Hypertension and target organ damage

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Chapter 1

Introduction

Introduction

Cardiovascular disease, and hypertension in particular, is an important cause of morbidity and mortality in the general population. Whereas major efforts have been undertaken to prove the benefits of antihypertensive treatment, our understanding of the pathophysiological processes that are associated with the pathogenesis of the disorder remains limited. When left untreated, hypertension may cause considerable damage to the cardiovascular system, leading to such complications as myocardial infarction, cerebrovascular accident, peripheral vascular disease and renal failure. More recently, other complications (e.g. open-angle glaucoma, retinal vascular occlusion) have been recognized as being linked to hypertension as well.

Before major complications occur, subclinical target organ damage (TOD) develops. This may include left ventricular hypertrophy, an increased intima-media thickness of larger vessels, glomerular obliteration and an increased intra-ocular pressure. For years, these events have been neglected and even today many physicians fail to seek properly for TOD in their patients. Yet, epidemiological studies have clearly shown that the presence of TOD enhances cardiovascular risk over and above that already associated with the elevated pressure. For instance, once left ventricular hypertrophy has developed as a result of long-standing hypertension, this complication becomes a risk factor in its own right and a predictor not only of further cardiac abnormalities[1], but also of other atherothrombotic events such as ischemic stroke.[2] Similarly, the presence of cerebrovascular abnormalities may raise cardiovascular risk over and above that conferred by hypertension itself. Although the brain and the heart are the prime targets of the hypertensive process, the kidney is also frequently affected. Evidence is accumulating that renal damage, once present, may also independently contribute to an increased cardiovascular risk.[3,4] This knowledge calls for a more elaborate work-up of hypertensive patients in order to identify those subjects who are particularly at risk.

Although not proven, it is generally assumed that target organ damage develops as a consequence of an elevated pressure and that it precedes the clinical manifestations of cardiovascular disease. In other words, TOD can be considered as an intermediate endpoint of the hypertensive process and, as such, could even serve as a substitute for morbidity and mortality in large-scale clinical trials. If this is true, it is important to know which components of blood pressure are most closely linked to TOD as it is well known that blood pressure is not a static phenomenon but varies greatly throughout the day. Although one measurement of blood pressure, taken at the office, already correlates reasonably well with cardiovascular damage and prognosis, substitution of casual pressure by 'usual blood pressure', defined as the average of multiple measurements on various occasions, improves the relationship considerably.[5] While it may be true that obtaining many blood pressure readings at the office is

more or less equivalent to 24-hour ambulatory measurements for assessing a patient's usual blood pressure and cardiovascular risk[6], the latter technique is much more practical and yields results within one day. Indeed, under a variety of conditions ambulatory blood pressure monitoring (ABPM) has proven to be superior to conventional blood pressure measurements not only for the diagnosis of hypertension but also for establishing its severity. Non-invasive ambulatory blood pressure measurements even appear to be a better predictor of outcome than clinic blood pressure. The development of rather cheap, validated devices which can be easily worn by the patient make ABPM an interesting tool to employ in clinical practice.

Blood pressure varies markedly over a 24-hour period. Some fluctuations are due to acute events, either physical or psychological. In addition, there is a circadian pattern: blood pressure is highest in the morning, falls slightly during the day and rises to a second peak in the late afternoon. It then falls again during the evening. When an individual goes to sleep, blood pressure falls markedly and stays at relatively low levels during the sleeping period. On awakening there is an acute rise in blood pressure, which has been refered to as the early morning peak. Data suggest that morbidity and mortality from cardiovascular and cerebrovascular disease is greatest in the hours around the awakening period. There are three types of information that ABPM can provide. First, it can give an estimate of the average or true blood pressure, which is the measure generally thought to be responsible for most of the adverse effects of high blood pressure. Secondly, it can describe the diurnal rhythm. Thirdly, it can give an estimate of the short-term variability of blood pressure.

Despite many investigations, the prognostic impact of 24-hour blood pressure variability has not yet been fully explored. It is easy to identify, in 24-hour blood pressure recordings, the presence or absence of a large nocturnal decline in pressure. Also, several studies have assessed the relationship between 24-hour averages of pressure and TOD or prognosis. However, it is only recently that the importance of nighttime blood pressure has been appreciated. For instance, the investigators from the Syst-Eur study have clearly shown that the best predictor of future cardiovascular complications in an elderly population with isolated systolic hypertension is nighttime pressure.[7] Others have pointed out that the magnitude of the nocturnal dipping of blood pressure is associated with, for instance, left ventricular hypertrophy.[8] So it is far from clear as to whether the precent dipping or the actual night time blood pressure are involved in cardiovascular load. These considerations have led us to start a series of investigations in which we have tried to explore the significance of the diurnal blood pressure profile in more detail. The results of this endeavour form the core of this thesis.

In Chapter 2 of this thesis the question is addressed whether patients with white coat hypertension have target organ changes. Although there are various ways to define white coat hypertension, it is usually considered as a condition in which the patient has elevated blood pressure in the office, but on average, has a normal pressure during 24-hour ambulatory monitoring. Whether or not this is a benign form of hypertension with a favourable course and low risk of complications is still a matter of dispute. While some argue that white coat

hypertension carries a relatively low risk compared to sustained hypertension[9], others take a more dim view based on the fact that white coat hypertension has characteristics similar to borderline hypertension and is associated with several metabolic risk factors.[10] To date, no outcome studies have been performed that were specifically designed to assess the effect of antihypertensive treatment on complication rates in patients with white coat hypertension. By establishing the degree of target organ damage in a group of patients with this condition, we wanted to provide additional evidence for or against the opinion that the white coat effect is a benign phenomenon.

Chapter 3 examines the relationship between 24-hour blood pressure and cardiorenal damage. In particular, the various components of the diurnal blood pressure profile (daytime pressure, nighttime pressure and the amount of nocturnal dipping) are related to left ventricular mass and renal function (serum creatinine and creatinine clearance). Since it is not well known whether the heart and the kidney respond differently to the burden of an elevated blood pressure burden, we performed additional analyses to evaluate if the relationship between blood pressure and degree of organ damage runs different courses for these two targets. Moreover, analyses were done in untreated and treated subjects, which allowed conclusions to be drawn about the potential modification of the pressure-damage relationship by antihypertensive treatment.

Chapter 4 focuses on the subclinical cerebral white matter leasions (WML) in hypertension. To this end, we measured white matter lesions and cognitive functions in a group of hypertensive subjects. In this study we wanted to determine which components of ambulatory blood pressure profile are best associated with the presence of WML in hypertensives. Chapter 5 continues on this topic but now looking at the common carotid intima-media thickness (IMT) as intermediate form of damage, as an increase in this measure is considered to represent a phase of the atherosclerotic process that precedes overt complications. This chapter also looks at the possibility that left-right differences may exist in IMT and in the occurrence of atherosclerotic cerebrovascular lesions. In Chapter 6 the changes in IMT are related to two candidate genes for atherosclerotic lesions: the ACE-gene and the gene encoding apolipoprotein E. Finally, in Chapter 7 the results of the various studies are summarized and put in perspective.

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Chapter 2

Is elevation of clinic blood pressure in patients with white coat hypertension who have normal ambulatory blood pressure associated with target organ changes?

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Abstract

Background

The issue as to whether white coat hypertension is a pathologically significant entity, with associated target organ changes, or that the condition carries the same risk for target organ involvement as normotension, is undecided. Previous studies which have shown pathological correlates between white coat hypertension and target organ damage have not controlled for the most obvious confounder, mean 24 hour blood pressure (BP).

Methods and results

In this study we retrospectively identified 33 ages and sex-matched pairs, one group with normal BP, the other with white coat hypertension. The white coat hypertensive group showed significantly greater left ventricular mass indexed for body surface area than normal controls (99.0 g/m^2 vs. 78.3 g/m^2, $p<0.001$). The population was then further matched for 24-hour mean BP (20 pairs), and was again compared for cardiac muscle changes. The significantly increased left ventricular mass index in the white coat population remained after controlling for 24-hour mean BP (101.1 g/m^2 vs. 81.0 g/m^2, $p<0.021$).

Conclusion

White coat hypertension is indeed associated with a larger left ventricular muscle mass than normotensives and these changes are independent of the actual 24-hour BP load, and may reflect increased BP lability, sympathetic nervous system derangement, or a genetic propensity in people with white coat hypertension to stress-related hypertensive reactions, as part of a pre-hypertensive state.

Introduction

White coat hypertension has long been recognised as an acute elevation of blood pressure (BP) occurring in the context of active third party BP measurement.[1] Variously termed 'white coat hypertension', 'clinical hypertension', or 'isolated clinic hypertension'[2] (we will herein refer to the phenomenon as white coat hypertension (WCH)), it has been assumed that the lack of sustained hypertension in these patients reflects a reactive sympathetic nervous system, and predicts a benign prognosis. A number of studies looking at evidence of target organ damage have given equivocal results; left ventricular hypertrophy and renal dysfunction have both been described as occurring in association with WCH[3–8], while other studies have not documented an association.[9–12] These studies have largely shown significantly higher mean BP in the WCH group, and it may be that the documented elevation of cardiac and renal indices of end-organ involvement found in these studies are simply a reflection of this higher BP in this group.[13]

In this case-control study, we identified a large cohort of patients with WCH defined on 24-hour ambulatory BP (ABP) monitoring, and an age and sex-matched normal cohort drawn from the normal population. Groups were compared for ABP profiles, and the presence of target organ involvement, namely the presence of myocardial hypertrophy. The groups were then further matched for 24-hour mean arterial BP, and the comparison for left ventricular mass index (LVMI) was then repeated, to determine if controlling for BP differences between the populations would remove the perceived differences in target organ involvement.

Subjects and Methods

Patient population

Patients were identified from a search of the database in the Blood Pressure Unit (Beaumont Hospital, Dublin, Ireland), which comprises patients referred to the hospital for investigation of hypertension. Patients referred to this service routinely have electrocardiography and echocardiography performed within 1 week of the ABP monitor being applied. Patients were selected if they met with the following definition of WCH, namely an elevation of the clinic BP, with or without an elevation of the initial BP (first hour) on the ABP monitor above 140 mmHg systolic and/or 90 mmHg diastolic, with a normalisation of the BP to below these figures within the next hour, and a subsequently normal BP mean for both daytime (systolic 135 mmHg, diastolic 85 mmHg) and night-time (systolic 125 mmHg, diastolic 70 mmHg) monitoring periods. Patients were excluded if the above definition was not met, or if the routine screening tests

were not performed. Also, patients were not included if they were documented as taking antihypertensive medication at any time prior to referral for the ABP monitor. Shift workers were excluded from the analysis.

Control population

Control patients were enrolled from a database of ABP in the normal population, the initial study of which has been described elsewhere.[14] Patients from this population have been routinely brought back for follow-up study, from 1995 onward, and had electrocardiography and echocardiography performed on the day of the ABP monitor. A total of 130 control subjects were available for cross matching. WCH patients were assigned age and sex-matched controls from this database. The matching procedure was undertaken without knowledge of the patients BP variables, by a physician told only: (1) which patient cohort the subject belonged to; and (2) age and sex. Only controls with a normal clinic, initial, daytime and nighttime ABP profile according to the above definition were enrolled. Again, patients were excluded if the screening data was deficient, or if they were taking medicines known to interfere with BP.

Echocardiography

Echocardiography was performed by a trained echo-cardiographer using a standard 2.5 MHz echocardiography transducer applied to the chest in the parasternal long and short axis planes, where measurements of wall thickness, and chamber size were made. To ensure there was no systematic observer bias, three M-mode tracings were printed from each of the videotaped studies; a trained technician then manually measured the chamber parameters, blinded to the case-control status of the patients, and took the mean dimensions for the three tracings as the measured variable. The left ventricular mass was calculated from these parameters using the formula of Devereux et al.[15] This was subsequently indexed for body surface area.

Blood pressure measurement

Clinic BP was measured in accordance with the recommendations of the British Hypertension Society.[16] For controls, all readings were required to be below 140 mmHg systolic and 90 mmHg diastolic. All case patients had an elevated clinic BP on referral from their general practitioner, and all had an elevated clinic BP again when measured in the Blood Pressure Unit prior to the affixing of the ABP monitor. The clinic pressures were measured in both cases and controls (after 5 min quiet sitting) by the Unit nurse, prior to affixing the ABP monitor. The lower reading was taken as the clinic pressure, and this value was entered into the database. Twenty-four ABP measurement was performed using the SpaceLabs 90207 (Redmond, WA, USA) ABP monitor.[17] Monitors were programmed to measure BP at 30-min intervals day and night. The monitor was removed the next day, and the data was transferred into a personal computer and loaded into a specialised software package (DABL).[18] The initial, daytime and night-time

systolic, diastolic and mean BP were calculated. The 'daytime' period was defined as the hours between 09.00 and 21.00 hours (excluding the initial period), and night-time as the hours between 01.00 and 06.00 hours. The 24-h period was defined as the total period of measurement time from application to removal of the monitor. Transition times (21.01 to 00.59 hours, and 06.01 to 08.59 hours) were not included in the estimation of day and night mean pressures, as these periods represent times during which bed rest is inconsistent and therefore cannot reliably be categorised.[19] Patients on night shift work, or within 4 weeks of completing night shift duty, were not included in the analysis. Recordings were not included if there were less than 14 valid readings during the day, or less than seven valid readings during the night. The validity criteria were those identified by the editing software, i.e., systolic BP, diastolic BP, diastolic BP <160 or >40 mmHg, systolic BP <260 or >50 mmHg. BP values not identified by the editing software were included in the analysis.[20]

Definitions and statistics

Clinical data was extracted from the database, in accordance with the following definitions. Family history of hypertension was defined as the reporting of hypertension in a first degree relative. A family history of vascular disease was present if one or more first-degree relatives had suffered a myocardial infarction, angina pectoris, a cerebrovascular accident or had been given a diagnosis of peripheral vascular disease. The presence of any other medical condition identified from the clinical review at time of monitoring was considered a potential confounder and this patients record was not included in the analysis.
The initial matched groups were compared for BP variables, and for left ventricular mass; the pairs were then further matched for BP by assigning a sex-specific sequential ranking code to the mean 24-hour BP for subjects in each group. The ranked pairs obtained were then compared for actual BP and age; corresponding BP values and age not differing by 2.5 mmHg and 2 years were deemed acceptably paired and this pairing was included in the age, sex and BP matched cohort. The secondary selection procedure was undertaken by an observer blinded to the left ventricular mass measurements of the cohort.
Group differences between variables was explored using the paired t-test. Where non-normal data was compared, the Wilcoxon Rank Sum method was used. Differences in proportions between paired variables were explored by calculation of the z statistic. A p-value of less than 0.05 was considered significant.

Results

Age and sex-matched population

A total of thirty-three age and sex-matched pairs were identified. The clinical data between groups are presented in Table 2.1. WCH patients were slightly

heavier than controls, but not significantly so. Body mass index (BMI) was comparable across the two groups. A family history of hypertension was found more frequently in the WCH patient population, but there was no difference in reported family history of vascular disease. The incidence of cigarette smoking was comparable between the two groups.

Table 2.1. Clinical characteristics of age and sex-matched patient population. Values are expressed as the mean (95% confidence intervals for the mean)

	Cases	Controls	p-value
n	33	33	
Age (yrs)	40.3	40.1	
Sex (M/F)	9/24	9/24	
Weight (kgs)	73.6 (67.9-79.4)	70.0 (65.9-74.1)	0.20
Height (cm)	166.6 (162.8-170.4)	168.8 (165.8-171.7)	0.23
Family history of hypertension	21/33	15/33	ns
History of vascular disease	15/33	17/33	ns
Smoker	13/33	13/33	ns

Intra-observer error for echocardiographic parameters was small; ANOVA testing showed no over-all difference in the mean LVM calculated from the three sets of measurements. Absolute differences across measurements did differ significantly from zero, with a mean difference of 2.4 grams (95% confidence interval 2.0–2.8). Accordingly, although significant, the absolute intra-observer variability was small.

The data pertaining to ABP are presented in Table 2.2. By definition, WCH patients had a significantly higher initial systolic and diastolic BP. Daytime and nighttime systolic BPs were significantly higher in the WCH patient group, although remaining within the normal range. There was no difference between the groups with respect to presence of nocturnal dipping of BP or heart rate. The LVMI is presented in Table 2.2. Both groups showed a LVMI within the normal range (<110 g/m^2), but the LVMI for white coat hypertensives was significantly greater than controls.

Table 2.2. Blood pressure and left ventricular mass data from age abd sex-matched cases and controls. All BP data are expressed as mmHg. Data values are expressed as means (95% confidence intervals for the mean).

	Cases	Controls	*P* value
Clinic SBP	162.2 (157.8-166.5)	110.6 (106.1-115.1)	<0.0001
Clinic DBP	102.0 (98.9-105.1)	69.6 (66.7-72.5)	<0.0001
Day SBP	125.4 (123.3-127.5)	117.1 (113.8-120.3)	<0.001
Day DBP	77.6 (76.0-79.2)	75.0 (72.9-77.1)	0.051
Night SBP	101.6 (98.9-104.3)	106.9 (104.0-109.8)	0.01
Night DBP	62.9 (60.8-64.9)	60.5 (58.5-62.4)	0.09
LVMI ($g.m^2$)	99.0 (88.2-109.8)	78.3 (71.3-85.3)	0.001

Age, sex and blood pressure matched population

Secondary matching identified 20 age, sex and BP matched pairs. BMI was not significantly different between the groups, and they were comparable for other clinical features (Table 2.3). The BP data are presented in Table 2.4. The groups were similar for 24-hour BP parameters, and only differed in the initial BP profile, with the WCH group having a higher initial systolic and diastolic pressure. End-organ data from the two groups shows persistence of the differences in left ventricular muscle mass index (Table 2.4), with the WCH group demonstrating significantly higher LVMI. A multiple regression model was fitted to the data with LVMI as the dependent variable, to determine possible confounding by the independent variables daytime and night-time systolic and diastolic BP, age, BMI, height, weight and sex. Additionally, the presence or absence of WCH was entered into the model as a covariate. The only significant predictors in the model were the presence or absence of WCH p= 0.011), and age (p= 0.01).

Table 2.3. Clinical characteristics of age, sex and BP-matched patient population. Values are expressed as the mean (95% confidence intervals for the mean)

	Cases	Controls	p value
N	20	20	
Age (yrs)	40.2	40.8	
Sex (M/F)	10/10	10/10	
Weight (kgs)	79.9 (72.5-87.4)	75.0 (70.0-80.0)	0.14
Height (cm)	170.5 (165.5-175.6)	173.1 (169.2-176.9)	0.37
Family history of hypertension	5/20	5/20	ns
History of vascular disease	9/20	10/20	ns
Smoker	8/20	9/20	ns

Table 2.4. Blood pressure data from age, sex and BP-matched cases and controls. All BP data are expressed as mmHg. Data values are expressed as means (95% confidence intervals for the mean)

	Cases	Controls	*p* value
Clinic SBP	160.2 (154.6-165.8)	116.2 (111.5-120.9)	<0.0001
Clinic DBP	102.3 (98.1-106.5)	73.0 (70.2-75.8)	<0.0001
Day SBP	125.2 (122.2-128.0)	123.0 (120.3-125.8)	0.17
Day DBP	77.2 (75.0-79.4)	78.8 (76.8-80.8)	0.18
Night SBP	108.0 (104.7-111.3)	105.3 (102.9-107.7)	0.10
Night DBP	63.1 (60.6-65.7)	62.8 (60.7-64.9)	0.81
LVMI ($g.m^2$)	101.1 (87.2-115.0)	81.0 (70.6-91.5)	0.021

Discussion

White coat hypertension as a clinically distinct entity has been recognised for some time.[21,22] As many as 20% of patients presenting for ABP monitoring with an elevated clinic measured BP may have a normal 24-h BP profile.[23] The debate continues as to whether the clinical condition of WCH represents a true pathological state, with associated morbidity, or a benign manifestation of a reactive sympathetic nervous system.[24]

There has been recent speculation that WCH is not an entirely benign entity. Left ventricular mass has been shown to be higher in elderly white coat hypertensives than in normal controls.[4,7] However the literature is at variance on the subject, with other reports suggesting that no significant left ventricular remodelling occurs in these patients.[11] It is interesting to note that previous comparative studies of WCH *vs.* normotension have shown higher 24-hour BPs in the WCH group.[6,7,11] This would at least suggest that within the normal range, WCH patients have a higher 24-hour BP load, occupying a higher pressure stratum than normotensives. The subtle changes in left ventricular mass could be accounted for by this BP discrepancy.[13] If this was the case, then one would expect that differences between normo- and white coat hypertensives with regard to target organ changes would disappear when the groups are further controlled for 24-hour BP. Indeed, two such studies,[9,10] comparing left ventricular mass between normotensive and WCH groups, where 24-hour BP was comparable across the two groups, showed no difference in structural heart changes. On the other hand, Glen *et al* [3] in a similarly designed study, with BP equivalence between the normal and WCH groups, showed evidence of functional cardiac derangement in the WCH group. This last study has however been criticised for having a very high cut-off point for the difference between normotension and hypertension, at 95 mmHg diastolic.[25,26] As discussed by Verdecchia et al.,[27] a high cut-off point may result in patients with borderline hypertension being included in the definition of WCH. As a result, end-organ damage may be ascribed to patients given the qualitative diagnosis of WCH, when in fact it is the quantitative, continuous variable of BP that is responsible

for end-organ changes. Our study specifically compares cardiac muscle mass between normal patients and white coat patients, and removes the possible confounding effect of differences in BP between the two groups. The fact that differences in muscle mass persisted when BP differences were removed from the equation is strong evidence that white coat hypertensives are indeed different from their normotensive counterparts.

Left ventricular hypertropy (LVH) has been well documented as an indicator of a poor prognosis in patients with hypertension.[28] It is possible that even with our strict matching of BP, minor differences in measured pressure might, over a protracted time period, give rise to the observed differences in left ventricular mass, but the minimal differences between the populations would make this very unlikely.

What aetiological mechanisms may be at work to cause cardiac changes in the presence of WCH but in the absence of sustained elevation of BP? Firstly, transient stress-related increases in BP, occurring throughout the course of the day may account for reactive changes in the vascular architecture of the heart, while not altering the mean BP load, as measured on 24-hour ABP monitoring. Thus, an increase in BP variability may account for the changes in target organs.[29] However, a number of studies have failed to show significant BP lability in patients with WCH.[30,31] Secondly, the presence of WCH may be a manifestation of an underlying dysfunctional sympathetic nervous system.[32,33] Left ventricular muscle hypertrophy has been ascribed to trophic activity of the sympathetic nervous system.[34] Again, however, sympathetic anomalies have not been definitively proven in WCH.[29,35] Finally, patients with WCH may have an underlying genetic propensity to an increased stress responsiveness of BP. This genetic tendency may also be expressed in subtle abnormalities of cardiac modelling. It is already known that children of hypertensive parents, without overtly elevated BP, may show structural cardiac muscle hypertrophy.[36] A prospective study showing that white coat hypertensives progress to sustained hypertension would provide good supportive evidence for this latter interpretation, and some evidence for this does exist.[37]

With respect to this study, it is always a concern that a retrospective case control study will be open to selection bias. It is possible that a particularly severe cohort of white coat hypertensives, with LVH, were selectively identified. However, the decision to perform the echocardiography was a protocol driven one, based on the referral BP, and without knowledge of the ABP monitoring result. The likelihood therefore of particularly a pathological cohort being identified is small.

A prevalence rate of 20% for WCH means that a significant number of patients in the community have a form of BP abnormality which carries a relatively low risk.[38] Our findings would concur with this interpretation. However, our results also suggest that WCH does describe a group of patients with a cardiovascular profile that is different from normal.

The only prospective study to date[25,39] had a relatively short follow-up period, and was unlikely to have shown either progression to sustained hypertension in

white coat hypertensives over this time period, and therefore would have been unlikely to have shown an excess of morbidity in these patients.
Further data are therefore required to determine the prognostic significance of our findings, with regard to mortality and end-stage organ failure.

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Chapter 3

Left ventricular hypertrophy and renal impairment are associated with reduced nocturnal blood pressure decline, even in adequately treated hypertensives

Rodríguez Hernández SA, Kroon AA, Atkins N, Lyons SP, Owens PE, de Leeuw PW, O'Brien ET

Abstract

Introduction
Patients with absence of a nocturnal blood pressure fall (non-dippers) may be at higher risk for cardiovascular and renal target organ damage. Left ventricular hypertrophy (LVH) and nephropathy have been reported to occur more frequently in non-dippers.

Objective
To assess whether a relation between nocturnal decline in blood pressure (BP) and target organ damage is still present during treatment, we determined the presence of LVH and renal damage in 479 treated hypertensives, selected from a database which comprises patients referred for treatment of hypertension to a large regional hospital.

Methods
LVH was determined by echocardiography. Nephropathy was assessed by measuring serum creatinine, creatinine clearance and microalbuminuria. For analysis, patients were divided in three groups: adequately treated (SBP<135 and DBP<85 mmHg, n=87), borderline treated (intermediate BP, n=193), and inadequately treated (SBP>140 and DBP>90 mmHg, n=199). Each BP group was subdivided into tertiles based of the degree of dipping.

Results
The mean percentage noctural fall in BP was 10%, 12%, and 16% in the adequately treated, borderline, and inadequately treated group, respectively. Patients in the inadequately and bordeline treated groups, as well as those with blunted nocturnal fall in the adequately treated group had significantly higher left ventricular mass index (LVMI). Only adequately treated subjects had a normal LVMI. No differences in renal function were observed between these groups. Reduced nocturnal dipping was associated with both a higher LVMI and impaired renal function.

Conclusion
In treated hypertensives, both LVMI and renal function are related to the degree of nocturnal fall in BP, but only cardiac damage is associated with overall inadequate treatment as based on daytime BP measurements. This suggests that antihypertensive treatment may not necessarily reduce cardiovascular risk and that the kidney is more resistant to hypertension-related damage than the heart.

Introduction

Several lines of evidence indicate that 24-hour ambulatory blood pressure (BP) is a far better predictor for target organ damage and cardiovascular risk than office blood pressure. In particular, nighttime pressure and the degree of nocturnal dipping are strongly related to prognosis in untreated essential hypertension.[1,2] Recently, Verdecchia et al. also demonstrated in treated hypertensives that ambulatory blood pressure monitoring (ABPM) is superior to office measurements for prediction of subsequent cardiovascular disease.[3] However, despite considerable improvements in antihypertensive therapy, cardiac and renal complications continue to occur. This may be related to insufficient reversal of target organ damage. Surprisingly, though, very little information is available with respect to the relationship between 24-hour or nocturnal BP and target organ damage in treated hypertensive patients. Also, it is not well known whether the degree of target organ damage in adequately treated hypertensives is proportional to the achieved level of blood pressure or not. This prompted us to address this question in more detail. Accordingly, we examined the association of ambulatory recorded blood pressure with cardiac and renal damage in a large group of treated patients with essential hypertension. Cardiac changes were assessed by echocardiography and renal damage was estimated from serum creatinine, creatinine clearance and micro-albuminuria.

Patients and Methods

Study population

Patients were identified from a search in the hypertension database of the Blood Pressure Unit from the Beaumont Hospital in Dublin (Ireland), which comprises patients referred to the hospital for treatment of hypertension. Patients referred to this service routinely have echocardiography performed within one week of the ABP monitor being applied. On the day the ABPM was executed, office BP was measured twice in sitting position. A check list was kept regarding the occurrence of familial hypertension in the first degree and the presence of cardiovascular morbidity due to hypertension. Furthermore, blood samples were taken for determination of serum creatinine, total cholesterol, HDL cholesterol, serum triglycerides, sodium, potassium, chloride, urate, glucose, urea and urine samples for protein, glucose, and microalbuminuria. All variables were determined by the standard procedures used at the routine laboratory. Microalbuminuria was measured using the dipstick method. Patients were only selected if they were treated with antihypertensive drugs. Patients were excluded if the above definition was not met, and if the routine tests were not adequately

performed. Furthermore, patients with a serum creatinine higher than 150 µmol/l were excluded from the study. After these criteria were applied, we included 479 treated hypertensive patients in this study.

Echocardiography

Echocardiography was performed by a trained technician using a standard 2.5 MHz transducer applied to the chest in the parasternal long and short axis planes for measurements of wall thickness and chamber size. Left ventricular mass (LVM) was calculated using the formula of Devereux et al.[4], and was indexed for body surface area. To ensure there was no systematic observer bias, the M-mode tracings were printed from each of the videotaped studies. A trained technician who was blinded to the patients then manually measured the chamber dimensions.

Definitions and statistics

For analysis, patients were devided in three groups: adequately treated (defined as daytime systolic BP <135 mmHg and daytime diastolic BP <85 mmHg), borderline treated (135 mmHg >= daytime systolic BP =<140 mmHg and 85 mmHg >= daytime diastolic BP =<90 mmHg), and inadequately treated hypertensives (daytime systolic BP >140 mmHg and daytime diastolic BP >90 mmHg), as measured by ABPM. Daytime was defined from 9:00 a.m. till 9:00 p.m. and nighttime as 1:00 a.m. till 6:00 a.m. Nocturnal fall in BP was calculated using the formula: [(MAP day - MAP night) / MAP day] x 100%, in which MAP represents mean arterial pressure. On the basis of the degree of nocturnal fall each treatment group was further subdivided into tertiles. Creatinine clearance was calculated according to the formula of Cockcroft and Gault.[5]

The data pertaining to the cases were uploaded into a commercially avialable spreadsheet, and this was in turn uploaded into a statistical program (SPSS Inc., Chicago, Illinois, USA). Differences between and within groups for normally distributed variables were explored with analysis of variance (one-way ANOVA) and, if necessary, followed by a t-test for independent samples. Differences in proportions were explored by calculation of the z-statistics. Bonferonni's method for correction of multiple testing was applied. Determinants of dipping were analyzed by means of linear regression. Unless indicated otherwise, all data are presented as mean ± standard deviation (SD). A p-value less than 0.05 was considered statistically significant.

Results

The main characteristics of all subjects were as follows: 37 % were males, body mass index (BMI) 28 $kg.m^{-2}$, office systolic BP 166±26 mmHg and diastolic BP 95±13 mmHg, mean daytime ambulatory systolic BP 148±19 mmHg and

diastolic BP 90±12 mmHg, mean nighttime ambulatory systolic BP 131±19 mmHg and diastolic BP 76±12 mmHg. Patients had been treated for a median period of 2.5 years (range 0.9 to 3.2 years) with the following medications: diuretics (39%), beta-blockers (38%), angiotensin-converting enzyme inhibitors (34%), calcium channel blockers (22%), angiotensin II receptor blockers (7%), and other drugs (6%). Forty percent of the patients received more than one antihypertensive drug (Figure 3.1)
The data concerning the nocturnal fall of BP and the parameters for cardiorenal target organ damage, subdivided according to treatment efficacy, are shown in Table 3.1. The percentage of dipping was highest in the inadequately treated group ($p<0.001$). This latter group had, by definition, the highest daytime MAP.

Table 3.1. Results of ambulatory blood pressure measurements and cardiorenal target organ damage in 479 treated hypertensive patients, subdivided in three groups on the basis of treatment efficacy

	Adequate*	Borderline	Inadequate	p-value#
Number	87	193	199	
Age (years)	53 ± 14	55 ± 12	53 ± 11	n.s.
Daytime MAP (mmHg)	92 ± 5	105 ± 6	122 ± 10	<0.001
Nighttime MAP (mmHg)	83 ± 9	92 ± 11	103 ± 14	<0.001
Dipping (%)	10 ± 10	12 ± 10	16 ± 9	<0.001
Serum creatinine (µmol/l)	93 ± 24	92 ± 24	91 ± 21	n.s
Creat. Clearance	83 ± 24	84 ± 28	88 ± 29	n.s
Microalbuminuria (µg/l)	25 ± 30	28 ± 29	34 ± 34	n.s
LVMI (g/m^2)	109 ± 38	124 ± 43	130 ± 38	<0.001

* for definition of treatment groups see method section; # one-way ANOVA; n.s. indicates not statistically significant; LVMI indicates left ventricular mass index.

However, nighttime MAP in this group was also significantly higher in comparison to the two other groups ($p<0.001$). Interestingly, subjects in this inadequately treated group more frequently used an angiotensin-converting enzyme inhibitor in comparison to the adequately treated group ($p=0.03$), whereas β-blockers ($p=0.004$) and combination treatments ($p=0.01$) were more frequently used in the adequately treated group (Figure 3.1).
Between the treatment groups we did not observe significant differences with regard to the measures for renal damage, i.e. serum creatinine, creatinine clearance, and microalbuminuria (Table 3.1). However, if treatment groups were further subdivided into tertiles based on the degree of nocturnal dipping of BP (Table 3.2), subjects with the smallest difference between day- and nighttime BP (Figure 3.2, panel A, tertile III) had a significantly lower creatinine clearance compared to the other two tertiles in all three treatment groups ($p=0.03$, $p=0.003$, and $p=0.05$, respectively). By multiple linear regression creatinine clearance was significantly associated with the degree of dipping ($\beta=0.51$, $p<0.001$) but not with treatment class. In other words, the amount by which BP fell during the night had a significant effect on creatinine clearance, but whether

or not patients were adequately treated had no further effect on this parameter of renal function.

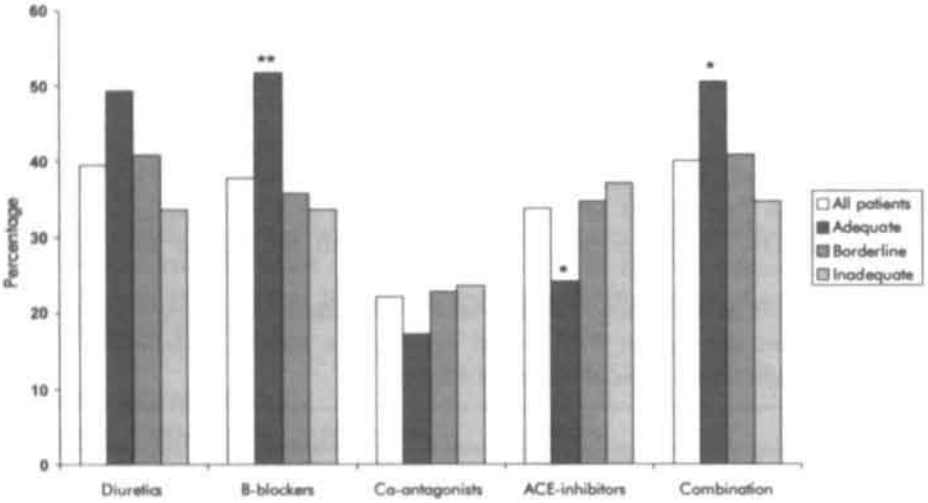

Figure 3.1. Frequency distribution of four main classes of antihypertensive drugs, represented for the total group (n=479) and subdivided in treatment efficacy groups: adequately treated, borderline treated, inadequately treated[&].
[&] for definition of treatment groups see method section; # combination therapy indicates treatment with two or more antihypertensive drugs;
* $0.01 \leq p < 0.05$; ** $p < 0.01$.

Table 3.2. Degree of noctural fall in blood pressure subdivided in tertiles per treatment group.

	Tertile I	Tertile II	Tertile III	p-value[#]
Adequately treated (SBP < 135 mm Hg and DBP < 85 mmHg)				
Number	29	29	29	
Dipping (%)	19 ± 4	11 ± 2	-1 ± 8	<0.001
Borderline treated (135 ≤ SBP ≤ 140 mmHg and 85 ≤ DBP ≤ 90 mmHg)				
Number	64	64	65	
Dipping (%)	22 ± 4	13 ± 5	2 ± 7	<0.001
Inadequately treated (SBP > 140 mmHg and DBP > 90 mmHg)				
Number	66	66	67	
Dipping (%)	25 ± 4	16 ± 3	6 ± 5	<0.001

[#] one-way ANOVA.

Results with regard to the presence of LVH were as follows: subjects with inadequately treated hypertension had the highest LVMI in comparison to the adequately treated group (Table 3.1; $p<0.001$). Moreover, as shown in Figure 3.2, panel B, within each treatment group there was a gradual, significant increase in LVMI going from subjects with the greatest percent dipping (tertile I)

to those who hardly changed at night (tertile III). By multiple linear regression we found a significant negative association between LVMI and the percentage of dipping in the whole group (β=-0.52, p=0.007). When the degree of dipping and treatment class were introduced into the regression model, both remained statistically significant, indicating that inadequate treatment produces extra damage over and above that associated with a low dipping state. No association was found between LVMI and creatinine clearance.

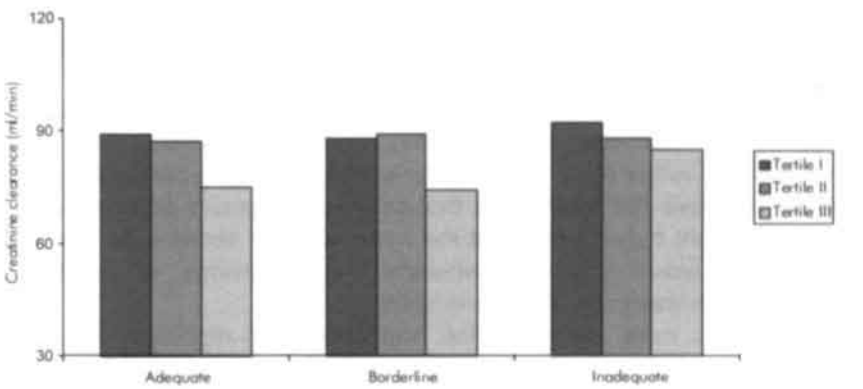

Figure 3.2. Creatinine clearance (panel A) and left ventricular mass index (panel B) represented in tertiles from the percentage of nocturnal fall in blood pressure per treatment efficacy group[&].
[&] for definition of treatment groups see method section.

Discussion

This study shows that both an increase in LVMI and a reduction in creatinine clearance are associated with a lesser degree of nocturnal blood pressure fall in treated hypertensive patients. Moreover, left ventricular hypertrophy, but not nephropathy, was associated with daytime BP measurements, i.e. subjects who were adequately treated had a significantly lower left ventricular mass than those who were not, but similar renal function.
Lowering of blood pressure in hypertensives should maximize patients' well-being and simultaneously lower the risk for pressure-related cardiovascular and renal complications. Therefore, our study was performed to evaluate if treated essential hypertensives achieve an acceptable lowering of their risk for the development of target organ damage. To this end, we assessed the presence of target organ damage in the heart and the kidneys of patients who had adequate or inadequate BP lowering during the day and the night. Data from controlled trials on antihypertensive treatment suggest that blood pressure goals, especially a systolic daytime level below 140 mmHg, are hardly met.[6] Our data suggest

that this is particularly deleterious for the heart, since only adequate treatment reduced LVMI significantly. With respect to hypertension-induced renal damage, one could have an even more pessimistic view, since renal function was similar in all groups irrespective of the adequacy of treatment. Apparently, adequate treatment does not lead to improvement of renal function as it does for the heart. However, it cannot be excluded from our data that the relative insensitivity of the kidney for lowering BP is a consequence of pre-existing nephropathy, present already before the start of treatment. Still, this possibility seems less likely, because LVMI had regressed, or was normal, in the adequately treated group in our study, and, generally, nephropathy develops as a rather late complication. Therefore, our data suggest that perhaps different pathophysiological mechanisms are involved in the development or prevention of target organ damage in the kidney and in the heart. This puts the observations of Collins et al. in perspective that treating hypertension improves prognosis, but does not return it to that of a normotensive population.[7] In this respect, one might hypothesize that the heart is more sensitive to the absolute height of the blood pressure, whereas renal damage is predominantly dependent on the variability of hypertension.

Only few studies have examined the implications of nighttime BP on target organ damage.[8-10] Morfis et al. have shown that nocturnal BP is a strong predictor of LVMI in treated hypertensives and normotensives. Grandi et al. have shown in never-treated essential hypertensives that a fall in nocturnal BP by more than 10% from daytime BP was not related to left ventricular morphology, and concluded that the nondipping status established on the basis of a single 24-hour BP monitoring does not identify hypertensive patients with greater cardiovascular damage. Indeed, in our study we observed a significantly higher degree of nocturnal BP fall in the inadequately treated group with the highest LVMI. However, the nighttime BP in this group was also significantly higher. This probably indicates that it is the absolute nighttime BP level, and not the percentage of dipping of BP, that is important for the development of cardiorenal target organ damage. Indeed, studies in rats have shown that blood pressure levels during sleep are more important for the development of cardiac hypertrophy.[11]

A limitation of our study may be the duration of antihypertensive treatment. However, patients in our study were treated for a minimum period of 11 months (median 30 months), which reduces the probability that the duration of the treatment confounded our results.

The differential effects of drugs on the target organ damage may be another limitation of this study. It is possible that different classes of drugs had another outcome on day and night blood pressure, and changed the "dipper-status": short-acting drugs may convert a person into a 'non-dipper', whereas long-acting drugs or taking drugs before bedtime may convert someone into a 'dipper'. In other words, β-blockers may cause a non-dipper status, and angiotensin-converting enzyme inhibitors and angiotensin II receptor blockers can induce a dipper-status.[12] In our study the use of β-blockers was significantly higher in the adequately treated group, and the use of angiotensin-converting

enzyme inhibitors was higher in the inadequately treated group. This indicates that, if differences in drug treatment influenced our results, it would make the conclusions of this study even stronger.

In conclusion, our data suggest that treatment of hypertension has disparate effects on the degree of target organ damage in heart and kidneys. On the basis of our results, we propose that the nighttime level of BP and not necessarily the degree of nocturnal fall in BP is the more important variable. We may have reached the point in which we have to give due attention to a 24-hour lowering BP strategy in order to prevent target organ damage to develop.

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Chapter 4

Blood pressure profile and its relation to cerebral white matter lesions in untreated hypertensives

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Abstract

Introduction
White matter lesions (WML) in the brain are common, both in normotensives and in hypertensives. The clinical significance of cerebral WML in hypertensives is not fully understood.

Objective
To investigate the relation between subcortical and periventricular WML and ambulatory blood pressure (BP) profiles in uncomplicated, untreated hypertensives.

Methods
In 96 untreated, uncomplicated hypertensive patients a MRI scan and 24-hour ambulatory BP monitoring was performed. The total volume of subcortical white matter lesions (sWML), the extent of periventricular white matter lesions (pWML), and the presence of subclinical cerebrovascular lesions were assessed. Characteristics of the ABPM were associated with presence and extent of the WML.

Results
The mean age of this group was 56±11 years, 59% were males, the daytime mean arterial pressure (MAP) was 118±14 mmHg, and the nighttime MAP 102±14 mmHg. Nocturnal BP fall, nor absolute daytime or nighttime BP levels or BP load were associated with WML in the total population. When subjects were subdivided into tertiles based on the percentage of dipping of BP at night, a significantly higher prevalence of pWML was observed in the tertile with the highest percentage of dipping (43% versus 31% and 26% respectively; $p=0.04$). Daytime MAP was comparable in these subgroups. No association with any of the BP parameters was found for the sWML.

Conclusion
In untreated, middle-aged, hypertensive patients without clinical complications WML, especially sWML, are a frequent finding. No association between these subcortical lesions and the circadian BP profile was found. The prevalence of pWML was, however, much lower in patients with a reduced nocturnal BP fall.

Introduction

Since the introduction of brain magnetic resonance imaging (MRI), the presence of cerebral lesions in the deep and subcortical white matter has been frequently reported. These so-called white matter lesions (WML) are a common finding in healthy, elderly people.[1] The presence of these WML has been taken as a prognostic factor for the development of stroke and cognitive impairment.[2-4] However, the pathogenesis of WML is only poorly understood. Studies have shown that age, hypertension, diabetes mellitus, and a history of stroke or heart disease are the most important factors related to the presence of WML.[1] In essential hypertension, the presence of WML has been associated with the severity of hypertension[5,6], the lack of blood pressure (BP) control in treated patients[7,8], and an increased nocturnal BP decline.[9,10] However, recent studies on ambulatory blood pressure monitoring (ABPM) and WML could not reproduce the latter finding, but rather stressed the association with the severity of high BP levels[11] and a reduced fall in nocturnal heart rate.[6] Relative tachycardia during the night may point towards an increased sympathetic drive or imbalance between the parasympathetic and sympathetic autonomic nervous system.
Taking all available evidence together, most data appear to have been obtained in elderly people, or in a wide range of ages. Moreover, few studies distinguished between cortical and periventricular WML. To further clarify the relation between BP and WML, we designed the present study in untreated, asymptomatic, middle-aged hypertensive patients. We aimed to investigate the association between the characteristics of the 24-hour ambulatory blood pressure profile and WML in the subcortical area and in the periventricular area of the brain in more detail.

Patients and Methods

Study population

Participants were selected from the hypertension outpatient clinic of the Department of Internal Medicine of the University Hospital of Maastricht. As part of the local protocol, ambulatory blood pressure measurements were performed in all patients. For the present analysis we selected 102 hypertensive patients who were between 40 and 80 years of ages, in whom no known cause of their hypertension was detected, and who were without apparent cardiovascular complications. This specific population was chosen because hypertension has been shown to be a major determinant of WML. Exclusion criteria were clinically documented ischemic or valvular heart disease, congestive heart failure, cerebrovascular accidents and/or transient ischemic attacks, chronic renal failure (serum creatinine >150 μmol/L), secondary hypertension, and claustro-

phobia. In all patients antihypertensive medication was discontinued for three weeks prior to the measurements. None of the subjects developed adverse events during this drug-free period. All patients gave their informed consent in writing, after which they were scheduled to undergo ambulatory BP measurements off-medication and a cerebral MRI. This study was part of another survey investigating the relationship between neuropsychological function tests and hypertension-induced target organ damage of the brain. The study was approved by the local medical ethics committee.

Blood Pressure Measurements and other covariates

Noninvasive ambulatory blood pressure measurements (Spacelabs 92127, Redmond, WA) were obtained starting on a morning weekday. The cuff was applied to the non-dominant arm. For analysis, daytime was defined from 7:00 a.m. till 11:00 p.m. and nighttime from 1:00 a.m. till 6:00 a.m. The apparatus was programmed to perform blood pressure measurements every 15 minutes during the day and every 30 minutes during the night. Subjects were instructed to adhere to their normal daily activities and regular sleeping hours. At the time of their visit to the hospital, office blood pressure of patients was recorded by means of a sphygmomanometer and their body mass index (BMI) was calculated from simultaneously performed measurements of height and weight. Information on risk factors like smoking was obtained with a standardized questionnaire, which was checked by a physician during the interview.

Neuroimaging

MR images were acquired on a Philips Intera NT, operating at 1.5 tesla. A scout sequence was used to align the subsequent scans. The MR examination protocol consisted of an axial proton density (PD) sequence, an axial T2-weighted fast spin-echo (FSE) sequence, and an axial T2-weighted fluid-attenuated inversion recovery (FLAIR) sequence.

All MR scans were post-processed in order to obtain estimates of the total volume of subcortical white matter lesions (sWML), the extent of periventricular white matter lesions (pWML), and the presence of subclinical cerebrovascular lesions. WML were scored according to the guidelines of Achten et al.[12]. For this purpose the axial PD, T2-weighted FSE, and FLAIR image stacks were aligned side by side on a computer screen using custom software (BIAS).[13] This software allowed for systematic inspection of synchonized MRI images stacks and manual demarcation of regions of interest (ROI). At each axial level, sWML were scored using predefined region of interest (ROI) masks, i.e. circles with a diameter of 2, 6 and 12 mm, respectively. Lesions were identified on the FLAIR image and then traced on both other images. If a lesion was present on all images a predefined ROI mask was fitted over the lesion, which approximated its size most closely. After inspection and demarcation of all sWML's in a stack the program generated an output file with the number and size of all lesions at each level of the scan. This information was then transferred into a statistical package to yield a total sWML volume score for each patient. In this procedure, the ROI's were

inflated to spheres with the same diameter, with corresponding volumes of 4.2, 113 and 905 mm^2, respectively.[12] Subcortical WML were processed by one medical investigator after satisfactory intra-class correlations (ICC) between 0.81 and 0.98 had been reached[14], based on the scoring of subsequent series of 10 random stacks by this investigator and an experienced neuroradiologist [PH]. Periventricular WML scores ranging between 0 and 3 were made for frontal and occipital periventricular regions ('caps') and the medial periventricular lining ('bands') separately, which were summed to an overall pWML score.[12] The ICC for both raters of pWML based on all stacks was 0.87 (Pearson's R=0.91). Finally, the presence of other cerebrovascular lesions (lacunar infarctions) was identified by the neuroradiologist.

Analysis of data

The data pertaining to the cases were uploaded into a commercially available spreadsheet, and this was in turn uploaded into a statistical program (SPSS Inc., Chicago, Ill.). The nocturnal fall in BP (dipping) was calculated using the formula: [(MAP daytime - MAP nighttime) / MAP daytime] x 100 %, in which MAP represents mean arterial pressure. On the basis of the degree of the nocturnal fall the whole group of patients was divided into tertiles. Differences between and within tertiles for normally distributed variables were explored with analysis of variance (one-way ANOVA) followed, if necessary, by a t-test for independent samples. Data which showed a skewed distribution were log-transformed before analysis. Differences in proportions were explored by calculation of the z-statistics. Bonferonni's method for correction of multiple testing was applied. Determinants of dipping were analyzed by means of (multiple) linear regression. Unless indicated otherwise, all data are presented as mean ± standard deviation (SD). A p-value less than 0.05 was considered statistically significant.

Results

Four patients were excluded from analysis because they were unable to complete the entire MRI procedure and withdrew prematurely. In addition, from two patients the MRI data had been accidentaly deleted from the computer. The characteristics of the remaining subjects (n=96) were as follows: 59% were males, 45% were current smokers and BMI averaged 29 kg/m^2. The average office BP was 166/95 mmHg, mean daytime ambulatory BP 157/97 mmHg and mean nighttime ambulatory BP 138/82 mmHg.

Table 4.1 shows the data for ambulatory BP and WML when patients were divided in tertiles based on the degree of their nocturnal blood pressure dip. No differences existed with respect to age, daytime MAP and daytime or nighttime heart rate. A significant difference in nighttime MAP was apparent, which was highest in the tertile with the least percentage dipping.

Only one subject of the whole group did not have sWML. The total volume of subcortical lesions was not different between the tertiles. Moreover, we did not

find an association between ambulatory BP variables and either the presence or the volume of sWML. The prevalence of pWML, on the other hand, exhibited a significant trend across the tertiles and highest in the tertile with the lowest nighttime diastolic BP or greatest amount of dipping. By multiple linear regression, we found a significant positive association between dipping of BP and the prevalence of pWML. The introduction of other potentially confounding variables in the regression model did not alter this relationship. Interestingly, in this subset of asymptomatic patients, the prevalence of lacunar cerebrovascular infarctions was also significantly higher in the group with the biggest nocturnal BP dip. Although the score of pWML also tended to be highest in the tertile with the largest BP dip during the night, this failed to reach statistical significance.

Table 4.1. White matter lesions and characteristics of the ambulatory measured blood pressure in essential hypertensive patients subdived into tertiles on the basis of their percentage decline in nocturnal blood pressure.

	Tertile I	Tertile II	Tertile III	p-value*
Number	32	32	32	n.s.
Age (years)	59 ± 9	54 ± 10	56 ± 12	n.s.
Daytime MAP (mmHg)	117 ± 14	119 ± 13	117 ± 15	n.s
Nighttime MAP (mmHg)	92 ± 12	102 ± 12	112 ± 19	<0.001
Dipping (%)	21 ± 4	14 ± 2	5 ± 7	<0.001
Daytime HR (beats/min)	81 ± 13	79 ± 10	80 ± 15	n.s.
Nighttime HR (beats/min)	67 ± 12	66 ± 8	67 ± 11	n.s
sWML (mm ^ 3)	1337 ± 3106	1861 ± 5588	806 ± 1811	n.s.
pWML [incidence, (%)]	43	31	26	0.037
pWML (score)	1.7 ± 1.7	1.5 ± 2.2	1.2 ± 1.7	n.s.
Lacunar infactions (%)	45	33	22	0.048

*, one-way ANOVA; n.s., indicates not statistically different; MAP, indicates mean arterial pressure; HR, indicates heart rate; s, indicates subcortical; p, indicates periventricular; WML, indicates white matter lesions.

Discussion

The present study shows that periventricular but not subcortical WML are related to 24-hour blood pressure variability. Patients with essential hypertension who have a large nocturnal fall in pressure apparently tend to have pWML more often than patients in whom the blood pressure dip during sleep is small or absent. With respect to sWML no clear relationship with blood pressure emerges. Silent lacunar infarctions, on the other hand, are again more frequently found in patients with larger falls in pressure during the night.
Results of previous studies concerning the prevalence of WML in patients with hypertension are conflicting. This may be related, at least in part, to differences in patient selection and MRI scoring systems.[15] As pointed out by Sierra and associates[6], most studies have been performed in elderly people or populations

with a wide age range. When these investigators examined a group of 66 untreated patients with essential hypertension who were between 50 and 60 years of age, they found a high prevalence of WML (just as we did). In addition, they showed that the severity of hypertension and not the circadian pattern of nocturnal blood pressure fall determined the presence of these lesions. The latter is in contrast to our present findings. Of course, the difference between these two studies could be related to the wider age range in our patient population. However, this is unlikely to be the sole explanation, not the least because the average age in our study was comparable to that in the patient group of Sierra et al. Also, the fact that patients had never been treated in the Sierra study, while they were temporarily off treatment in ours, cannot fully account for the observed differences. There is, however, one fundamental difference between the two studies. Sierra and coworkers divided their patients on the basis of the presence of lesions in the brain and then compared the groups with and without WML for various clinical, biochemical and blood pressure characteristics. This is, in fact, a retrospective approach of the data. In our study, we have taken blood pressure as the discriminating factor to divide patients. In other words, by departing from the blood pressure profile we tried to 'prospectively' assess the impact of pressure on the prevalence of WML. In doing so, we identified the relationship between the nocturnal BP dip and the prevalence of pWML. Because the score of pWML was not significantly different across the tertiles of BP dip (even though there was a trend), it seems possible that dividing patients simply on the basis of presence or absence of lesions may obscure the effect of BP.

It remains speculative why a larger nocturnal BP decline is associated with a greater prevalence of WML. Interestingly, the prevalence of silent lacunar infarctions was also higher when nocturnal BP was lower. Maintained, or even exaggerated, falls in nocturnal blood pressure were also observed in patients with lacunar infarctions as recently described by Boreas (Thesis, University of Maastricht, 2001). There are at least two possible explanations for these findings. The first is that the presence of WML or lacunar infarcts causes greater day-to-night variability of blood pressure. Although there are no data in the literature to support or reject this hypothesis, we cannot a priori exclude this possibility. However, a second, and perhaps more intriguing explanation could be that patients with reduced nocturnal dipping are relatively protected against the deleterious effects of an elevated pressure. One could hypothesize that larger shifs in pressure throughout a 24-hour period put a greater burden on autoregulatory systems in the brain, which may then decompensate at a faster pace than in patients in whom such shifts are smaller. Impairment of autoregulation of periventricular vessels with increasing age and hypertension, alone or combined, makes the brain more susceptible for hypoxia and could explain the observed relationship. Since both WML and lacunar infarcts are thought to have a microcirculatory origin, this possibility needs further attention. However, even then it remains elusive why we failed to show an association between BP and the prevalence of subcortical WML.

In conclusion, our data in an untreated uncomplicated hypertensive population show a relationship between nocturnal blood pressure decline and degree of WML in the periventricular area of the brain.

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Chapter 5

Is there a side predilection for cerebrovascular disease?

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Abstract

Background

In studies on carotid artery intima media thickness (IMT) and stroke, researchers implicitly assume that cerebrovascular abnormalities show a symmetrical distribution.

Methods and results

To evaluate whether there is a difference in IMT between the two carotids we compared left and right common carotid artery IMT measured by B-mode ultrasonography in a group of 102 untreated hypertensive patients. The average IMT showed a significant difference between both sides (left: 0.75 ± 0.11 mm; right 0.71 ± 0.11 mm; $p<0.001$). This was associated with a higher cross sectional area of IMT and a higher flow velocity at the left side. Arterial diameters, however, were not different. Because a higher IMT may be associated with an increased risk of non-lacunar stroke, we also assessed whether there is a side preference with respect to cerebrovascular accidents. To this end, we explored our population-based Stroke Registry of 1843 subjects and, indeed, found a significantly higher incidence of non-lacunar cerebrovascular stroke at the left side, while lacunar infarcts were symmetrically distributed.

Conclusion

Our findings suggest a predilection for cerebrovascular disease at the left side, which may be related to greater intimal damage in the left carotid artery.

Introduction

An increase in carotid artery intima media thickness (IMT) not only coincides with other risk factors such as hypercholesterolemia, hypertension and diabetes mellitus, but also correlates independently with clinical endpoints such as myocardial infarction and peripheral atherosclerosis.[1-7] Recent data have confirmed the relationship between IMT and stroke, especially of the non-lacunar subtype.[8] Thus, an increase in IMT can be considered as a marker of cardiovascular risk. Usually, one average measurement from the left and right common carotid artery for the determination of IMT.[9] However, it is not known whether this is justified, as differences may exist in IMT between both arteries. Indeed, during routine assessment we frequently noted a left-right difference in IMT. Other studies also suggest differences between left and right IMT[5,9], but it is not clear whether there is a systematic difference in favour of one side. The present study was performed to investigate this possibility in more detail. In the first part we systematically compared left and right IMT in a group of hypertensive patients who had been referred to our hospital for evaluation of their elevated blood pressure. The second part comprised a retrospective analysis of the Maastricht Stroke Registry, which contains data on all stroke patients admitted to our hospital since 1988. This was done to assess whether there is a side preference in the occurrence of non-lacunar stroke. We hypothesised that atherosclerotic complications as they relate to an increased IMT may progress at a faster pace in the thickest carotid artery and, hence, that hemispheric infarcts may occur more often at that side of the brain.

Subjects and Methods

Study 1: IMT measurements

Patients were selected from the hypertension outpatient clinic of the Department of Internal Medicine of the University Hospital of Maastricht. As part of the local protocol IMT measurements were performed in all patients. For the present analysis we selected 102 untreated hypertensive patients in whom no known cause for their hypertension could be detected and who were without apparent cardiovascular complications. This specific population was chosen because hypertension is a major determinant of IMT and stroke[10, 11] and because the absence of cardiovascular complications would render secondary changes of IMT less likely. Blood pressure was measured before each IMT measurement after 5 minutes of rest in sitting position. Measurements of the IMT of the posterior wall as well as of lumen diameters of the left and right common carotid artery were obtained 1 cm proximal to the bulb from an anterolateral

and posterolateral view (SONOS 5500; Agilent-Philips; linear array transducer, 3-11 MHz). The left and right artery were investigated in random order.
End diastolic B-mode images of the IMT were analysed offline with an automated edge-tracking method (M'ath, version 2.0.1; Metris, France).[12] The average IMT was measured over a length of 10 mm, and the mean of both the anterolateral and posterolateral view at each side was calculated and used for further analyses. In addition flow velocity indices, i.e. mean velocity (cm/sec), pulsatility index (PI) and resistance index (RI), were derived from the Doppler spectrum. PI and RI were calculated as follows: PI= (S-D)/MN and RI= (S-D)/S, in which S and D indicate systolic and diastolic velocity (cm/sec), respectively, and MN mean velocity (cm/sec). The cross sectional area of IMT (CSA-IMT) was calculated according to the formula: CSA-IMT = x IMT x (IMT+D), in which D is lumen diameter (mm).[9] Measurements were performed by four trained operators, none of whom was aware of the purpose of the study. Although patients were informed why they were investigated, they did not know that this comprised evaluation of a left-right difference in IMT.

Study 2: Stoke registry

The Maastricht Stroke Registry is a large database containing prospectively entered clinical, neuroradiological and outcome data of all patients that have been admitted to our hospital with a stroke.[10] The University Hospital is the only hospital in the Maastricht region and has an adherent population of approximately 200.000 people. The Registry started in 1988 and at the time of this study had data on 1843 patients. We explored the Registry and compared the frequency of territorial large vessel atherosclerotic cerebral infarcts and cardioembolic stroke in both hemispheres. In addition, we looked at side differences for small deep lacunar infarcts. This type of infarct would not be expected to occur more frequently on one side, because it is generally caused by local, intra-cerebral small vessel disease.
A *territorial infarct* was defined as an acute stroke syndrome with CT or MRI findings compatible with infarction involving the cortex, or in the absence of a specific lesion, as a clinically identified cortical syndrome consisting of unilateral motor and/or sensory symptoms and signs in combination with signs of cortical dysfunction with or without visual field defect, or as isolated monoparesis or as isolated cortical dysfunction (usually dysphasia). Patients with a large subcortical infarct were included in the territorial infarct group because of similar pathogenesis. Territorial infarct patients with a potential cardioembolic stroke source were assigned to the cardioembolic stroke subgroup. Such patients had at least one of the following cardiac features: chronic or intermittent ECG confirmed atrial fibrillation; left ventricular myocardial infarction within six weeks preceding stroke; left ventricular or atrial thrombus; left ventricular aneurysm; left ventricular akinetic segment; cardiomyopathy; mitral or aortic valve abnormalities (endocarditis, mitral stenosis, prosthetic aortic or mitral valves); and in young patients without any other specific stroke cause: atrial septal defect, ventricular septal defect. Patients with a non-cardioembolic symptomatic territorial infarct (presumably large vessel disease, i.e. atherothrombosis or

artery-to-artery embolism whether or not confirmed by non-invasive carotid studies) were assigned to the atherothrombotic infarct subgroup. A *lacunar infarct* (LACI) was defined as an acute stroke syndrome with a CT or MRI lesion compatible with occlusion of a single perforating artery, i.e., a small, subcortical, sharply marginated hypo-dense (CT) or hyper-intense (MRI) lesion with a diameter smaller than 15 mm (small deep infarct), or as a specific lacunar syndrome (i.e., unilateral motor and/or sensory symptoms and signs that completely involved at least 2 of 3 body parts (face, arm, leg) without disturbance of consciousness or language, visual field defect, or other signs of cortical dysfunction) in the absence of a specific lesion on neuroimaging. A potential cardioembolic stroke cause was not taken into account when assigning patients to this infarct subgroup. Patients with a 'rare' stroke cause, such as arterial dissection, vasculitis, fibromuscular dysplasia, hematological disorder, etc. were not included in the study because of small numbers and heterogeneity in stroke cause.

The study was approved by the Medical Ethical Committee of the Maastricht University Hospital, and performed according to the institutional guidelines. All subjects, or, if necessary, the next of kin gave written informed consent to use patient data for this type of scientific evaluation.

Statistical analysis

Differences in IMT between the left and right carotid were determined using *t*-tests for paired samples. The concordance between left and right IMT was analysed by linear regression. Bland-Altman analysis was used to assess systematic differences between both sides.[13] Proportional differences between stroke subgroups were determined using Chi-tests. Data are shown as mean ± standard deviation (SD), unless indicated otherwise. A p-value less than 0.05 was considered statistically significant.

Results

Study 1

Mean age of the 102 hypertensive subjects was 56±11 years, 60 % were male, and body mass index averaged 29±6 $kg.m^{-2}$. Office systolic and diastolic blood pressures were 165±7 and 94±8 mmHg, respectively. A close relationship was found between the IMT of both sides (regression equation $y=0.5755x+0.2844$; $R^2=0.4528$; $p<0.001$; Figure 5.1). Table 5.1 shows the IMT of the left and right carotid artery: 0.75±0.11 mm and 0.71±0.11 mm, respectively; $p<0.001$. The mean left-right difference was 0.03±0.09 mm with no systematic deviation at any level of average IMT (Figure 5.2). There was no significant difference in luminal diameter between both carotids (Table 5.1). Differences in IMT between the left and right side remained significant after correction for the diameter. As

shown in Table 5.1 the cross-sectional area of the intima-media complex was greater at the left side as well. Mean blood flow velocities in the left and right carotid artery at the side where the IMT measurements were performed were 41.1±9.4 cm/sec versus 39.3±9.8 cm/sec, respectively (p=0.004). No differences between left and right arteries could be found with respect to PI and RI (Table 5.1).
Results were similar for all four observers.

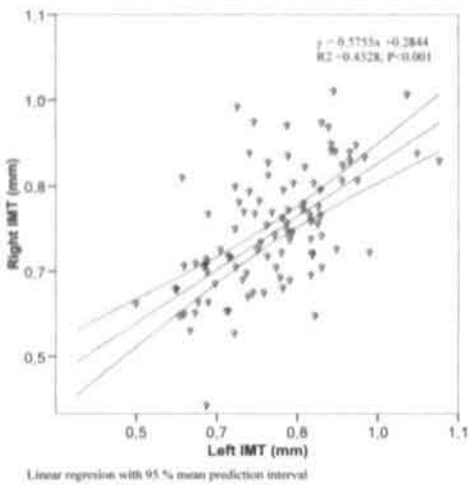

Figure 5.1. Left versus right sided carotid intima-media thickness (IMT), n=102 (regression line and 95% mean confidence interval).

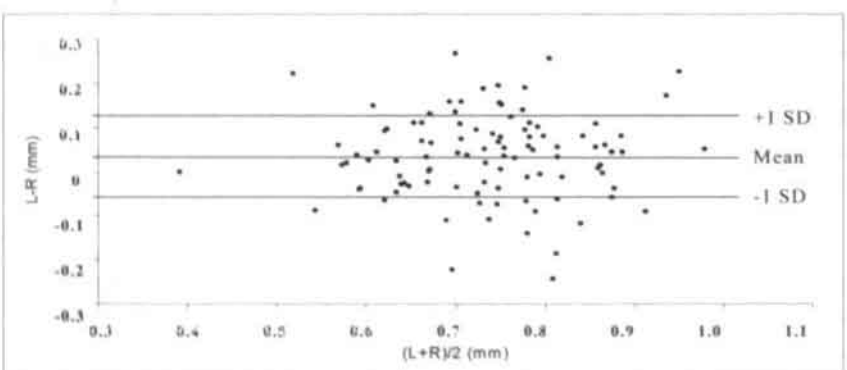

Figure 5.2. Bland Altman plot of the difference between left (L) and right (R) intima media thickness as a function of their means (n=102).

Table 5.1. Echo Doppler characteristics of left and right common carotid artery

	Left	Right	p value
IMT (mm) †	0.75 ± 0.11	0.71 ± 0.11	<0.001 *
Lumen (mm)	7.5 ± 1.0	7.6 ± 1.0	n.s.‖
PI ‡	1.18 ± 0.39	1.19 ± 0.40	n.s.‖
RI §	0.64 ± 0.09	0.64 ± 0.10	n.s.‖
Mean velocity (cm/sec)	41.1 ± 9.4	39.3 ± 9.8	0.004*
CSA-IMT (mm^3)	10.6 ± 3.0	9.9 ± 2.7	0.001*

* t-test for paired samples; † intima media thickness; ‡ pulsatility index; § resistance index; ‖ not significantly different.

Study 2

The median age of the patients (n=1843) in this database was 72 years (range 25-99), 51% was male and 50% was known with hypertension (systolic blood pressure •160 mmHg and/or diastolic blood pressure •90 mmHg). Lacunar infarcts appeared to be symmetrically distributed (Table 5.2). In contrast, for non-lacunar strokes we found a predilection for side: both atherosclerotic and cardioembolic stroke subtypes were significantly more frequent in the left hemisphere: Chi=9.81, OR 1.39 (95% CI 1.13-1.70), and Chi=7.49, OR 1.46 (95% CI 1.11-1.92), respectively (Table 5.2). Stroke severity, based on the Rankin score, was not different for left-sided and right-sided strokes.

Table 5.2. Distribution of stroke subtypes in the Maastricht Stroke Registry

Subtypes	Number	Left (%)	Right (%)
Lacunar	721	357 (49.9)	364 (50.1)
Cardioembolic	319	188 (58.9)*	131 (41.1)
Atherosclerotic	803	463 (57.7)*	340 (42.3)
All strokes	1843	1008 (54.7)	835 (45.3)

* p<0.001 for left/right difference

Discussion

The present study shows that in untreated essential hypertensives a difference exists between IMT of the left and right carotid artery, with higher values on the left side. Also, cross-sectional area (a marker of vascular mass) of the intima-media complex was larger on the left then on the right side. In most published studies IMT values are seldomly reported for the left and right carotid separately. However, in studies in which data are actually given for both sides, the IMT of the left common carotid tends, on average, to be larger than on the right.[9,15] Although the clinical significance of this asymmetry is not yet apparent, it has been shown recently that a thicker intima-media complex is associated with non-

lacunar strokes.[8] Accordingly, we hypothesized that if the left-right differences in IMT were to be a general phenomenon, one could expect a side preference for the occurrence of non-lacunar stroke as well. This was, indeed, borne out by the anatomical predilection of non-lacunar strokes in the left hemisphere in our Stroke Registry. Non-lacunar strokes can be divided in atherosclerotic and cardioembolic subtypes. Generally, lacunar strokes are caused by local obstruction, and not by embolism, whereas most cardioembolic strokes occur in the absence of carotid disease.

Therefore, the similarity between cardioembolic and atherosclerotic strokes with regard to predilection to the left hemisphere in our study suggests a role for hemodynamic factors. These may cause more cardiac emboli to enter the left carotid system, and also more often affect the left carotid artery structure. Since we also observed a significant difference in mean flow velocity between the two carotids, hemodynamic factors may, indeed, contribute to differences in vascular pathology. Our data suggest that shear forces, which are strong determinants of adaptive intima media thickening, are different in both arteries and that the left and right carotid are exposed differentially to the early atherosclerotic process. The fact that it takes longer for the blood to flow through the aortic arch could also play a role as atherosclerotic lesions at this side may cause embolic stroke.[14]

To the best of our knowledge, no data have been published with regard to side predilection of strokes. Even though we compared stroke rates in a different population than the one in which we obtained IMT measurements, our data are in accordance with the assumption that atherosclerotic lesions develop earlier on the left side.

One could argue that the differences in the site of strokes may have resulted from admission bias in this particular cohort of patients: left hemispheric infarcts may be more symptomatic, and rated by physicians as more severe. However, the initial stroke severity in our Registry was similar between left and right hemispheric infarcts, which makes such a bias less likely.

In conclusion, we have shown an increased IMT at the left side and suggest that this may predispose to a higher prevalence of left hemispheric non-lacunar infarcts. Further prospective studies investigating the relationship of IMT and stroke in the same cohort are necessary to provide support for these results and warrant reporting data from both left and right carotid arteries when performing IMT measurements. Our findings may set the stage for an early, differential preventive strategy for left or right carotid artery disease. This may be highly relevant as the left hemisphere is dominant in most people.

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Chapter 6

Effect of polymorphisms of the angiotensin-converting enzyme and apolipoprotein E on carotid intima-media thickness in untreated hypertensives

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Abstract

Background
Several studies have assessed the relationship between intima-media thickness (IMT) and the angiotensin-converting enzyme (ACE) insertion/deletion (I/D) polymorphism and/or the apolipoprotein (Apo) E polymorphism. Interaction between several polymorphisms is thought to be more important than the effect of a single gene. Recently, it has been suggested that asymmetry in carotid IMT is present.

Objective
To investigate the individual effects of the aforementioned polymorphisms and their interaction on the common carotid artery IMT at both left and right side, in hypertensive patients at risk for development of carotid artery atherosclerosis.

Methods
This cross-sectional study incorporated 109 untreated, hypertensive subjects, in which IMT of the common carotid artery was assessed ultrasonographically (B-mode). To correct for differences in lumen diameter the cross-sectional area of the IMT was calculated. Left-to-right differences of the IMT was assessed and related to the individual and interactive effects of the ACE D allele and the Apo E4 allele.

Results
A significant association of the ACE D allele was found with the mean IMT of both sides, and, left or right carotid IMT separately. Left carotid IMT was significantly thicker than right. For both sides the D allele showed a gene-dose effect. The Apo E polymorphism did not influence IMT, alone or in the presence of an ACE D allele.

Conclusion
The ACE D allele is a determinant of the IMT of the common carotid artery in hypertensive subjects. There is no synergy with the Apo E4/* genotype. Significant differences in IMT between the left and right carotid artery are present, which may be relevant for side predilection of stroke.

Introduction

An increase in carotid artery intima-media thickness (IMT) not only coincides with other risk factors like hypercholesterolemia, hypertension and diabetes mellitus, but also correlates independently with clinical endpoints such as myocardial infarction, stroke and peripheral atherosclerosis.[1-8] Measurements of carotid artery IMT, and probably also of femoral artery IMT[9,10] are generally considered, therefore, to provide information about the risk of future atherosclerotic complications.[11]

The mechanisms leading to increased IMT are not well understood but may involve genetic factors. Among the latter polymorphisms in the angiotensin-converting enzyme (ACE) gene and the gene encoding for apolipoprotein (Apo) E rank high. Several studies have addressed the association between these candidate genes and the carotid IMT, but mostly with conflicting results.[7,12-23] As far as the association between atherosclerotic complications and the insertion/deletion (I/D) polymorphism of the ACE gene is concerned, recently performed meta-analyses remained inconclusive.[24,25] Even though the presence of the ACE D allele seem to be associated with atherosclerotic complications, this is not true for all populations. The Apo E gene has 3 common alleles, APO*E2, APO*E3, and APO*E4.[26] Apo E plays a pivotal role in the transport of lipoproteins and is involved in numerous processes in the arterial wall.[27,28] Studies on this gene indicated that either the presence of the E4 or the E2 allele are associated with an increased carotid IMT.[29] However, it has been suggested that the E2 allele may have a protective effect with regard to morbidity and mortality of atherosclerosis[30,31], and that an excess of subjects with one or two apo E4 alleles is found among populations with high cardiovascular risk.[18,32]

Evidence is accumulating that analyses of additive effects and/or synergism between candidate genes, especially in the presence of the ACE D allele, are more important than single-gene studies.[10,33,34] The relationship between carotid IMT and the ACE I/D and/or Apo E polymorphisms has been assessed in only a few studies[7,20], but the combined effect of these risk alleles has not been investigated.

Therefore, the aim of the present study was to investigate the interaction of the ACE I/D and Apo E polymorphisms on the carotid IMT in a group of untreated hypertensive subjects. Since we recently found that there is a difference in left and right carotid artery IMT in such patients, we also examined whether this difference is genotype-dependent.

Methods

Study population

Participants were selected from the hypertension outpatient clinic of the Department of Internal Medicine of the University Hospital of Maastricht. As part of the local protocol, IMT measurements were performed in all patients. For the present analysis we selected 109 hypertensive patients who were between 40 and 80 years of age, in whom no known cause of their hypertension could be detected, and who were without apparent cardiovascular complications. This specific population was chosen because hypertension is a major determinant of IMT and stroke and because the absence of cardiovascular complications would render secondary changes of IMT less likely.[35, 36] Exclusion criteria were clinically documented ischemic or valvular heart disease, congestive heart failure, cerebrovascular accidents and/or transient ischemic attacks, chronic renal failure, or secondary hypertension. None of the participants were members of the same family. In all patients antihypertensive medication was discontinued for tree weeks prior to the measurements. None of the subjects developed adverse events during this drug-free period, and all participants gave their informed consent in writing. This study was part of another survey investigating the relationship between neuropsychological function tests and hypertension-induced target organ damage of the brain. The study was approved by the local medical ethics committee.

Blood Pressure Measurements and other covariates

Noninvasive ambulatory blood pressure measurements (Spacelabs 92127, Redmond, WA) were obtained starting on a morning weekday. The cuff was applied to the non-dominant arm. For analysis, daytime was defined from 7:00 a.m. till 11:00 p.m. and nighttime from 1:00 a.m. till 6:00 a.m. The apparatus was programmed to perform blood pressure measurements every 15 minutes during the day and every 30 minutes during the night. Subjects were instructed to adhere to their normal daily activities and regular sleeping hours. Office blood pressure was measured by means of a random zero sphygmomanometer on both arms in sitting position during the visit.
The body mass index (BMI) was calculated as weight divided by height square. Information on risk factors like smoking was obtained with a standardized questionnaire, which was checked by a physician during the interview.

Carotid artery intima-media thickness

Ultrasound measurements were performed in all patients in the recumbent position. Measurements of the IMT of the posterior wall of the left and right common carotid artery were obtained 1 cm proximal to the bulb from an anterolateral and posterolateral view (SONOS 5500; Agilent-Philips; linear array transducer, 3-11 MHz). The left and right arteries were investigated in random order. Enddiastolic B-mode images of the IMT were analyzed offline

with an automated edge-tracking method (M'ath, version 2.0.1; Metris, France).[37] Offline, the frame showing the narrowest diameter in the enddiastolic phase was selected and stored as a bitmap file for further processing. The average IMT was measured over a length of 10 mm, and the mean of both the anterolateral and posterolateral view at each side was calculated and used for further analysis. To correct the IMT for differences in lumen diameter the cross sectional area of IMT (CSA-IMT) was calculated according to the formula: CSA-IMT = π x IMT x (IMT+D), in which D is the lumen diameter (mm).[11] Measurements were performed by four trained operators, none of who was aware of the purpose of the study. Although patients were informed why they were investigated, they did not know that this also comprised evaluation of a left-right difference in IMT.

Genetics

The ACE I/D and the apo E polymorphisms were determined at the Cardiovascular Genotyping (CAGT) laboratory of the Department of Internal Medicine of the University Hospital Maastricht. DNA was extracted from whole blood using the QIAamp® Blood Kit (Qiagen Inc., Valencia, CA).[38] Genotyping was performed using a multilocus genotyping assay for candidate markers of cardiovascular disease risk (Roche Molecular Systems Inc., Alameda, CA). Briefly, each DNA sample is amplified using two multiplex polymerase chain reactions, and the alleles are genotyped simultaneously using an array of immobilized, sequence-specific oligonucleotide probes. This array of probes is blotted on plastic strips and after staining genotypes can be scored based on blue (positive) and white (negative) bands. Each blue band, representing a specific genotype, was scored by specific software (counting the pixel intensity of each band) and manually checked.

Statistical Analysis

The data pertaining to the cases were uploaded into a commercially available spreadsheet, and this was in turn uploaded into a statistical program (SPSS Inc., Chicago, Illinois, USA). Differences in IMT between the left and right carotid artery were determined using the *t*-test for paired samples. Differences between genotype groups were tested by analysis of variance (one-way ANOVA), using Bonferroni's method for post hoc multiple comparisons between genotype classes. Genotype frequencies between groups were compared using a *Chi*-test and tests for Hardy-Weinberg equilibrium were carried out using standard methods. The interaction between the ACE and the apo E polymorphisms on IMT was tested using the following model: $IMT = b_0 + b_1X + b_2Y + b_3XY$, in which b represents a constant variable, X the risk allele of polymorphism X, Y the risk allele of polymorphism Y, and XY the interaction between the two. The mean coding approach was also used to test for interaction[39]. The latter method was added because it does not imply any genetic hypothesis.
Based on literature, we decided to perform the primary analyses with regard to the Apo E polymorphism on subjects with one or two Apo E4 alleles (Apo E4/*) versus

the remaining subjects, which were those with the Apo E3/3, E3/2 and E2/2 combinations, the Apo E4/* group being the one with increased risk of intima-media thickening. Secondary analyses were done in two other groups: Apo E3 or E2 / E3 or E2 versus the remaining combinations, in which the Apo E3-2/E3-2 subjects are supposed to be those with the lowest cardiovascular risk.
All data are presented as mean ± standard deviation (SD), unless indicated otherwise. A p-value less than 0.05 was considered statistically significant.

Results

The mean age of the 109 subjects was 56±11 years, 60 % were male, body mass index averaged 29±6 kg/m^2, and 46% were current smokers. Office systolic and diastolic blood pressures off treatment were 165±7 and 94±8 mmHg, respectively, and mean 24 h ambulatory measurements 152±20 and 93±12 mmHg, respectively.
The genotype distributions of both the ACE I/D and the Apo E polymorphisms were in Hardy-Weinberg equilibrium. Results of the IMT measurements are shown in Table 6.1. The mean IMT of all subjects was 0.73±0.10 mm. A significant difference between the IMT of the left and right carotid artery was found: 0.75±0.11 mm and 0.71±0.11 mm, respectively ($p<0.001$). Lumen diameter was not significantly different for both carotids. Differences in IMT between the left and right side remained significant after calculation of the CSA-IMT (Figure 6.1). Both subjects with the Apo E4/* and those with the Apo E_R/* genotype had significantly thicker common carotid IMT at the left side in comparison to the right side (Table 6.1). However, no difference in either left or right CSA-IMT was observed between both genotype groups (Figure 6.1, panel A). In the presence of one or two ACE D alleles, left carotid IMT was also significantly thicker than the right one (Table 6.1). Moreover, a dose effect of the D allele was observed in the left ($p=0.001$) as well as in the right carotid artery ($p=0.03$), not only for the uncorrected IMT but also for the CSA-IMT (Figure 6.1, panel B). In univariate analysis, the D allele was significantly associated with the mean carotid IMT ($p=0.04$) and with the left carotid IMT ($p=0.03$), but not with the IMT at the right side. Apo E allele groups were not associated in univariate analysis with any of the IMT values. This was true when alleles were grouped either as Apo E4/* or as Apo E3-2/E3-2.
Table 6.2 shows the IMT data in relation to both the ACE I/D and the Apo E alleles. Using the linear regression model, no evidence for interaction between the ACE and the Apo E gene was found. Since there is uncertainty with respect to the pattern of inheritance (recessive, co-dominant or dominant), we re-analyzed the data using the mean coding approach. This analysis showed a significant deviation from the population mean in the presence of two D alleles of the mean carotid IMT ($p=0.05$) and of the left carotid IMT ($p=0.03$). This did not change significantly in the presence of an Apo E4 allele or E3-2 alleles.

Table 6.1. Intima media thickness subdivided by Apo E and ACE I/D polymorphism.

	Left (mm)	Right (mm)	Mean	p-value *
All subjects	0.75 ± 0.12	0.72 ± 0.11	0.73 ± 0.10	p= 0.001
Apo E genotype				
E4/* (n=29)	0.76 ± 0.09	0.71 ± 0.11	0.73 ± 0.09	p= 0.004
ER# (n=68)	0.75 ± 0.12	0.72 ± 0.12	0.73 ± 0.11	p= 0.031
ACE genotype				
II (n=23)	0.67 ± 0.13	0.68 ± 0.09	0.68 ± 0.10	n.s.
ID (n=53)	0.75 ± 0.11	0.71 ± 0.11	0.73 ± 0.10	p= 0.007
DD (n=33)	0.78 ± 0.11	0.74 ± 0.10	0.76 ± 0.09	p= 0.024

* paired t-test t-test left versus right IMT; #R, indicates all remaining (non Apo E4/*) genotypes, i.e. Apo E3/E3, E3/E2, and E2/2.

Table 6.2. Intima media thickness in relation to both the ACE I/D and Apo E polymorphisms.

	ACE I/D	Apoliprotein E	
		Apo E4/*	Apo ER#
Mean IMT	II	0.79 ± 0.05	0.67 ± 0.16
Left IMT (mm)		0.78 ± 0.10	0.68 ± 0.17
Right IMT (mm)		0.80 ± 0.01	0.67 ± 0.18
Mean IMT	ID	0.73 ± 0.09	0.73 ± 0.10
Left IMT (mm)		0.76 ± 0.09	0.74 ± 0.11
Right IMT (mm)		0.70 ± 0.11	0.71 ± 0.11
Mean IMT	DD	0.73 ± 0.09	0.77 ± 0.09
Left IMT (mm)		0.76 ± 0.09	0.79 ± 0.11
Right IMT (mm)		0.70 ± 0.12	0.75 ± 0.09

#R, indicates all remaining (non Apo E4/*) genotypes, i.e. Apo E3/E3, E3/E2, E2/2.

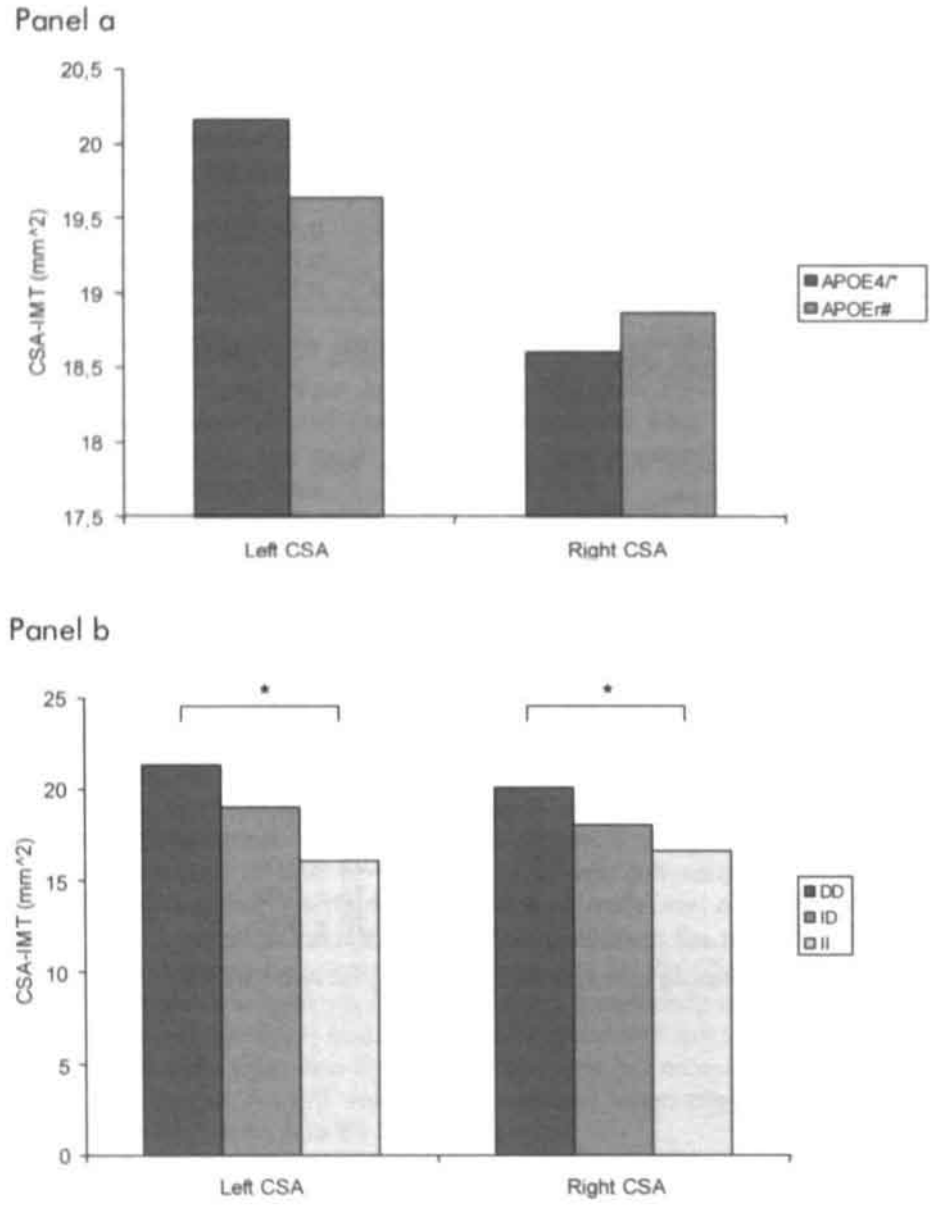

Figure 6.1. Cross sectional area of the intima-media thickness (CSA-IMT) of the left and right common carotid artery (CCA) in relation to the apolipoprotein (Apo) E polymorphism (panel A) and the angiotensin-converting enzyme (ACE) insertion/deletion (I/D) polymorphism (panel B).
Apo E_x indicates all the remaining (non-Apo E4/*) polymorphisms, i.e. Apo E3/E3, E3/E2, and E2/E2.

Discussion

In the present study we investigated the individual and interactive influences of two polymorphisms on the IMT of the common carotid artery of untreated hypertensive subjects. Our data show an association of the ACE D allele with the mean carotid IMT, and, left or right carotid IMT separately. The Apo E polymorphism did not influence IMT, either alone or in the presence of an ACE D allele.

Several studies have reported on relationship between the ACE I/D polymorphism and carotid IMT.[10, 12, 14-17, 19-23] From these, approximately half described a positive association between the ACE D allele and the carotid IMT, in a Japanese[15, 17], Italian[12], Chinese[22], and Finish population[16], respectively. Most of these studies were performed in healthy, middle-aged populations. Therefore, it is difficult to compare the results of these studies with ours. As a matter of fact, only one study is comparable to ours: Jeng et al.[22] also observed a positive association with the presence of the ACE D allele in hypertensive, middle-aged subjects, who are at increased risk for development of carotid artery atherosclerotic pathology. Recently, Balkestein et al.[10] showed in the population-based FLEMENGHO study the same association with the D allele, however, only with regard to the femoral IMT and not the carotid artery IMT. Nevertheless, these data confirm that the presence of the ACE D allele is an important genotypic marker for the development of atherosclerosis. However, selection of the study population, e.g. with or without additional cardiovascular risk factors, has a substantial impact on the outcome of the different association studies.

With respect to the functional changes associated with the presence of the ACE D allele one can only speculate. The presence of the ACE D allele is associated with higher systemic ACE levels[40], which may stimulate the local generation of angiotensin II [41], which, in turn, can promote intima-media thickening. So far, however, no study has provided convincing evidence to support the assumption that more angiotensin II is formed locally.

The Apo E gene was one of the first of which polymorphisms associated with cardiovascular disease were studied thoroughly. This gene influences lipoprotein metabolism and the plasma concentration of total cholesterol, LDL cholesterol, apo B, and apo E, and it confers a risk for coronary heart disease.[29] In our study, we did not find an association between the Apo E polymorphisms and carotid IMT. Several authors have studied the relation between IMT and Apo E in the past.[7, 13, 18, 20, 31] Among these, the recent study by Slooter et al. is the most powerful one. These authors found in the population-based Rotterdam study of 5401 elderly subjects (mean age 69 years) that carriers of the E2/E3 genotype had a slightly, but significantly, thinner IMT (mean difference –0.02 mm) than the most common E3/E3 group. Subjects with the Apo E4/E4 genotype had a bit more carotid atherosclerosis, which, however, was not statistically significant. So, while apo E helps to explain interpopulation differences of lipids and coronary heart disease, the findings of our study corroborate the general conclusions of the Rotterdam group that the Apo E4/* genotype is not an important risk factor for carotid IMT. In our study, we did not observe an inverse relation with the E3/E2

combination, which may be due to the limited number of patients included in our study. However, if present, the small protective effects of this allele combination does not seem to justify population screening as a risk marker.

The hypothesis underlying this study was that interactions between candidate genes might influence carotid IMT by the growth stimulating effect of angiotensin II and the effects of (apo)lipoproteins on the vascular wall. More and more, the concept is emerging that genetic interactions between loci, rather than single genes, make up the genetic basis for cardiovascular disease. This concept was elegantly illustrated in the study of Balkestein et al.[10] who showed an interaction between the ACE D allele, the α-adducin 460Trp allele, and the aldosterone synthase –344T allele on the IMT of the femoral artery. There are no other studies that looked into the interaction of the ACE D allele and the Apo E4/* genotype. Although theoretically sound, we did not find an additive or synergistic effect for the E4 allele in the presence of the ACE D allele. Firstly, this may be due to the age of the population studied. The effect of genetic factors on cardiovascular disease may not be linear with age and different genes may act at different times during life.[42] This could play a role in our study, since Slooter et al.[31] found a significant effect of the Apo E genotype at relatively old age. Secondly, environmental factors may mask or enhance the effect of certain genes. This is probably not an important explanation for the lack of interaction in our study, since we assessed IMT in subjects with relatively mild hypertension and few risk factors for cardiovascular disease. Thirdly, genes may have a different impact on cardiovascular events, carotid, or femoral IMT. Although the latter possibility cannot be excluded, data from the literature do not suggest a differential effect of either the ACE D allele or the Apo E4 allele for several outcome parameters.

In accordance with our previous findings, the present study shows that a difference exists between IMT of the left and right carotid artery, with higher values on the left side. Also, cross-sectional area (a marker of vascular mass) of the IMT was larger on the left than on the right side. In most published studies IMT values are seldomly reported for the left and right carotid separately. However, in studies in which data are actually given for both sides, the IMT of the left common carotid tends, on average, to be larger than on the right[11, 43]. Although the clinical significance of this asymmetry is not yet apparent, it has been shown recently that a thicker intima-media complex is associated with non-lacunar strokes[44]. Interestingly, the presence of an ACE D allele adds significantly, in an allele-dose dependent way, to this left-to-right difference. Unfortunately, it is not know whether subjects with the ACE I/D or D/D genotype are more prone to develop a stroke at the left hemisphere. Further prospective studies investigating this relation between IMT, side predilection for stroke, and genetic predisposition are ,therefore, necessary.

In conclusion, our study shows that the D allele of the ACE gene is associated in a gene-dose effect with increased common carotid IMT. Moreover, this effect was more pronounced at the left common carotid artery, and not affected by different Apo E alleles. This study further indicates that the Apo E gene is not an important marker for carotid atherosclerosis.

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Chapter 7

General discussion

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General discussion

Under normal circumstances blood pressure varies considerably throughout the day. Perhaps the most striking and most consistent finding is the nocturnal decline in blood pressure. Although this has been amply demonstrated, the question has not been resolved whether this reflects a true circadian rhythm or that it is the result of variations in physical activity and rest. Whereas many studies tend to support the latter explanation, it is evident that lack of activity cannot be the sole explanation for a fall in pressure during sleep as the magnitude of the day-night difference in pressure becomes less and sometimes even disappears, for instance, in severe hypertension[1] or when renal function is impaired.[2]

It has been suggested that a blunted day-night rhythm could be due to volume retention during sleep. While this may be true for some patients, several arguments plea against this possibility as being very important. Indeed, the normal rhythm may be preserved in patients with primary aldosteronism and the loss of nocturnal hypotension in patients on hemodialysis does not seem to be related to fluid gain, but rather to the severity of hypertension.[2] Furthermore, patients with essential hypertension in general do not display an exaggerated fall in sleeping pressures while they often do exhibit a reversal of the diurnal cycle in sodium excretion.[3]

From a hemodynamic point of view the fall in pressure during the night may be related to a decline in cardiac output or in peripheral vascular resistance or in both. Data on this issue are controversial. While some investigators observed a parallel decrease in arterial pressure and cardiac output[4,5], others failed to find any consistent change in cardiac output.[6] From personal experience, however, we know that it is extremely difficult, if not impossible, to obtain reliable estimates of cardiac output in sleeping subjects without causing some arousal. There is a need, therefore, for studies that will throw more light on the systemic hemodynamic alterations during sleep. Nevertheless, at the present time available data favour a reduction in vascular resistance rather than in cardiac output as the cause of the nocturnal blood pressure fall.[7]

Clinical significance of the diurnal blood pressure variations

In recent years the interest in diurnal blood pressure variations has shifted from a pathophysiological paradigm into an epidemiological one. Nowadays, many researchers look upon the nocturnal blood pressure fall as a phenomenon with prognostic significance. Others emphasize that blood pressure surges during the day and the degree of the so-called blood pressure load determine the development of target organ damage. Despite these subtle differences in opinion, it has become clear that blood pressure variabililty has a much greater impact than originally thought.

The most convincing way to show that blood pressure variability is important, is by demonstrating that cardiovascular prognosis in the long run is related to one or more components of the diurnal pressure profile. Interestingly, in this respect nighttime pressure seems to be a rather powerful predictor of cardiovascular prognosis. This has been demonstrated, for instance, in the Syst-Eur trial for an elderly population with isolated systolic hypertension.[8] Others have pointed out that the magnitude of the nocturnal dipping of blood pressure is associated with target organ damage.[1] The latter investigators have divided their patients in so-called dippers and non-dippers based on the degree of the nocturnal blood pressure fall. Arbitrarily, a fall in pressure by more than 10% from daytime values was defined as dipping. However, while we do not want to underestimate the importance of the dipping phenomenon, there are problems with this definition. Except for uncertainty about the reproducibility of the degree of dipping, a dichotomous approach taking an arbitrary cut-off point is difficult to justify. Our policy, therefore, has become to treat the nocturnal pressure fall as a continuous rather than a dichotomous variable and to analyse all our data as such. If it is true that nocturnal pressure is even more important for prognosis than daytime pressure, one may even entertain the hypothesis that it is not noctural dipping, but rather daytime rising, which bears the prognostic information. This does not alter the described relationships between dipping and prognosis in numerical terms, but conceptually it may be a better approach of the truth.

Hypertension and target organ damage

During the natural history of essential hypertension progressive vascular disease develops which ultimately will cause target organ damage and overt clinical sequelae such as myocardial infarction, cerebrovascular accidents, peripheral arterial disease and loss of renal function. In epidemiological surveys and intervention trials these clinical complications are usually referred to as 'hard'endpoints. However, in this respect the term 'hard' refers more to the patients' experience than to the degree of accuracy that can be established with conventional diagnostic procedures. Indeed, with modern imaging techniques and refined laboratory methods it has become possible to measure objectively also a number of subclinical endpoints. For instance, renal damage can be detected by measuring micro-albuminuria and creatinine clearance, cardiac damage is demonstrable with echocardiographic methods as well as magnetic resonance imaging (MRI) procedures and cerebral damage can be detected by CT-scanning and MRI. While these abnormalities do not produce symptoms by themselves, they herald future complications of the hypertensive process and act as risk factors in their own right. In other words, the presence of left ventricular hypertrophy (LVH), carotid artery disease or reduced renal function, to mention just a few, all predict the occurrence of further cardiovascular complications over and above the risk already brought about by the elevated blood pressure.[9-12] It is, therefore, no longer justified to dismiss the subclinical or intermediate endpoints as being of lesser importance. Even the widely held opinion that

intermediate endpoints play no role in assessing the therapeutic value of antihypertensive drugs is a testimony of incogent neglect.
The cardiovascular endpoints of hypertension can be classified in various ways, namely by pathogenesis, by topography and by severity. All three aspects have been highlighted by McFate Smith in his report on the outcome of the USPHS trial.[13] The USPHS investigators demonstrated a lot of acumen in categorizing the cardiovascular events met in their trial, both in terms of pathogenesis and of impact. Their pathogenesis-based subdivision of hypertensive organ damage into straightforward hypertensive events and those mediated through atherosclerosis (table 7.1) still makes sense today, even though such a strict distinction is no longer tenable in the light of more recently obtained evidence. Nevertheless, it is interesting that with modern antihypertensive treatment we have been able to substantially reduce cerebral infarction and (to a lesser extent) myocardial complications but we are not so good in reverting microcirculatory complications. As a matter of fact, congestive heart failure and renal insufficiency which are both related to microcirculatory disturbances, now are leading causes of hypertension-related morbidity and mortality.

Table 7.1. The two major substrates of hypertensive organ damage.

Atherosclerotic
Cerebral infarction
Myocardial infarction
Plaque formation in larger vessels
Hypertensive
Cerebral hemorrhage
Left ventricular hypertrophy and congestive heart failure
Aortic dissection
Encephalopathy or retinopathy, grade III or IV
Microcirculatory complications (e.g. renal insufficiency, malignant hypertension, lacunar cerebral infarction etcetera)

Adapted from[13]

The topical categorization of endpoints from the USPHS with some tiny modifications still serves as a template for the investigations which form the basis of the present thesis and which concentrate on four major targets of the hypertensive process: the heart, the brain, the kidneys and the larger vessels, in particular the carotid artery.

The brain

Hypertension is associated with an increased incidence of cerebral infarcts and hemorrhages. According to several inventories, atherothrombotic brain infarction is by far the commonest substrate of completed stroke, perhaps occurring with a frequency that is 20 times higher than that of intracerebral hemorrhage. However, besides territorial (or cortical) strokes, the hypertensive patient is prone to develop small vessel abnormalities leading to subcortical

(lacunar) infarcts. In addition, it is increasingly being recognized that white matter lesions occur more frequently in a hypertensive population than in normotensive controls.

The heart

Long-standing hypertension is a major risk factor for the development of coronary complications and heart failure. Clinically, hypertensive heart disease manifests itself through the sequelae of cardiac hypertrophy and/or the symptoms and signs of coronary insufficiency. Both of these conditions may lead to ischemic events, arrhythmias and congestive heart failure. Hypertrophic responses of the heart do not only occur in response to pressure overload, but may be related to volume overload as well. In hypertension both pressure and volume effects seem to play a role.
Not uncommonly, hypertensive patients may have anginal complaints or other signs of myocardial ischemia in the face of angiographically normal coronary arteries. In these cases the imbalance between oxygen supply and demand is thought to be related to increased coronary resistance at the microvascular level. While the impediment to flow does not necessarily lead to symptoms at rest, it may do so after pacing or exercise.

The kidneys

In most recent hypertension trials, renal dysfunction appeared to be a rather uncommon endpoint. However, amongst others this may be due to suboptimal detection methods. Another explanation could be that a decline in renal function is a rather late complication, which can manifest itself only when the patient survives other circulatory threats on the heart and the brain. Anyway, in dialysis units hypertension has certainly become a more important cause of end-stage renal disease than intrinsic renal abnormalities.

Large vessels

The vascular complications associated with hypertension usually arise from atherosclerotic changes. Although the large arteries are not always regarded as end organs in their own right, they are particularly prone to injury in the presence of high pulsatile pressure and turbulence, and certain levels of shear stress. In this sense, they are involved in true end organ damage, as exemplified by such major catastrophes as dissecting aneurysm and occlusive peripheral artery disease. Before plaque formation and other frank atherosclerotic lesions become visible, one may see several other abnormalities such as reduced distensibility and compliance of the vascular wall, increased pulse wave velocity and thickening of the intima-media complex. However, even though the presence of these features correlates reasonably well with the risk of complications, it has not been established without doubt that these changes are true forerunners of atherosclerosis.

Pressure-related determinants of target organ damage in hypertension

Epidemiological studies clearly show that a high blood pressure is not the only determinant of target organ damage, as hypercholesterolemia, diabetes, smoking and several other factors markedly enhance the risk of complications. Nevertheless, pressure remains one of the major risk factors. As we have seen above, though, the variations in pressure make it difficult to pinpoint one particular aspect of the elevated pressure as the culprit. In this regard, two lines of research are prevalent in the literature which both employ 24-hour ambulatory blood pressure monitoring (ABPM) as tool. One focuses on establishing a true diagnosis of hypertension and on verifying whether patients with high office pressures but low blood pressure values on ABPM behave differently as far as risk is concerned as compared to those with high values on both accounts. The other line of research tries to assess which component of the blood pressure pattern (daytime pressure, nighttime pressure, dipping, total variability etcetera) carries the best prognostic information. In this thesis we have followed both lines.

The issue that has arisen from the study of daily blood pressure profiles and that forms the core of Chapter 2 of this thesis is that of white coat hypertension. The identification of patients who show this phenomenon has led to the question whether this condition runs a benign course or not. Perloff and coworkers were among the first to show that patients with low ambulatory pressures relative to their clinic pressure have a lower risk.[14,15] Later on, Verdecchia and colleagues reported a similar event rate per 100 patients-years for patients with white coat hypertension and normotensive persons, 0.49 and 0.47, respectively.[16] In contrast, the event rate in hypertensive dippers was 1.79, and 4.99 per 100 patients-years in non-dippers. The investigators of the prospective population-based Ohasama study reported that the ambulatory pressure was a better predictor of morbidity than screening pressure.[17] Redon et al. examined subjects with refractory hypertension.[18] They showed that subjects in the lowest tertile of ambulatory blood pressure had a significantly lower rate of morbidity over the next 4 years, despite similar clinic pressures. Investigators from the Northwick Park Hospital in London performed a 10 years follow-up study with ABPM.[19] One of their findings was that only 11% of the patients with white coat hypertension had left ventricular hypertrophy, as compared with 38% of the patients with sustained hypertension. Similar differences were seen in carotid artery thickening. Taken all data together, the picture emerges of white coat hypertension being a relatively benign condition with a favourable course.

As far as target organ damage in patients with white coat hypertension is concerned data from the literature are conflicting but this may be related to insufficient control of 24-hour blood pressure, which was higher than in the control population in most studies. Therefore, we adopted a different policy in our work and matched patients with controls on the basis of the 24-hour blood pressure avarages. This analysis shows that white coat hypertension is, indeed, associated with a larger left ventricular mass. Although the difference did not extend into the pathological range, these results call at least for some caution. As the studied population was, on average, 40 years of age, it may be that with

time LVH develops with the associated enhancement of risk. Obviously, only long-term follow-up data over periods of at least two decades will be able to provide the final verdict about the prognostic significance of white coat hypertension. For the time being, we believe that the condition is less innocent than has been assumed hitherto.

In chapter 3 and 4 of this thesis we have investigated the relationship between 24-hour blood pressure variability and target organ damage in heart, kidneys and brain respectively. With modern imaging techniques we were able to show a high percentage of white matter lesions (WML) in patients with essential hypertension who were temporarily off treatment. However, no clear relationship between the presence of these abnormalities and reduced nocturnal dipping was found. In fact, the reverse was true in that most lesions occurred in those patients with the greatest nocturnal fall in blood pressure. Based on these data it seems less likely that cerebral WML can be explained by the severity of hypertension alone. It is true that compared to patients without WML, those with this type of lesions have higher 24-hour systolic and diastolic blood pressure.[20] Thus, an elevated blood pressure per se may be a risk factor for the lesions to appear but the severity of hypertension or, for that matter, the lack of nocturnal dipping (or daytime rising) does not have an additional effect. Maintained, or even exaggerated, falls in nocturnal blood pressure were also observed in patients with lacunar infarctions as recently defended by Boreas in her thesis (University of Maastricht, 2001). There are at least two possible explanations for these findings. The first is that the presence of WML or lacunar infarcts causes greater day-to-night variability of blood pressure but a second, and perhaps more intriguing explanation could be that patients with reduced nocturnal dipping are relatively protected against the deleterious effects of an elevated pressure. One could hypothesize that larger shift in pressure put a greater burden on autoregulatory systems in the brain, which may then decompensate at a faster pace than in patients in whom such shifts are smaller.

Contrary to the brain, the heart is quite sensitive to differences in 24-hour or nighttime pressure. As we showed in Chapter 3 left ventricular mass in treated patients is still higher as nighttime blood pressure is higher and this is true for patients with reduced dipping as well. Compared to well-controlled patients those who are inadequately treated fare even worse. Thus, our data suggest that cardiac damage closely follows the blood pressure burden. Surprisingly, though, this is not true for renal damage, measured by means of the creatinine clearance. In other words, in treated patients the kidney seems to be more resistant to the detrimental effects of hypertension or the reversal thereof by medication than the heart. Although our studies do not allow to construct a 'vulnerability' order, they suggest at least that such an order could be: heart-kidney-brain (in descending order of sensitivity). The disparate effects of blood pressure on the various organs may be related to different pathophysiological mechanisms or, stated another way, to atherosclerotic or purely hypertensive complications. The latter (glomerulosclerosis, cerebral white matter matter lesions) seem to be more qualitative (i.e. less dependent upon the severity of hypertension), while the atherosclerotic lesions are more quantitative (i.e. very

much dependent upon the height of pressure). This hypothesis could also explain why in the major hypertension trials the effect of treatment is much greater on (atherothrombotic) stroke and myocardial infarction than on microvascular complications, e.g. in the kidney.

Atherothrombotic cerebral infarction is related to carotid artery pathology and this is the reason why we also included studies on this part of the vasculature. Because an increase in the IMT of the common carotid artery may be a premonitory sign of carotid atherosclerosis, we evaluated whether IMT measurements could tell us anything about macrocirculatory cerebral complications of hypertension. Interestingly, we found a left-right difference in IMT (greater at left side) which has been noted before by others but which has not been elaborated on further. In our analysis, we were able to show also that atherothrombotic brain infarctions occur more frequently on the left side, thereby lending support for the hypothesis that both an increase in IMT and the occurrence of ischemic stroke are part of the same pathophysiological entity. Since lacunar infarcts show a symmetrical distribution, this again favours a different pathophysiology through changes in the local microcirculation.

Any review of atherosclerotic complications in hypertension would not be complete without paying at least some attention to genetic factors. Although various individual genes and genetic factors have been linked to the development of essential hypertension, a combination of multiple genes most likely contributes to the development of the disorder in a particular patient. It is extremely difficult, therefore, to accurately determine the relative contributions of each of these genes. Moreover, it is now well recognised that many common disorders, such as hypertension, arise from complex interactions between genes and environmental factors.

In intron 16 of chromosome 17 the presence (I) or absence (D) of a 287 base pair DNA fragment has been described.[21] This polymorphism correlates with higher plasma ACE activity in subjects with one or two D-alleles. It has been shown that almost 50% of the interindividual variation in plasma ACE levels is determined by the ACE genotype.[22] Numerous studies investigating the association between this polymorphism and blood pressure variation are conflicting. In meta-analyses, a correlation has been found with macro- and microvascular (renal) atherosclerotic complications but not with hypertension.[23,24] A more recent meta-analysis[25] has confirmed this observation, although, the association between the D-allele and ischemic heart disease could only be shown in smaller studies, using selected subgroups. Large studies failed to demonstrate this association, indicating the importance of the selection of subjects with the same genetic background. Whether there is a causal relationship between the presence of atherosclerotic disease and higher circulating ACE levels, or even more important, tissue ACE levels, is still a matter of debate. Whatever the background may be, our studies clearly show a relationship between the presence of the D-allele and a thicker intima-media complex, notably on the left side. In fact, the left-to-right difference is far greater in DD than in II individuals. This shows, as far as we know for the first time, a differential effect of genetics on different parts of the circulation. At the same

time these data underscore our limited knowledge in this area. While we thought that the Apo E gene, which has been linked to atherosclerosis before, would show a relationship with IMT, this was not borne out by the facts. Future studies, therefore, have to focus upon other genes which may, either alone or in concert with the ACE gene, modify the IMT and, thereby, the risk of stroke.

Limitations and perspectives

Obviously, there are a number of limitations with respect to the studies that form part of this thesis. The patients were selected from a tertiary referral hospital and the results of the various studies, therefore, need not to be valid for all hypertensive subjects. Moreover, it is not possible anymore to study untreated patients over a prolonged period of time. Thus, studies had to be carried out in treated individuals or in patients who were temporarily off treatment. Whether medication could have influenced some of the results will remain unresolved. Finally, all measurements were obtained at only one point in time. This means that we are not well informed about the consistency of our data over time.
For the future, many additional studies are necessary which address exactly these points. Many more patients with different genetic background and degrees of atherosclerosis need to be studied. Other genetic markers have to be taken into account as well. We should also be prepared to go back to pathophysiology and decipher why purely hypertensive and atherosclerotic complications differ in their sensitivity to the effects of blood pressure. This may also pave the way for other treatment modalities and the development of new drugs.
Prospective therapeutic hypertension trials would benefit from better standardization of end organ damage criteria. This applies to clinical evaluation as well as ancillary techniques. In the hospital setting, numerous techniques are available for a rather accurate assessment of the nature of end organ damage encountered in the course of the hypertensive process but these need to be implemented at a larger scale. Finally, ambulatory blood pressure and assessment of nighttime blood pressure may become indispensible in the evaluation of the hypertensive patient.

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Chapter 8

Summary

Summary

This thesis is dedicated to the problem of target organ damage (TOD) in patients with essential hypertension. High blood pressure is one of the, if not the most important risk factor for cardiovascular complications such as myocardial infarction, cerebrovascular accident, and peripheral vascular disease. The kidney plays a special role in that this organ may be both culprit and victim of the hypertensive process. TOD is generally considered an intermediate endpoint, the presence of which accelerates the risk of overt cardiovascular events. The purpose of the studies described in this thesis was to shed more light on the relationship between blood pressure variability and TOD. The first aspect of this relationship concerns the problem of white coat hypertension (WCH) and is covered in **Chapter 2**. The question raised in that chapter was whether WCH should be considered as a variant of 'true' hypertension with its TOD or as a rather innocent phenomenon without TOD. The results of our retrospective analysis clearly show that WCH is associated with a larger left ventricular mass than normotensives and that these changes are independent of blood pressure load as measured by 24-hour recordings. Thus, WCH does not appear to be an entirely benign condition, but larger prospective follow-up studies are necessary to determine long-term risk of morbidity and mortality associated with this abnormality.

In **chapter 3** certain target organ changes (in heart and kidney) are related to blood pressure variability. Because a lot of data already exist with respect to such changes in untreated patients, the data in **chapter 3** focus on treated patients. This was primarily driven by the question whether long-term treatment would be able to reduce TOD. Based on the 24-hour blood pressure profile we divided our patients into three groups of adequately treated, inadequately treated and borderline treated blood pressure. TOD was measured by echocardiography (heart) and serum creatinine, creatinine clearance and micro-albuminuria (kidney). Interestingly, the results of our analysis show different patterns for the heart and the kidney. When related to the severity of hypertension, in this case expressed as the degree of treatment adequacy, left ventricular mass is quite sensitive to the prevailing ambulatory blood pressure level while renal function is not. In other words, patients who are inadequately treated have higher left ventricular mass but similar renal function as those who are adequately treated. Apparently, renal damage is less easy to revert than cardiac damage. The data, however, also show that reduced nocturnal dipping was associated with both a higher cardiac mass and impaired renal function. This means that a higher nocturnal pressure or, stated differently, a smaller day-to-night difference in pressure, causes greater damage in both target organs. As shown in **Chapter 4**, the latter phenomenon is quite opposite to what occurs in the brain. Indeed, when untreated patients were investigated for the presence of so-called white matter lesions, an abnormality supposedly related to

hypertension and aging, these lesions appeared to occur less often in the presence of a blunted day-night rhythm. We speculated that smaller swings in blood pressure may protect the brain from microvascular damage in this respect. Thus, the results from chapters 2 to 4 illustrate the heterogeneous response of target organs to the hypertensive process. Certainly, it is not justified anymore to assume a simple pressure-damage relationship in hypertension.

Whereas in Chapter 4 attention was paid to a microcirculatory complication, in Chapter 5 a macrocirculatory effect is discussed, namely changes in carotid artery wall. Although we did not consider plaque formation, we looked at the intima-media thickness (IMT) which probably predicts future vascular events. Interestingly, we found a left-to-right difference with higher values occurring at the left side. Because we speculated that this could mean that atherothrombotic cerebrovascular accidents would also occur more frequently in the left hemisphere we explored the database of the Maastricht Stroke Registry which contains the data of all stroke patients who have been admitted to our hospital since 1988. Our analysis, indeed, indicated that atherothrombotic cerebral infarcts are significantly more often localized in the left hemisphere whereas lacunar infarcts show a symmetrical distribution. This underscores, once again, the impact of different pathological and pathophysiological processes: even distribution of microvascular complications (white matter lesions) but asymmetric distribution of macrocirculatory complications.

In Chapter 6 the side preference of carotid artery wall abnormalities is taken a step further by relating these to certain genetic polymorphisms. While we had expected the changes in IMT to be related to the Apo E gene (which has been linked to atherosclerosis), we did not find evidence for such a relationship. On the contrary, the ACE gene did have an effect. The presence of one or two D-alleles of this gene significantly increased the IMT and the left-to-right difference in IMT. The ACE gene has also been implicated in the pathogenesis of atherosclerosis and our data lend further support to that hypothesis.

Finally, in Chapter 7 the results of the various studies are discussed in the light of data from the literature. We propose to make a clear distinction between microcirculatory and macrocirculatory complications and to consider the possibility that the various components of the 24-hour blood pressure profile may have a different impact on the various target organs of the hypertensive process.

Chapter 9

Samenvatting

Samenvatting

Dit proefschrift is gewijd aan het probleem van eindorgaanschade bij patiënten met essentiële hypertensie. Hoge bloeddruk is een van de, zo niet de, belangrijkste risicofactor voor cardiovasculaire complicaties zoals myocard infarct, cerebrovasculaire accidenten en perifeer vaatlijden. De nieren spelen een belangrijke rol bij het ontstaan en bij de gevolgen van hypertensie. Eindorgaanschade wordt algemeen beschouwd als een tussenstadium. Het is enerzijds een uiting van onvoldoende gereguleerde hypertensie, anderzijds bepaalt het de verdere cardiovasculaire prognose. Het doel van de studies in dit proefschrift is om meer helderheid te verkrijgen over de relatie tussen bloeddrukvariabiliteit en eindorgaanschade. Het eerste aspect van bloeddrukvariabiliteit heeft betrekking op het probleem van "witte jas hypertensie". Dit wordt beschreven in **hoofdstuk 2**. De vraag die we probeerden te beantwoorden in dit hoofdstuk was of witte jas hypertensie als een variant van "echte" hypertensie met eventueel eindorgaanschade moet worden beschouwd of dat dit toch gezien moet worden als een onschuldig fenomeen zonder eindorgaanschade. De resultaten van onze retrospectieve analyses tonen duidelijk, dat witte jas hypertensie is geassocieerd met een toegenomen linker ventrikelmassa en dat deze verandering onafhankelijk is van de mate van bloedrukbelasting gemeten met behulp van een 24-uurs ambulante bloeddrukmeter. Kortom witte jas hypertensie lijkt niet een volledig onschuldig fenomeen te zijn. Grotere prospectieve studies zijn evenwel nodig om de lange termijn risico's ten aanzien van morbiditeit en mortaliteit vast te leggen.

In **hoofdstuk 3** is de orgaanschade in hart en nieren gerelateerd aan de variabiliteit van de bloeddruk. Omdat er in dit opzicht genoeg gegevens bestaan bij onbehandelde hypertensieven, hebben wij in deze studie behandelde hypertensieven onderzocht. De vraagstelling hierbij was of lange termijn behandeling met bloeddrukverlagende medicamenten in staat zouden zijn om eindorgaanschade te doen verminderen. Op basis van 24-uurs bloeddrukregistraties werden de patiënten in drie groepen onderverdeeld: adequaat behandeld, inadequaat behandeld en borderline behandeld. Eindorgaanschade werd gemeten met behulp van echografie van het hart en de bepaling van het serum-kreatinine, de berekening van de kreatinineklaring en het bepalen van microalbuminurie. Uit de analyses kwam naar voren dat het effect van bloeddrukverlaging verschillend was voor het hart en de nieren. De ernst van hypertensie, in dit geval uitgedrukt in de mate waarin de bloeddruk onder een bepaalde streefwaarde kwam, was sterk bepalend voor de ernst van linker ventrikel hypertrofie terwijl dat voor de nierfunctie uitgedrukt in kreatinineklaring geen gevolgen had. Met andere woorden, patiënten die adequaat behandeld zijn hebben een lagere linker ventrikel massa, maar een even verslechterde nierfuncties als de adequaat behandelden. Schijnbaar is nierschade minder makkelijk terug te draaien dan hartschade. Niet

tegenstaande dit gegeven vonden we wel een omgekeerde verband tussen de mate van nachtelijke bloeddrukdaling en schade, zowel voor linker ventrikel hypertrofie als voor nierfunctie. Dit betekent dat hogere nachtelijke drukken, of anders verwoord, kleinere dag-nacht verschillen in bloeddruk, grotere schade aan beide doelorganen aanrichten. Zoals beschreven in **hoofdstuk 4**, is het bovenbeschreven fenomeen geheel omgekeerd voor de schade van hoge bloeddruk in de hersenen. Sterker nog, onbehandelde patiënten met hypertensie hebben minder witte stof schade, een afwijking die werd gemeten met MRI, dan bij een afwezige nachtelijke bloeddruk daling. Op grond hiervan veronderstellen wij dat kleinere veranderingen in bloeddruk tussen dag en nacht beschermend zouden kunnen werken voor de hersenen. De resultaten van **hoofdstuk 3 en 4** illustreren de heterogene respons van eindorganen op het hypertensieve proces. Hier volgt ongetwijfeld uit dat het niet meer vanzelfsprekend is te veronderstellen dat er een simpele druk-schade relatie bestaat bij hypertensie.

Terwijl in **hoofdstuk 3 en 4** aandacht is besteed aan de microcirculatoire complicaties van hoge bloedruk, worden in **hoofdstuk 5** de macrocirculatoire gevolgen nader belicht, in het bijzonder de dikte van de binnenbekleding van de vaatwand van de halsslagader (het zogenaamde intima-media complex). De dikte van het intima media complex, gemeten met echografie, hangt samen met toekomstige vasculaire complicaties: hoe dikker dit complex, hoe meer kans op gegeneraliseerde atherosclerotische vaatschade. Interessant was onze bevinding van een links-rechts verschil: de linker halsslagader was meestal dikker dan de rechter. Om te onderzoeken of deze toegenomen vaatschade samenhangt met atherotrombotische herseninfarcten aan de linkerkant, hebben wij gebruikt gemaakt van de Maastricht Stroke Registry. Dit is een bestand dat gegevens bevat van alle patiënten die sinds 1988 onderzocht zijn in ons ziekenhuis wegens een herseninfarct. Onze analyses toonden inderdaad aan dat atherotrombotische infarcten vaker voorkomen in de linker hemisfeer, terwijl lacunaire infacten een gelijke verdeling tussen links en rechts tonen. Dit benadrukt nogmaals het belang van verschillende pathologische en pathofysiologische processen die het gevolg zijn van hoge bloeddruk: gelijke verdeling van microvasulaire complicaties (witte stof laesies), maar een ongelijke verdeling van macrocirculatoire complicaties.
In **hoofdstuk 6** zijn de veranderingen in het intima-media complex verder geanalyseerd door deze te relateren aan genetische polymorfismen. Terwijl wij gedacht hadden een relatie te vinden tussen intima-media dikte en het Apo E gen (een gen dat is gerelateerd aan atherosclerose), vonden wij een associatie met het ACE insertie/deletie polymorphisme. Bij aanwezigheid van een of twee D-allelen van het ACE-gen vonden wij een significante toename van de intima-media dikte en met name aan de linker kant. Deze bevindingen ondersteunen de reeds eerder geformuleerde hypothese dat er een relatie bestaat tussen het ACE-gen en de ontwikkeling van atherosclerose.

Tot slot hebben wij in **hoofdstuk 7** de resultaten van de verschillende onderzoeken uit dit proefschrift becommentarieerd tegen het licht van gegevens uit de literatuur. Wij suggereren dat het belangrijk is een duidelijk onderscheid te maken tussen microcirculatoire en macrocirculatoire complicaties van hoge

bloeddruk en om de mogelijkheid in acht te nemen dat de verschillende componenten van de 24-uurs bloeddrukregistratie verschillende aangrijpingspunten kunnen hebben op de verschillende doelorganen die betrokken zijn in het hypertensieve proces.

Dankwoord

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Curriculum vitae

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Sergio Alejandro Rodríguez Hernández werd geboren op 19 maart 1971 te Bogotá Colombia. Op Curaçao, Nederlandse Antillen, behaalde hij in 1987 zijn MAVO diploma aan het Amador Nita College. In 1989 volgden de HAVO en in 1991 het VWO diploma aan het Peter Stuyvesant Lyceum te Curaçao. Aansluitend begon hij zijn studie Geneeskunde aan de Rijksuniversiteit Limburg. Hij volgde een aantal co-schappen in het buitenland; Chirurgie (Vall d'Hebron Hospital Departamento de Cirugia General Barcelona-Spanje; Prof. dr. G. Kootstra en Prof. dr. M. Almengol), Oogheelkunde (Istituto di Clinica Oculistica dell' Università di Firenze, Firenze-Italia; Mw. Dr. M. Beintema en Prof. dr. R. Frosini), Gynaecologie & Obstetrie (Sint Elizabeth Hospital Willemstad- Curaçao Nederlandse Antillen; Dr. G. Dunselman en drs. F. Capello) en Kindergeneeskunde (Hospital Clinico San Cecilio Granada-Spanje; Dr. W. Gerver en Prof.dr. G. Galdó Muñoz). Van 1994 tot en met 1997 was hij student-assistent bij de afdeling Interne Geneeskunde (Prof. dr. P.W. de Leeuw en Dr. M.M.E. Krekels) van het academisch ziekenhuis Maastricht. In 1996 ontving hij de studentenprijs binnen het Dr. Dekker programma van de Nederlandse Hartstichting. Dit maakte het mogelijk om gedurende 3 maanden te werken op de afdeling Cardiologie (Blood Pressure Unit) van het Beaumont Hospital te Dublin-Ierland. Hier werd de basis gelegd voor twee artikelen uit dit proefshrift (Prof. dr. E. O'Brien). In 1999 werd het artsexamen behaald aan de Universiteit Maastricht, waarna hij als AGNIO werkzaam was in het St. Maartens Gasthuis te Venlo, afdeling Interne Geneeskunde (Dr. A. Luik). Dit werd onderbroken om zijn onderzoek te kunnen afmaken. De overige studies die in dit proefschrift beschreven zijn werden uitgevoerd tijdens een aanstelling als arts-onderzoeker bij de vakgroep Psychiatrie en Neuropsychologie (Prof. dr. J. Jolles en Dr. M. van Boxtel) in samenwerking met de vakgroep Interne Geneeskunde (Prof. dr. P. W. de Leeuw en Dr. A.A. Kroon) in de periode 2000-2002. Op dit moment is hij AGNIO Interne Geneeskunde in het academisch ziekenhuis Maastricht.