards arterial remodeling as an important phenomenon that is already present in early stages of hypertension. With regard to renal hemodynamics, borderline hypertension is characterized by a parallel decline in systemic and renal blood flow with advancing age and increased renal vascular resistance. Data on hemodynamics in prehypertensives was, unfortunately, scarce. Therefore, we analyzed in Chapter 6 data from young male medical students who were either normotensive or prehypertensive and compared them to age and sex-matched patients with established hypertension. We found that, in addition to elevated peripheral vascular resistance and increased arterial stiffness, prehypertensive individuals showed elevated levels of renal vascular

resistance, whereas glomerular filtration relatively intact. We observed an elevated renal perfusion in prehypertensives whereas renal fraction and filtration fraction were not different from normotensives and hypertensives. These results suggest that despite the fact that blood pressure had not yet reached hypertensive levels, arterial remodeling already was already present in prehypertensives and that renal hemodynamics are altered to a high flow, high resistance state. These changes in renal hemodynamics may predispose to enhanced susceptibility to the damaging effects of elevated blood pressure pulsatility. However, this needs to be confirmed in appropriate experiments. Unfortunately, we did not study large artery remodeling directly using methods such as pulse-wave velocity measurement or ultrasonography, but these findings support the hypothesis that arterial remodeling may be an early driving force for the development of hypertension and hypertensive renal target organ damage. Future studies are required to evaluate the link between macrovascular remodeling and renal hemodynamics in different stages of hypertension. Also, it would be of interest to investigate what processes underlie the rise in renal vascular resistance and to assess whether mechanisms of macrovascular arterial remodeling are also active in the renal microcirculation.

Unifying systemic and renal arterial remodeling and hemodynamics

We showed that alterations in renal hemodynamics occur in participants with prehypertension, suggesting that a disturbance of the regulation of vasoconstriction and/or vasodilation of peripheral systemic arteries and renal blood vessels are one of the early mechanisms in the development of essential hypertension, and possibly precede a persistent elevation of blood pressure. We also showed that (maladaptive) carotid artery remodeling commences before hypertension has been established and persists once this remodeling is established. As described earlier, these findings are supported by other studies which also found that arterial remodeling sometimes precede the development of overt hypertension. For instance it has recently been demonstrated in normotensive young adults that PWV was predictive of incident hypertension four years later.[53] However, these data need to be confirmed in selected populations. Also, it remains to be investigated whether this results from changes in the structure and composition of the arterial wall or that functional changes (e.g. endothelial dysfunction, impaired vasodilatory capacity etc.) precede structural arterial remodeling. Likewise, the biological stimuli responsible for these changes need to be established. Based on the findings of this thesis and available data, we hypothesize that arterial remodeling may not only be an adaptation to already elevated blood pressure but in itself may be a factor in the development of essential hypertension, which then ultimately initiates a self-perpetuating cycle of increasing blood pressure and more arterial remodeling.

Clinical implications

The findings of this thesis have implications for future clinical patient care. First of all, our findings show that arterial remodeling and change in renal hemodynamics occur before overt hypertension has developed and therefore contribute to cardiovascular risk in patients who are currently not eligible for treatment according to clinical practice guidelines. Since there are indications that these early alterations may precede (and possibly contribute to) the development of hypertension, identification and possibly treatment of pre-hypertensive patients or selected patients who show signs of early arterial remodeling, may prevent future development of overt hypertension and subsequent target organ damage and may reduce future cardiovascular mortality. However, with the ever rising costs of modern healthcare in Western countries, cost-effectiveness and efficacy should be major considerations in these decisions. Nevertheless, since currently the majority of hypertension-related costs and disease burden are generated by the late cardiovascular and renal consequences, earlier intervention may yet prove to be cost-effective and beneficial. These aspects need to be investigated further.

In recognition of growing evidence indicating that earlier intervention in the process of hypertension is required, the recently published ACC/AHA-guidelines on the treatment of hypertension lowered the cut-off levels for hypertension for nearly all patient categories, from 140/90 mmHg to 130/80 mmHg and set this blood pressure as general treatment goal. [54] The question remains, however, whether the cut-off value and treatment goals for hypertension should be arbitrarily lowered -yet again-, or that a different approach of individual cardiovascular risk assessment is more appropriate, in which blood pressure is used as a continuous measure and is combined with other markers such as (early) arterial remodeling, hemodynamics, and cardiovascular risk factors.[16]

Unfortunately, approximately only 50% of patients achieve the current treatment goal for blood pressure of 140/90 mmHg due to various reasons such as medication adherence or resistance to therapy. Raising the level by lowering treatment goals, may prove to be infeasible and lead to a rise in the number of people who are not on-target, especially in patients with high-grade established hypertension. However, since there is still progression of arterial remodeling even at pre-hypertensive levels, it can be questioned whether achieving treatment at blood-pressure treatment goal is sufficient for preventing cardiovascular disease progression. For instance, it has been demonstrated that IMT progresses in young, well controlled grade-1 hypertensive patients as well as in young individuals with white coat hypertension.[55] This suggests that treatment of hypertension alone may not be sufficient to completely prevent progression of arterial remodeling, and shows that novel treatment strategies are required. In order to develop such novel treatments, a thorough understanding of the complex pathophysiological mechanisms underlying hypertension and arterial

remodeling is still of utmost importance.

Arterial remodeling has already been shown to be a possible target for drug-treatment, independently of blood pressure, albeit with varying results. [56-58] We showed in Chapter 3 that use of ARB was associated with a reduction of CWT and CWS, although this needs to be further explored. Other mechanisms described in Chapters 2 and 5 may also be suitable future treatment targets. For example, we are currently evaluating the effects of inhibition of vascular calcification by administration of menaquinone-7 (vitamin K2) in a randomized, placebo controlled trial. [59] Other candidates such as metalloproteinase inhibitors and cross-link breakers may also be interesting candidates to treat arterial remodeling, although again more research is required. [60,61]

Perspectives for future research

The findings in this thesis provide interesting data on the pathophysiology of arterial remodeling and its role in systemic and renal hemodynamics in essential hypertension. However, many questions still need to be addressed in order to improve our understanding of these complex mechanisms. This is especially the case for the earlier stages of essential hypertension. For instance, it is still incompletely understood which pathophysiological mechanisms drive the increased resistance of resistance arteries as observed in pre-hypertensives and hypertensives. It is also not known whether eutrophic remodeling of resistance arteries progresses towards outward, hypertrophic remodeling in the course of the disease and, -if so- what drives these changes. In order to systematically study arterial remodeling in various stages of hypertension, longitudinal clinical studies have to be designed in which well selected and documented participants of different age-groups are monitored with regard to arterial characteristics of different vascular regions as well as systemic and/or renal hemodynamics, using standardized measurement protocols. Especially challenging, but worthwhile, is further exploration of functional and structural remodeling in the hypertensive process of the renal vasculature and other not readily accessible vascular beds. These studies would lead to better understanding of the link between macrovascular arterial remodeling and renal hemodynamics. The data obtained by careful study of different vascular beds in different phases of hypertension allow for the development of computational models in collaboration with biophysicists, enabling assessment of the effects of blood pressure pulsatility on longitudinal arterial remodeling. Finally, in-vitro studies assessing the biochemical and histopathological properties of different arterial regions under different levels of pulsatility may lead to improved insights in the driving factors of arterial remodeling as was shown in the study by Bloksgaard et al. that evaluated changes in the microarchitecture of collagen and elastin induced by blood pressure pulsatility in both pig and human arteries. [62]

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Summary

Introduction

Hypertension is one of the most important risk factors for cardiovascular disease and mortality, and is one of the leading causes of end-stage renal failure requiring dialysis. Since worldwide approximately one in three to four individuals have hypertension, the disorder is considered to be a major public health problem. Although extensive research on essential hypertension has identified several major pathophysiological mechanisms, our understanding of the exact etiology of essential hypertension remains incomplete, especially with regard to the earlier phases of hypertension. In this thesis we therefore aimed to evaluate several questions regarding essential hypertension focusing on one of its major pathophysiological mechanisms: arterial remodeling and its renal and systemic consequences. Arterial remodeling is the term for alterations in structure and function of arteries and involves changes in vessel or lumen diameter, rarefaction, hypertrophy of the intimal or medial layers, altered ratio between collagen and elastin, as well as endothelial dysfunction, altered vasodilatory response, and vascular tone. Generally, the process of arterial remodeling is thought to be a complex and actively regulated adaptation to changing arterial hemodynamics, however, there is also increasing evidence that arterial remodeling may be a cause of hemodynamic change and possibly contributes to the development of essential hypertension. To gain insight in whether or not this is indeed the case, the longitudinal study of arterial remodeling and hemodynamics as well as the underlying mechanisms in association with the etiology of essential hypertension remains an important area of research.

Arterial remodeling in essential hypertension

First, we summarized in Chapter 2 some of the major pathophysiological and biological mechanisms underlying arterial remodeling such as hypertrophy of the arterial media by proliferation and differentiation of vascular smooth muscle cells, disturbances in the balance between elastin- and collagen fibers, and extracellular matrix calcification due to impaired regulation of soft-tissue calcification. We described how monogenetic diseases like Pseudoxanthoma Elasticum, Keutel-syndrome, and Marfan's disease cause specific alterations to these key regulators of arterial remodeling, in order to evaluate the extent of these mechanisms in arterial remodeling in other cardiovascular diseases, including essential hypertension. We also reviewed the hemodynamic consequences of arterial remodeling and the hypothesis of the detrimental effects of increased blood pressure pulsatility associated with increased arterial stiffness.

Next, we evaluated in Chapter 3 how remodeling of the carotid artery changes over time, comparing hypertensive patients to normotensive participants from a single primary care facility. We found that in hypertensive patients carotid remodeling is outward hypertrophic and did not change significantly over time, despite that

blood pressure declined over time. Furthermore, remodeling was maladaptive in hypertensives, although over time circumferential wall tension (CWT) and stress (CWS) fell, possibly as a result from blood pressure treatment. These results suggest that once hypertension is established, the structural changes to the carotid artery are not readily reversed. In contrast to the findings in hypertensives, normotensives had significant carotid enlargement and increased wall thickness over time, although blood pressure did not change. Circumferential wall tension also rose significantly. Nevertheless since CWS did not change significantly over time and even tended to decrease over time, this carotid remodeling could still be termed adaptive. These findings indicate that hypertrophic outward carotid remodeling possibly precedes the development of hypertension. We also evaluated which factors predict longitudinal arterial remodeling and found that male sex, age, smoking (pack years), and pulse pressure were predictors of maladaptive carotid remodeling. Finally we found that use of angiotensin-receptor blockers but not general use of antihypertensive drugs was associated with longitudinal decline in CWS.

Arterial remodeling and the kidneys

In this thesis we also evaluated the interrelationship between arterial remodeling, hemodynamics and the kidneys. The kidneys play an important role in the etiology of essential hypertension and cardiovascular disease. They are the primary regulator of volume and salt homeostasis and are central to the regulation of the renin-angiotensin system; one of the most important systems in the regulation of blood pressure. It is therefore not surprising that impairments in any of these renal regulatory systems can lead to a rise in blood pressure. On the other hand, the kidney is itself vulnerable to damaging effects of high blood pressure as can be observed in the development of renal organ damage in patients with hypertension. It has been proposed that arterial remodeling may be one of the linking mechanisms between the development of hypertension and cardiovascular disease on one hand, and impaired kidney function or damage on the other. As described in Chapter 2, end-stage renal disease (ESRD) and advanced chronic kidney disease (CKD) are characterized by markedly elevated cardiovascular risk as well as significantly increased arterial stiffness and patients often display extensive arterial calcification. This accelerated arterial remodeling in ESRD has been attributed to the detrimental effects caused by renal failure such as an impaired homeostasis of calcium and phosphate, uremia, high levels of inflammation, and oxidative stress. However, although in earlier phases of chronic kidney disease these mechanisms play a less important role, mild to moderate CKD is still associated with elevated arterial stiffness. However, it is less clear whether arterial stiffness is associated with accelerated decline in kidney function over time since there have been relatively few longitudinal studies investigating this question in a population without established kidney disease. Therefore, in Chapter 4 we prospectively assessed the

association between arterial stiffness (measured as the carotid-femoral Pulse Wave Velocity [PWV]) and change in estimated glomerular filtration rate (eGFR) over time in patients from a single primary care facility without overt kidney disease but with cardiovascular risk factors including hypertension. We showed that PWV and age were independently predictive of annual decline of eGFR over time. Moreover we showed that this association was amplified in patients aged 62 years or older. On the other hand, change in eGFR was not associated with changing PWV. These results support the hypothesis that elevated blood pressure pulsatility, which results from arterial stiffening, leads to renal damage, possibly by disturbances in renal autoregulation or impaired intrarenal hemodynamics.

Renal and systemic hemodynamics in the etiology of essential hypertension

We aimed to further investigate the role of the renal hemodynamics in the etiology of essential hypertension in relation to systemic hemodynamics or arterial remodeling. Decades of extensive research have shown that in established hypertension, renal hemodynamics are characterized by a reduction in renal blood flow (RBF), increase in filtration fraction (FF) and elevated renal vascular resistance (RVR), whereas alterations in systemic hemodynamics involve elevated peripheral vascular resistance, normal or slightly decreased cardiac output, and increased arterial stiffness. However, it is not clear which of these processes are responsible for the initiation and later development of essential hypertension. Therefore the primary objective was to evaluate whether there are distinct changes in patients who were still in an early phase of hypertension (i.e. prehypertension or borderline hypertension) compared to either patients with established hypertension or normotensive individuals.

In Chapter 5 we first summarized the available literature on the systemic and renal hemodynamic characteristics in both established essential hypertension as well as in borderline hypertension and prehypertension. Generally, borderline hypertension is characterized by increased peripheral vascular resistance that can occasionally be accompanied by an elevated heart rate and/or cardiac index (i.e. hyperkinetic heart). With regard to renal hemodynamics there seems to be an age-related decline in renal perfusion, but this is proportionate to declining systemic flow suggesting that there's no preferential vasoconstriction in the renal vasculature. Less is known about prehypertension, however, the limited studies available show with regard to systemic hemodynamics elevated peripheral vascular resistance, increased arterial stiffness but unchanged cardiac index. Data on renal hemodynamics in prehypertension in relation to cardiac output was unfortunately scarce. Therefore, in Chapter 6 we studied in an age and sex-matched cohort of 20 normotensive, 20 prehypertensive and 20 hypertensive young males, what differences exist with regard to systemic and renal hemodynamics. We measured glomerular filtration rate (GFR), renal vascular resistance (RVR), and

renal plasma flow (RPF) and related these measures of renal hemodynamics to markers of systemic hemodynamics such as cardiac index (CI), total peripheral resistance (TPR), plasma volume (PV), and stroke-volume to pulse pressure ratio (SV/PP) which is a marker of general vascular stiffness. Also, we measured the hormones noradrenalin, aldosterone, and active plasma renin. We mainly found that with regard to systemic hemodynamics, prehypertension seemed to take an intermediate position between normotension and established hypertension: declining trend in cardiac index, rising peripheral resistance, and rising trend in arterial stiffness. Plasma volume, however, was significantly lower in prehypertensives and hypertensives. Glomerular filtration rate had a decreasing trend across groups, whereas renal vascular resistance rose, although these findings were most pronounced in established hypertension. Remarkably, renal plasma flow was significantly increased in prehypertensives in comparison to both normotensives and hypertensives while filtration fraction and renal fraction were comparable across all groups. These findings possibly suggest renal hyperperfusion in prehypertension, despite increase in renal resistance and may reflect some kind of autoregulatory mechanism to maintain intraglomerular pressure. One can conclude from Chapters 5 and 6 that the hemodynamic changes found in established essential hypertension can already be observed in a phase in which blood pressure has not yet risen to a hypertensive level. Elevated peripheral vascular resistance and increased arterial stiffness are hallmarks of active arterial remodeling, a process that may be driven by factors other than elevated blood pressure. Similarly, the kidneys in the prehypertensive individual display elevated vascular resistance but not out of proportion to systemic changes in vascular resistance while intrarenal perfusion is increased, possibly as a, yet unknown, adaptive mechanism.

Main findings in this thesis

In this thesis we assessed different questions regarding arterial remodeling and systemic and renal hemodynamic aspects in patients with both early and advanced stages of essential hypertension. Our main findings in this thesis were:

- There are multiple complex and interdependent pathophysiological mechanisms involved in (hypertensive) arterial remodeling. Studying monogenetic diseases associated with specific vascular phenotypes can help in our understanding of these mechanisms.
- Essential hypertension is associated with outward hypertrophic, maladaptive carotid remodeling that, once established, remains relatively constant over time.
- Lowering blood pressure seem to reduce circumferential wall stress longitudinally, but not structural carotid remodeling in hypertensives.
- In normotensives carotid remodeling occurs even before overt hypertension is

established.

- Carotid-femoral Pulse Wave Velocity is independently associated with accelerated age-related decline of estimated glomerular filtration rate, especially in individuals aged 62 years or older.
- Prehypertension is associated with increasing peripheral vascular resistance, elevated arterial stiffness with no changes in cardiac output and no signs of an overactive sympathetic nervous system. Prehypertension seems to be an intermediate stage between normotension and hypertension in which arterial remodeling already commences before blood pressure reaches hypertensive levels.
- Renal hemodynamics in prehypertensives are characterized by hyperperfusion while renal vascular resistance rises, possibly reflecting an adaptive mechanism. There is no preferential vasoconstriction and glomerular filtration rate and filtration fraction remain relatively unchanged.

Nederlandstalige samenvatting

Introductie

Hoge bloeddruk (hypertensie) is één van de meest belangrijke risicofactoren voor (sterfte aan) hart- en vaatziekten en is daarnaast één van de belangrijkste oorzaken van nierfalen. Aangezien wereldwijd ongeveer één op de drie à vier personen hoge bloeddruk heeft, is het een aanzienlijk probleem voor de volksgezondheid. In de afgelopen tientallen jaren, heeft uitgebreid wetenschappelijk onderzoek naar het ontstaan van hypertensie onze kennis over deze aandoening aanzienlijk vergroot en zijn er meerdere biologische mechanismen gevonden die hierin een rol spelen. Desondanks is de exacte manier waarop hoge bloeddruk ontstaat nog steeds grotendeels onbekend. Vooral met betrekking tot de vroegere fases van hypertensie, waarin de bloeddruk nog niet in hoge mate is gestegen. Om een beter inzicht in het ontstaan van hoge bloeddruk te krijgen is het van belang om diverse afwijkingen ten opzichte van normaal te bestuderen in de verschillende fases van hoge bloeddruk. Daarom hebben wij in dit proefschrift onderzoek gedaan naar één van de belangrijkste mechanismen die een rol speelt in het ontstaan en in stand houden van hypertensie en daarnaast bijdraagt aan de schadelijke gevolgen van hoge bloeddruk: arteriële vaatwand remodelering.

Arteriële vaatwand remodelering bij essentiële hypertensie

Onder arteriële vaatwand remodelering worden de veranderingen in structuur en functie van de slagaders bedoeld zoals veranderingen in de diameter van het vat, dikte van de vaatwand, het aantal en soort cellen en eiwitten die in de vaatwand aanwezig zijn en de manier waarop het vat reageert op specifieke prikkels door te vernauwen (vasoconstrictie) of te verwijden (vasodilatatie). Deze veranderingen worden verondersteld een zorgvuldig gereguleerde reactie te zijn op veranderde omstandigheden in bloeddruk of bloeddoorstroming in de slagaders. Echter, er zijn ook steeds meer aanwijzingen dat arteriële vaatwand remodelering al optreedt voordat de bloeddruk of de bloeddoorstroming verandert en wellicht zelfs hiervoor verantwoordelijk is. In **hoofdstuk 2** van dit proefschrift hebben wij enkele van de meest belangrijke mechanismen van arteriële vaatwand remodelering samengevat zoals verdikking (hypertrofie) van de vaatwand door toename van het aantal gladde spiercellen, een verstoorde verhouding tussen elastische en stugge vezels en verkalkingen in de vaatwand. Aan de hand van genetische aandoeningen die gekenmerkt worden door specifieke afwijkingen in verschillende regulatiemechanismen wordt de rol van deze mechanismen in ziekten zoals hoge bloeddruk verhelderd. Daarnaast worden de gevolgen van arteriële vaatwand remodelering op de hoogte en regulatie van bloeddruk beschreven in hoofdstuk 2. Omdat het niet geheel duidelijk was of er verschillen zijn in de manier waarop de vaatwand in de tijd verandert tussen patiënten met hoge bloeddruk enerzijds en mensen met een normale bloeddruk anderzijds,

hebben wij hier in **hoofdstuk 3** onderzoek naar gedaan. Wij hebben met echografisch onderzoek de halsslagaders van mensen met hoge bloeddruk (hypertensie) vergeleken met mensen met een normale bloeddruk. We vonden dat bij mensen met hypertensie de halsslagader groter (qua diameter) was en een dikkere vaatwand had dan die van mensen met normale bloeddruk. Daarbij stond er een hogere spanning op de vaatwand dan bij mensen met normale bloeddruk. Enkele jaren later bleken de veranderingen in de vaatwand nog steeds aanwezig, ondanks dat de bloeddruk beter was behandeld bij deze hypertensieve patiënten. De mechanische spanning op de vaatwand nam echter wel af. Opmerkelijk genoeg vonden we dat bij mensen met normale bloeddruk, zowel de dikte van de vaatwand als de diameter significant toenamen na enkele jaren, ondanks dat de bloeddruk niet steeg. Deze bevinding ondersteunt de hypothese dat arteriële vaatwand remodelering voorafgaat aan stijging van de bloeddruk. Verder vonden wij dat het mannelijke geslacht, hogere leeftijd, roken en bloeddruk (polsdruk) belangrijke voorspellers waren voor vaatwand remodelering en dat gebruik van specifieke bloeddrukmedicatie (angiotensinereceptorblokkers) de mechanische spanning op de vaatwand kan doen afnemen.

Arteriële vaatwand remodelering en de nieren

In dit proefschrift onderzochten we ook de wederzijdse relatie tussen arteriële vaatwand remodelering en de nieren. De nieren spelen een uitermate belangrijke rol in het ontstaan en voortbestaan van hoge bloeddruk en hart- en vaatziekten. De nieren zijn een van de belangrijkste regelaars van de bloeddruk, doordat ze nauwkeurig de hoeveelheid vloeistof en zout in de bloedvaten beïnvloeden. Daarnaast regelt de nier de hoeveelheid van het eiwit renine dat er voor zorgt dat het belangrijkste regulatiesysteem van bloeddruk (het renine-angiotensine-aldosteron systeem, afgekort: RAAS) wordt aangestuurd. Het is dus niet verrassend dat een verstoring in een van deze mechanismen leidt tot hoge bloeddruk. Aan de andere kant is de nier zelf heel kwetsbaar voor de schadelijke effecten van hoge bloeddruk en verstoringen in haar bloeddorstrooming. Dit is goed te zien aan het feit dat veel mensen met hypertensie schade aan de nieren ontwikkelen en dat het hebben van hypertensie één van de belangrijkste risicofactoren is om eindstadium nierfalen te ontwikkelen. Arteriële vaatwand remodelering is verondersteld een belangrijke verbindende factor te zijn tussen hoge bloeddruk en hart- en vaatziekten enerzijds en een verstoorde werking van de nieren anderzijds. Zoals in hoofdstuk 2 werd beschreven wordt vergevorderd nierfalen gekenmerkt door een aanzienlijk verhoogd risico op hart- en vaatziekten en is er vaak sprake van fors toegenomen vaatstijfheid. Daarnaast hebben patiënten met nierfalen vaak uitgebreide vaatwandverkalkingen. Deze uitingen van versnelde arteriële vaatwand remodelering kunnen echter worden toegeschreven aan de veranderingen die de falende nieren teweegbrengen zoals een verstoorde balans tussen calcium en fosfaat in het bloed, verhoogde concentraties van afvalproducten

zoals ureum en een hogere mate van ontsteking in het lichaam. Echter, zelfs in vroegere fases van nierfalen waarin deze mechanismen nauwelijks of hoogstens beperkt een rol spelen, is er al sprake van toegenomen vaatwandstijfheid. Het was echter onduidelijk of deze verhoogde vaatwandstijfheid op lange termijn ook leidt tot vermindering van de nierfunctie of schade aan de nieren. Dit hebben wij daarom in hoofdstuk 4 verder onderzocht. Wij vroegen ons af of verhoogde vaatwandstijfheid van de aorta (gemeten als polsgolfsnelheid; PWV) voorspellend was voor een snellere jaarlijkse afname van de nierfunctie dan verwacht op basis van leeftijd. We bestudeerden deze effecten over een periode van enkele jaren bij patiënten uit een huisartsenpraktijk die wel risicofactoren voor hart- en vaatziekten hadden (waaronder hypertensie), maar geen tekenen van schade aan de nieren bij aanvang van het onderzoek. We vonden inderdaad dat een toename van de polsgolfsnelheid en een hogere leeftijd onafhankelijke voorspellers waren voor een versnelde jaarlijkse achteruitgang van nierfunctie, terwijl nierfunctie zelf niet voorspellend was voor veranderingen in vaatwandstijfheid. Dit effect was het meest uitgesproken bij patiënten die ouder waren dan 62 jaar. Deze bevindingen ondersteunen de hypothese dat arteriële vaatwand remodellering en de gevolgen hiervan kunnen leiden tot schade aan de nieren. Naar aanleiding van deze bevindingen vroegen wij ons af of de regulatie van de bloeddorstroming in de nier mogelijk verstoord zou kunnen zijn.

Veranderingen in bloeddorstroming in het ontstaan van hoge bloeddruk

We onderzochten vervolgens welke rol de bloeddorstroming in de nieren speelt in het ontstaan van hoge bloeddruk en in welke relatie deze staat tot arteriële vaatwand remodellering en de bloeddorstroming in de rest van het lichaam (systemische hemodynamiek). Eerder onderzoek bij patiënten met hoge bloeddruk heeft namelijk aangetoond dat de bloeddorstroming in de nieren afneemt, de vaatweerstand van de nieren toeneemt en de filtratie fractie (dit is de hoeveelheid bloed die door de nieren stroomt en die gefilterd wordt door de nier) eveneens toeneemt. Hypertensie kenmerkt zich wat betreft de systemische hemodynamiek door een verhoogde perifere vaatweerstand, een toegenomen vaatstijfheid en een normaal (of licht verlaagd) hartminuutvolume. Het is echter niet geheel duidelijk welke van deze observaties verantwoordelijk zijn voor het ontstaan en in stand houden van hoge bloeddruk. Daarom was ons doel om mensen die zich nog in een vroege fase van hoge bloeddruk bevonden te vergelijken met enerzijds patiënten met gevorderde hypertensie en anderzijds gezonde vrijwilligers zonder hoge bloeddruk. In hoofdstuk 5 hebben wij allereerst de beschikbare literatuur samengevat over onderzoek verricht naar de systemische hemodynamiek en de hemodynamiek van de nieren bij zowel patiënten met hoge bloeddruk als patiënten met een voorstadium van hypertensie. Algemeen kenmerkt zich de vroege fase van hypertensie door een toegenomen

perifere vaatweerstand, soms vergezeld van een toegenomen hartfrequentie en/of hartminuutvolume. Met betrekking tot de bloeddorstrooming in de nieren lijkt er sprake van een leeftijd-gerelateerde afname in renale doorbloeding die vergelijkbaar is met afname van de bloeddorstrooming in de rest van het lichaam. Helaas vonden we maar weinig gegevens over deze waarden bij personen in een nog vroegere fase van het hypertensieve proces. Daarom vergeleken we in hoofdstuk 6 de bloeddorstrooming van 20 jonge mannen met normale bloeddruk met 20 jonge mannen met prehypertensie (een systolische bloeddruk tussen 130 – 139 mm Hg en diastolische bloeddruk tussen 80 en 89 mm Hg) en 20 jonge mannelijke patiënten met bestaande hoge bloeddruk. We vroegen ons af of er verschillen tussen de groepen waren in de doorbloeding van de nieren, de nierfunctie, de vaatweerstand, het hartminuutvolume, het plasmavolume en de vaatwandstijfheid. Daarnaast maten we de bloedconcentraties van de hormonen noradrenaline, aldosteron en de plasmarenine-activiteit. We vonden dat prehypertensie een intermediaire positie inneemt tussen normale bloeddruk en hypertensie. Er was sprake van een dalende trend in het hartminuutvolume en een stijgende trend in de perifere vaatweerstand en de vaatstijfheid. Wel hadden mensen met prehypertensie en hypertensie een lager plasmavolume dan mensen met normale bloeddruk. De nierfunctie liet een dalende trend zien bij prehypertensieve en hypertensieve personen, maar de renale vaatweerstand nam toe. Opmerkelijk genoeg was er bij prehypertensieve personen sprake van een toegenomen bloeddorstrooming van de nieren in vergelijking met de andere twee groepen. Hierbij was er geen verschil in filtratie fractie en renale fractie tussen de groepen. Uit de bevindingen van hoofdstuk 5 en 6 kunnen we concluderen dat de veranderingen die we observeerden bij patiënten met hypertensie al aanwezig zijn in een vroege fase waarin bloeddruk nog niet is gestegen. Een verhoogde perifere vaatweerstand en vaatstijfheid zijn kenmerkend voor arteriële vaatwand remodelering en zijn dus al aanwezig voordat bloeddruk stijgt. Dit suggereert dat dit proces wordt aangestuurd door andere factoren dan alleen hoge bloeddruk. Verder hebben de nieren bij prehypertensie weliswaar een toegenomen vaatweerstand, maar is de bloeddorstrooming juist toegenomen. Dit is mogelijk een compensatiemechanisme om de filtratie in stand te houden, echter de precieze werking ervan is nog onbekend.

Belangrijkste conclusies uit dit proefschrift

- Er zijn verschillende complexe en met elkaar samenhangende mechanismen betrokken bij arteriële vaatwand remodelering. Door genetische aandoeningen te bestuderen die specifieke afwijkingen in een van deze mechanismen veroorzaken kunnen wij beter begrijpen welke rol deze mechanismen spelen in aandoeningen zoals hoge bloeddruk.
- Essentiële hypertensie is geassocieerd met verdikking en uitzetting van de

halsslagaders, waarbij de wand onder relatief hoge mechanische spanning staat. Als deze afwijkingen eenmaal zijn gevormd herstellen ze ook niet meer in de loop van de tijd.

- Verlagen van bloeddruk lijkt de mechanische spanning van de vaatwand te verlagen, maar niet de structurele veranderingen aan de vaatwand.
- Bij mensen met een normale bloeddruk krijgen de halsslagaders na verloop van tijd een grotere diameter en een dikkere vaatwand, ondanks dat bloeddruk niet stijgt in diezelfde periode. Dit suggereert dat arteriële vaatwand remodelling al optreedt voordat bloeddruk stijgt en reflecteert mogelijk een verouderingsverschijnsel.
- Vaatwandstijfheid leidt tot een versnelling van de leeftijd-gerelateerde achteruitgang van de nierfunctie, vooral bij mensen ouder dan 62 jaar.
- Prehypertensie kenmerkt zich door verhoogde perifere vaatweerstand en arteriële vaatwandstijfheid maar niet door veranderingen in hartminuutvolume of tekenen van een hyperactief sympathisch zenuwstelsel. Prehypertensie lijkt een intermediaire fase te zijn tussen normale bloeddruk en hypertensie waarbij pathologische arteriële vaatwand remodelling al lijkt op te treden.
- De doorbloeding van de nieren is toegenomen bij prehypertensieven terwijl de vaatweerstand is toegenomen. Dit weerspiegelt mogelijk een compensatiemechanisme.

Valorization addendum

Introduction

The results presented in this thesis contribute to our knowledge about the etiology of high blood pressure (hypertension) and its associated cardiovascular outcome. In addition to their scientific value, these results also have potentially beneficial implications for society.

The cost of hypertension and cardiovascular disease

As already mentioned in Chapter 1 of this thesis, hypertension is one of the most important risk factors for cardiovascular diseases such as stroke, myocardial infarction, heart failure, and peripheral artery disease and is one of the leading causes of death. In addition, hypertension is a major cause of chronic kidney disease and dialysis and is involved in the development of vascular dementia. With prevalence rates of hypertension ranging between 22 – 46%, amounting to more than one billion affected people worldwide, hypertension is a significant public health problem. It has been estimated that 92 million disability-adjusted life-years (i.e. the number of life-years lost due to illness and disability) can be directly attributed to hypertension.[1] In the Netherlands, the costs of cardiovascular disease were € 11.6 billion in 2015, which corresponds to 12.3% of the total health-care related costs (RIVM, Statline Statistics) (Table 1). Although medication-treatment of hypertension accounted for ‘only’ € 694.3 million (0.6%) of total cardiovascular costs, 44% of cardiovascular costs were due to blood-pressure-related diseases such as coronary artery disease, stroke, and peripheral artery disease (Table 1). In a similar manner, kidney failure, for which hypertension is the second-most important risk factor, was also responsible for € 738 million (Table 1). Since it has been estimated that hypertension is directly attributable for 54% of

Table 1. Health care costs related to cardiovascular diseases in the Netherlands in 2015

Cost item	Costs (million)
Total health care	€ 94,424.2
Total cardiovascular diseases	€ 11,572.8
Hypertension (pharmacotherapy)	€ 694.3
Coronary heart disease	€ 2,406.1
Stroke	€ 1,636.2
Peripheral artery disease	€ 1,076.0
Kidney failure	€ 738.5

Source: Rijksinstituut voor Volksgezondheid en Milieu (RIVM) - Statline

<https://statline.rivm.nl/#/RIVM/nl/dataset/50040NED/table?ts=1536508597364>

the disease-burden of stroke and 47% of ischemic heart disease,[1] the total (indirect) costs of hypertension are therefore much higher. These numbers also show that the large majority of hypertension-associated costs are caused by its complications and late consequences. Since the prevalence of hypertension is rising worldwide, it is expected that, if left untreated, the socio-economic burden of cardiovascular diseases will rise to immense proportions. Therefore, developing efficient prevention and treatment strategies is a major priority in order to reduce ever expanding healthcare costs and morbidity.

Implications of understanding pathophysiological mechanisms

In order to develop novel preventive and therapeutic strategies, a thorough understanding of the mechanisms underlying the development of hypertension and its progression to hypertensive target-organ damage is required. Since arterial remodeling is thought to be a major pathophysiological factor in the development of both hypertension and hypertensive target-organ damage, the focus of this thesis was to investigate its role in different stages of essential hypertension. The findings in this thesis have not only confirmed this hypothesis (Chapters 3 and 4) but also show that changes in the vasculature such as increased renal and systemic arterial resistance occur relatively early in the hypertensive process (i.e. prehypertension) (Chapters 5 and 6). Although we have not presented research on novel treatment strategies in this thesis, these results have improved our understanding of the pathophysiology of essential hypertension. In the next paragraphs we will discuss the possible application and consequences of our findings in more detail.

Arterial remodeling as potential target for therapeutic intervention

In chapter 2 we reviewed several important mechanisms underlying arterial remodeling, learning from genetic diseases that are characterized by defects in specific regulatory proteins and which lead to a distinct vascular phenotype. These mechanisms could be a target for future interventions to reduce arterial remodeling and possibly also the development of hypertension and hypertensive target-organ damage. Since we found in this thesis that maladaptive arterial (carotid) remodeling is a relatively early phenomenon which is not easily reversible despite lowering of blood pressure (Chapter 3) and that aortic remodeling independently accelerates the decline in kidney function (Chapter 4), slowing down maladaptive arterial remodeling may have significant health benefits. One of the pathophysiological mechanisms, vascular calcification, is of particular interest in this regard. A key regulator of vascular calcification is the Vitamin-K-dependent Matrix Gla Protein (MGP) and absence or impaired functionality of MGP has been shown to induce extensive arterial calcification (Chapter 2). In line with these findings, our group has shown that inhibition of Vitamin-K by coumarin derivatives

induces peripheral arterial calcification. [2] Based on these findings we are currently investigating whether supplementation of high-dose Vitamin K2 (menaquinone) reduces pre-existent arterial calcification. [3] If this hypothesis is confirmed, Vitamin K2 might become a novel pharmacotherapeutic option directly targeting arterial remodeling. As already mentioned in Chapter 2, in addition to vascular calcification, the other pathways may prove to be possible potential therapeutic targets, although further research is still required.

Prehypertension: to treat or not to treat

As already stated in Chapter 5, when studying the etiology of essential hypertension, one would ideally follow individuals before they develop hypertension. Since this is practically not feasible we focused in this thesis on participants who are in an early phase of the hypertensive process and can be labeled as being prehypertensive. We demonstrated in Chapter 6 that even in young prehypertensive volunteers, systemic and renal vascular resistance are elevated in comparison to normotensive controls, suggesting that arterial remodeling occurs even before high blood pressure has been established. Similar findings could be observed in Chapter 3 where active carotid artery remodeling was observed in still normotensive participants. Our results suggest that early intervention may prove to be beneficial in either slowing down maladaptive arterial remodeling or the development of complications. These findings are in agreement with other studies that showed that prehypertension is in itself a risk factor for developing overt hypertension as well as elevated cardiovascular risk (Chapter 5). [4] When considering that the majority of hypertension-related costs are generated by its late complications such as stroke or myocardial infarction, it is logical to assume that early pharmacotherapeutic intervention in the prehypertensive stage could be beneficial. In line with this, the 2017 ACC/AHA-guidelines lowered blood pressure thresholds for treatment-eligible Stage I hypertension to 130-139/80-89 mmHg if these patients have a concomitant cardiovascular disease or condition. [5] However, there are several implications of lowering the cut-off values defining high blood pressure. First of all it results in a significant rise in the prevalence of patients with hypertension and of the number of people that are now recommended to receive antihypertensive medication. For instance, in the US this would mean that 63% of people aged between 45 and 75 years would be labeled as being hypertensive, reflecting an increase of almost 27%. [6] From an economic perspective, the costs of hypertension are therefore also expected to rise significantly, but in the long term this may lead to lower costs resulting from fewer strokes or myocardial infarctions. A study evaluating the economic impact of implementing the 2017 ACC-AHA guidelines in Switzerland estimated an annual increase of € 60.3 million (22%) of the costs of antihypertensive treatment. [7] When extrapolating these numbers for the Netherlands, this would mean an increase of € 152 million per year for the treatment

of hypertension. In addition to elevated costs, labeling previously healthy people with a diagnosis is not without harm as Pickering pointed out, referring to a study by Haynes et al. in which labeling steelworkers with the diagnosis of hypertension was associated with a significant increase in absenteeism of work, as well as lower quality of marital and home life. [8] Such unintended psychosocial effects should be considered in implementing a screening or preventive program. Secondly, patients already being treated for hypertension would require more intense treatment in order to reach the lower treatment goals. Even with current cut-off values, management of hypertension remains a clinical challenge since only 50% of patients with established hypertension reach the desired blood-pressure goal. [9] Low adherence to pharmacotherapy and lifestyle intervention are major issues for many patients. It can be hypothesized that this problem may be even greater when a fairly asymptomatic disease requires intense treatment that is not without adverse side-effects. These considerations need to be evaluated before any public health campaign or screening program can be initiated. Nevertheless, our results and other evidence suggest that early treatment and prevention may give rise to a significant health benefit. Therefore cost-effectiveness has to be carefully studied before any recommendation can be made whether or not to screen and treat prehypertension.

Conclusion

In this thesis we investigated the role of arterial remodeling in the development of hypertensive target-organ damage in various stages of essential hypertension, including prehypertension. The results of this thesis have contributed to our understanding of the pathophysiology of essential hypertension and may have beneficial implications for future treatment and management strategies.

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Dankwoord

Dankwoord

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Curriculum Vitae

Curriculum Vitae

Barry van Varik was born on June 11, 1981 in Breda, The Netherlands. He attended secondary school at Trevianum in Sittard and graduated in 2001. In 2002 he started his study of Medicine at Maastricht University. From the the second year of his studies, he participated in the student honors-program “Onderzoekstraject Geneeskunde” where he began doing scientific research at the department of physiology under the supervision of prof. dr. U. Schotten. After obtaining his master degree (cum laude) in 2007 he continued doing scientific research, now on the subject of arterial remodeling and hemodynamics in hypertension under the supervision of prof. dr. A.A. Kroon, prof. dr. P.W. de Leeuw, and dr. R.J.M.W. Rennenberg of the Maastricht University Medical Centre. In 2008 he graduated from medical school (cum laude) and continued his research as PhD-student at the same department. This work finally led to this PhD thesis. In December 2013 he started his residency in Internal Medicine at the Maastricht University Medical Centre (under supervision of prof. dr. C.D.A. Stehouwer and prof. dr. R.P. Koopmans) and starting in 2016 at the Zuyderland Medical Centre in both Sittard-Geleen and Heerlen (supervisors dr. B.J. Looij and dr. J. Buijs). He will start his subspecialization in Vascular Medicine (also under supervision of prof. dr. A.A. Kroon) in May 2019. Barry is married to Jolyn Nijsink and together they live in the village of Eckelrade, surrounded by the beautiful hills and countryside of South Limburg.

List of publications

List of publications

Rennenberg, R. J. M. W., van Varik, B. J., Schurgers, L. J., Hamulyak, K., Cate, Ten, H., Leiner, T., et al. (2010). Chronic coumarin treatment is associated with increased extracoronary arterial calcification in humans. *Blood*, 115(24), 5121–5123.

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Oral presentations

European Society of Hypertension (ESH) 2018, Barcelona (Spain): Differences in long-term changes in carotid remodeling between normotensive and hypertensive persons in a primary care population

Regional Endocrinology Rounds, April 14, 2016, Thorn (The Netherlands): A nervous patient (case-report)

Vascular rounds 2015, Thorn (The Netherlands): A patient with therapy resistant hypertension (case report)

European Society of Hypertension (ESH) 2014, Athens (Greece): Long term changes in carotid intima-media thickness and arterial Remodeling are associated with antihypertensive treatment status

European Society of Hypertension (ESH) 2011, Milan (Italy): the longitudinal association

between arterial stiffness and renal damage in a primary care population

Artery Society meeting 2009, Cambridge (United Kingdom). Arterial calcification after chronic use of vitamin-k antagonists.