Age-related changes in vascular structure and function
Determinants and cardiovascular risk

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Determinanten en cardiovasculair risico

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1

General introduction

The principal function of the arterial system is to deliver an adequate supply of blood to tissues and organs. In performing this primary conduit function, the arteries transform the pulsatile flow generated by ventricular contraction into a continuous flow of blood in the periphery. This latter cushioning function is dependent on the viscoelastic properties of the arterial walls. The structural elements of the vessel wall that are important in determining the vessel's distensibility are elastin, which is very stretchable and is important to pulsatile behavior, and collagen, which in contrast can resist stress. With aging, there is thinning and fracturing of elastin and increased collagen deposition, resulting in an increased stiffening of the vessel walls more pronounced in the central, predominantly elastic arteries, compared to the distal, predominantly muscular arteries[1, 2].

In older subjects the stiffening of the central arteries speeds the pulse wave, causing an early return of the pressure wave in late systole, with a consequent increase of systolic blood pressure, a decrease of diastolic blood pressure, and therefore a wide pulse pressure[3, 4].

In the past, vascular stiffening and an increase in systolic and pulse pressure have been considered as a part of normal aging. However, increasing evidence points to arterial stiffness as a critical precursor of disease. Although arterial stiffness increases with age[5] independently of the presence of cardiovascular risk factors or other associated conditions, the extent of this increase may depend on several environmental or genetic factors. High arterial stiffness has been associated with hypertension[6, 7], diabetes mellitus[5], end-stage renal disease[8] and atherosclerosis[9, 10]. Emerging evidence has also shown an association between increased arterial stiffness and incident cardiovascular disease in patients with hypertension[11-13] and end-stage renal disease[14]. Growing insights into the mechanisms underlying stiffness make it a potential target for intervention and prevention of cardiovascular disease. Recent progress in non-invasive techniques enables simple measurements of arterial stiffness and the number of publications performed to study these processes has increased in the last years. However, determinants of high arterial stiffness and its clinical implications have been exiguously investigated in large population-based studies.

The aim of this thesis is to study determinants and clinical implications of arterial stiffness. All the studies presented in this thesis are based on the Rotterdam Study, a population-based cohort study among subjects aged 55 years and older.

The first part of this thesis focuses on determinants of arterial stiffness. In chapter 2.1 the association between the angiotensin-converting enzyme insertion/deletion polymorphism and arterial stiffness is described. Chapter 2.2 focuses on the association between C- reactive protein and arterial stiffness. The relation between impaired fasting glucose and arterial stiffness is presented in chapter 2.3. Chapter 2.4 examines whether alcohol consumption is related with functional properties of the vessel walls. The studies described in chapter 3 concern the relation between

arterial stiffness and blood pressure levels. In chapter 3.1 the relation between aortic stiffness and postural blood pressure changes is examined. Chapter 3.2 deals with the role of different blood pressure components in predicting cardiovascular disease. The predictive role of arterial stiffness is investigated in chapter 4. Finally, in the general discussion, chapter 5, the main findings of this thesis are discussed.

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2

Determinants of arterial stiffness

2.1

Angiotensin-converting enzyme gene insertion/deletion polymorphism and carotid stiffness

Abstract

The insertion/deletion (I/D) polymorphism of the ACE gene may be involved in structural arterial changes. Aim of the present study was to assess the relationship between the ACE I/D gene and vessel wall stiffness among older adults.
The study was conducted within the Rotterdam Study, a population-based cohort study including subjects aged 55 years and older. The II, ID and DD genotypes of the ACE gene were determined in all subjects. The distensibility coefficient (10^{-3}/kPa) of the carotid artery and the carotid-femoral pulse wave velocity were measured during the third phase of the Rotterdam Study (1997-1999) and were used as measure of arterial stiffness. Data on both carotid stiffness and the ACE genotype were available for 3001 participants. After adjustment for age and gender, subjects with the ID and DD genotype had higher carotid stiffness compared to subjects with II genotype, [distensibility coefficient (10^{-3}/kPa) 10.24 (95% CI, 10.06-10.43), 10.27 (95% CI,10.02-10.52), 10.65 (95% CI,10.37-10.93) respectively (ID versus II genotype, p= 0.017), (DD versus II genotype, p= 0.037)]. In stratified analyses, the association was strongest in subjects younger than 70 years. No difference was seen for pulse wave velocity among genotypes.
In conclusion, the results of this population-based study show that the ACE ID/DD genotypes are associated with higher common carotid stiffness.

Introduction

One of the characteristics of the aging cardiovascular system is stiffening of the vessel wall. Arterial stiffness has been shown to be an independent predictor of cardiovascular morbidity and mortality in patients with essential hypertension [1,2] and end-stage renal disease [3,4]. This association may be explained by increased cardiac afterload [4,5], decreased coronary artery perfusion [6], promotion of plaque rupture [7]. Arterial stiffness increases with age [8] but also hypertension [9,10], atherosclerosis [11,12], diabetes mellitus [8] and end–stage renal disease [13] are conditions associated with increased vessel wall stiffness.

Genetic factors may be involved in the development of arterial stiffness. The angiotensin-converting enzyme (ACE) genotype has been implicated in structural changes of the vessel wall [14,15]. The ACE genotype has an I/D polymorphism in intron 16, which has been previously found to be associated with cardiovascular diseases and atherosclerosis [16-19].

Two previous studies [20,21] reported a relationship between the ACE I allele and increased arterial stiffness in patients with hypertension and type 2 diabetes, whereas no association was found in healthy controls. To our knowledge, only one study investigated the association between the ACE I/D polymorphism and arterial stiffness in a general population [22]. The results of this study which was conducted among young adults, suggest that the ACE D allele predisposes to a decreased compliance of elastic arteries. The aim of the present study is to assess the role of the ACE I/D polymorphism in determining arterial stiffness in a population-based study among older adults.

Subjects and Methods

This study was conducted within the framework of the Rotterdam Study, an ongoing prospective population-based cohort study among subjects aged 55 years or over, living in Ommoord, a suburb of Rotterdam, The Netherlands. The rationale and design of the Rotterdam Study have been described elsewhere [23]. Baseline data were collected from 1990 to 1993. The third examination phase took place from 1997 to 1999. The Medical Ethics Committee of Erasmus Medical Center approved the study and written consent was obtained from all participants.

Cardiovascular risk factors

Information on cardiovascular risk factors was collected during the third follow up examination. Data on drug use and smoking habits were obtained during the home interview. Smoking was classified as never, former or current smoking.

At the research center, blood pressure was measured twice on the right arm using a random-zero sphygmomanometer. The average of the two blood pressure values was used in the analyses. Hypertension was defined as a blood pressure level ≥160/100 mmHg and/or the use of antihypertensive medication. Body mass index [weight/height^2] was calculated. Serum total cholesterol and high-density lipoprotein (HDL) cholesterol values were determined by an automated enzymatic procedure (Boehringer Mannheim System). Diabetes mellitus was defined as use of anti-diabetic medication and/or a fasting serum glucose level ≥7.0 mmol/l.

Arterial Stiffness

Arterial stiffness was measured by two different methods, i. e. the distensibility coefficient (DC) of the common carotid artery as measure of common carotid arterial stiffness and the carotid-femoral pulse wave velocity (PWV) as measure of aortic stiffness. Both measures were obtained on the same day, in the same room. Subjects were instructed to refrain from smoking and from taking coffee, tea or pain medications on the day of measurement, and from taking alcohol on the day of measurements and the day before.

Common carotid distensibility was assessed with the subjects in supine position, the head tilted slightly to the contralateral side for the measurement in the common carotid artery. The vessel wall motion of the right common carotid artery was measured by means of a duplex scanner (ATL Ultramark IV, operating frequency 7.5 MHz) connected to a vessel wall movement detector system. The details of this technique have been described elsewhere [24,25]. After 5 minutes of rest, a region at 1.5 cm proximal to the origin of the bulb of the carotid artery was identified using B-mode ultrasound. The displacement of the arterial walls was obtained by processing the radio frequency signals originating from two selected sample volumes positioned over the anterior and posterior walls. The end-diastolic diameter (D), the absolute stroke change in diameter during systole (ΔD), and the relative stroke change in diameter (ΔD/D) were computed as the mean of four cardiac cycles of three successive recordings. Blood pressure was measured twice at the upper arm with a Dinamap automatic blood pressure recorder during the measurement session. The mean was taken as the subjects reading. Pulse pressure (ΔP) was defined as the difference between systolic and diastolic blood pressure. Mean arterial pressure was calculated by the following formula: diastolic blood pressure + 1/3 pulse pressure(systolic blood pressure-diastolic blood pressure). The cross-sectional arterial wall distensibility coefficient was calculated according to the following equation: distensibility coefficient = $(2\Delta D/D)/\Delta P(10^{-3}/kPa)$ [26]. In the present study, measurements were restricted to the right side to save time. In previous studies no differences could be detected between arterial wall properties of the right and left common carotid artery (SK Samijo, unpublished results, 1997).

Carotid-femoral PWV was measured with the subjects in supine position. Blood pressure was measured twice with a sphygmomanometer after five minutes of rest, and the mean was taken as the subject's reading. Mean arterial pressure was calculated. Carotid-femoral PWV was assessed with an automatic device (Complior, Colson) [27] that assessed the time delay between the rapid upstroke of the feet of simultaneously recorded pulse waves in the carotid and the femoral artery. The distance between the recording sites in the carotid and the femoral artery was measured with a tape. PWV was calculated as the ratio between the distance measured and the foot-to-foot time delay and was expressed in meters per second. The average of at least 10 successive measurements, to cover a complete respiratory cycle, was used in the analysis. In a reproducibility study in 47 subjects the intra-class correlation coefficient was 0.80 both for DC and carotid- femoral PWV.

Population for analysis

Of the 4024 subjects who underwent the physical examination of the third phase, PWV was measured in 3550 subjects whereas common carotid distensibility was measured in 3098 subjects. Missing information on measures of arterial stiffness was almost entirely due to logistic reasons. Information on PWV measurements and ACE gene polymorphism was available for 3406 subjects, information both on common carotid distensibility and ACE gene polymorphism was available for 3001 participants.

Genotype Investigations

The II, ID and DD genotypes were determined at baseline, with blood samples, using the polymerase chain reaction technique (PCR) using a PE9600 PCR machine according to the method of Lindpaintner et al. [28] with some modifications. The result of amplification were 319-bp and 597-bp amplicons for the D and I alleles respectively. Because the D allele in heterozygous samples is preferentially amplified, there is a tendency of misclassification for about 4 to 5 percent of ID genotypes to DD. In order to avoid this misclassification, a second independent PCR has been performed with a primer pair that recognizes an insertion specific sequence. The reaction yielded a 335-bp amplicon only if the I allele was present. In the post PCR analyses, 10 μl of PCR product was loaded on 3% agarose gel. Two independent investigators have read pictures from each gel and all ambiguous samples were analysed a second time.

Data Analysis

ACE genotype was tested as three class variables (presence of 0, 1, or 2 D alleles). The Hardy-Weinberg equilibrium was tested by a Chi-square test. Statistical analysis of differences in means or frequencies of characteristics among the genotypes was compared with a one-way analysis of variance and Chi-square analysis, respectively. The association of ACE genotype with arterial stiffness was next investigated by analysis of variance after adjustment for potential confounding variables. Subsequently, analyses were conducted in strata of age, gender, hypertension, body mass index, diabetes mellitus and smoking habits. Two categories of age were defined, subjects younger than 70 years, and subjects aged 70 and over. The median value of body mass index (26.7 kg/m^2) was used to define two strata. Linear regression analysis was used to test interaction between age and ACE genotype.

Results

Characteristics of the study subjects (3001 subjects with common carotid distensibility measurement) are presented in table 1. The distribution of the ACE genotype was consistent with Hardy-Weinberg equilibrium. None of the traits differed significantly between those with or without a successful genotype.

Table 1. Characteristics of the study population (n = 3001)

	Men (1263)	Women (1738)
Age (years)	71.7±6.5	72.1±6.9
Body mass index (kg/m^2)	26.1±3.2	27.1±4.3
Systolic blood pressure (mmHg)	134.1±19.1	132.1±19.6
Diastolic blood pressure (mmHg)	73.9±9.4	67.7±9.3
Mean arterial pressure (mmHg)	94.1±11.4	89.2±11.5
Heart rate (bpm)	71.7±12.6	74.6±11.6
Hypertension (%)	33.8	36.4
Total cholesterol (mmol/l)	5.5±0.95	6.06±0.96
HDL cholesterol (mmol/l)	1.25±0.33	1.51±0.41
Current smokers (%)	18.1	14.3
Diabetes mellitus (%)	7.8	6.9
Distensibility coeffcient (10^{-3}/kPa)	10.9±4.2	9.9±4.1
Pulse wave velocity (m/sec)	13.9±3.1	13.06±2.8
ACE II genotype (%)	22.5	22.5
ACE ID genotype (%)	50.8	49.8
ACE DD genotype (%)	26.7	27.7

Data are expressed as percentage or mean ± standard deviation.

Table 2. Distensibility coefficient (10-3/kPa) of common carotid artery and pulse wave velocity (m/s) according to ACE genotype.

		ACE genotype		Anova *p*
Model adjusted for	II (n=675)	ID (n=1508)	DD (n=818)	
		Distensibility coefficient, mean and 95% CI		
age and gender	10.65 (10.37-10.93)	10.24[a] (10.06-10.43)	10.27[b] (10.02-10.52)	0.047
age, gender, MAP, HR	10.58 (10.34-10.82)	10.24[c] (10.08-10.40)	10.33 (10.11-10.54)	0.058
age, gender, MAP, HR, tot chol, HDL chol, smoking, BMI, DM	10.62 (10.38-10.86)	10.28[d] (10.12-10.44)	10.35 (10.13-10.57)	0.034
	II (n=777)	ID (n=1704)	DD (n=925)	
		Pulse wave velocity, mean and 95% CI[e]		
age and gender	13.45 (13.26-13.65)	13.53 (13.40-13.66)	13.57 (13.39-13.74)	0.729
Age, gender, MAP, HR	13.50 (13.33-13.68)	13.56 (13.44-13.67)	13.49 (13.33-13.65)	0.989
Age, gender, MAP, HR, tot chol, HDL chol, smoking, BMI, DM	13.51 (13.34-13.69)	13.48 (13.37-13.60)	13.50 (13.34-13.66)	0.685

MAP: mean arterial pressure; HR: heart rate; tot chol: total cholesterol; HDL chol: HDL-cholesterol, BMI: body mass index; DM: diabetes mellitus. CI: Confidence interval.

[a] P value (0.017) of subjects with ID genotype versus subjects with II genotype.

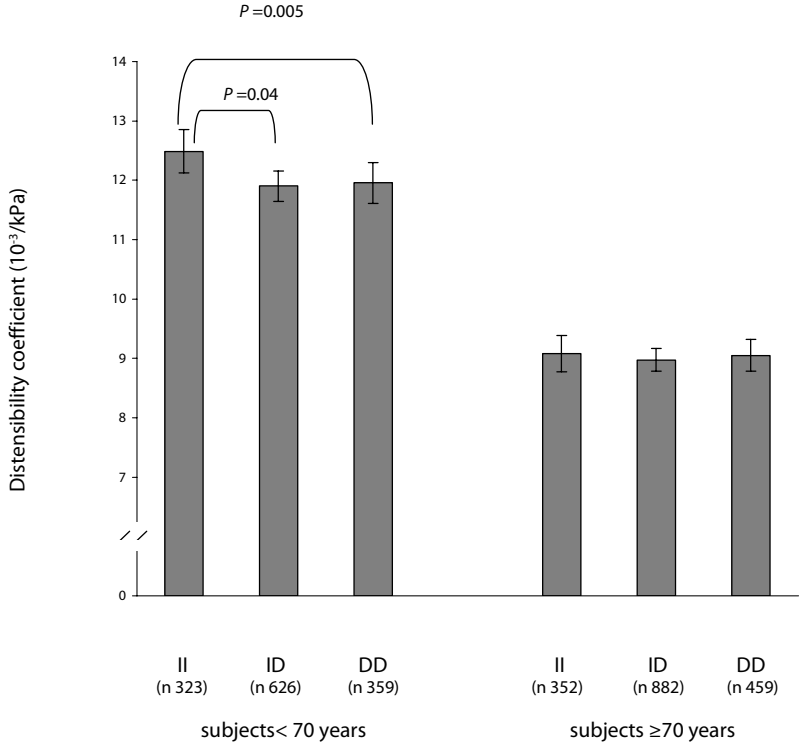
[b] P value (0.037) of subjects with DD genotype versus subjects with II genotype.

[c] P value (0.021) of subjects with ID genotype versus subjects with II genotype.

[d] P value (0.010) of subjects with ID genotype versus subjects with II genotype.

[e] Pulse wave measurements were available for 3406 subjects.

Figure 1. Mean values of distensibility coefficient of the common carotid artery by ACE genotype through age categories. Bars represent means, lines represent 95% confidence intervals. Means are adjusted for age, gender, mean arterial pressure and heart rate.



P = 0.005, distensibility coefficient of the subjects with ID genotype significantly different from subjects with II genotype. *P* = 0.04, distensibility coefficient of the subjects with DD genotype significantly different from subjects with II genotype. No statistical significant difference between ID and DD genotype. No statistical significant difference between genotypes in the other age category.

Subjects with the ID and DD genotype had a higher stiffness of the common carotid artery compared to subjects with the II genotype, while no difference was found between the ID and DD genotypes (table 2). Pulse wave velocity was not different in the three genotype groups (table 2).

In analyses stratified for age (figure 1) 1308 subjects were younger than 70 years and 1693 subjects were aged ≥70 years. Subjects younger than 70 years with the ID and DD genotype had higher stiffness of the common carotid artery compared to subjects with the II genotype after adjustment for age, gender, mean arterial pressure and heart rate. The distensibility coefficients (10^{-3}/kPa) of II, ID and DD genotype were respectively 12.48 (95% CI, 12.11-12.84), 11.90 (95% CI,11.64-12.16) and 11.96 (95% CI,11.62-12.31). The interaction between age and the ACE ID genotype was statistically significant (p for interaction = 0.041). When

we adjusted also for total and HDL cholesterol, smoking, body mass index and diabetes mellitus, results maintained statistically significant. We did not find any difference in carotid stiffness between genotypes in the subjects aged ≥70 years. In analyses stratified for gender, hypertension, body mass index, diabetes mellitus and smoking habits, no difference in the effect of genotype on stiffness was seen between the strata (data not shown).

Discussion

In this population-based study we found that the presence of the ACE ID and DD genotype was associated with higher stiffness of the common carotid artery. The association was strongest in subjects younger than 70 years old. No relation was found between ACE genotype and arterial stiffness measured as carotid-femoral pulse wave velocity.

Some aspects of this study need to be discussed. Information on arterial stiffness was not available for all participants which was primarily due to logistic reasons. Therefore, we believe that this will not have biased the results. Secondly, by calculating the distensibility coefficient, distension of the common carotid artery is adjusted for pulse pressure measured in the brachial artery. We thereby assume that pulse pressure measured in the brachial artery is representative of pulse pressure in the carotid arteries. In dogs it has been demonstrated that pulse pressure in the brachial artery is linearly related to blood pressure in the carotid artery over a wide range of blood pressure. However, it is known that the arterial pressure waves undergo transformation in the arterial tree and therefore the pulse pressure is higher in the brachial artery than in more central vessels like the carotid artery. On the other hand, non-invasive cuff-based measurement of blood pressure underestimates pulse pressure. Several investigators compared non-invasively measured elastic arterial properties and showed the validity of brachial pressure to measure pulse pressure [29,30]. Thirdly, the distensibility coefficient has a strong correlation with mean arterial pressure. A higher mean arterial pressure in the artery stretches the elastin and collagen fibres in the arterial wall, making the arteries less distensible. Consequently, we repeated the analysis after adjustment for mean arterial pressure.

Our results are in agreement with the results of a previous study which showed that higher stiffness of the common carotid artery was associated with the ACE D allele in a small group of young adults[22]. Two other studies assessed the involvement of the ACE I/D polymorphism in arterial stiffness [20,21]. Benetos et al. [20] reported that aortic stiffness, assessed by measuring aortic pulse wave velocity, was similar among the three ACE I/D genotypes in normotensive subjects whereas it was slightly higher among hypertensive subjects with the II genotype. Taniwaki

et al. [21] reported that the I allele of the ACE gene was associated with stiffening of the large arteries, such as the carotid artery and the aorta in patients with type 2 diabetes mellitus. In our study, the sample was definitely too small to see significant differences among strata of blood pressure and diabetes mellitus. We found that the association was strongest in subjects younger than 70 years and no longer present in the oldest age category.

We found no association between ACE genotype and carotid-femoral pulse wave velocity. The measure of carotid distensibility is a local measure of stiffness that gives information on an elastic artery, while carotid-femoral pulse wave velocity reflects the vessel wall stiffness of several territories providing information on both elastic and muscular arteries. There may be differences between various types of arteries with respect to the contribution of each of these components, and genetic determinants may have a different outcome according to the type of artery studied. It has been shown that in elastic arteries, hypertrophy of the wall is predominantly due to intima thickening, whereas in muscular arteries this phenomenon mainly reflects remodelling of the media [31] which might consequently induce primarily an increased distensibility of the muscular arteries. We cannot exclude that the difference in findings for the distensibility coefficient and pulse wave velocity is due to differences in the validity or reproducibility of the measurement. The validity of both the distensibility coefficient and pulse wave velocity has been shown in studies that examined associations of these measures with cardiovascular risk factors and cardiovascular disease [1,2,8,10,12] . We found that the reproducibility of both measures was adequate. Therefore, we do not think that this is a likely explanation for our results.

The mechanisms that may modulate the relation between ACE gene and vascular stiffness are not completely clear. Higher circulating levels and tissue ACE activity are present in subjects with the D compared to the I allele [32-34]. ACE catalyzes the conversion of angiotensin I to angiotensin II and the breakdown of bradykinin to kinin degradation products. Both angiotensin II and bradykinin are potent peptide hormones that play a role in vascular wall homeostasis reducing vascular tone, vascular smooth muscle cell growth and production of extracellular matrix [35-38]. These processes may then lead to progressive degeneration of arterial media with fractures and fragmentation of elastic lamellae, increased collagen and calcium content and dilation and hypertrophy of the large arteries with subsequent increased arterial stiffness. Hence, chronic exposure to high levels of circulating and tissue ACE may predispose to increased arterial stiffness. Finally, it has been shown that treatment with ACE inhibitors may increase vascular compliance and thereby reduce arterial stiffness, independently from blood pressure levels. The results confirm the role of ACE in the development of arterial stiffness.

In summary, the results of our population-based study show that the presence of the ACE ID and DD genotype was associated with higher stiffness of the common carotid artery. The association was strongest in subjects younger than 70 years old.

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2.2

C-reactive protein and arterial stiffness

Abstract

Arterial stiffness is one of the characteristics of vascular aging. Increases in pulse pressure, which reflects an increase in the stiffness of the large arteries, are associated with elevated C-reactive protein levels. This may suggest a role of inflammation in the development of arterial stiffness. We investigated the relation between measures of arterial stiffness and C-reactive protein within the framework of the Rotterdam Study, a population-based cohort study including subjects aged 55 years and older. The carotid-femoral pulse wave velocity and the distensibility coefficient of the carotid artery were used as measures of arterial stiffness. Data on both arterial stiffness and C-reactive protein were available for 866 participants.
In adjusted models, levels of C-reactive protein were linearly associated with pulse wave velocity (regression coefficient 0.081, 95% CI 0.001-0.161). Adjusted mean values of pulse wave velocity were significantly different across tertiles of C-reactive protein, being higher in the highest tertile of C-reactive protein. However, no significant association between C-reactive protein and carotid distensibility was observed.

Introduction

Arterial stiffness increases with age [1], but also hypertension [2], atherosclerosis [3] and diabetes mellitus [4] are conditions associated with increased vessel wall stiffness. Several studies have suggested that subjects with cardiovascular disease have increased arterial stiffness compared to subjects without cardiovascular disease [5, 6]. Moreover, high arterial stiffness has been shown to be an independent predictor of cardiovascular morbidity and mortality in patients with essential hypertension [7] and end-stage renal disease [8].

Increased levels of inflammatory markers, particularly C- reactive protein (CRP) are associated with atherosclerosis [9, 10] and higher risk of cardiovascular events [11-13]. A recent study found that increases in pulse pressure, which reflects a gradual increase in the stiffness of the large arteries, are associated with elevated CRP levels [14]. This association might also suggest a role of inflammation in the pathogenesis of arterial stiffness.

To study the hypothesis that high CRP levels are associated with arterial stiffness, we conducted a cross-sectional study examining the association between serum CRP levels and measures of arterial stiffness.

Subjects and Methods

Study subjects

This study was conducted within the framework of the Rotterdam Study, an ongoing prospective population-based cohort study composed of 7983 men and women aged 55 years and over, living in Ommoord, a suburb of Rotterdam, The Netherlands. Its overall aim is to investigate he incidence and determinants of chronic disabling diseases. The rationale and design of the Rotterdam Study have been described elsewhere [15]. Baseline data were collected from 1990 to 1993. The third examination phase took place from 1997 until 1999. During this phase information on cardiovascular risk factors was collected, measurements of arterial stiffness and atherosclerosis were obtained and blood samples were taken for the measurement of CRP. The Medical Ethics Committee of the Erasmus Medical Center approved the study and written consent was obtained from all participants.

Arterial Stiffness

Arterial stiffness was measured by two different methods, i. e. the distensibility coefficient (DC) of the common carotid artery as a measure of common carotid arterial stiffness and the carotid-femoral pulse wave velocity (PWV) as a measure of aortic stiffness. Both measures were obtained on the same day, in the same

room. Subjects were instructed to refrain from smoking and from taking coffee, tea or pain medications on the day of measurement, and from taking alcohol on the day of measurements and the day before. In a reproducibility study in 47 subjects the intra-class correlation coefficient was 0.80 both for the distensibility coefficient and the carotid-femoral pulse wave velocity.

Carotid distensibility

Common carotid distensibility was assessed with the subjects in supine position, the head tilted slightly to the controlateral side for the measurement in the common carotid artery. The vessel wall motion of the right common carotid artery was measured by means of a duplex scanner (ATL Ultramark IV, operating frequency 7.5 MHz) connected to a vessel wall movement detector system. The details of this technique have been described elsewhere [16, 17]. After five minutes of rest, a region at 1.5 cm proximal to the origin of the bulb of the carotid artery was identified using B-mode ultrasound. The displacement of the arterial walls was obtained by processing the radio frequency signals originating from two selected sample volumes positioned over the anterior and posterior walls. The end-diastolic diameter (D), the absolute stroke change in diameter during systole (ΔD), and the relative stroke change in diameter (ΔD/D) were computed as the mean of four cardiac cycles of three successive recordings. Blood pressure was measured twice at the upper arm with a Dinamap automatic blood pressure recorder during the measurement session. The mean was taken as the subjects reading. Pulse pressure (ΔP) was defined as the difference between systolic and diastolic blood pressure. Mean arterial pressure was calculated by the following formula: diastolic blood pressure + 1/3 (systolic blood pressure-diastolic blood pressure). The cross-sectional arterial wall distensibility coefficient was calculated according to the following equation: distensibility coefficient = $(2\Delta D/D)/\Delta P$ (10^{-3}/kPa) [18]. In the present study, measurements were restricted to the right side to save time. In previous studies no differences were detected between arterial wall properties of the right and left common carotid artery (SK Samijo, unpublished results, 1997).

Pulse wave velocity

Carotid-femoral pulse wave velocity (PWV) was measured with the subjects in supine position. Blood pressure was measured twice with a sphygmomanometer after five minutes of rest, and the mean was taken as the subject's reading. Mean arterial pressure was calculated by the following formula: diastolic blood pressure + 1/3 (systolic blood pressure-diastolic blood pressure). Carotid-femoral PWV was assessed with an automatic device (Complior, Colson) [19] that assessed the time delay between the rapid upstroke of the feet of simultaneously recorded pulse waves in the carotid and the femoral artery. The distance between the recording sites in the carotid and the femoral artery was measured with a tape. PWV

was calculated as the ratio between the distance measured and the foot-to-foot time delay and was expressed in meters per second. The average of at least 10 successive measurements, to cover a complete respiratory cycle, was used in the analysis.

Cardiovascular risk factors

Data on drug use and smoking habits were obtained during the home interview. Smoking status was classified as current, past or never smoker. At the research center, blood pressure was measured twice on the right arm using a random-zero sphygmomanometer. Body mass index [weight (kg)/height^2 (m)] was calculated. Serum total cholesterol and high-density lipoprotein (HDL) cholesterol values were determined by an automated enzymatic procedure (Boehringer Mannheim System). Diabetes mellitus was defined as the use of anti-diabetic medication and/or a fasting serum glucose level ≥7.0 mmol/l. Prevalent cardiovascular disease was defined as a history of myocardial infarction or stroke. Information on cardiovascular disease was assessed during a home interview. A history of myocardial infarction and stroke was confirmed by reviewing the medical records from the general practitioner and/or medical specialist or by ECG. Occurrence of myocardial infarction or stroke was reported by general practitioners in the research area. Research physicians verified all information by checking patient records of the general practitioner. In addition, discharge reports and letters of medical specialists were obtained for hospitalized patients.

C- reactive protein

Non-fasting blood was collected in tubes containing 0.129 mol/L of sodium citrate. The ratio of blood to sodium citrate was 9:1. Plasma was collected after centrifugation for 10 minutes at 3000 rotations per minute at 4° C. Subsequently, platelet-free plasma was obtained by centrifugation for 10000 rotations per minute and was immediately frozen and stored at -80° C. All tubes were stored on ice before and after blood sampling. C-reactive protein was measured by a nephelometric method (Dade-Behring). The detection limit of the assay was 0.2 mg/L; the inter and intra-assay coefficient of variation for the method used were both 3.24%.

Measures of Atherosclerosis

Ultrasonography of both carotid arteries was performed with a 7.5-Mhz linear-array transducer and a duplex scanner (ATL UltraMark IV). Common carotid intima-media thickness (IMT) was determined as previously described [20].

Aortic atherosclerosis was diagnosed by radiographic detection of calcified deposits in the abdominal aorta on a lateral abdominal film. The extent of abdominal aortic atherosclerosis was scored according to the length of the involved area (with scores 0 to 5 corresponding to 0, ≤1 cm, 1 to 2.5 cm, 2.5 to 4.9 cm, 5.0 to 9.9 cm and ≥10.0 cm) [21].

Population for analysis

Of the 4024 subjects who underwent the physical examination of the third phase of the Rotterdam Study, aortic stiffness measured as PWV was measured in 3550 subjects whereas common carotid distensibility was measured in 3098 subjects. Missing information on both measures was almost entirely due to logistic reasons. For the present study, levels of CRP were assessed in a randomly selected age- and sex- stratified sample of 970 participants. Outliers (values >3 standard deviations of the population distribution) of logarithmically transformed CRP (n= 21) were excluded since they might indicate the presence of an active inflammatory disease leaving 949 subjects for analysis. Finally, data on C-reactive protein and PWV were available for 866 subjects; data on C-reactive protein and the carotid distensibility were available for 677 subjects.

Data Analysis

The association between C-reactive protein and arterial stiffness was investigated by linear regression analysis (with PWV and DC as the dependent variable) and next investigated by analysis of covariance where mean values of PWV were compared across tertiles of CRP levels. Cut-off points for tertiles of CRP were 1.67 mg/L and 3.40 mg/L. Analyses were adjusted for age, gender, mean arterial pressure, heart rate, body mass index, total cholesterol, high-density lipoprotein cholesterol, diabetes mellitus, smoking status (current, past or never smoker) and previous cardiovascular disease. Additional adjustments were made for intima media thickness and aortic calcification in the analyses of PWV, only for intima media thickness in the analyses of DC.

Results

Baseline characteristics of the population are shown in table 1. After adjustment for age and gender, levels of CRP were linearly associated with PWV (regression coefficient 0.138, 95% confidence interval, 0.063-0.213) and remained significant after adjustment for confounders and after additional adjustment for measures of atherosclerosis (table 2). C-reactive protein was not associated with carotid

Table 1. Baseline characteristics of the population

Variable	n = 866
Men (%)	47.6
Age (years)	70.9± 5.4
Pulse wave velocity (m/s)	13.4± 3
Distensibility coefficient (10^{-3}/kPa)	10.6± 4.2
Mean arterial pressure (mm Hg)	107.2±12.6
Heart rate (bpm)	75.2±15.1
Diabetes mellitus (%)	6.5
Body mass index (kg/m^2)	26.8± 3.7
Current smokers (%)	15.7
Total cholesterol (mmol/L)	5.8± 0.95
HDL-cholesterol (mmol/L)	1.39±0.39
Previous cardiovascular disease (%)	13.9
C-reactive protein (mg/L) *	2.34 (1.64 to 3.12)
Intima media thickness (mm)	0.87±0.14
Aortic calcification (%)†	19.7

Values are means ± SD for continuous variables and percentages for dichotomous variables.
* For data with a skewed distribution, the median and interquartile range are shown.
† Defined as a calcification score (range 0 to 5) larger than 3.

distensibility (table 2). Increasing tertiles of CRP were significantly associated with increasing levels of PWV (figure 1). Mean values of PWV adjusted for age gender were 12.98 m/s (95% confidence interval, 12.66-13.30) in the first tertile of CRP, 13.49 m/s (95% confidence interval, 13.17-13.81) in the second tertile and 13.82 m/s (95% confidence interval, 13.50-14.15) in the last tertile. Corresponding mean values of PWV in fully adjusted models were 13.08 (95% confidence interval, 12.77-13.45) in the first tertile of CRP, 13.56 m/s (95% confidence interval, 13.24-13.89) in the second tertile and 13.59 (95% confidence interval, 13.24-13.94) in the last tertile of CRP. Associations of cardiovascular risk factors and measures of atherosclerosis with pulse wave velocity are given in table 3.

Discussion

In this population-based study we found that in older adults CRP levels are associated with pulse wave velocity, a measure of arterial stiffness. No association was found between CRP levels and carotid distensibility.

Some aspects of this study need to be discussed before interpreting these data. Firstly, the cross-sectional design may limit our ability to infer a causal relationship between measures of aortic stiffness and inflammatory mediators. Secondly, serum levels of CRP were measured only once, therefore intraindividual variation, as has

Table 2. Multiple linear regression (β) coefficients and 95% confidence intervals describing the association between C-reactive protein (independent variable) and measures of arterial stiffness (dependent variable)

Pulse wave velocity (m/s)	β	95% confidence interval
Model 1	0.138	(0.063, 0.213)
Model 2	0.099	(0.024, 0.172)
Model 3	0.115	(0.039, 0.190)
Model 4	0.088	(0.006, 0.170)
Distensibility coefficient (10^{-3}/kPa)		
Model 1	-0.005	(-0.175, 0.064)
Model 2	-0.003	(-0.104, 0.097)
Model 3	-0.003	(-0.110, 0.104)
Model 4*	0.001	(-0.092, 0.122)

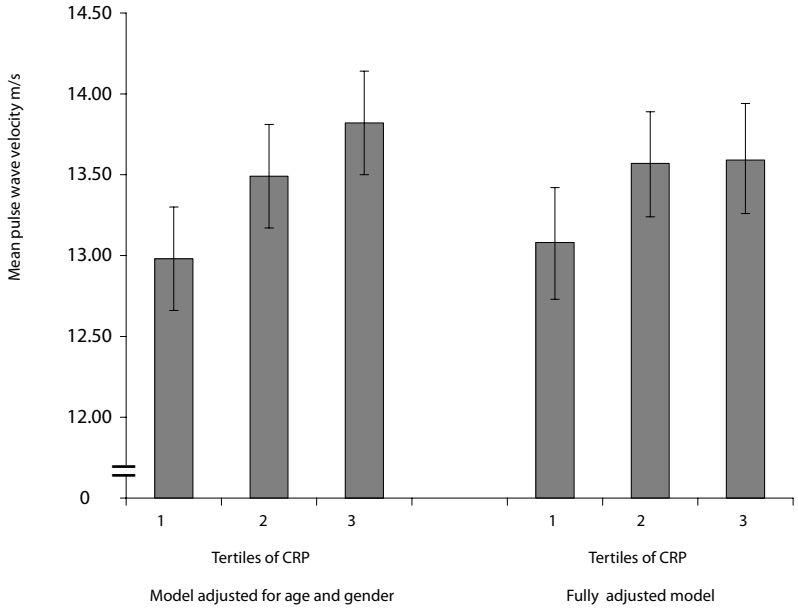
Model 1 Adjusted for age and gender.
Model 2 Adjusted for age, gender, mean arterial pressure and heart rate.
Model 3 Adjusted for age, gender, mean arterial pressure, heart rate, body mass index, total cholesterol, high-density lipoprotein cholesterol, diabetes mellitus, smoking and previous cardiovascular disease.
Model 4 Adjusted for age, gender, mean arterial pressure, heart rate, body mass index, total cholesterol, high-density lipoprotein cholesterol, diabetes mellitus, smoking, previous cardiovascular disease, intima media thickness and aortic calcification score.
*Aortic calcification score is not included in this model.

Table 3. Associations of cardiovascular risk factors and measures of atherosclerosis with pulse wave velocity

	Model 1		Model 2	
	β	95% CI	β	95% CI
Men (yes/no)	0.87	0.49, 1.24	0.43	0.21, 0.85
Age (years)	0.18	0.14, 0.21	0.13	0.09, 0.17
Mean arterial pressure (mmHg)	0.08	0.06, 0.09	0.07	0.06, 0.09
Heart rate (bpm)	0.03	0.02, 0.04	0.02	0.01, 0.03
Diabetes mellitus (yes/no)	1.12	0.35, 1.89	0.70	-0.07, 1.49
Body mass index (kg/m^2)	0.05	-0.001, 0.10	0.04	-0.10, 0.01
Current smokers (yes/no)	-0.18	-0.31, 0.35	0.04	-0.37, 0.28
Total cholesterol (mmol/L)	-0.02	-0.22, 0.18	0.09	-0.29, 0.11
HDL-cholesterol (mmol/L)	0.01	-0.02, 0.05	0.00	-0.04, 0.03
Previous cardiovascular disease (yes/no)	0.71	0.16, 1.25	0.40	-0.15, 0.96
Intima media thickness (mm)	2.20	0.77, 3.62	0.88	-0.56, 2.33
Aortic calcification *	0.46	0.32, 0.60	0.33	0.19, 0.47

Model 1 is adjusted for age and gender.
Model 2 is adjusted for age, gender, mean arterial pressure, heart rate, body mass index, total cholesterol, high-density lipoprotein cholesterol, diabetes mellitus, smoking, previous cardiovascular disease, intima media thickness and aortic calcification score.
*Defined as a calcification score (range 0 to 5) larger than 3

Figure 1. Mean values of pulse wave velocity by tertiles of CRP. Bars represent means, lines represent 95% confidence intervals. Means are adjusted for age and gender, and further for mean arterial pressure, heart rate, body mass index, total cholesterol, high-density lipoprotein cholesterol, diabetes mellitus, smoking, previous cardiovascular disease, intima media thickness and aortic calcification score.



been reported [22], cannot be taken into account. However, such variation will likely result in an underestimation of the true relationship. Thirdly, measures on arterial stiffness were not available for all participants. Because this was primarily due to logistic reasons and therefore random, we believe that this will not have biased the results.

In the present study, we found that high CRP levels were independently associated with increased arterial stiffness. These results are in agreement with the ones of a recent study [14], which showed that wide pulse pressure, a consequence of increased stiffness of the large arteries, is associated with elevated CRP levels. However, pulse pressure is considered to be a surrogate measure of arterial stiffness; in our study we used the pulse wave velocity and the distensibility coefficient of the common carotid artery which are direct measures of arterial stiffness.

Several mechanisms may explain the role of CRP in the pathophysiology of arterial stiffness. Increased levels of CRP have been found to be associated with insulin resistance variables [23], diabetes mellitus [24, 25], and high blood pressure levels [26], which are major determinants of arterial stiffness. High CRP levels, therefore, could contribute to increased PWV, by being responsible for metabolic

and hemodynamic changes that lead to arterial stiffness. However, after inclusion of cardiovascular risk factors in the analyses, the estimates remained statistically significant. This indicates an independent association between CRP levels and PWV.

Another possible mechanism may be the effect of CRP on endothelial dysfunction. Several studies have shown that increased levels of inflammation markers, particularly CRP, are associated with endothelial dysfunction [23, 27], and the inflammation reaction is known to inhibit the endothelium-dependent vasodilatation [28]. The vascular endothelium releases vasoactive substances; one of these, nitric oxide, has a major influence on basal arteriolar tone and blood pressure [29, 30]. Moreover, agonists that stimulate endothelial nitric oxide release, such as acetylcholine, also reduce muscular artery stiffness in vivo [31, 32]. It has also been shown that basal nitric oxide production influences positively muscular arteries distensibility in vivo, and that the effect of acetylcholine on large arteries is mainly nitric oxide-mediated [33]. These findings support a major role of endothelium in regulating arterial distensibility, suggesting that impaired endothelial function may alter the mechanical properties of the vessel walls leading to increased arterial stiffness.

It could also be speculated that arterial stiffness affects CRP levels. Elevation of pulse pressure, which reflects an increase in the stiffness of the large arteries, inhibits acetylcholine-induced endothelium-dependent relaxation by generating reactive oxygen species [34], which may stimulate inflammatory pathways [35]. Moreover, high levels of pulse pressure are associated with greater flow reversal during diastole [36], which can increase the expression of adhesion molecules [37].

Increased CRP levels play a role in the development of atherosclerosis [9, 10], which has also been related to arterial stiffness [3]. As reported in previous studies, measures of common carotid intima-media thickness and radiographically assessed calcifications of the abdominal aorta reflect atherosclerosis [21, 38]. In the present study, measures of intima-media thickness and the aortic calcification score were used as indicators of atherosclerosis in adjusted models. To evaluate whether the association between CRP levels and arterial stiffness was independent of atherosclerosis, we included both variables in models where PWV was tested. Because carotid distensibility is a local measure of arterial stiffness, we included only intima-media thickness in models where carotid distensibility was tested. After adjustment for atherosclerosis, the association between CRP levels and increased PWV remained statistically significant, supporting that the association of CRP with arterial stiffness is independent of atherosclerosis.

CRP levels were strongly associated with increased PWV, whereas no association was found between CRP levels and common carotid distensibility. The measure of carotid distensibility is a local measure of stiffness which gives information about an elastic artery, while carotid-femoral pulse wave velocity reflects the vessel

wall stiffness of several territories providing information about both elastic and muscular arteries. Arterial stiffness depends on structural and functional properties of the vessel wall. Such properties are not uniform along the arterial tree, and there may be differences between elastic and muscular arteries. Therefore the association between CRP levels and arterial stiffness may be different according to the different structure of the vessel wall. However, this remains hypothetical, other explanations are possible.

In conclusion, in this population-based study, we found that increased levels of CRP, were associated with arterial stiffness independently of cardiovascular risk factors and atherosclerosis. Considering the cross sectional design of the present study, mechanisms remain speculative and require further study. However, the association we found between CRP levels and arterial stiffness may be useful to understand better the biochemical mechanisms responsible for the development of arterial stiffness.

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2.3 Impaired fasting glucose and arterial stiffness

Abstract

Aims. Several studies have found increased arterial stiffness in diabetic subjects. Impaired fasting glucose frequently occurs in elderly non-diabetic subjects but it is not fully established whether this condition is also associated with increased arterial stiffness. We studied the association between impaired fasting glucose and arterial stiffness in 2987 subjects, aged 60 years or over.

Methods. The study was part of the Rotterdam Study, a population-based cohort study. Arterial stiffness was assessed by measuring common carotid arterial distensibility.

Results. In the total cohort, common carotid distensibility decreased with increasing impairment of glucose metabolism. Under 75 years of age, subjects with impaired fasting glucose were comparable to subjects with a normal glucose tolerance with respect to arterial stiffness. Above 75 years of age, subjects with impaired fasting glucose had stiffer arteries than subjects with a normal glucose tolerance, reaching the same arterial stiffness as diabetic subjects. The mean difference (95% confidence interval; p-value) in distensibility coefficient between subjects with a normal respectively an impaired glucose tolerance and between subjects with a normal glucose tolerance and diabetic subjects was 0.1 (-0.04-0.05; p=0.88) and 1.2 (0.7-1.7; p<0.001) under 75 years of age and 0.7 (0.2-1.2; p=0.007) and 0.8 (0.3-1.4; p=0.002) above 75 years of age, respectively.

Conclusion. Our findings indicate that impaired fasting glucose is related to increased arterial stiffness in elderly subjects. An advanced stage of arterial stiffness, comparable with that of diabetic subjects, is only reached at high age.

Abbreviations: ARIC, Atherosclerosis Risk in Community; HDL, high-density lipoprotein; IFG, impaired fasting glucose; D, end-diastolic diameter; ΔD, absolute stroke change in diameter; ΔD/D, relative stroke change in diameter; ΔP, pulse pressure; MAP, mean arterial pressure.

Introduction

Stiffening of the arteries is more pronounced in diabetic subjects than in non-diabetic subjects [1-5]. Furthermore, in subjects with type 2 diabetes mellitus, the presence of insulin resistance is positively associated with arterial stiffness [6]. Some studies suggest that a positive association of an impaired glucose metabolism with arterial stiffness is not confined to diabetic subjects. [6-11] However, these studies have been relatively small. Most of these studies have been confined to young or middle-aged subjects [6-9]. Impairment in glucose metabolism frequently occurs in elderly non-diabetic subjects [12, 13]. Increased arterial stiffness is associated with increased cardiovascular risk [14, 15]. Therefore, it is of importance to know whether an impaired glucose metabolism is accompanied by arterial stiffening in elderly non-diabetic subjects. One study addressed the relation between an impaired glucose metabolism and peripheral arterial stiffness in elderly subjects[10]. Recently, the relation between an impaired glucose metabolism and central arterial stiffness was assessed in the same study population[11]. These relatively small studies showed increased femoral and brachial artery stiffness but not increased carotid artery and central artery stiffness in subjects with impaired glucose metabolism as compared to subjects with a normal metabolism. The aim of the present study was to examine, in a large population-based cohort, whether arterial stiffness, as assessed by common carotid artery distensibility, is increased in elderly non-diabetic subjects with an impaired glucose metabolism, relative to subjects with a normal glucose metabolism.

Subjects and methods

Subjects

The Rotterdam Study. This is a population-based cohort study that aims at assessing the occurrence of and risk factors for chronic diseases in the elderly. The rationale and design of the Rotterdam study have been described in detail elsewhere [16]. The third examination phase started in March 1997 and used the same protocol as was used at the baseline examinations. The data collection comprised an extensive home interview and subsequent visits to the study center for clinical examinations. For the present study, the first 3011 participants who attended the third examination phase and had a measurement of arterial stiffness were eligible. Information on all variables used in the present study was collected during the third examination phase. The Medical Ethics Committee of the Erasmus University approved the study and written consent was obtained from all participants.

Clinical and laboratory methods

Cardiovascular risk factors. Information on cardiovascular risk factors was collected at the research center. Anthropometric measures were obtained while the subject was wearing lightweight clothes and no shoes, and included height, weight, and waist and hip circumference. Body mass index (weight/height^2) and the waist-to-hip ratio were calculated. For participants who were not known to have diabetes mellitus, fasting blood samples were obtained by venapuncture with minimal stasis using a 12- gauge Butterfly needle. Non-fasting blood samples were obtained from diabetic participants. Serum total cholesterol and high-density lipoprotein (HDL) cholesterol were determined using an automatic enzymatic procedure (Boehringer Mannheim, Mannheim, Germany). Glucose was enzymatically determined by the Hexokinase method (Boehringer Mannheim, Mannheim, Germany).

Carotid artery atherosclerosis. As an indicator of atherosclerosis in the carotid artery we used the presence of plaques in the common carotid artery assessed by on-line evaluation of the ultrasonographic images. Ultrasonography of both carotid arteries was performed with a 7.5 MHz linear-array transducer (Ultramark IV, ATL, Bothell, Washington, USA). Plaques were defined as a focal widening relative to adjacent segments, with protrusion into the lumen and composed of either only calcified deposits or a combination of calcified and noncalcified material. No attempt was made to quantify the size of the lesions. Severity of plaques in the common carotid artery was graded as 0 (no plaques) or 1 (presence of plaques at the far or near wall of the left or right common carotid artery).

Glucose status. Information on history of diabetes mellitus and use of blood glucose lowering medication was obtained during a home interview. Additionally, information on prescription of blood glucose lowering medication was obtained from the pharmacy. Glucose status was classified into three categories: non-diabetic subjects with normal fasting glucose concentrations, non-diabetic subjects with impaired fasting glucose (IFG), and subjects with diabetes mellitus. IFG is a recently defined diagnostic category based on a fasting plasma glucose concentration [17]. Analogous to the World Health Organization criteria of impaired glucose tolerance it represents a metabolic stage intermediate between normal glucose homeostasis and diabetes and is associated with the insulin resistance syndrome [17, 18]. Normal glucose tolerance was defined as a fasting glucose level below 6.1 mmol/l, without a history of diabetes mellitus and without the use of blood glucose lowering medication. IFG was defined as a fasting serum glucose level between 6.1 and 6.9 mmol/l without a history of diabetes mellitus and without the use of blood glucose lowering medication [17]. Diabetes mellitus was defined as a history of diabetes mellitus and/or the use of blood glucose lowering medication and/or a fasting serum glucose level equal or greater than 7.0 mmol/l [17].

Arterial stiffness. Arterial stiffness was assessed at the research center by measuring common carotid artery distensibility and expressed as the distensibility coefficient. A lower distensibility coefficient indicates increased arterial stiffness. Subjects were instructed to refrain from smoking and from taking coffee, tea, alcohol or pain-medication on the day of measurement, and from taking alcohol on the day before. The vessel wall motion of the right common carotid artery was measured with a Duplex scanner with an operating frequency of 7.5 MHz (ATL Ultramark IV, ATL, Bothell, Washington, USA) connected to a vessel wall movement detector system. The details of this technique have been described elsewhere [19, 20]. Briefly, this system enables the transcutaneous assessment of the displacement of the arterial walls during the cardiac cycle and, hence, the time-dependent changes in arterial diameter relative to its diastolic diameter at the start of the cardiac cycle. Subjects were placed in supine position, with the head tilted slightly to the contralateral side for the measurements in the common carotid artery. A region at 1.5 cm proximal to the origin of the bulb of the carotid artery was identified using B-mode ultrasonography. The displacement of the arterial walls was obtained by processing the radio frequency signals originating from two selected sample volumes positioned over the anterior and posterior walls. The end-diastolic diameter (D), the absolute stroke change in diameter during systole (ΔD), and the relative stroke change in diameter (ΔD/D) were computed as the mean of four cardiac cycles of three successive recordings. Blood pressure was measured twice at the upper arm with a Dinamap automatic blood pressure recorder during the measurement session. The mean was taken as the subjects reading. Pulse pressure (ΔP) was defined as the difference between systolic and diastolic blood pressure. Mean arterial pressure (MAP) was calculated by adding 1/3 pulse pressure to the diastolic blood pressure. The cross-sectional arterial wall distensibility coefficient was calculated according to the following equation [21]: distensibility coefficient = $(2\Delta D/D)/\Delta P$ (10^{-3}/kPa). With this system a wall displacement of a few micrometers can be resolved [19] and D, ΔD, ΔD/D, and the distensibility coefficient can be assessed reliably [20]. The arterial wall properties, as determined in this way, are defined as the relative changes in arterial cross-sectional area, expressed in terms of diameter, for a change in pressure. They reflect a combination of passive elastic properties and active components induced by smooth muscle cells. We performed a reproducibility study in 47 subjects, which showed an intra-class correlation coefficient of 0.80 for the distensibility coefficient. In the present study, measurements were restricted to the right side to save time. In previous studies no differences could be detected between arterial wall properties of the right and left common carotid artery (unpublished results).

Data analysis

Population for analysis. Of all participants who attended the follow-up examination, information on common carotid distensibility was available for 77%. Missing information on common carotid distensibility was mainly due to logistic reasons. The first 3011 participants with information on common carotid distensibility were eligible for the present study. We excluded 24 subjects from whom non-fasting blood was drawn (without having a history of diabetes as reason of drawing non-fasting blood) leaving 2987 subjects to be included in the analyses. A sub-analysis in which we evaluated possible determinants of reduced arterial distensibility was performed on a sub-population of 2816 subjects with complete information on all determinants related to arterial distensibility. Missing information on determinants was mainly due to logistic reasons. In the analysis with fasting glucose as determinant diabetic subjects with non-fasting glucose were excluded (n=77).

Statistical analysis. Characteristics of subjects with a normal glucose tolerance, subjects with IFG and diabetic subjects were calculated and tested for differences between groups after adjustment for age using one way analyses of covariance for continuous characteristics and logistic regression analyses for dichotomous characteristics. Before addressing the association between glucose status and arterial distensibility, fasting glucose and other potential determinants were related to common carotid distensibility in the total cohort (including non-diabetic with a normal and impaired glucose tolerance and diabetic subjects) using multiple linear regression analysis. The other potential determinants were age, gender, MAP, total cholesterol, HDL-cholesterol, body mass index and waist-to-hip ratio. Analyses were adjusted for age, gender and MAP, except when one of these variables was the determinant of interest.

The effect of glucose status on arterial distensibility was evaluated using one-way analyses of covariance. Differences in mean distensibility coefficient between subjects with a normal glucose tolerance, subjects with IFG and diabetic subjects were tested adjusted for age, gender, and MAP. Additional adjustment for the presence of plaques in the common carotid artery was made to evaluate whether the association between impaired fasting glucose and arterial distensibility persisted independently of atherosclerosis. Because arterial stiffening is a slow process taking years to develop, analyses were performed for the total cohort and within age-strata (a priori cut-off point of 75 years of age). All analyses were performed using the statistical package SPSS 8.0 for Windows 95 (SPSS Inc., Chicago, Illinois, USA).

Results

Characteristics. The characteristics of subjects with a normal glucose tolerance, subjects with IFG and diabetic subjects are presented in table 1. Diabetic subjects were significantly older than subjects with a normal glucose tolerance. After adjustment for age, subjects with IFG and diabetes mellitus tended to have higher levels of cardiovascular risk factors as compared to subjects with a normal glucose tolerance, except for total cholesterol that was lower in diabetic subjects than in subjects with a normal glucose tolerance and subjects with IFG. Diabetic subjects had a higher prevalence of plaques in the common carotid artery as compared to both subjects with a normal glucose tolerance and subjects with IFG, adjusted for age.

Determinants of arterial stiffness. In the total cohort, age, gender, MAP, fasting glucose, HDL-cholesterol, body mass index, and waist-to-hip ratio were all significantly associated with the distensibility coefficient after adjustment for age, gender

Table 1. Characteristics of the study population by glucose status.

Characteristic	Normal glucose tolerance n=2209	Impaired fasting glucose n=422	Diabetes mellitus n=356
Age (years)	72 (60-101)	72 (61-93)	74 (61-91)[b,c]
Men (%)[a]	41	43	47[e]
Systolic blood pressure (mmHg)	131 ± 19	137 ± 20[d]	140 ± 17[e]
Diastolic blood pressure (mmHg)	70 ± 10	73 ± 10[d]	71 ± 9[e]
Mean arterial pressure (mmHg)	90 ± 12	94 ± 13[d]	94 ± 10[e]
Total cholesterol (mmol/l)	5.9 ± 1.0	5.9 ± 1.0	5.6 ± 0.9[e,f]
HDL-cholesterol (mmol/l)	1.4 ± 0.4	1.4 ± 0.5[d]	1.2 ±0.3[e,f]
Glucose (mmol/l)	5.3 ± 0.4	6.4 ± 0.2[d]	8.8 ± 2.5[e,f]
Body mass index (kg/m^2)	26.2 ± 3.8	28.0 ± 4.2[d]	28.3 ± 4.3[e]
Waist-to-hip ratio	0.91 ± 0.10	0.94 ± 0.10[d]	1.0 ± 0.09[e,f]
Distension (μm)	324 ± 109	311 ± 111	308 ± 107
Diameter (mm)	7.8 ± 1.0	8.0 ± 1.0[d]	8.1 ± 0.9[e]
Presence of plaques in the common carotid artery (%)[a]	14.3	16.1	24.7[e,f]
Distensibility coefficient (10^{-3}/kPa)	10.9 ± 4.4	9.8 ± 4.4[d]	8.8 ± 3.6[e,f]

Values are given as mean ± standard deviation except for age that is given as mean (range) and categorical variables[a] that are given as percentage. HDL-cholesterol = high-density lipoprotein cholesterol.
[b] $p < 0.05$ diabetics versus normal glucose tolerance, [c] $p < 0.05$, diabetics versus impaired fasting glucose, [d] $p < 0.05$ impaired fasting glucose versus normal glucose tolerance, adjusted for age, [e] $p < 0.05$, diabetics versus normal glucose tolerance, adjusted for age, [f] $p < 0.05$, diabetics versus impaired fasting glucose, adjusted for age.

Table 2. Multiple linear regression beta-coefficients (95% C.I.) describing the association of various variables with the common carotid arterial distensibility coefficient (10^{-3}/kPa).

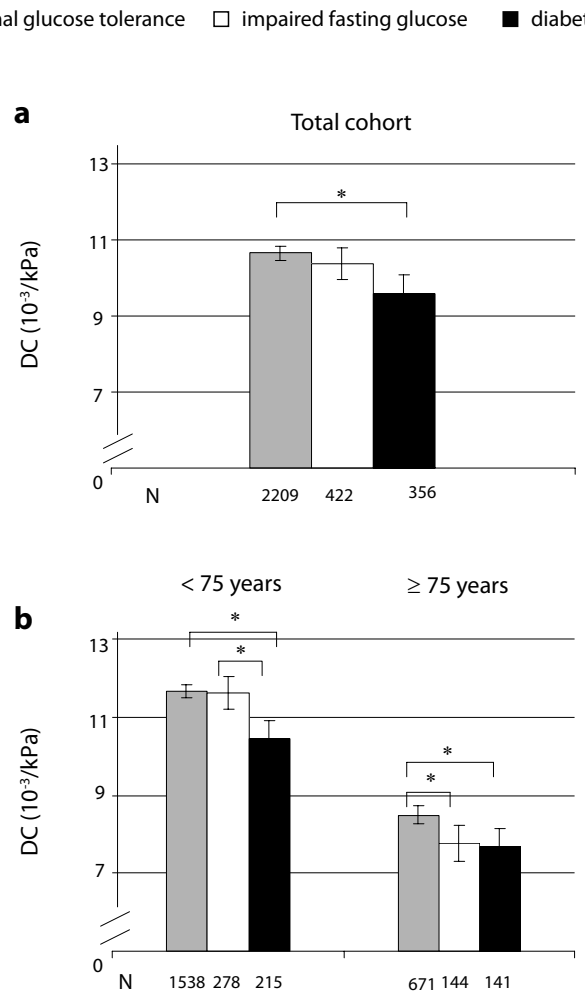
Variable	beta-coefficient[a] (95% CI)
Age (years)[b]	-0.28 (-0.30 ; -0.26)
Men[c]	-0.89 (-1.18 ; -0.60)
Mean arterial pressure (mmHg)[d]	-0.17 (-0.18 ; -0.16)
Fasting glucose (mmol/l)[e]	-0.29 (-0.39 ; -0.19)
Total cholesterol (mmol/l)[e]	-0.06 (-0.19 ; 0.07)
HDL-cholesterol (mmol/l)[e]	0.76 (0.44 ; 1.09)
Body mass index (kg/m^2)[e]	-0.09 (-0.12 ; -0.06)
Waist-to-hip ratio[e]	-2.58 (-3.99 ; -1.17)

[a] Increase in distensibility coefficient (10^{-3}/kPa) for every unit increase of the independent variable. [b] Adjusted for gender; [c] adjusted for age; [d] adjusted for age and gender; [e] adjusted for age, gender and mean arterial pressure.
n = number of subjects. HDL-cholesterol = high-density lipoprotein cholesterol.
CI = confidence interval.

and MAP where appropriate (Table 2). Total cholesterol was not associated with the distensibility coefficient.

Arterial stiffness and glucose status. Figure 1 shows the mean distensibility coefficient of the common carotid artery for subjects with a normal glucose tolerance, subjects with IFG and diabetic subjects for the total cohort and in strata of age. All analyses were adjusted for age, gender and MAP. In the total cohort, adjusted common carotid artery distensibility coefficients (10^{-3}/kPa) of subjects with a normal glucose tolerance, subjects with IFG and diabetic subjects were 10.7 (standard error: 0.07), 10.4 (0.16) and 9.6 (0.18) respectively. The difference in distensibility coefficient between subjects with a normal glucose tolerance and subjects with IFG was not significant (mean difference in distensibility coefficient (95% C.I; p-value): 0.3 (-0.07 to 0.6; p=0.12)). The difference in distensibility coefficient between subjects with a normal glucose tolerance and diabetic subjects was highly significant (mean difference: 1.1 (0.3 to 1.3; p<0.001)). Adjusted common carotid artery distensibility coefficients (10^{-3}/kPa) of subjects with a normal glucose tolerance, subjects with IFG and diabetic subjects under 75 years of age were 11.7 (standard error: 0.07), 11.6 (0.21) and 10.5 (0.24) and above 75 years of age were 8.5 (0.11), 7.8 (0.24) and 7.7 (0.25) respectively. Subjects under 75 years of age with IFG were comparable with subjects with a normal glucose tolerance with respect to arterial stiffness (mean difference: 0.01 (-0.4 to 0.5; p=0.88)), while diabetic subjects under 75 years of age had significantly increased arterial stiffness as compared to subjects with a normal glucose tolerance (mean difference: 1.2 (0.7 to 1.7; p<0.001)). Above 75 years of age, arterial stiffness of subjects with IFG was of the same order as arterial stiffness of diabetic subjects and both were significantly higher than arterial stiffness of subjects with a normal glucose tolerance

Figure 1a, b. Mean distensibility coefficient (95% confidence intervals) in subjects with normal glucose tolerance, subjects with impaired fasting glucose and diabetic subjects of the Rotterdam Study in the total cohort (**a**) and in strata of age (**b**) adjusted for age, gender and mean arterial blood pressure. The number of subjects within each group is indicated at the lower end of the respective bar. DC = distensibility coefficient. * p < 0.01.



(mean difference between subjects with IFG and subjects with a normal glucose tolerance: 0.7 (0.2 to 1.2; p=0.007) and between diabetic subjects and subjects with a normal glucose tolerance: 0.8 (0.3 to 1.4; p=0.002)). Results were similar for men and women and after additional adjustment for the presence of plaques in the common carotid artery (data not shown).

Discussion

The results of this population-based study in elderly subjects indicate that among subjects under 75 years of age, subjects with IFG are comparable to subjects with a normal glucose tolerance with respect to arterial stiffness. Above 75 years of age, arterial stiffness of subjects with IFG reaches that of diabetic subjects and both groups have increased arterial stiffness as compared to subjects with a normal glucose tolerance.

Some methodological issues need to be discussed. Firstly, by calculating the distensibility coefficient, distension of the common carotid artery is adjusted for pulse pressure measured in the brachial artery. We thereby assume that pulse pressure measured in the brachial artery is representative of pulse pressure in carotid arteries. In dogs, it has been demonstrated that pulse pressure in the brachial artery is linearly related to blood pressure in the carotid artery over a wide range of blood pressures [21]. However, it is known that the arterial pressure waves undergo transformation in the arterial tree and therefore pulse pressure is higher in the brachial artery than in more central vessels like the carotid artery [22]. On the other hand, non-invasive cuff-based measurement of blood pressure underestimates pulse pressure [23]. Several groups showed the validity of the use of brachial pressures [24-26]. Secondly, in analyses with arterial distensibility, a measure highly dependent on blood pressure, adequate correction for blood pressure is of the utmost importance. The distensibility coefficient is calculated by dividing the relative distension by pulse pressure. Despite this correction, the distensibility coefficient has a strong negative association with MAP. A higher MAP in the artery stretches the elastin and collagen fibers in the arterial wall, making the artery less distensible. Blood pressure is one of the major determinants of arterial stiffness and also part of the insulin-resistance syndrome. Therefore, all analyses were adjusted for MAP.

The insulin resistance syndrome consists of insulin resistance, compensatory hyperinsulinemia, obesity (especially abdominal or visceral obesity), dyslipidemia of the high-triglyceride and/or low-HDL type, and hypertension [27, 28]. IFG is a metabolic stage, intermediate between normal glucose homeostasis and diabetes and is associated with insulin resistance [17, 18]. Studies concerning insulin resistance and an impaired glucose metabolism in relation to arterial stiffness are scarce and mainly confined to diabetic subjects or young to middle-aged healthy subjects [6, 7, 9]. In subjects with type 2 diabetes mellitus, the presence of insulin resistance, as assessed with the euglycaemic hyperinsulinaemic clamp technique, is associated with increased arterial stiffness [6]. Also, in young healthy non-diabetic subjects, insulin resistance, as assessed with the euglycaemic hyperinsulinaemic clamp technique, is associated with increased arterial stiffness independent of MAP [7]. In healthy non-diabetic middle-aged women, variables of the insulin resistance syndrome were

found to be associated with reduced arterial distensibility, after adjustment for MAP[8]. The ARIC study examined fasting glucose levels in relation to arterial stiffness in non-diabetic subjects aged 45 to 64 years and reported higher indexes of arterial stiffness when the fasting glucose level was above normal [9]. Recently, an impaired glucose metabolism was found to be related to increased femoral and brachial artery stiffness, but not to carotid artery stiffness[10].

This is the first large study in which arterial stiffness of elderly subjects with IFG is compared with that of elderly subjects with a normal glucose tolerance and elderly diabetic subjects. We only found increased arterial stiffness in subjects with IFG among subjects aged 75 years or over, which is in contrast with the results of the ARIC study. The ARIC Study, however, did not adjust for MAP. When we re-analyzed our data without adjustment for MAP, we found similar results as the ARIC study in subjects under 75 years of age. In that analyses, mean age and sex adjusted distensibility coefficients (10^{-3}/kPa) of subjects with a normal glucose tolerance, subjects with IFG and diabetic subjects were 11.8 (standard error: 0.10), 11.1 (0.24) and 10.0 (0.28) respectively. All groups were significantly different from each other. However, for reasons explained in the previous paragraph, we attach more value to results adjusted for MAP. Henry and colleagues also found no relation between an impaired glucose metabolism and carotid artery distensibility coefficient after correction for MAP in subjects with a mean age of 68.5 years[10]. In the same study population, subjects with an impaired glucose metabolism were found to have values of central arterial stiffness intermediate between those of subjects with a normal glucose metabolism and subjects with diabetes mellitus type 2. However, differences in arterial stiffness between subjects with an impaired glucose metabolism and subjects with a normal glucose metabolism were not significant after adjustment for MAP[11]. However, we did not hypothesize a priori such a difference in association between younger and older subjects. Therefore, we cannot exclude that these results are due to chance. Some studies suggest gender-differences in the relation of diabetes and insulin-resistance with arterial stiffness [5, 7] but data are contradictory. We did not find gender-differences in the association of IFG with reduced arterial distensibility.

We also examined other potential determinants of arterial stiffness. Several variables evaluated are part of the insulin-resistance syndrome (fasting glucose, HDL-cholesterol, body mass index and waist-to-hip ratio). We found parameters of the insulin resistance syndrome to be strongly associated with arterial stiffness in elderly subjects, which is in accordance with a previous study of our group in healthy non-diabetic middle aged women [8]. Total cholesterol, which is not part of the insulin-resistance syndrome, was not associated with arterial distensibility. The finding of a significant association between fasting glucose as continuous variable and increased arterial stiffness in the total cohort is in agreement with the trend in increasing arterial stiffness from subjects with a normal glucose tolerance, subjects

with IFG to diabetic subjects in the total cohort. This might suggest a role of high glucose levels in determining arterial stiffness also in subjects with normal glucose metabolism. However, the analysis with fasting glucose as continuous variable included subjects with diabetes mellitus who were newly diagnosed in the third examination phase on the basis of their fasting glucose level, and had both high levels of fasting glucose and increased arterial stiffness.

The association of arterial stiffness with atherosclerosis is still subject to debate [29-32]. We additionally adjusted the associations of IFG and diabetes mellitus with arterial distensibility for the presence of atherosclerosis in the common carotid artery. Additional adjustment did not alter the results. This suggests that the associations of IFG and diabetes mellitus with increased arterial stiffness are in part independent of atherosclerosis. A relation between fasting glucose levels and arterial stiffness independent of atherosclerosis may be explained by hyperglycaemia leading to increased arterial stiffness by collagen cross linking due to non-enzymatic glycation [33, 34].

An impaired glucose metabolism is a frequent condition in elderly non-diabetic subjects [12, 13]. In our study population, 13.7% of subjects under 75 years of age and 15.1% of subjects above 75 years had IFG on the basis of recently developed diagnostic criteria. Arterial stiffness is a process that generally develops slowly taking years to reach advanced stages. The present study is the first study in which a sub-analysis on the eldest elderly is performed. Our results showed that above 75 years of age, non-diabetic subjects with an impaired glucose metabolism reach the same arterial stiffness as diabetic subjects. Increased arterial stiffness is associated with increased cardiovascular risk [14, 15] and recent evidence suggests opportunities to treat arterial stiffness induced by hyperglycaemia in the near future [35]. Therefore, it is important to recognize that healthy non-diabetic elderly subjects with high fasting glucose levels reach the same arterial stiffness as diabetic subjects at high age. We found that in subjects with IFG, arterial stiffness has not yet reach advanced stages under 75 years of age. In this group, early treatment of hyperglycaemia may prevent advanced arterial stiffness.

In conclusion, the results of this population-based study show that IFG is related to increased arterial stiffness in non-diabetic elderly men and women.

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2.4

Alcohol consumption and arterial stiffness

Abstract

Background. Light to moderate alcohol consumption has been associated with lower risk of cardiovascular disease. The protective effect of alcohol could involve arterial properties as arterial stiffness and distensibility.
Methods. The relation between alcohol and arterial stiffness was studied within the framework of the Rotterdam Study, a population-based study in individuals aged 55 and older. The present study included 3178 subjects participating in the third examination phase. Arterial stiffness was measured by two different methods, i. e. the carotid-femoral pulse wave velocity and the distensibility coefficient of the common carotid artery. Categories of alcohol consumption were defined as follow; 0 drinks per day (non-drinkers), up to 1 drink, 1 to 2 drinks, and more than 2 drinks. Multivariate logistic regression analysis was used to compute odds ratios and corresponding 95% confidence intervals of high arterial stiffness.
Results. According to predefined categories of alcohol consumption odds ratios for high pulse wave velocity were 0.64 (95% CI, 0.36-1.15), 0.71 (95% CI, 0.39-1.31) and 0.92 (95% CI, 0.51-1.66). Corresponding estimates in women were 0.68 (95% CI, 0.50-0.93), 0.56 (95% CI, 0.37-0.86) and 0.88 (95% CI, 0.57-1.34). Odds ratios for low carotid distensibility coefficient were 0.92 (95% CI, 0.50-1.69), 0.77 (95% CI, 0.40-1.45) and 0.91 (95% CI, 0.49-1.68) in men; corresponding estimates in women were 0.74 (95% CI, 0.55-0.99), 0.67 (95% CI, 0.45-0.99) and 0.73 (95% CI, 0.49-1.09).
Conclusions. Moderate alcohol consumption is associated with lower arterial stiffness independently of cardiovascular risk factors and atherosclerosis in women; in men, although the same trend was observed, the estimates lacked statistical significance. We found the association to be U-shaped and stronger for wine consumption.

Introduction

Light to moderate alcohol consumption seems to have a protective effect on the cardiovascular system. Higher cardiovascular morbidity and mortality have been shown in heavy and non-drinkers compared to moderate drinkers resulting in an U-shaped association [1-3]. The mechanism underlying this beneficial effect of moderate alcohol consumption is still incompletely understood. Increase of arterial stiffness, which is one of the characteristics of the aging cardiovascular system [4], and is associated with cardiovascular risk factors as hypertension [5, 6] and diabetes mellitus [7], has been considered as possible mechanism. The results obtained in the studies on the relation between alcohol consumption and arterial stiffness, however, are inconsistent. In middle-aged Japanese men alcohol consumption was found to be associated with high aortic stiffness measured as pulse wave velocity [8, 9]. Conversely, in another study alcohol consumption was shown to be associated with reduced arterial stiffness [10]. Recent studies found a J-shaped association between alcohol consumption and arterial stiffness in men aged 40-80 years [11] and an inverse association in healthy postmenopausal women [12]. We hypothesized that moderate drinkers could have less stiff arteries compared with non or heavy drinkers; therefore we investigated the relation between alcohol consumption and arterial stiffness within the framework of the Rotterdam Study, a population-based study in individuals aged 55 and older.

Methods

Population

This study was conducted within the framework of the Rotterdam Study, an ongoing prospective population-based cohort study among subjects aged 55 years and over, living in Ommoord, a suburb of Rotterdam, The Netherlands. The rationale and design of the Rotterdam Study have been described elsewhere [13]. The third examination phase took place from 1997 until 1999. During this phase, information on cardiovascular risk factors was collected, measurements of arterial stiffness and atherosclerosis were obtained and alcohol consumption was assessed as part of the interview at the study center. The Medical Ethics Committee of the Erasmus Medical Center approved the study and written consent was obtained from all participants.

Arterial Stiffness

Arterial stiffness was measured by two different methods, i. e. the carotid-femoral pulse wave velocity (PWV) as measure of aortic stiffness and the distensibility coefficient (DC) of the common carotid artery as measure of common carotid

arterial stiffness. Both measures were obtained on the same day, in the same room. Subjects were instructed to refrain from smoking and from taking coffee, tea or pain medications on the day of measurements, and from taking alcohol on the day of measurements and the day before.

Carotid-femoral pulse wave velocity

Carotid-femoral pulse wave velocity (PWV) was measured with the subjects in supine position. Blood pressure was measured twice with a sphygmomanometer after five minutes of rest, and the mean was taken as the subject's reading. Mean arterial pressure was calculated by the following formula: diastolic blood pressure + 1/3 (systolic blood pressure-diastolic blood pressure). Carotid-femoral PWV was assessed with an automatic device (Complior, Colson) [14] that assessed the time delay between the rapid upstroke of the feet of simultaneously recorded pulse waves in the carotid and the femoral artery. The distance between the recording sites in the carotid and the femoral artery was measured with a tape. PWV was calculated as the ratio between the distance measured and the foot-to-foot time delay and was expressed in meters per second. The average of at least 10 successive measurements, to cover a complete respiratory cycle, was used in the analysis.

Distensibility coefficient of the common carotid artery

Common carotid distensibility was assessed with the subjects in supine position, the head tilted slightly to the controlateral side for the measurement in the common carotid artery. The vessel wall motion of the right common carotid artery was measured by means of a duplex scanner (ATL Ultramark IV, operating frequency 7.5 MHz) connected to a vessel wall movement detector system. The details of this technique have been described elsewhere [15, 16]. After five minutes of rest, a region at 1.5 cm proximal to the origin of the bulb of the carotid artery was identified using B-mode ultrasound. The displacement of the arterial walls was obtained by processing the radio frequency signals originating from two selected sample volumes positioned over the anterior and posterior walls. The end-diastolic diameter (D), the absolute stroke change in diameter during systole (ΔD), and the relative stroke change in diameter ($\Delta D/D$) were computed as the mean of four cardiac cycles of three successive recordings. Blood pressure was measured twice at the upper arm with a Dinamap automatic blood pressure recorder during the measurement session. The mean was taken as the subjects reading. Pulse pressure (ΔP) was defined as the difference between systolic and diastolic blood pressure. Mean arterial pressure was calculated by the following formula: diastolic blood pressure + 1/3 (systolic blood pressure-diastolic blood pressure). The cross-sectional arterial wall distensibility coefficient was calculated according to the following equation: distensibility coefficient = $(2\ \Delta D/D)/\Delta P$ (10^{-3}/kPa) [17]. In the present study, measurements were restricted to the right side to save time. In

previous studies no differences could be detected between arterial wall properties of the right and left common carotid artery (SK Samijo, unpublished results, 1997). In a reproducibility study in 47 subjects the intra-class correlation coefficient was 0.80 both for the distensibility coefficient and the carotid-femoral pulse wave velocity.

Alcohol consumption

Alcohol consumption was assessed as part of the interview at the study center. Participants reported the number of alcoholic beverages they consumed weekly in each of four categories: beer, wine, liquor, and moderately strong alcohol types. The latter category contained predominantly fortified wines, namely sherry and port. Non-drinkers were considered abstainers. By adding the number of drinks of specific alcoholic beverages consumed per week, the total mean daily consumption of alcohol in drinks per day was calculated. Since most of the moderately strong alcoholic drinks were wine types, this category was combined with the wine category in the analyses. The alcohol consumption was divided into daily consumption of 0 drinks (non-drinking), up to 1 drink, 1 to 2 drinks, and more than 2 drinks.

Cardiovascular risk factors

At the research center, blood pressure was measured twice on the right arm using a random-zero sphygmomanometer. Body mass index [weight /height^2] was calculated. Diabetes mellitus was defined as use of anti-diabetic medication and/or a fasting serum glucose level ≥7.0 mmol/l [18]. Serum total cholesterol and high-density lipoprotein (HDL) cholesterol values were determined by an automated enzymatic procedure (Boehringer Mannheim System). Information on smoking habits was obtained during the interview.

Measure of intima- media thickness

Ultrasonography of both carotid arteries was performed with a 7.5-Mhz linear-array transducer and a duplex scanner (ATL UltraMark IV). Common carotid intima-media thickness (IMT) was determined as previously described [19].

Population for analysis

Of the 4024 subjects who underwent the physical examination during the third phase of the Rotterdam Study, arterial stiffness as assessed by means of PWV was determined in 3550 subjects whereas common carotid distensibility was measured in 3098 subjects. Missing information on both measures was almost entirely due to logistic reasons. Non-drinkers could have an adverse risk profile, while past-drinkers may have stopped drinking due to health problems, eventually cardiovascular disease. Therefore, to avoid bias, past drinkers were excluded form the analyses

leaving 3178 subjects with data both on alcohol consumption and PWV; data on alcohol consumption and carotid distensibility were available for 2973 subjects.

Statistical Analysis

Logistic regression analysis adjusted for age was used to compute odds ratios (OR) and corresponding 95% confidence intervals (95% CI) of high arterial stiffness (being in the last tertile of PWV for PWV measurements, being in the first tertile of DC for DC measurements) for categories of alcohol consumption, using non-drinkers as the reference category. The cut-off point for the last tertile of PWV was 14.25 m/s. The cut-off point for the first tertile category of DC was 8.20 (10^{-3}/kPa). Analyses were performed in men and women separately. The logistic regression analyses were repeated with adjustment for mean arterial pressure, heart rate, body mass index, diabetes mellitus, smoking habits, total cholesterol and high- density lipoprotein. Additional adjustment for atherosclerosis was made using measures of intima media thickness. Subsequently, we performed the analysis of variance after adjustment for age to compare mean values of measures of stiffness within categories of alcohol consumption. Furthermore, analyses were conducted for each specific type of alcoholic beverage, separately, with additional adjustment for the other types of beverage.

Results

Baseline characteristics of the population are shown in table 1. After exclusion of past drinkers, data on both alcohol consumption and PWV were available for 3178 subjects, of these 57% was woman. Mean age among men was 71.5±6.7 years, 72.1±6.8 years among women. In men, 4.2% of the subjects were non-drinkers, 41% consumed up to one glass per day, 21.1% 1 to 2 glasses per day and 33.7% more than 2 glasses per day. In women, 14.9% of the subjects were non-drinkers, 59.3% consumed up to one glass per day, 13.4% 1 to 2 glasses per day and 12.4% more than 2 glasses per day. Data on alcohol consumption and DC were available for 2973 subjects. Mean values and corresponding 95% confidence intervals of pulse wave velocity and distensibility coefficient according to categories of alcohol consumption are shown in figures 1-4. Table 2 presents odds ratios for high arterial stiffness for categories of alcohol consumption with adjustment in men and women. Arterial stiffness was significantly lower in moderate drinkers compared to non-drinkers. In men, age adjusted odds ratios for high PWV were 0.64 (95% CI, 0.36-1.15) for consumption of up to 1 drink per day, 0.71 (95% CI, 0.39-1.31) for 1-2 drinks per day and 0.92 (95% CI, 0.51-1.66) for more than 2 drinks per

Table 1. Characteristics of the study population (n=3178)

	Men (1367)	Women (1811)
Age (years)	71.5±6.4	72.1±6.8
Body mass index (kg/m^2)	26.3±3.1	27.1±4.2
Systolic blood pressure (mmHg)	135.6±19.1	133.2±19.6
Diastolic blood pressure (mmHg)	73.9±9.5	68.1±9.2
Mean arterial pressure (mmHg)	107.1±12.4	106.4±13.1
Heart rate (bpm)	73.2±14.7	76.6±14.2
Total cholesterol (mmol/l)	5.6±0.9	6.04±0.9
HDL- cholesterol (mmol/l)	1.3±0.3	1.5±0.4
Current smokers (%)	17.3	14.7
Diabetes mellitus (%)	7.3	4.4
Intima media thickness (mm)	0.91±0.15	0.87±0.14
Non drinkers (%)	4.1	14.7
Beer consumption (%)	57.1	2.4
Wine consumption (%)	72.2	81.7
Liquor consumption (%)	76.6	16.4
Pulse wave velocity (m/s)	13.9±3.1	13.1±2.8
Distensibility coefficient (10^{-3}/kPa)*	10.4±4.1	10.3±4.1

Values are expressed as percentage or mean ± standard deviation. HDL cholesterol: high- density lipoprotein cholesterol. *Data on distensibility coefficient and alcohol consumption are available for 2973 subjects.

day, compared to non-drinkers. Corresponding estimates in women were 0.68 (95% CI, 0.50-0.93), 0.56 (95% CI, 0.37-0.86) and 0.88 (95% CI, 0.57-1.34). Odds ratios for low DC were 0.92 (95% CI, 0.50-1.69), 0.77 (95% CI, 0.40-1.45) and 0.91 (95% CI, 0.49-1.68). Corresponding estimates in women were 0.74 (95% CI, 0.55-0.99), 0.67 (95% CI, 0.45-0.99) and 0.73 (95% CI, 0.49-1.09). After adjustment for cardiovascular risk factors and measures of atherosclerosis associations lacked significance but presented the same trend. Table 3 presents odds ratios of high arterial stiffness according to specific alcoholic beverage.

Discussion

In this large population-based study we found an U-shaped association between alcohol consumption and arterial stiffness; moderate alcohol consumption is associated with reduced arterial stiffness in this population of elderly men and women.

Some aspects of this study need to be discussed. Firstly, the cross-sectional design may limit our ability to infer a causal relationship between measures of arterial stiffness and alcohol consumption. Secondly, information on alcohol intake may have introduced misclassification in exposure; specifically we are afraid

Table 2. Odds ratio of high arterial stiffness according to mean daily alcohol consumption

	Pulse wave velocity (m/s)					
	OR*	95% CI	OR †	95% CI	OR ‡	95% CI
Men						
Non-drinker (60)	1.00	(reference)	1.00	(reference)	1.00	(reference)
Up to 1 drink (559)	0.64	0.36-1.15	0.60	0.31-1.15	0.61	0.32-1.15
1-2 drinks (288)	0.71	0.39-1.31	0.69	0.35-1.37	0.73	0.38-1.42
More than 2 drinks (460)	0.92	0.51-1.66	0.82	0.42-1.58	0.89	0.46-1.69
Women						
Non-drinker (281)	1.00	(reference)	1.00	(reference)	1.00	(reference)
Up to 1 drink (1069)	0.68	0.50-0.93	0.69	0.48-.098	0.68	0.48-0.95
1-2 drinks (242)	0.56	0.37-0.86	0.60	0.37-0.98	0.59	0.37-0.95
More than 2 drinks (222)	0.88	0.57-1.34	0.87	0.53-1.43	0.91	0.57-1.46
	Distensibility coefficient (10^{-3}/kPa)					
Men						
Non-drinker (54)	1.00	(reference)	1.00	(reference)	1.00	(reference)
Up to 1 drink (497)	0.92	0.50-1.69	0.76	0.38-1.50	0.77	0.40-1.48
1-2 drinks (255)	0.77	0.40-1.45	0.65	0.32-1.33	0.64	0.32-1.29
More than 2 drinks (399)	0.91	0.49-1.68	0.88	0.44-1.77	0.89	0.45-1.74
Women						
Non-drinker (249)	1.00	(reference)	1.00	(reference)	1.00	(reference)
Up to 1 drink (932)	0.74	0.55-0.99	0.82	0.59-1.14	0.82	0.59-1.13
1-2 drinks (208)	0.67	0.45-0.99	0.69	0.44-1.08	0.72	0.47-1.11
More than 2 drinks (199)	0.73	0.49-1.09	0.80	0.51-1.27	0.79	0.50-1.23

* Model adjusted for age.
† Model adjusted for age, mean arterial pressure, heart rate, diabetes mellitus, smoking habits, body mass index, total cholesterol and high- density lipoprotein cholesterol.
‡ Model adjusted for age, mean arterial pressure, heart rate, diabetes mellitus, smoking habits, body mass index, total cholesterol and high- density lipoprotein cholesterol and intima media thickness.
OR: Odds ratio. CI: Confidence interval.

of underreporting of the level of alcohol consumption among heavy drinkers [20] affecting our results. Thirdly, non-drinkers may not be the most appropriate category; abstainers could have an adverse risk profile, while past-drinkers may have stopped drinking due to health problems, eventually cardiovascular disease. Thus the use of non-drinkers as reference category has been suggested to amplify the benefits of light-to-moderate alcohol consumption [21]. Therefore, we excluded the past drinkers from the analyses. Finally, measures on arterial stiffness and data on alcohol consumption were not available for all participants; because this was

Table 3. Odds ratio of high arterial stiffness according to specific alcoholic beverage consumption

	High PWV		Low DC	
	OR	95% CI	OR	95% CI
Daily beer consumption				
Non-drinker (n=2661)	1.00	(reference)	1.00	(reference)
Up to 1 drink (n=364)	0.90	0.65-1.23	0.93	0.67-1.29
1-2 drinks (n=86)	1.29	0.73-2.27	1.20	0.67-2.12
More than 2 drinks (n=67)	1.09	0.57-2.07	0.60	0.32-1.36
Daily wine consumption				
Non-drinker (n=1970)	1.00	(reference)	1.00	(reference)
Up to 1 drink (n=846)	0.83	0.67-1.03	0.98	0.78-1.22
1-2 drinks (n=210)	0.74	0.55-0.99	0.87	0.63-1.20
More than 2 drinks (n=152)	0.95	0.65-1.38	1.05	0.73-1.53
Daily liquor consumption				
Non-drinker (n=716)	1.00	(reference)	1.00	(reference)
Up to 1 drink (n=1843)	0.86	0.68-.109	0.88	0.70-1.11
1-2 drinks (n=371)	1.04	0.72-1.51	1.11	0.76-1.64
More than 2 drinks (n=248)	1.33	0.86-2.05	0.70	0.44-1.12

Model adjusted for age, gender, mean arterial pressure, heart rate, diabetes mellitus, smoking habits, body mass index, total cholesterol and high density lipoprotein cholesterol, intima media thickness and other type of beverage. OR: Odds ratio. CI: Confidence interval. PWV: pulse wave velocity, DC: distensibility coefficient.

primarily due to logistic reasons and therefore random, we believe that this will not have biased the results.

Previous results on the relation between alcohol and arterial stiffness are inconsistent. Longitudinal studies in Japanese men aged 35-59 years found that alcohol consumption was a risk factor for increased aortic stiffness [8, 9]. Conversely, other studies showed that alcohol consumption was associated with decreased pulse wave velocity in the general population [10] and in patients with diabetes mellitus type 2 [22]. Recent studies found a J-shaped association between alcohol consumption and arterial stiffness in men aged 40-80 years [11] and an inverse association in healthy postmenopausal women [12]. In the present study, we found an U-association between alcohol consumption and arterial stiffness in women; in men we observed the same trend, however, the estimates lacked statistical significance.

Several cardiovascular risk factors may mediate the association between alcohol consumption and arterial stiffness. Moderate alcohol consumption decreases the risk of diabetes mellitus type 2 [23], whereas the association with high blood pressure levels may be J-shaped [24]. Both diabetes mellitus and hypertension are determinants of arterial stiffness [5-7]. Therefore, moderate alcohol consumption might reduce arterial stiffness by interference with the factors responsible for the increase in arterial stiffness in such cardiovascular risk factors as diabetes and

Figure 1. Mean values and corresponding 95% confidence interval of pulse wave velocity according to glasses of alcohol daily consumed (men).

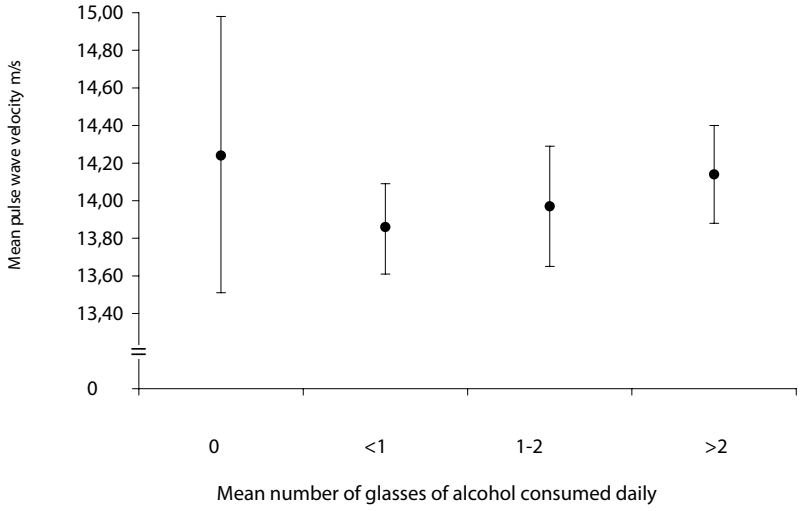


Figure 2. Mean values and corresponding 95% confidence interval of pulse wave velocity according to glasses of alcohol daily consumed (women). * P= 0.05 different from the reference group

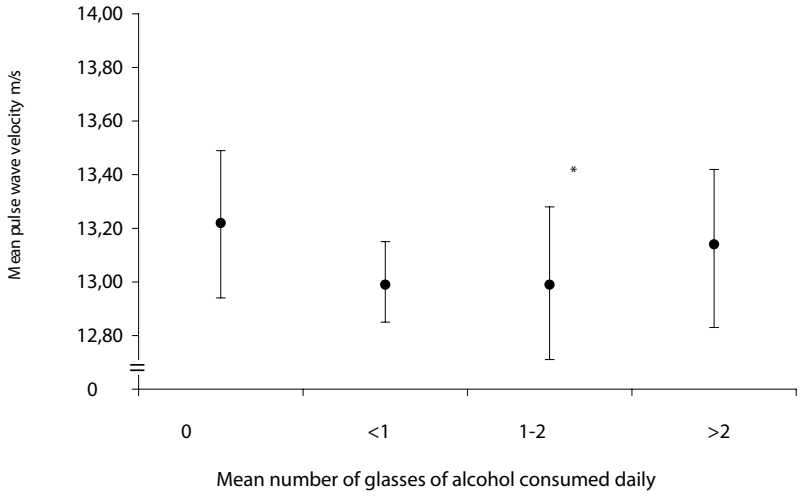


Figure 3. Mean values and corresponding 95% confidence interval of distensibility coefficient according to glasses of alcohol daily consumed (men).

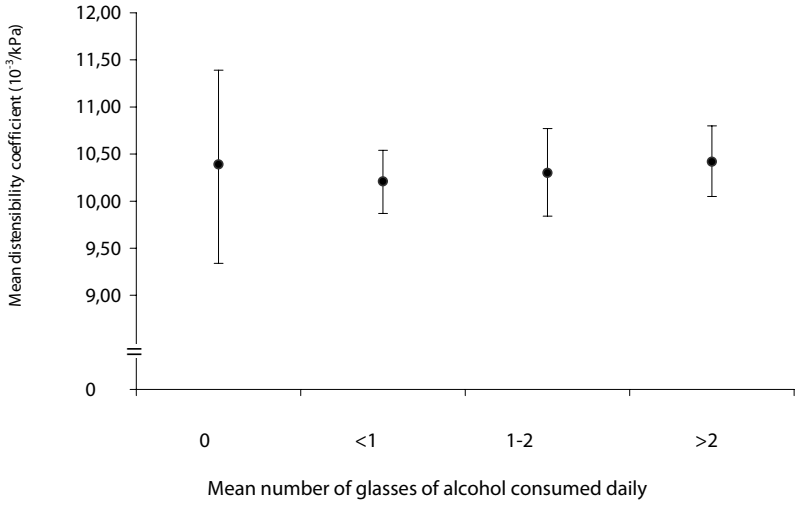
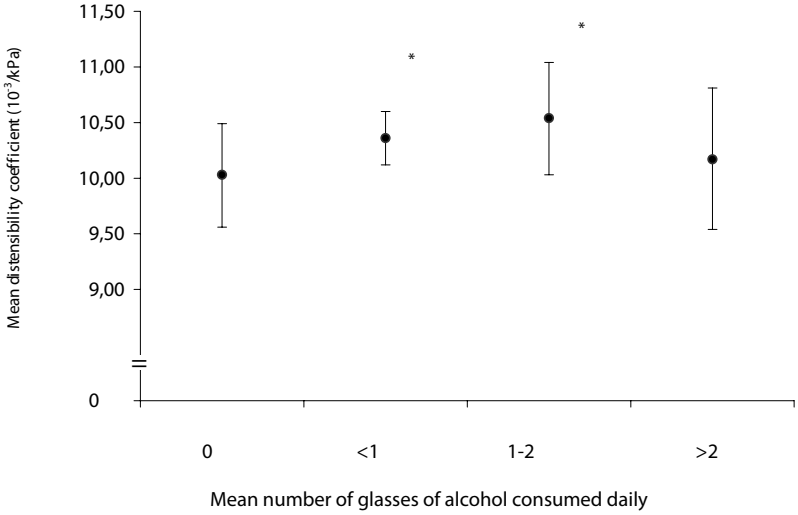


Figure 4. Mean values and corresponding 95% confidence interval of distensibility coefficient according to glasses of alcohol daily consumed (women). * P< 0.05 different from the reference group



hypertension. However, this seems to be unlikely because in fully adjusted models the estimates remained statistically significant. Therefore, also the alcohol-induced increase in HDL cholesterol [25] cannot completely explain the results obtained.

Although it is known that atherosclerosis may increase arterial stiffness [26] and has an inverse association with moderate alcohol consumption [27], previous studies [8-12] did not evaluate whether the association between alcohol consumption and arterial stiffness was mediated by atherosclerosis. For this reason, we performed analyses with additional adjustment for intima-media thickness. Also in these models, estimates remained unchanged suggesting that the association is independent of atherosclerosis.

Alcohol exposure increases the production of vasoactive substances, like nitric oxide, thereby inducing endothelium-dependent vasodilatation [28, 29]. Exposure of blood vessels to alcohol can promote nitric oxide generation and subsequent vasodilatation [30, 31]. Moreover, nitric oxide can convey vasoprotection by inhibiting platelet aggregation and platelet adhesion to the vascular wall [32, 33]. In this way, the vascular wall is not only protected against thrombosis, but also against the release of platelet-derived growth factors that stimulate smooth muscle proliferation and its production of matrix molecules. Whether such mechanisms are involved needs further investigation.

It is still unclear whether specific alcohol beverages are equivalent in the ability to affect artery wall stiffness. In analyses stratified for type of beverage we found an U-shaped association of wine consumption with both PWV and DC, although the association was only significant for PWV. In the effect on artery wall stiffness, non-alcoholic components of wine have to be considered as well. For example, in vitro, flavonoids have shown to influence vessel wall tension [34], while quercetin, a powerful antioxidant, relaxes the aortic rings and tannins have strong vasodilating properties.

In conclusion, in this large population-based study of older adults we found an U-shaped association between alcohol consumption and arterial stiffness in women; in men, the same trend was observed but the estimates lacked statistical significance. The association is independent of cardiovascular risk factors and atherosclerosis and seems to be stronger for wine consumption.

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3

Arterial stiffness and blood pressure levels

3.1

Arterial stiffness as underlying mechanism for orthostatic hypotension

Abstract

Background Aging is associated with orthostatic hypotension; it is not well known whether arterial stiffening, one of the characteristics of the aging vascular system, is involved in the pathogenesis of orthostatic hypotension.
Methods We investigated the relation between arterial stiffness and orthostatic hypotension within the framework of the Rotterdam Study, a population-based study in individuals aged 55 and older. The present study included 3362 subjects participating in the third examination phase. The carotid-femoral pulse wave velocity was used as measure of arterial stiffness. Orthostatic hypotension was assessed with blood pressure measurements in supine and standing position.
Results Odds ratios for orthostatic hypotension increased through quartiles of pulse wave velocity; age and gender adjusted odds ratio for orthostatic hypotension in the last quartile of pulse wave velocity was 1.76 (95% CI, 1.35-2.29) when compared with the first quartile (reference). In fully adjusted models estimates remained statistically significant. In subjects with higher stiffness we observed a higher drop in blood pressure but no significant change of the heart rate.
Conclusions Arterial stiffness is associated with orthostatic hypotension. The drop in blood pressure levels is associated with increasing levels of stiffness and the small response of heart rate to orthostatic challenge may support the hypothesis of a reduced baroreflex due to stiff arteries.

Introduction

Age and abnormalities in blood pressure homeostasis may predispose to orthostatic hypotension, a very common condition among older persons [1-3] which is associated with syncope [4], falls [5] and stroke [6], leading to hospitalisation and functional impairment.

Orthostatic challenge is responsible for redistribution of the blood volume in the lower parts of the body and for decreases in blood pressure and cardiac preload. In response, carotid and aortic arterial wall receptors activate the baroreflex, leading to vasoconstriction, increasing peripheral arterial resistance, heart rate and myocardial contractility. Determinants of orthostatic hypotension are not yet completely known, and it is not clear whether arterial stiffening, one of the characteristics of the aging vascular system, is involved in the pathophysiology of orthostatic hypotension. Reduced arterial compliance is associated with a reduction of the cardiovagal baroreflex sensitivity, a key mechanism for short-term arterial blood pressure regulation [7]. A recent relatively small study [8] found that arterial stiffness of the upper limbs was significantly higher in patients with orthostatic hypotension and a history of falls; however, the relation between reduced functional properties of the vessel wall and a decreased hemodynamic response to orthostatic challenge has never been investigated in healthy elderly. We postulated that age-related vascular changes might be implicated in determining impaired hemodynamic response to orthostatic challenge in a population of elderly. To study whether arterial stiffness was associated with orthostatic hypotension, we investigated the relation between pulse wave velocity and orthostatic hypotension in a large population-based study.

Methods

Population

This study was conducted within the framework of the Rotterdam Study, an ongoing prospective population-based cohort study among subjects aged 55 years and over, living in Ommoord, a suburb of Rotterdam, The Netherlands. The rationale and design of the Rotterdam Study have been described elsewhere [9]. The third examination phase took place from 1997 until 1999. During this phase, information on cardiovascular risk factors was collected; measurements of blood pressure, arterial stiffness and atherosclerosis were obtained. The Medical Ethics Committee of the Erasmus Medical Center approved the study and written consent was obtained from all participants.

Arterial Stiffness

Arterial stiffness was measured by the carotid-femoral pulse wave velocity (PWV). Subjects were instructed to refrain from smoking and from taking coffee, tea or pain medications on the day of measurements, and from taking alcohol on the day of measurements and the day before. PWV was measured with the subjects in supine position. Carotid-femoral PWV was assessed with an automatic device (Complior, Colson) [10] that assessed the time delay between the rapid upstroke of the feet of simultaneously recorded pulse waves in the carotid and the femoral artery. The distance between the recording sites in the carotid and the femoral artery was measured with a tape. PWV was calculated as the ratio between the distance measured and the foot-to-foot time delay and was expressed in meters per second. The average of at least 10 successive measurements, to cover a complete respiratory cycle, was used in the analysis.

Blood pressure and orthostatic hypotension

Blood pressure was measured with the patient in supine position after 5 minutes rest. Blood pressure was measured twice with a sphygmomanometer, and the mean was taken as the subject's reading. Mean arterial pressure was calculated by the following formula: diastolic blood pressure + 1/3 (systolic blood pressure-diastolic blood pressure). Measurements were repeated in standing position after 1, 3 and 5 minutes. According to the consensus Committee of the American Autonomic Society and the American Academy of Neurology[11], orthostatic hypotension was defined as a decline in systolic blood pressure ≥20 mm Hg and/or a decline in diastolic blood pressure of ≥10 mm Hg at any of the three standing blood pressure measurements.

Cardiovascular risk factors

Body mass index [weight (kg)/height $(m)^2$] was calculated. Diabetes mellitus was defined as use of anti-diabetic medication and/or a fasting serum glucose level ≥7.0 mmol/l [12]. Serum total cholesterol and high-density lipoprotein (HDL) cholesterol values were determined by an automated enzymatic procedure (Boehringer Mannheim System). Information on smoking habits and use of antihypertensive medication was obtained during the interview.

Measure of carotid intima media thickness

Ultrasonography of both carotid arteries was performed with a 7.5-Mhz linear-array transducer and a duplex scanner (ATL UltraMark IV). Common carotid intima-media thickness (IMT) was determined as previously described [13].

Population for analysis

Of the 4024 subjects who underwent the physical examination of the third phase, arterial stiffness measured as PWV was measured in 3550 subjects. Missing information was almost entirely due to logistic reasons. Data both on orthostatic hypotension and PWV were available for 3362 subjects.

Statistical Analysis

Differences in means or frequencies of characteristics between subjects with and without orthostatic hypotension were compared using a one-way analysis of variance and Chi-square analysis, respectively. Logistic regression analysis was performed to asses whether PWV, included as continuous variable in the model, was associated with orthostatic hypotension. In this model, PWV was included as the determinat and orthostatic hypotension the outcome. Logistic regression analysis was performed to compute odds ratios (OR) and corresponding 95% confidence intervals (95% CI) within quartiles of PWV, where the first quartile was considered as reference. Cut-off points for quartiles of PWV were 11.3 m/s, 13.1 m/s and 15.2 m/s for the first, second and third quartile, respectively. Analyses were adjusted for age, gender, mean arterial pressure, heart rate, body mass index, total cholesterol, high-density lipoprotein cholesterol, diabetes mellitus, smoking status, use of antihypertensive medication and IMT. Furthermore, analysis of covariance adjusted for age, gender and heart rate at baseline were performed, whereby mean values of PWV and change in heart rate with corresponding 95% CI were compared across categories of drop in systolic and diastolic blood pressure.

Results

Baseline characteristics of the population are shown in table 1. Subjects with orthostatic hypotension were older, had higher systolic and diastolic blood pressure levels, presented a higher prevalence of diabetes mellitus and had a higher intima media thickness. In subjects with and without orthostatic hypotension, PWV was 14.2±3.3 m/s and 13.3±2.9 m/s, respectively. PWV was significantly associated with orthostatic hypotension [OR 1.04 (95% CI, 1.01-1.08)] in fully adjusted models. Odds ratios for orthostatic hypotension increased through quartiles of PWV (table 2); age and gender adjusted odds ratio for orthostatic hypotension in the last quartile of PWV was 1.76 (95% CI, 1.35-2.29) when compared with the first quartile (reference). In the fully adjusted model, the odds ratio in the last quartile was 1.38 (95%CI, 1.02-1.87) when compared with the reference category. There was no drop in systolic blood pressure in 1644 subjects; in 904 subjects the drop was between 1-10 mmHg, in 474 subjects the drop was between 11-19 mmHg and in 340 subjects the drop was ≥20 mmHg (figure 1). Corresponding mean

Table 1. Characteristics of the study population

Characteristics	Subjects without OH (n=2638)	Subjects with OH (n=724)	p-value
Age (years)	71.6±6.5	73.4±7.1	0.0001
Men (%)	42.6	41.2	ns
Body mass index (kg/m^2)	26.8±3.8	26.5±3.8	ns
Systolic blood pressure (mmHg)	149.9±23.1	165.3±26.5	0.0001
Diastolic blood pressure (mmHg)	78.9±11.2	84.2±12.8	0.0001
Mean arterial pressure (mmHg)	105.6±12.4	108±13.4	0.0001
Heart rate (bpm)	69.7±12.2	69.9±12.8	ns
Total cholesterol (mmol/l)	5.8±0.9	5.8±0.9	ns
HDL cholesterol (mmol/l)	1.4±0.4	1.4±0.3	ns
Current smokers (%)	15.1	17.7	ns
Diabetes mellitus (%)	5.6	8.1	0.013
Use of antihypertensive medication (%)	19.6	23.9	0.013
Intima media thickness (mm)	0.86±0.14	0.89±0.15	0.0001
Pulse wave velocity (m/s)	13.3±2.9	14.2±3.3	0.0001

Values are expressed as percentage or mean ± standard deviation.
OH: orthostatic hypotension. HDL cholesterol: high-density lipoprotein cholesterol.

values of PWV and 95% CI in these groups of subjects were 13.30 (13.13-13.46) m/s, 13.54 (13.36-13.72) m/s, 13.67 (13.41-13.94) m/s and 14.03 (13.66-14.40) m/s, respectively (test for trend p=0.017). There was no drop in diastolic blood pressure in 1065 subjects; in 1594 subjects the drop was between 1-10 mmHg, in 537 subjects the drop was between 11-19 mmHg and in 166 subjects the drop was ≥20 mmHg (figure 2). Corresponding mean values of PWV and 95% CI in these groups of subjects were 13.29 (13.09-13.50) m/s, 13.42 (13.29-13.56) m/s, 13.82 (13.57-14.08) m/s and 14.29 (13.81-14.77) m/s, respectively (test for trend p=0.01). Finally, there was no significant increase in heart rate consequent to orthostatic challenge in subjects with a high drop in blood pressure compared with subjects without drop in blood pressure (figure 3 and 4).

Discussion

In this large population-based study arterial stiffness was associated with orthostatic hypotension in the elderly, the decline in both systolic and diastolic blood pressure levels consequent to the standing position was associated with increasing levels of stiffness.

Several issues need to be discussed before interpreting these results. Firstly, the cross-sectional design may limit our ability to infer a causal relationship between measures of arterial stiffness and orthostatic hypotension. Secondly, measures of

Table 2. Odds ratios of orthostatic hypotension within quartiles of PWV

	OR*	95% CI	OR†	95% CI
Quartiles of PWV				
1st quartile PWV (n=843)	1.00	(reference)	1.00	(reference)
2nd quartile of PWV (n=835)	1.29	1.00-1.67	1.21	0.92-1.59
3rd quartile of PWV (n=844)	1.52	1.17-1.96	1.35	1.03-1.79
4th quartile of PWV (n=840)	1.76	1.35-2.29	1.38	1.02-1.87

* Model adjusted for age and gender.
† Model adjusted for age, gender, mean arterial pressure, heart rate, body mass index, total cholesterol, HDL cholesterol, diabetes mellitus, smoking, use of antihypertensive medication and intima media thickness.
OR: Odds ratio. CI: Confidence interval. PWV: pulse wave velocity.

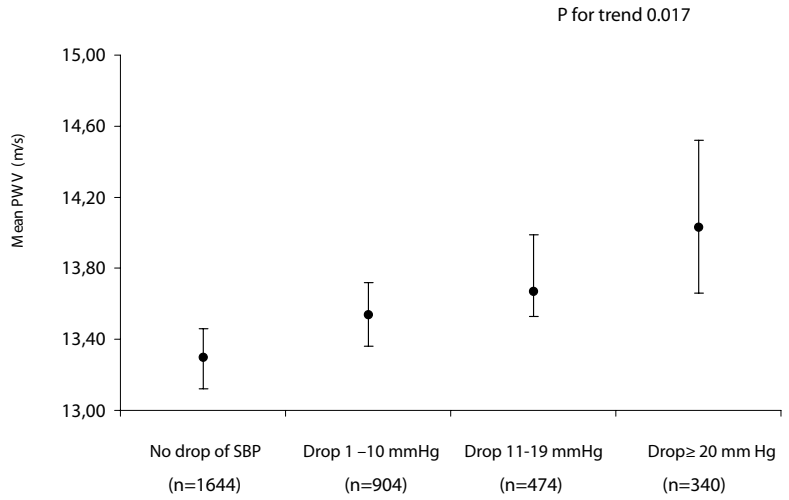
PWV were not available for all participants; however, since this was primarily due to logistic reasons and therefore random, we believe that this will not have biased the results. Thirdly, information on blood pressure levels obtained on a single occasion was used; the use of multiple blood pressure measurements or the use of ambulatory monitoring might have improved accuracy and precision.

A recent study [8] performed in a relatively small group of elderly patients, found that arterial stiffness of the upper limb was significantly higher in patients with orthostatic hypotension and a history of falls; similarly, aortic pulse wave velocity tended to be higher in patients with orthostatic hypotension, but this difference was not statistically significant. In the present population-based study high arterial stiffness was associated with orthostatic hypotension. The drop in blood pressure levels consequent to orthostatic challenge was positively correlated with increasing stiffness, whereas heart rate response was small. These results support the hypothesis that arterial stiffness plays a role in the occurrence of orthostatic hypotension.

It has been shown that age-related decrease in arterial compliance is related with a decrease in cardiovagal baroreflex [7]. The activation of baroreceptors is the first step in the baroreflex response, modulating sympathetic and parasympathetic activity. The arterial tree plays an important role in this mechanism since the detection of blood pressure changes involves receptors located in the arterial wall. Vessel wall stiffness in barosensitive regions of the vascular bed may play an important role in reducing baroflex sensitivity by mechanically restricting the stretch and relaxation of baroceptors during physiologic changes of arterial pressure. Since these receptors are located inside the arterial wall and are triggered by stretch, arterial stiffness may impair this activation and induce a decline in baroreceptor acitivity [14].

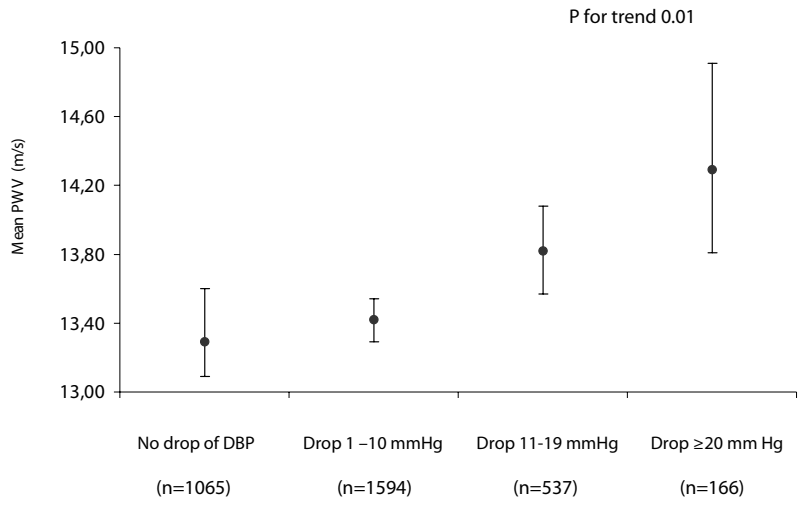
The absence of increased heart rate contemporary with a decline in blood pressure level is compatible with a dysfunction of the baroreflex. Moreover, since orthostatic challenge generates sympathetic-dependent vasoconstriction, arterial

Figure 1. Mean PWV and postural changes of systolic blood pressure.



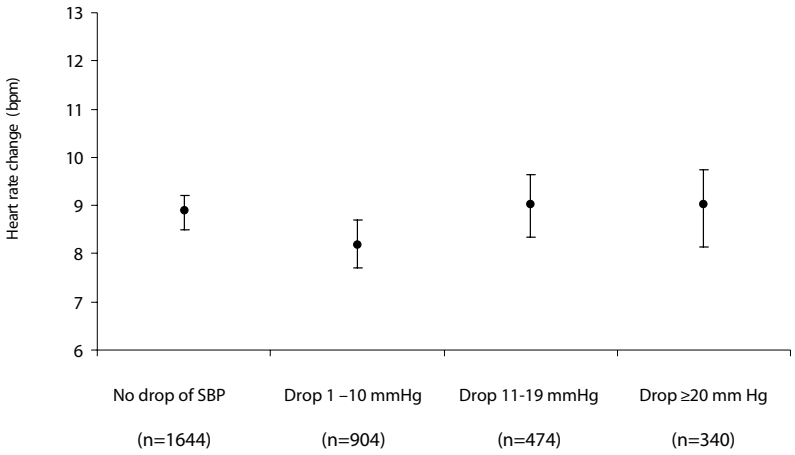
Points represent mean levels of pulse wave velocity; lines represent 95% confidence intervals. Means are adjusted for age, gender and systolic blood pressure levels at baseline. SBP: systolic blood pressure. PWV: pulse wave velocity.

Figure 2. Mean PWV and postural changes of diastolic blood pressure.



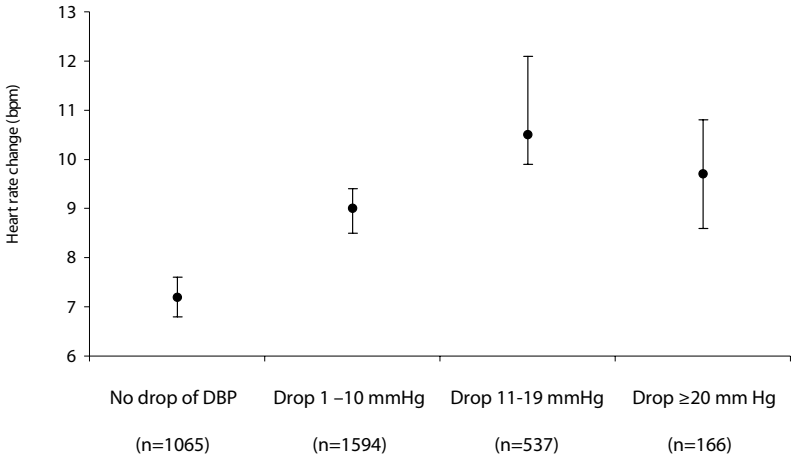
Points represent mean levels of pulse wave velocity; lines represent 95% confidence intervals. Means are adjusted for age, gender and diastolic blood pressure levels at baseline. DBP: diastolic blood pressure. PWV: pulse wave velocity.

Figure 3. Mean heart rate change and postural changes of systolic blood pressure.



Points represent mean change of heart rate; lines represent 95% confidence intervals. Means are adjusted for age, gender and heart rate at baseline. SBP: systolic blood pressure.

Figure 4. Mean heart rate change and postural changes of diastolic blood pressure.



Points represent mean change of heart rate; lines represent 95% confidence intervals. Means are adjusted for age, gender and heart rate at baseline. DBP: diastolic blood pressure.

stiffness might reduce the vasoconstricting potential of the arterial wall.

Orthostatic hypotension has been found to be associated with diabetes mellitus [15], atherosclerotic lesions and the use of antihypertensive drugs [16], which are also conditions associated with arterial stiffness. Therefore, one possible question is

whether the association between stiffness and orthostatic hypotension is totally mediated by these comorbidities. However, the association between arterial stiffness and orthostatic hypotension remained significant also after additional adjustments for cardiovascular risk factors and carotid intima media thickness which is a measure of atherosclerosis, confirming an independent etiologic role of arterial stiffness in determining orthostatic hypotension.

In conclusion, in this large population-based study arterial stiffness was associated with orthostatic hypotension in the elderly. We were able to detect an association between arterial stiffness and postural blood pressure changes; the drop in blood pressure levels was linearly associated with increasing levels of stiffness and the small response of heart rate to orthostatic challenge may support the hypothesis of a baroreflex dysfunction due to stiff arteries. However, considering the cross-sectional design of the present study, mechanisms remain speculative and require further studies. If this hypothesis is valid, arterial stiffness could be a potentially modifiable factor in determining baroreflex dysfunction and might be considered a therapeutic target.

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3.2

Pulse pressure and cardiovascular events

Abstract

Objectives: To compare the strength of the relative risks of systolic, diastolic and pulse pressure as predictors of myocardial infarction, stroke and all-cause mortality in older adults.
Design: Prospective cohort study.
Setting: The Rotterdam Study, a Dutch population-based study.
Participants: Total of 4,234 subjects aged 55 years and older with no previous myocardial infarction or stroke at baseline.
Measurements: Blood pressure levels at baseline. First myocardial infarction and stroke, all-cause mortality during follow-up.
Results: During follow-up, 205 subjects had a myocardial infarction (average follow-up period 7 years), 137 subjects had a stroke (average follow-up period 6.1 years) and 748 subjects died. One standard deviation difference in systolic, diastolic and pulse pressure was associated with relative risks of myocardial infarction of 1.24 (95% confidence interval 1.06-1.46), 1.07 (0.92-1.25) and 1.25 (1.07-1.48), respectively. Corresponding relative risks for stroke were 1.59 (1.37-1.86), 1.27 (1.10-1.48) and 1.48 (1.27-1.72). For all-cause mortality the corresponding relative risks and 95% CI were 1.21 (1.11-1.31), 1.06 (0.99-1.14) and 1.20 (1.10-1.31).
Conclusion: The results of this study suggest that in a population of apparently healthy older adults pulse pressure is not a better predictor of cardiovascular events and all-cause mortality than systolic blood pressure.

Introduction

The role of pulse pressure in predicting cardiovascular morbidity and mortality in patients with impaired left ventricular function [1] and with hypertension [2-6] has been shown. There is increasing evidence that pulse pressure in older age is an independent predictor of risk of cardiovascular disease in the general population [7-11]. The Framingham Heart Study has shown that at any level of systolic blood pressure, lower levels of diastolic blood pressure were associated with an increased risk of coronary artery disease, suggesting that pulse pressure is an important component of risk [9]. Two recent studies conducted in older adults have reported discordant data regarding the predictive role of pulse pressure. Franklin et al. [12] using follow-up data from the Framingham Heart Study found that with increasing age there was a gradual shift from diastolic blood pressure to systolic blood pressure and then to pulse pressure as predictors of coronary heart disease. From 60 years of age and on, pulse pressure became superior to systolic blood pressure. On the other hand, other studies [13] [14, 15]showed that although all measures of blood pressure were related to the risk of cardiovascular events in older adults, systolic blood pressure remained the best single predictor. The aim of the present study was to assess the role of various blood pressure components as predictors of myocardial infarction, stroke and all-cause mortality in older adults.

Methods

Subjects

This study was conducted within the framework of the Rotterdam Study, an ongoing prospective population-based cohort study among subjects aged 55 years or over, living in Ommoord, a suburb of Rotterdam, The Netherlands. The rationale and design of the Rotterdam Study have been described elsewhere [16]. In total, 7983 (response rate 78%) subjects agreed to participate and were interviewed at home. The participants visited the study center for physical examinations. Baseline data were collected from March 1990 to July 1993. The Medical Ethics Committee of Erasmus Medical Center approved the study and written consent was obtained from all participants. We excluded subjects with missing data on blood pressure (n= 975), which was mostly due to living in a nursing home. We also excluded subjects who used antihypertensive medications at baseline (n=2228) and subjects with previous myocardial infarction (n= 871) or a previous stroke (n= 255). The numbers missing are overlapping and the total number of persons available for the present analyses was 4234. In this study the endpoints were a first myocardial infarction, a first stroke and all-cause mortality during follow-up.

Risk Factor Measurements

Medical records of patients with a self-reported previous myocardial infarction or stroke were checked to verify the diagnosis. Myocardial infarction was defined as a self-reported myocardial infarction verified by hospital discharge data, written information from the subject's general practitioner or electrocardiogram measurements. A neurologist reviewed all suspected stroke cases reported. Data on indication for use of blood pressure-lowering medication were collected during the interview by a physician at the research center. Systolic (SBP), first Korotkoff phase and diastolic (DBP), fifth Korotkoff phase, blood pressure were measured in duplicate on the right arm using a random-zero sphygmomanometer, after the participant had been seated for at least five minutes. The mean of the two blood pressure values was used in the analyses. Pulse pressure (PP) was calculated (SBP-DBP). Serum total cholesterol and high-density lipoproteins (HDL)-cholesterol values were determined by an automated enzymatic procedure in a non-fasting blood sample. Diabetes mellitus was defined as a non-fasting or postload blood glucose levels above 11.0 mmol/l and/or use of anti-diabetic medication. Body mass index [weight (kg)/height^2 (m)] was calculated. Data on smoking habits were obtained during the home interview. Smoking was classified as never, former or current smoking.

Incident Cardiovascular Events

Myocardial infarction

After the baseline examination, the general practitioners in the research area reported incident cardiovascular events. An incident myocardial infarction was considered to have occurred when the event led to a hospitalisation and the hospital discharge record indicated a diagnosis of a new myocardial infarction on the basis of signs and symptoms, electrocardiographic recordings and repeated laboratory investigations during hospital stay. Research assistants verified all information by checking medical records at the general practitioners' offices since in all cases discharge reports from medical specialists were obtained. Subsequently, two research physicians independently coded all reported events; codes were assigned according to the International Classification of Diseases, 10th edition. Codes on which the research physicians disagreed were discussed in order to reach consensus. Finally, an expert in the field of cardiology reviewed all events and verified whether the research physicians had correctly applied the coding rules. In case of disagreement between the medical expert and the research physicians, the expert's judgement was considered final. Follow-up was complete in 97% of the participants until 1 January 2000.

Stroke

For incident stroke, when an event was reported, additional information including the date of the possible stroke was obtained by interviewing the general practitioner and scrutinising information from hospital discharge records and/or neuroimaging in the case of admission or referral. Medical information was checked and evaluated by an experienced neurologist. An incident stroke was considered to have occurred when the event led to a hospitalisation and the hospital discharge record indicated the diagnosis of a new stroke; the clinical diagnosis was based on signs, symptoms and neuroimaging investigations during hospital stay, or in case of no hospitalisation, sign and symptoms associated with the event obtained from the general practitioner were highly suggestive of a stroke according to the neurologist. Events were coded according to the International Classification of Diseases, 10th edition. Follow-up was complete in 98.1% of the participants until 1 January 1998.

All-cause mortality

Information on vital status was acquired at regular intervals from the municipal authorities of Rotterdam. In addition, general practitioners in the study district of Ommoord provided computerised reports on the deaths of the participants on a regular basis. General practitioners outside the study region were contacted yearly to obtain information on vital status. Follow-up was complete in 98.8% of the participants until 1 January 2000.

Data Analysis

Primary analyses assessed the relation of single and dual blood pressure component, as continuous variables, with myocardial infarction, stroke and total mortality. Cox's proportional hazard regression analysis was carried out to estimate relative risks (RR) with corresponding 95% confidence intervals (CI). To compare the association of different blood pressure components with a particular end point, relative risks and correspondent 95% confidence intervals were estimated for a 1-SD difference in each component which was 22.4 mmHg for SBP, 11.6 mmHg for DBP and 18.1 for PP. This is a standardized comparison of RR and is necessary since each blood pressure components is measured on a different scale [13-15]. Moreover the relative risks of systolic blood pressure and pulse pressure were compared with a T test to assess whether the estimates were significantly different. Secondary analyses consisted of including two blood pressure components to asses the relation simultaneously with adjustment for each other. Adjustments were made for age, gender, and additionally for body mass index, total cholesterol, HDL cholesterol, diabetes mellitus and smoking. To study whether the association of blood pressure with incident events varied by age and gender stratified analyses

were done. Assumptions for proportional hazards models were checked by log-log plot models.

Results

Table 1 shows the baseline characteristics of the study population. During follow-up, 205 subjects had a myocardial infarction (average follow-up period 7 years), 137 subjects had a stroke (average follow-up period 6.1 years) and 748 subjects died (average follow-up period 7 years). One standard deviation difference in SBP, DBP and PP was associated with relative risks of myocardial infarction of 1.24 (95% CI 1.06-1.46), 1.07 (95% CI 0.92-1.25) and 1.25 (95% CI 1.07-1.48), respectively (Table 2). Corresponding relative risks for stroke were 1.59 (95% CI 1.37-1.86), 1.27 (95% CI 1.10-1.48) and 1.48 (95% CI 1.27-1.72). For all-cause mortality the corresponding relative risks and 95% CI were 1.21 (1.11-1.31), 1.06 (0.99-1.14) and 1.20 (1.10-1.31), respectively. Estimates of systolic blood pressure and pulse pressure were not statistically different when tested. In models including both SBP and PP, the predictive value of SBP did not change substantially, whereas the predictive value of PP became weaker for all the endpoints studied. The strength of the association of SBP and PP with myocardial infarction, and of SBP and DBP with stroke became higher in older subjects (Table 3), whereas the association of all the three blood pressure components with all-cause mortality became somewhat weaker with age. The relative risks of myocardial infarction and all-cause mortality were comparable in men and women (data not shown). For stroke, the predictive role of DBP was lower, and the one of PP higher in women than in men (women RR 1.74, 95% CI 1.34-2.25), (men, RR 1.39, 95% CI 1.06-1.82).

Table 1. Baseline Characteristics of the Study Population (n = 4234)

Characteristics	mean ± SD or %
Age (years)	67.9±8.9
Men (%)	40.3
Systolic blood pressure (mm Hg)	136.8±21.6
Diastolic blood pressure (mm Hg)	73.2±11.3
Pulse pressure (mm Hg)	63.6±17.3
Body mass index (kg/m^2)	25.7±3.4
Total cholesterol (mmol/L)	6.6±1.2
HDL cholesterol (mmol/L)	1.4±0.3
Current smokers (%)	25.2
Diabetes mellitus (%)	7.8

Data are presented as mean (SD) or percentages (%)

Table 2. Relative Risk of Myocardial Infarction, Stroke and Death Associated with a 1-Standard Deviation Increment of Blood Pressure Components

	Myocardial infarction		Stroke		Total mortality	
	RR (95% CI)*	RR (95% CI)†	RR (95% CI)*	RR (95% CI)†	RR (95% CI)*	RR (95% CI)†
Single term model						
Systolic blood pressure	1.30 (1.13-1.49)	1.24 (1.06-1.46)	1.61 (1.36-1.90)	1.59 (1.37-1.86)	1.17 (1.09-1.26)	1.21 (1.11-1.31)
Diastolic blood pressure	1.15 (0.97-1.29)	1.07 (0.92-1.25)	1.33 (1.12-1.57)	1.27 (1.10-1.48)	1.10 (1.02-1.19)	1.06 (0.99-1.14)
Pulse pressure	1.27 (1.10-1.47)	1.25 (1.07-1.48)	1.49 (1.26-1.76)	1.48 (1.27-1.72)	1.16 (1.08-1.24)	1.20 (1.10-1.31)
Dual term model						
Systolic blood pressure	1.36 (1.12-1.64)	1.34 (1.10-1.65)	1.66 (1.36-2.03)	1.64 (1.31-2.05)	1.20 (1.09-1.31)	1.24 (1.11-1.38)
Diastolic blood pressure	0.99 (0.82-1.19)	0.94 (0.77-1.14)	0.96 (0.79-1.17)	1.00 (0.80-1.25)	0.96 (0.88-1.04)	0.96 (0.87-1.07)
Systolic blood pressure	1.34 (1.02-1.76)	1.20 (0.89-1.61)	1.55 (1.15-2.10)	1.64 (1.17-2.29)	1.12 (0.98-1.28)	1.16 (0.99-1.36)
Pulse pressure	1.01 (0.76-1.34)	1.09 (0.80-1.48)	1.05 (0.78-1.43)	0.99 (0.70-1.41)	1.05 (0.92-1.20)	1.05 (0.89-1.23)
Diastolic blood pressure	1.16 (1.01-1.34)	1.10 (0.94-1.28)	1.25 (1.07-1.46)	1.29 (1.08-1.53)	1.06 (0.99-1.13)	1.08 (1.00-1.17)
Pulse pressure	1.28 (1.10-1.49)	1.27 (1.08-1.50)	1.51 (1.28-1.57)	1.49 (1.24-1.79)	1.15 (1.07-1.24)	1.19 (1.09-1.29)

Results based on 4234 subjects, 205 myocardial infarctions, 137 strokes, 748 deaths.
RR: Relative risk. CI: Confidence interval. *Adjusted for age and gender. †Adjusted for age, gender, total cholesterol, HDL cholesterol, diabetes mellitus, smoking habits, body mass index.

Table 3. Relative Risk of Myocardial Infarction, Stroke and Total Mortality Associated with a 1-Standard Deviation Increment of Blood Pressure Components in Different Categories of Age

	Myocardial infarction		Stroke		Total mortality	
	<70 years	≥70 years	<70 years	≥70 years	<70 years	≥70 years
	124 events	81 events	44 events	93 events	210 events	538 events
	RR (95% CI)	RR (95% CI)	RR (95% CI)	RR (95% CI)	RR (95% CI)	RR (95% CI)
Single term model						
Systolic blood pressure	1.23 (1.01-1.51)	1.30 (1.01-1.70)	1.55 (1.15-2.08)	1.66 (1.33-2.07)	1.27 (1.10-1.47)	1.17 (1.06-1.29)
Diastolic blood pressure	1.14 (0.94-1.38)	1.08 (0.84-1.40)	1.25 (0.92-1.69)	1.38 (1.12-1.71)	1.17 (1.01-1.36)	1.06 (0.97-1.17)
Pulse pressure	1.20 (0.97-1.48)	1.32 (1.01-1.73)	1.51 (1.11-2.05)	1.52 (1.21-1.90)	1.24 (1.06-1.46)	1.17 (1.06-1.30)
Dual term model						
Systolic blood pressure	1.23 (0.95-1.60)	1.40(1.00-1.96)	1.63 (1.11-2.39)	1.61 (1.21-2.12)	1.27 (1.04-1.55)	1.21 (1.06-1.38)
Diastolic blood pressure	1.00 (0.78-1.28)	0.89 (0.65-1.22)	0.92 (0.63-1.95)	1.05 (0.80-1.38)	1.00 (0.82-1.22)	0.95 (0.84-1.07)
Systolic blood pressure	1.23 (0.85-1.79)	1.13 (0.69-1.83)	1.41 (0.80-2.48)	1.77 (1.17-2.69)	1.28 (0.96-1.69)	1.10 (0.91-1.32)
Pulse pressure	0.99 (0.67-1.47)	1.19 (0.72-1.95)	1.21 (0.62-2.01)	0.92 (0.60-1.40)	0.99 (0.73-1.35)	1.10 (0.91-1.32)
Diastolic blood pressure	1.11 (0.92-1.35)	1.06 (0.82-1.36)	1.48 (1.08-2.02)	1.46 (1.17-1.84)	1.13 (0.98-1.31)	1.05 (0.95-1.15)
Pulse pressure	1.18 (0.95-1.46)	1.31 (1.00-1.72)	1.19 (0.89-1.60)	1.34 (1.08-1.67)	1.21 (1.03-1.42)	1.17 (1.05-1.29)

Results based on 2740 subjects <70 years and 1584 subjects ≥70 years. RR: Relative risk. CI: Confidence interval.
Models adjusted for age, gender, total cholesterol, HDL cholesterol, diabetes mellitus, smoking habits, body mass index.

Discussion

The predictive value of pulse pressure is similar to that of systolic blood pressure for myocardial infarction and all-cause mortality in older subjects. Diastolic blood pressure did not predict myocardial infarction. All the blood pressure components were predictors of stroke but systolic blood pressure was a slightly better predictor than pulse pressure. Both systolic blood pressure and pulse pressure predicted all-cause mortality. The association of systolic blood pressure and pulse pressure with myocardial infarction became stronger in older subjects while for stroke these associations did not change.

The relative risks of myocardial infarction and all-cause mortality were similar in men and women, whereas for stroke the predictive role of diastolic blood pressure was lower, and the one of pulse pressure higher in women than in men.

The present study has some limitations. Firstly, were used information on blood pressure levels obtained on a single occasion. The use of multiple blood pressure measurements or the use of ambulatory monitoring would improve accuracy and precision. Subjects with a previous myocardial infarction or stroke and subjects who used anti-hypertensive medication at baseline were excluded. However, participants may have started taking anti-hypertensive medication after baseline causing misclassification of blood pressure levels and an underestimation of the associations.

Recent findings from the Framingham Heart Study [12] showed that with increasing age, there was a gradual shift in predictive value from diastolic blood pressure to systolic blood pressure and then to pulse pressure. From 60 years and older pulse pressure became superior to systolic blood pressure and diastolic blood pressure in predicting coronary heart disease. In this study [12], relations with coronary heart disease were estimated for a 10 mmHg increase in blood pressure level. This comparison remains dubious since each blood pressure component has a different scale, a 10 mmHg change in systolic blood pressure is not comparable to a 10 mmHg change in pulse pressure. Other studies[13-15] using a methodology similar to the one we used, concluded that systolic blood pressure predicted coronary and cerebrovascular events and all-cause mortality better than diastolic blood pressure and pulse pressure, however, these conclusions were based on a very small difference in estimates. In the present study the predictive roles of systolic blood pressure and pulse pressure for myocardial infarction were similar in a large population of older adults.

Increased pulse pressure levels were also associated with stroke, although systolic blood pressure was a slightly better predictor. The association between pulse pressure and stroke was already reported by other studies in hypertensive patients and in population based studies [1, 13, 17]. Thus while the effect of pulse pressure is likely to be stronger than or equal to that of systolic blood pressure in relation

to coronary events, this is not the case for stroke. This finding is consistent with large artery stiffness contributing to myocardial infarction risk in older subjects [3, 8, 12]. The underlying mechanism may be the poor coronary perfusion associated with low diastolic blood pressure since the coronary perfusion has a volume flow which is regulated by the diastolic blood pressure [18]. Additionally, a high systolic blood pressure causes a disproportionate increase in end-diastolic stress, which is the principal hemodynamic factor that promotes the development of cardiac hypertrophy, increased ventricular oxygen consumption, and can compromise the capacity for coronary perfusion [19-22]. Alternatively high pulse pressure may be a consequence of atherosclerosis [23, 24] and not causally related to cardiovascular disease.

A recent study [25] showed that the association of pulse pressure and risk of mortality was complex because it depended on age and systolic and diastolic blood pressure levels. Although we did not evaluate the effect of pulse pressure depending on levels of systolic and diastolic blood pressure, the conclusion of this study was not different from the present study. The use of pulse pressure in clinical practice is not recommended.

In conclusion, in this population-based study, the predictive roles of systolic blood pressure and pulse pressure for myocardial infarction and all-cause mortality were similar. Increased pulse pressure levels were also associated with stroke, although systolic blood pressure was a slightly better predictor. These results suggest that in a population of older adults not using antihypertensive medication, pulse pressure is not a better predictor of cardiovascular events than systolic blood pressure.

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4

Consequences of arterial stiffness

4.1

The prognostic value of arterial stiffness

Abstract

Background - Arterial stiffness has been associated with the risk of cardiovascular disease in selected groups of patients. We evaluated whether arterial stiffness is a predictor of coronary heart disease and stroke in a population-based study among apparently healthy subjects.

Methods - The present study included 2835 subjects participating in the third examination phase of the Rotterdam Study. Arterial stiffness was measured as aortic pulse wave velocity and carotid distensibility. Cox's proportional hazard regression analysis was carried out to compute relative risks.

Findings- During follow–up period, 101 subjects developed coronary heart disease (mean follow-up period 4.1 years) and 63 subjects developed a stroke (mean follow–up period 3.2 years). The risk of cardiovascular disease increased with increasing aortic pulse wave velocity. Relative risks and corresponding 95% confidence intervals of coronary heart disease for subjects in the second and upper tertile of aortic pulse wave velocity, compared to subjects in the reference category were 1.72 (0.91-3.24) and 2.45 (1.29-4.66), respectively, in models adjusted for age, gender, mean arterial pressure and heart rate. Corresponding estimates for stroke were 1.22 (0.55-2.70) and 2.28 (1.05-4.96). Estimates decreased slightly after adjustment for cardiovascular risk factors and intima media thickness. Aortic pulse wave velocity provided also additional prognostic value above cardiovascular risk factors and carotid intima media thickness. Measures of carotid distensibility were not significantly associated with cardiovascular disease.

Interpretation- Aortic pulse wave velocity is an independent predictor of cardiovascular disease in apparently healthy subjects.

Introduction

Aging [1] induces structural and functional changes of the vessel wall, which result in decreased vascular distensibility and elevated arterial stiffness. As consequence of arterial stiffness, systolic blood pressure increases causing a rise in left ventricular workload [2] and subsequent hypertrophy, and diastolic blood pressure decreases leading to an impaired coronary perfusion [3]. Arterial stiffness can be evaluated by measuring the pulse wave velocity (PWV) between two sites in the arterial tree, with a higher PWV indicating stiffer arteries. Alternatively, the relative change in lumen diameter during the cardiac cycle and normalized with respect to the driving pulse pressure provides a measure of arterial distensibility. Several studies have shown that aortic PWV and carotid distensibility are predictors of cardiovascular events in patients with hypertension [4-6], and end-stage renal disease [7,8]. Only one study examined the association between arterial stiffness and risk of cardiovascular events in the general population [9]. In this study an association was found between carotid stiffness and cardiovascular events. However, this study was conducted only in men and included a small number of events. The present study is the first to investigate whether aortic PWV and carotid distensibility are predictors of incident coronary heart disease and stroke in a large population of apparently healthy subjects.

Methods

Study population

This study was conducted within the framework of the Rotterdam Study, an ongoing prospective population-based cohort study comprising 7983 men and women aged 55 years and over, and living in Ommoord, a suburb of Rotterdam, The Netherlands. The rationale and design of the Rotterdam Study have been described elsewhere [10]. The overall aim of the study is to investigate the incidence and determinants of chronic disabling diseases. Baseline data were collected from 1990 to 1993. The third examination phase took place from 1997 until 1999. During this phase measurements of cardiovascular risk factors, atherosclerosis and arterial stiffness were conducted. Subjects in nursing homes did not visit the research center and were not invited for these measurements. The Medical Ethics Committee of Erasmus Medical Center approved the study and written consent was obtained from all participants.

Measures of arterial stiffness

Pulse wave velocity

Carotid-femoral pulse wave velocity (PWV), a measure of aortic stiffness, was calculated with the subjects in supine position. PWV was assessed with an automatic device (Complior® Artech Medicla, Pantin - France) [11] that measured the time delay between the rapid upstroke of the feet of simultaneously recorded pulse waves in the carotid and the femoral artery. The distance between the recording sites in the carotid and the femoral artery was measured with a tape. PWV was calculated as the ratio between the distance measured and the foot-to-foot time delay and was expressed in meters per second.

Carotid distensibility

Common carotid distensibility was assessed with the subjects in supine position, the head tilted slightly to the contralateral side for the measurement in the common carotid artery. The vessel wall motion of the right common carotid artery was measured by means of a duplex scanner (ATL Ultramark IV, operating frequency 7.5 MHz) connected to a vessel wall movement detector system. The details of this technique have been described elsewhere [12]. After five minutes of rest, a region at 1.5 cm proximal to the origin of the bulb of the carotid artery was identified using B-mode ultrasound. The displacement of the arterial walls was obtained by processing the radio frequency signals originating from two selected sample volumes positioned over the anterior and posterior walls. The end-diastolic diameter (D), the absolute stroke change in diameter during systole (ΔD), and the relative stroke change in diameter (ΔD/D) were computed as the mean of four cardiac cycles of three successive recordings. The cross-sectional arterial wall distensibility coefficient was calculated according to the following equation: distensibility coefficient = $(2\Delta D/D)/\Delta P$ $(10^{-3}/kPa)$ [13].

Cardiovascular risk factors

Information on cardiovascular risk factors was collected during the third follow up examination. Data on drugs use and smoking habits were obtained during the home interview. Smoking was classified as never, former or current smoking. Systolic (first Korotkoff phase) and diastolic (fifth Korotkoff phase) blood pressure were measured twice on the right arm using a random-zero sphygmomanometer, after the participant had been seated for at least five minutes. The mean of the two blood pressure values was used in the analyses. Pulse pressure was defined as the difference between systolic and diastolic blood pressure. Mean arterial pressure was calculated as diastolic blood pressure + 1/3 pulse pressure. Body mass index [weight (kg)/height^2 (m)] was calculated. Serum total cholesterol and high-density lipoproteins (HDL) cholesterol values were determined by an

automated enzymatic procedure (Boehringer Mannheim System). Diabetes mellitus was defined as a history of diabetes mellitus and/or the use of blood glucose lowering medication and/or a fasting serum glucose level equal or greater than 7.0 mmol/l [14]. Information on history of coronary heart disease and stroke was given by the general practitioner.

Measure of carotid intima media thickness

Ultrasonography of both carotid arteries was performed with a 7.5-MHz linear-array transducer and a duplex scanner (ATL UltraMark IV). Common carotid intima-media thickness (IMT) was determined as previously described [15]. The maximum common carotid IMT was determined as the average of the maximum IMT of near- and far wall measurements over a length of 1 cm, and the average of left and right maximum common carotid IMT was computed.

Incident cardiovascular events

Subjects participating in the Rotterdam study are continuously monitored for the occurrence of cardiovascular events through automated linkage with the files from general practitioners in the study district. When myocardial infarction, coronary artery bypass grafting, percutaneous transluminal coronary angioplasty and stroke was reported, the research assistants collected additional information from medical records of the general practitioner and in addition, obtained information from the hospital discharge records or nursing home records including letters from medical specialists. For the diagnosis of cardiac events, two research physicians independently coded all reported events; codes were assigned according to the International Classification of Diseases, 10th edition [16]. Finally, an expert in the field of cardiovascular disease reviewed all events and verified the coding rules. In case of a stroke medical information was checked and evaluated by two research physicians and an experienced neurologist. Endpoints in the present study were first incident coronary heart disease and stroke. Coronary heart disease was defined as the occurrence of a fatal or non-fatal myocardial infarction (ICD-10 code I21), a percutaneous transluminal coronary angioplasty, a coronary artery bypass graft, other forms of acute (I24) or chronic ischemic heart disease (I25), sudden cardiac death (I46 and R96), and death due to ventricular fibrillation (I49) and congestive heart failure (I50). Follow-up was complete until 1 January 2003. ICD-10 codes for stroke were (I61, I63, I64). Follow-up was complete until 1 January 2002. Differences of duration of follow-up periods were due to logistic reasons.

Population for analysis

A total of 4024 subjects visited the research center during the third phase of the Rotterdam Study, PWV was measured in 3550 subjects and distensibility coefficient was measured in 3098 subjects. Missing information on PWV and distensibility

coefficient was almost entirely due to logistic reasons. Of the 3550 subjects with a measurement of PWV, 69 subjects (1.9%) were excluded from the analyses because of quality control of the PWV recordings, leaving 3481 subjects. We excluded subjects with previous coronary heart disease and previous stroke, leaving 2835 subjects for PWV analyses. Of these, information on distensibility coefficient was available in 2265 subjects. Data on risk factors and intima media thickness were available in more than 95% of subjects.

Statistical Analysis

Cox's proportional hazard regression analysis, adjusted for age and gender, was carried out to estimate relative risks with corresponding 95% confidence intervals (CI) for coronary heart disease and stroke associated with tertiles of PWV and distensibility coefficient. Missing value indicators were used to take care of missing values in covariates. Cut-off points for gender specific tertiles of PWV were 12.3 m/s and 14.6 m/s for the second and third tertile in men and corresponding cut-off points in women were 11.9 m/s and 14.2 m/s. Cut-off points for tertiles of distensibility coefficient were 8.8 10^{-3}/kPa and 12.7 10^{-3}/kPa for the second and third tertile in men; corresponding cut-off points in women were 7.8 10^{-3}/kPa and 11.3 10^{-3}/kPa. Reference categories were subjects in the first tertile of PWV and subjects in the last tertile of distensibility coefficient. Analyses were subsequently adjusted for mean arterial pressure and heart rate and additionally for body mass index, total cholesterol, HDL cholesterol, diabetes mellitus, smoking status, use of anti-hypertensive medication and IMT. We repeated the analyses in strata of age and gender. In these analyses coronary heart disease and stroke were combined to increase the number of events. Receiver-operating characteristics (ROC) curves were used to compute the prognostic values of different sets of cardiovascular risk indicators. Differences in the predicted values between models were estimated by comparing the areas under the ROC curve, taking correlation between the areas into account [17].

Results

Table 1 presents the baseline characteristics of the study population. During a mean follow–up period of 4.1 years, 101 subjects developed coronary heart disease, whereas 63 subjects developed a stroke during a mean follow–up period of 3.2 years. The risk of coronary heart disease increased with increasing PWV up to an age and gender adjusted relative risk of 2.44 (95% CI, 1.33-4.45) for subjects with the highest aortic PWV compared to the reference group (table 2). Estimates remained statistically significant after additional adjustment for cardiovascular risk factors and IMT. Compared with the reference categories, the risk of stroke in the

Table 1. Baseline characteristics of the study population (n=2835)

Characteristics	Mean ± SD or %
Age (years)	71.7±6.7
Men (%)	39.2
Systolic blood pressure (mmHg)	143.1±21.2
Diastolic blood pressure (mmHg)	75.7±10.7
Mean arterial pressure (mm Hg)	106.7±12.5
Heart rate (bpm)	73.9±11.9
Body mass index (kg/m^2)	26.6±3.8
Total cholesterol (mmol/L)	5.8±0.9
High-density lipoprotein cholesterol (mmol/L)	1.4±0.4
Current smokers (%)	15.5
Diabetes mellitus (%)	7.1
Use of antihypertensive medication (%)	20.9
Intima media thickness (mm)	0.86±0.14
Pulse wave velocity (m/sec)	13.3±2.9
Distensibility coefficient (10^{-3}/kPa)*	10.6±4.4

Data are presented as mean (SD) or percentages (%).
*Measures of distensibility coefficient were available in 2265 subjects.

upper tertiles of PWV was 2.34 (1.13-4.82), in models adjusted for age and gender. In fully adjusted models the estimates were slightly decreased. Table 3 shows how the risk of coronary heart disease and stroke increased in subjects with the lowest carotid distensibility coefficient, although estimates were not statistically significant. The risk of cardiovascular disease did not differ across strata of age and gender. For example, multivariate adjusted relative risks for the highest PWV compared to the reference group, were 2.21 (1.02-4.75) and 2.01 (1.13-3.57) among subjects younger than and older than 70 years of age, respectively. Estimates tended to be somewhat higher in men (2.22; 1.22-4.04) than in women (1.84; 0.94-3.60). The distensibility coefficient was not associated with incident cardiovascular disease in any of the subgroups. The area under the ROC curve (table 4) was 0.70 for the model based on cardiovascular risk factors and IMT. When PWV was added to this model the discriminatory power improved to 0.72 (p 0.04) indicating the independent and significant additional prognostic value of PWV.

Discussion

In the present study we found that aortic PWV is a strong predictor of coronary heart disease and stroke. The predictive role is independent of cardiovascular risk factors and carotid IMT. Moreover, aortic PWV has additive prognostic value for

Table 2. Relative risks of coronary heart disease and stroke associated with tertiles of pulse wave velocity

		Model 1 RR 95% CI	Model 2 RR 95% CI	Model 3 RR 95% CI
	Subjects/CVD			
1st tertile PWV	956/26	1.00 (reference)	1.00 (reference)	1.00 (reference)
2nd tertile PWV	946/46	1.50 (0.92-2.44)	1.39 (0.84-2.29)	1.37 (0.83-2.29)
3rd tertile PWV	933/85	2.40 (1.51-3.83)	2.02 (1.22-3.34)	2.01 (1.21-3.34)
	Subjects/CHD			
1st tertile PWV	956/15	1.00 (reference)	1.00 (reference)	1.00 (reference)
2nd tertile PWV	946/32	1.71 (0.92-3.19)	1.72 (0.91-3.24)	1.57 (0.83-2.97)
3rd tertile PWV	933/54	2.44 (1.33-4.45)	2.45 (1.29-4.66)	2.07 (1.08-3.98)
	Subjects/stroke			
1st tertile PWV	956/17	1.00 (reference)	1.00 (reference)	1.00 (reference)
2nd tertile PWV	946/37	1.24 (0.57-2.70)	1.22 (0.55-2.70)	1.20 (0.56-2.75)
3rd tertile PWV	933/66	2.34 (1.13-4.82)	2.28 (1.05-4.96)	2.15 (0.98-4.71)

Model 1: adjusted for age, gender. Model 2: Model 1 + mean arterial pressure and heart rate. Model 3: Model 2 + diabetes mellitus, smoking habits, body mass index, total cholesterol, high-density lipoprotein cholesterol, use of anti-hypertensive medication and intima media thickness. PWV: pulse wave velocity. CHD: coronary heart disease. CVD: cardiovascular disease(CHD and stroke combined). RR: relative risk. CI: confidence interval.

cardiovascular disease above risk factors and carotid IMT. The prognostic value of carotid distensibility is less consistent.

Before interpreting the results, some methodological issues need to be discussed. Firstly information on measures of stiffness was not available for all subjects, however, this was mostly due to logistic reasons, which is likely to be random and thus will not have biased our estimates. Secondly, subjects who did not visit the research center could have had stiffer arteries. If so, this will have altered the distribution of arterial stiffness and may have led to an underestimation of the relative risks. Thirdly, subjects may have started anti-hypertensive treatment after baseline, which may have affected arterial stiffness and underestimated the risk estimates. Advantages of our study are its prospective nature, the population-based setting with a large number of subjects and the inclusion of the two most commonly used measures of arterial stiffness.

Previous studies investigated the clinical consequences of arterial stiffness. Studies conducted in patients with end-stage renal disease, a population with high mortality rate, have shown that aortic PWV is a strong predictor of cardiovascular and overall mortality [8]. Also in middle-aged hypertensive patients, aortic PWV was associated with a high risk of cardiovascular events and mortality [4-6]. In these studies, the predictive value of PWV was independent of cardiovascular risk fac-

Table 3. Relative risks of coronary heart disease and stroke associated with tertiles of carotid distensibility coefficient

		Model 1 RR 95% CI	Model 2 RR 95% CI	Model 3 RR 95% CI
	Subjects/CVD			
1st tertile DC	755/59	2.12 (1.27-3.55)	1.48 (0.83-2.64)	1.37 (0.75-2.47)
2nd tertile DC	755/40	1.52 (0.91-2.53)	1.29 (0.76-2.17)	1.23 (0.72-2.09)
3rd tertile DC	755/25	1.00 (reference)	1.00 (reference)	1.00 (reference)
	Subjects/CHD			
1st tertile DC	755/32	1.81 (0.94-3.49)	1.32 (0.71-2.59)	1.32 (0.68-2.54)
2nd tertile DC	755/28	1.67 (0.89-3.13)	1.18 (0.56-2.47)	1.12 (0.52-2.39)
3rd tertile DC	755/16	1.00 (reference)	1.00 (reference)	1.00 (reference)
	Subjects/stroke			
1st tertile DC	755/29	2.13 (0.96-4.76)	1.47 (0.60-3.61)	1.39 (0.55-3.52)
2nd tertile DC	755/12	1.03 (0.44-2.42)	0.88 (0.36-2.11)	0.86 (0.35-2.09)
3rd tertile DC	755/10	1.00 (reference)	1.00 (reference)	1.00 (reference)

Model 1: adjusted for age, gender. Model 2: Model 1 + mean arterial pressure and heart rate. Model 3: Model 2 + diabetes mellitus, smoking habits, body mass index, total cholesterol, high-density lipoprotein cholesterol, use of anti-hypertensive medication and intima media thickness. DC: distensibility coefficient. CHD: coronary heart disease. CVD: cardiovascular disease(CHD and stroke combined). RR: relative risk. CI: confidence interval

tors. The role of carotid stiffness in predicting cardiovascular mortality has been recently investigated in a relatively small study which included 367 elderly men [9]. No association was found between carotid distensibility and cardiovascular mortality, whereas the Young's modulus of the carotid artery, a measure of stiffness, which provides information about the elastic wall material independently of the vessel geometry, was a predictor of cardiovascular mortality. However, the additive prognostic value of this measure above cardiovascular risk factors and measures of atherosclerosis was statistically not significant. To the best of our knowledge, our study is the first prospective study showing that aortic PWV is a strong predictor of coronary heart disease and stroke in a large general population of apparently healthy subjects.

In the present study, aortic PWV was associated with risk of coronary heart disease and stroke whereas carotid distensibility was not. Although age- and gender adjusted estimates for distensibility coefficient were increased, the estimates attenuated after further adjustment for mean arterial pressure and heart rate. We do not know why measures of the distensibility coefficient were not associated with increased cardiovascular risk in adjusted models.

The carotid distensibility coefficient is a measure of local and focal vascular stiffness, rather than a segmental as covered by the aortic PWV, and may be modu-

Table 4. Prognostic value of different sets of cardiovascular risk markers

	Area under the ROC curve (95%CI) Cardiovascular disease
Age, gender and risk factors	0.69 (0.65-0.73)
Age, gender, risk factors and IMT	0.70 (0.67-0.74)
Age, gender, risk factors, IMT and PWV	0.72 (0.68-0.75)*

* P = 0.04 versus previous model containing risk factors and IMT. IMT: intima media thickness. PWV: pulse wave velocity. Cardiovascular diseaseis coronary heart disease and stroke combined. Risk factors are mean arterial pressure, heart rate, diabetes mellitus, smoking habits, body mass index, total cholesterol, high-density lipoprotein cholesterol, use of anti-hypertensive medication.

lated by plaques. When calculating the distensibility coefficient, the distension of the common carotid artery is adjusted for brachial pulse pressure. In dogs a good correlation was found between pulse pressures as assessed in the brachial artery and as assessed in the common carotid artery, over a wide range of pressures [28]. Therefore, the difference in pulse pressure between the brachial and the carotid artery, if any, is not likely to have affected the validity of the measurement of the distensibility coefficient in subjects with a restricted age range.

Several mechanisms may explain the association between aortic PWV and cardiovascular disease. Arterial stiffening may lead to early pulse wave reflection and to early valve closure with an increase of central systolic blood pressure, a decrease of diastolic blood pressure and a consequent increase of pulse pressure [18]. The elevation of systolic blood pressure increases myocardial oxygen demand, reduces ejection fraction and increases ventricular load, inducing left ventricular hypertrophy[19], a risk factor for both coronary heart disease and stroke. Moreover, since myocardial blood supply depends largely on diastolic blood pressure[3], the contemporary decrease of diastolic blood pressure can compromise coronary perfusion resulting in subendocardial ischemia [3,20]. Aortic PWV is also associated with stroke. A raised pulse pressure due to increased vascular stiffness may induce arterial remodeling, increasing the wall thickness and the development of plaques [21,22], and is also a predictor of stroke [23]. Furthermore, aortic stiffness is caused by structural changes, including fibrosis, medial smooth muscle necrosis, breaks in elastin fibers, calcifications, and diffusion of macromolecules into the arterial wall, which have also been described at the site of the cerebral vasculature [24]. Additionally, stiffer arteries may contribute to rupture and ulceration of atherosclerotic plaques [25,26], especially when an inhomogeneity in stiffness in and around the plaque is present, increasing the risk of plaque rupture, and, hence, embolism followed by cardiovascular events[27].

The prognostic value of several cardiovascular risk factors decreases with age, due to selective survival and the influence of co-morbidity on risk factors levels [29,30]. Blood pressure levels may decrease with age due to congestive heart

failure or a general decrease in well-being. In contrast, vascular stiffness steadily increases with age and can be accelerated by co-morbid diseases. In the present study, high aortic PWV increased the risk of cardiovascular events in a population of apparently healthy elderly, and remains a predictor also in older subjects. Moreover, adding measures of stiffness to models containing cardiovascular risk factors and carotid IMT improved significantly the prognostic value for cardiovascular events demonstrating additional information of arterial stiffness in stratification of cardiovascular risk. The measurement of PWV is non-invasive, easy to apply and allows a functional assessment of the arterial tree. Given the relatively low costs and the low burden to the subject, we think the measurement should be considered as an additional tool for the identification of subjects at high risk of cardiovascular disease.

In conclusion, this is the first prospective population-based study showing that aortic PWV is a strong predictor of coronary heart disease and stroke among apparently healthy subjects. The measure of aortic PWV improved the prognostic value above cardiovascular risk factors and atherosclerosis demonstrating additional information of arterial stiffness in risk stratification. The results of the present study indicate that arterial stiffness should no longer be considered as an innocent expression of vascular aging, but as a sign of increased cardiovascular risk.

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5

General discussion

The structural elements of the vessel wall that are particularly important in determining the vessel's stiffness are elastin and collagen. Elastin is very stretchable [1] and is important to pulsatile behavior, but not of great importance in determining vessel wall strength. When elastin fibers are stretched and release they return promptly to their original state. Collagen fibers are much stiffer and can resist stresses > 100 times the fracture stress of elastin fibers but are less extensible [2, 3]. The vessel wall is considered to act in a biphasic manner with the elastin fibers important in determining stiffness at low distending pressures, and collagen in determining stiffness at high distending pressures. In the normal arterial tree, the large arteries act as a buffering system that is dependent on vessel compliance. During systole, the stroke volume is rapidly infused into the arterial tree, with only 30% to 40% resulting in forward flow, whereas the rest is stored in the large arteries and subsequently released into the periphery during diastole. This buffering action essentially converts the pulsatile flow at the level of the aorta to continuous flow in the capillaries (the Windkessel effect). With aging, there is a thinning and fracturing of elastin and increased collagen deposition in the tunica media and therefore an increased vessel wall stiffness [4, 5]. Alterations in the buffering capability of the large arteries, mediated through changes in arterial compliance, have hemodynamic consequences and important effect on organ function. The increase in arterial stiffness is responsible for earlier wave reflections and changes in pressure wave contours [6, 7]. The arterial pressure waveform is derived from the complex interaction of the left ventricular stroke volume, the physical properties of the arterial tree, and the characteristics of the fluid in the system. At the time of left ventricular ejection, a pressure wave is initiated that is propagated forward in the blood as well as by the aortic and arterial walls. When arteries are compliant and pulse wave velocity is relatively slow, reflected waves return to the central aorta in diastole, augmenting diastolic blood pressure and, therefore, coronary blood flow, which occurs predominantly during diastole. When arteries are stiffer and pulse wave velocity is higher, reflected waves arrive earlier and augment central systolic blood pressure, rather than diastolic blood pressure, increasing left ventricular workload and compromising coronary blood flow [8, 9] . Because left ventricular ejection remains stable or even decreases with age, arterial stiffness is the principal factor responsible for increased systolic blood pressure, decreased diastolic blood pressure and, therefore, high pulse pressure during aging[10].

Arterial stiffness has been exiguously investigated in large population-based studies. The studies presented in this thesis are based on the Rotterdam Study and focus on two measures of arterial stiffness: carotid-femoral pulse wave velocity as measure of aortic stiffness and the distensibility coefficient of the common carotid artery as measure of carotid stiffness. The Rotterdam Study is a population-based cohort study among subjects aged 55 years and older at baseline. In total, 7983 subjects agreed to participate and were included in the study. Baseline data were

collected from March 1990 to July 1993. During the third examination phase (from 1997 until 1999) measurements of arterial stiffness were obtained. Of the 4148 subjects who were eligible for physical examination carotid-femoral pulse wave velocity was measured in 3550 subjects and distensibility of the common carotid artery was measured in 3098 subjects.

Determinants

Age is the main determinant of changes in the viscoelastic vessel wall properties and is associated with vascular stiffness [4, 5, 11]. However, the extent of these changes may depend on several cardiovascular risk factors. Besides age, hypertension and diabetes mellitus can alter arterial structure and reduce vascular elasticity. In subjects with hypertension [12, 13] the principal structural modification of the vessel wall is hypertrophy of the media with a considerable development of extracellular matrix, mainly at the site of the central but not peripheral arteries. Nonenzymatic glycation due to raised blood glucose and consequent collagen cross linkage lead to alterations in the mechanical arterial properties in diabetes mellitus [14, 15]. It has been reported that high cholesterol levels alter the endothelial function, leading to a decreased relaxation of the arterial vessels but at the moment the results of the literature are controversial [6, 16-18]. However, within our population study we found high-density lipoprotein cholesterol to be associated with carotid stiffness, whereas no association was found between serum cholesterol levels and measures of pulse wave velocity [19].

Traditional risk factors can only explain part of the incidence of cardiovascular events. Many studies have focused on the role of inflammation in the development of atherosclerosis and cardiovascular disease. C-reactive protein (CRP), a marker of acute inflammation, is associated with atherosclerosis [20, 21] whereas results on the predictive value of CRP are not consistent. [22-24, 25]. A recent study found that increases in pulse pressure, which reflect a gradual increase in stiffness of the large arteries, are associated with elevated CRP levels [26]. In the study described in chapter 2.2, the association between CRP and arterial stiffness is discussed. Increased levels of CRP have been found to be associated with major determinants of arterial stiffness as insulin resistance variables [27], diabetes mellitus [28, 29], and high blood pressure levels [30]. Therefore, it could be speculated that CRP levels would contribute to increased arterial stiffness, by being associated with metabolic and hemodynamic changes that lead to arterial stiffness. However, since associations remain after taking these factors into account in statistical models, other mechanisms might be involved. High CRP levels are associated with endothelial dysfunction [27, 31]. The vascular endothelium releases nitric oxide, a substance that has a major influence on basal arteriolar tone and blood pressure [32, 33] inducing arterial

distensibility [34]. Moreover, agonists that stimulate endothelial nitric oxide release, such as acetylcholine, also reduce muscular artery stiffness in vivo [35, 36]. It may be that high CRP levels impairs endothelial function and subsequently alters the mechanical properties of the vessel walls leading to increased arterial stiffness.

Increased arterial thickness, the presence of plaques and decreased carotid distensibility often coexist in the same subjects [37-39] and the relationships among different alterations of the arterial wall, that is, hypertrophy (increased media thickness), atheromatosis (plaques) and stiffness (decreased compliance), remain to be explored. Atherosclerosis and arterial stiffness are both related to an unfavourable cardiovascular risk profile [40, 41] and it has been speculated that arterial stiffness may play a role in the development of atherosclerosis or vice versa. Studies evaluating the relation between arterial stiffness and atherosclerosis have reported conflicting results [42-44]. The observed difference in the impact of independent determinants of these vascular entities, as body mass index and high levels of cholesterol that are strongly associated with atherosclerosis but not clearly associated with vascular stiffness [45], may suggest that the two alterations are at least partly independent entities of vascular damage.

The genetic background of arterial stiffness is starting to be explored and several recent studies have suggested that arterial mechanisms are influenced by genes, as those related to the renin-angiotensin aldosterone system. The angiotensin-converting enzyme (ACE) gene has been implicated in structural changes of the vessel wall [46, 47]. The ACE gene has an I/D polymorphism in intron 16, which has been previously found to be associated with cardiovascular diseases and atherosclerosis [48-51]. However, results between the ACE I/D polymorphism and vascular stiffness are not consistent. It has been found that aortic stiffness was similar among the three ACE I/D genotypes in normotensive subjects whereas it was slightly higher among hypertensive subjects with the II genotype [52]. In another study the I allele of the ACE gene was associated with stiffening of the large arteries in patients with diabetes mellitus type 2 compared with subjects without diabetes mellitus [53]. The results of our study presented in chapter 2.1 are in contrast with these two previous studies but in agreement with a recent study [54] which showed that higher stiffness of the common carotid artery was associated with the ACE D allele in a small group of young adults. We found higher stiffness of the common carotid artery was present in subjects with the ACE D allele. Higher circulating levels and tissue ACE activity are present in subjects with the D compared to the I allele [55-57]. ACE catalyzes the conversion of angiotensin I to angiotensin II and the breakdown of bradykinin to kinin degradation products. Both angiotensin II and bradykinin are potent peptide hormones that play a role in vascular wall homeostasis reducing vascular tone, vascular smooth muscle cell growth and production of extracellular matrix [58-61]. These processes may lead to progressive degeneration of arterial media as fractures and fragmentation of elastic

lamellae, increased collagen and calcium content and dilation and hypertrophy of the large arteries with subsequent increased arterial stiffness. Other candidate genes related to cardiovascular aging, particularly those related to elastin, collagen and telomere length [62-64] may offer new insights into genetic patterns influencing the pathogenesis of arterial stiffness.

Different lifestyles may also influence arterial elastic properties. Cigarette smoking is a known risk factor for cardiovascular disease however data on arterial compliance are less consistent. Reduced small vessels compliance has been demonstrated in smokers compared to non-smokers [65], whereas others [66] were only able to demonstrate short-term changes in arterial distensibility, but not long-term effects. Physical exercise has also been shown to influence arterial functional properties. Senior endurance-trained athletes demonstrated less stiff arteries than sedentary men of the same age [67]. Light to moderate alcohol consumption seems to have a protective effect on the cardiovascular system [68-70]. However, few studies investigated the relation between alcohol consumption and arterial stiffness and it is not clear whether alcohol consumption has an association with elastic properties of the vessel wall. Alcohol consumption increased pulse wave velocity in middle-aged Japanese men [71], whereas other authors showed that alcohol consumption was associated with lower arterial stiffness [72]. Recent studies found a J-shaped association between alcohol consumption and arterial stiffness in men aged 40-80 years [73] and an inverse association in healthy postmenopausal women [73]. We have found an U-shaped association, independent of cardiovascular risk factors and atherosclerosis, between alcohol consumption and arterial stiffness in women. In men, the same trend was observed, although the estimates lacked statistical significance (chapter 2.4). The association seems to be stronger for wine consumption. However, we do not believe that these results should encourage alcohol consumption.

Prognostic value of arterial stiffness

In the past, vascular stiffening and the increase in systolic and pulse pressure have been considered as a part of normal aging. Besides its strong relation with age, arterial stiffness is also associated with hypertension [74, 75], diabetes mellitus [11] and atherosclerosis [42, 76]. Changes in the arterial walls, which lead to reductions of arterial compliance, may precede the onset of clinically apparent disease, and identify individuals at risk before disease onset (symptoms due to disease are, in general, late manifestations of alterations in organ function). The ability to predict alterations in vascular structure and function before the onset of clinical diseases has potential advantages. Aortic stiffness has been found to be a predictor of cardiovascular disease in selected groups of patients with hypertension [77-79], and

patients with end-stage renal disease [80, 81]. However, the previous studies have been performed in selected categories of patients with a high cardiovascular risk and the results cannot be extrapolated to the general population. The role of carotid stiffness in predicting cardiovascular mortality has been recently investigated in a relatively small study that included 367 elderly men [82]. In this study, different parameters of carotid stiffness were included. No association was found between carotid distensibility and cardiovascular mortality, whereas the Young's elastic modulus, a measure of stiffness, which provides information about the elastic wall material independently of the vessel geometry [83, 84], was a predictor of cardiovascular mortality. However, the additive prognostic value of this measure above cardiovascular risk factors and measures of atherosclerosis was not statistically significant. In chapter 4.1 we show that aortic pulse wave velocity is a strong predictor of cardiovascular disease among apparently healthy subjects. The association is present both in men and women and in younger and older subjects. The measure of aortic pulse wave velocity improves the prognostic value above cardiovascular risk factors and carotid intima media thickness, which is a measure of atherosclerosis. These results show the additional information of arterial stiffness in risk stratification also in older age and indicate that arterial stiffness should no longer be considered as an innocent expression of vascular aging but as a sign of increased cardiovascular risk. In our study we were not able to find an association between carotid stiffness and incident cardiovascular disease. Age- and gender adjusted risk estimates for carotid stiffness were increased but the estimates attenuated after further adjustment.

Clinical implications and future research

Vascular stiffening and the subsequent increase in systolic and pulse pressure have been considered in the past as a part of normal aging. To date, determinants of arterial stiffness and its clinical consequences have been exiguously investigated in large population-based studies. Besides aging, also hypertension, diabetes mellitus, atherosclerotic lesions and recently, specific genes have been found to be associated with structural changes of the vessel wall that lead to stiffer arteries. However, a large part of the variability in arterial stiffness is yet unknown. Large populations based studies should investigate whether age-related hormonal changes, nutritional patterns, physical activity and possible interactions of genetic and environmental factors can influence the functional vascular properties.

It is not completely clear whether vascular stiffening is a generalized process or whether several measures of stiffness obtained in different vascular territories may provide distinct information. We found different associations when we studied stiffness measured at various sites of the arterial tree. In the proximal aorta and

its main branches, elastin is the main component, whereas in the distal muscular arteries the collagen to elastin ratio is reversed and in peripheral arteries collagen predominates. Differences in elastic and muscular contents of the arterial wall could lead to differences in vascular stiffening at various sites. This, in combination with the different methods used in our studies, could also partially explain the results obtained. The measure of the carotid distensibility is a local measure of stiffness that provides information on the functional properties at a specific site of the arterial tree and may be strongly influenced by the presence of atherosclerotic plaques near the site of measurement. The measure of the pulse wave velocity provides information on generalized stiffness; however, this measure combines measurements of elastic and muscular arteries, making it impossible to evaluate differences in functional properties between the two types of arteries. Future research should investigate the relation between risk factors and structural and functional modifications at different sites of the arterial tree.

Different non-invasive measures of atherosclerosis are currently used in stratification of cardiovascular risk. The ankle- arm index, which is the ratio between systolic blood pressure of the tibial posterior artery and systolic blood pressure of the brachial artery, predicts cardiovascular disease [85, 86]. Carotid intima media thickness is a predictor of coronary heart disease in the general population, independent of traditional risk factors and other measures of atherosclerosis [87]. Recently, the prognostic value of coronary calcification has been investigated within the framework of the Rotterdam Coronary Calcification Study and has been shown to be a strong and independent predictor of cardiovascular disease (Rozemarijn Vliegenthart, personal communication). However, both measures of intima media thickness and coronary calcification require expansive devices and may require extensively trained personnel. Aortic pulse wave velocity, a relatively new technique, can measure non-invasively and we have shown that this measure is predictive of cardiovascular disease in the general population, with an additive value above other known risk factors also in older age. The strength of the associations with incident cardiovascular disease, the relatively low cost, ease of use, and acceptability to patients may suggest the measurements of arterial stiffness to identify individuals at higher cardiovascular risk. However, the role of aortic pulse wave velocity in the prediction of primary cardiovascular disease, in relation with other risk factors and co-morbid situations as atherosclerosis, needs to be confirmed in other studies including different populations.

Several studies have focused on treatment of arterial stiffness and most attention has been paid to antihypertensive drugs. Considering the local actions of angiotensin II on arterial stiffening (fibrosis, collagen synthesis), drugs interfering with the renin-angiotensin-aldosterone system (RAAS) are important candidates. Recent studies have shown blood pressure-independent effects of ACE inhibitors [88] on arterial stiffness. Additional potential candidates along the RAAS axis are

aldosterone antagonists. Aldosterone is an important mediator of fibrotic changes at the level of the heart and recent data suggest that similar effects may occur in the arterial wall of rats [89] and humans [90].

Another approach to reduce stiffness is to circumvent hemodynamic effects and to target directly the molecular events leading to arterial stiffening. A potential molecular target in this respect is the formation of advanced glycation end-products (AGE). These products are responsible for the arterial stiffening in conditions such as diabetes[91] and aging [92] in animal models. Drugs interfering with the formation of AGE, have been shown in animal models to reverse arterial stiffening without influencing blood pressure levels [92, 93] . A more futuristic approach is the pharmacologic interference with collagen metabolism. This is a particularly difficult target in view of the extremely low turnover of this vessel wall component. Early attempts in experimental models of hypertension [94] were promising, but have not been developed in the clinical setting. In conclusion, arterial stiffness should no longer be considered as an innocent expression of vascular aging but as a sign of increased cardiovascular risk. This should encourage future research to get more insights into the pathophysiology of this process, to confirm the role of arterial stiffening in risk prediction and to evaluate therapeutic possibilities.

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6

Summary

Arterial stiffness increases with age, but also hypertension, diabetes mellitus and atherosclerosis are associated with increased vessel wall stiffness. Several studies have suggested that subjects with cardiovascular disease have increased arterial stiffness compared to subjects without cardiovascular disease. The prognostic value of high vessel wall stiffness has been generally investigated in relatively small studies or in studies which have been performed in a limited number of patients at risk. The aim of the studies described in this thesis was to search for new insight into factors that are involved in the etiology of arterial stiffness and to investigate the clinical consequences of stiffer arteries in a large population of apparently healthy elderly. All the studies presented in this thesis are based on the Rotterdam Study, a population-based cohort study including 7983 subjects aged 55 years and older, at baseline.

Chapter 1 gives a general introduction to this thesis. Chapter 2 focuses on determinants of arterial stiffness. In chapter 2.1 the association between the angiotensin-converting enzyme (ACE) insertion/deletion (I/D) polymorphism and arterial stiffness is investigated. The ACE I/D polymorphism is suggested to be involved in structural arterial changes and has been previously found to be associated with cardiovascular diseases and atherosclerosis. We found that the presence of the ACE ID and DD genotype was associated with higher stiffness of the common carotid artery. We found that the association was strongest in subjects younger than 70 years and no longer present in the oldest age group. Possibly, older subjects with the ACE D genotype and therefore with a higher cardiovascular risk already died before entering the study causing selective mortality. No relation was found between ACE I/D polymorphism and carotid-femoral pulse wave velocity. In chapter 2.2 C-reactive protein (CRP), a marker of inflammation, was studied in relation to arterial stiffness. Increased levels of CRP have been found to be associated with insulin resistance variables, diabetes mellitus, and high blood pressure levels, which are major determinants of arterial stiffness. High CRP levels, therefore, could contribute to high stiffness, by being associated with metabolic and hemodynamic changes that lead to arterial stiffness. However, we found that increased levels of CRP were associated with arterial stiffness independently of cardiovascular risk factors and atherosclerosis. These results indicate an independent association between CRP levels and arterial stiffness that may be useful to understand better the biochemical mechanisms responsible for the development of arterial stiffness. The relation between impaired fasting glucose and arterial stiffness is presented in chapter 2.3. We compared arterial stiffness of subjects with impaired fasting glucose with arterial stiffness of non-diabetic subjects without impaired fasting glucose and with that of diabetic subjects. Subjects with impaired fasting glucose under 75 years of age were found to have arterial stiffness comparable with non-diabetic subjects without impaired fasting glucose, while diabetic

subjects had stiffer arteries. Above 75 years of age, arterial stiffness of subjects with impaired fasting glucose reached that of diabetic subjects.

Chapter 2.4 describes the association between alcohol consumption and arterial stiffness. Light to moderate alcohol consumption seems to have a protective effect on the cardiovascular system. We found an U-shaped association between alcohol consumption and arterial stiffness in women. In men, the same trend was observed, although the estimates lacked statistical significance. The association was independent of cardiovascular risk factors and atherosclerosis and seemed to be stronger for wine consumption. These results suggest that moderate alcohol consumption may positively influence arterial properties.

The studies described in chapter 3 concern the relation between arterial stiffness and blood pressure levels. In chapter 3.1 the relation between aortic stiffness and postural blood pressure changes is examined. Age-related vascular changes might be implicated in determining impaired hemodynamic response to orthostatic challenge. We found that arterial stiffness was independently associated with orthostatic hypotension. We also found an association between arterial stiffness and postural blood pressure changes; a higher drop in blood pressure levels was associated with higher levels of stiffness, whereas a small response of heart rate to orthostatic challenge was observed, supporting the hypothesis of a reduced baroreflex due to stiff arteries. However, mechanisms remain speculative and require further studies. If this hypothesis is valid, arterial stiffness could be a potentially modifiable factor in determining baroreflex dysfunction and might be considered a therapeutic target.

Chapter 3.2 deals with the role of different blood pressure components in predicting cardiovascular disease. There is increasing evidence that pulse pressure, a surrogate measure of arterial stiffness, is an independent predictor of cardiovascular disease in the older adults. Reports of the Framingham Study show that from 60 years of age and on, pulse pressure became superior to systolic blood pressure in predicting coronary heart disease, on the other hand other epidemiological studies have reported discordant data. We investigated the predictive role of different blood pressure components for cardiovascular disease and mortality and we found that pulse pressure is comparable but not stronger than systolic blood pressure in predicting myocardial infarction and all-cause mortality, whereas systolic blood pressure was a slightly better predictor of stroke. In chapter 4 we investigated whether different measures of arterial stiffness are predictors of coronary heart disease and stroke in a large population of apparently healthy elderly. Arterial stiffness was measured as carotid-femoral pulse wave velocity as a measure of aortic stiffness and common carotid distensibility as a measure of carotid stiffness. We found that aortic stiffness was a strong predictor of coronary heart disease and stroke, whereas the prognostic role of carotid stiffness was less consistent. Moreover, additional information on aortic stiffness improved the prognostic value

for cardiovascular events in models containing cardiovascular risk factors. Vessel wall stiffening causes a gradual reduction of the peripheral amplification and a premature return of reflected waves in late systole, increasing systolic blood pressure and decreasing diastolic blood pressure. The elevation of systolic blood pressure increases the myocardial oxygen demand, reduces the ejection fraction and increases the ventricular load, inducing left ventricular hypertrophy. Furthermore, stiffer arteries may contribute to rupture and ulceration of atherosclerotic plaques increasing the risk of cardiovascular events. In the general discussion, chapter 5, the main findings of this thesis are discussed. The results indicate that generalized arterial stiffness should no longer be considered as an innocent expression of vascular aging but as a sign of increased cardiovascular risk. The measurement of pulse wave velocity is non-invasive, easy to apply, allows a functional assessment of the arterial tree and should be considered as an additional tool for the identification of subjects at high risk of cardiovascular disease.

Samenvatting

Verstijving van de slagaders is een aan veroudering gerelateerd proces. Hiernaast zijn ook hoge bloeddruk, diabetes mellitus en atherosclerose geassocieerd met structurele en functionele vasculaire veranderingen die tot verstijving van de slagaders leiden. Verschillende onderzoeken naar de gevolgen van verstijving van slagaders hebben aangetoond dat slagaderverstijving geassocieerd is met een verhoogd risico op cardiovasculaire ziekten. De voorspellende waarde van slagaderverstijving is doorgaans onderzocht in relatief kleine onderzoeken of in onderzoeken met geselecteerde patiëntengroepen met een hoog risico. Het doel van dit proefschrift was om nieuwe inzichten te verkrijgen in factoren die betrokken zijn bij het ontstaan van slagaderverstijving en de gevolgen van verstijving van slagaders te onderzoeken in een prospectief onderzoek in de algemene bevolking. Alle studies beschreven in dit proefschrift zijn uitgevoerd binnen het Erasmus Rotterdam Gezondheid Onderzoek (ERGO) , een onderzoek onder mannen en vrouwen van 55 jaar en ouder.

Na een algemene inleiding in hoofdstuk 1, worden in hoofdstuk 2 vier studies beschreven die ingaan op determinanten van verstijving van slagaders. In het onderzoek beschreven in hoofdstuk 2.1 is de relatie tussen verstijving van slagaders en het insertie/deletie (I/D) polymorfisme van het angiotensin-converting enzyme (ACE) gen onderzocht. Diverse studies suggereren dat het ACE I/D polymorfisme geassocieerd is met atherosclerose en cardiovasculaire ziekten. Onze resultaten laten zien dat deelnemers met het D-allel van dit polymorpisme een grotere vaatwandstijfheid van de arteria carotis communis hadden ten opzichte van deelnemers met het I allel. De associatie was sterker onder de jongste deelnemers en niet langer aanwezig in de oudste groep. Het is mogelijk dat oudere personen met het D-allel een hogere cardiovasculair risico hebben en reeds overleden zijn voor de start van het onderzoek. Geen relatie werd gevonden tussen het I/D ACE polymorpisme en vaatwandstijfheid van de aorta. In hoofdstuk 2.2 worden de resultaten van een studie gepresenteerd waarin de relatie werd onderzocht van C-reactief proteïne (CRP), een onstekingsparameter, en vaatwandverstijving. Hogere CRP waarden waren geassocieerd met parameters van het insuline resistentie syndroom, en met diabetes mellitus en hoge bloeddruk. CRP was ook geassocieerd met vaatwandstijfheid van de aorta, onafhankelijk van cardiovasculaire risicofactoren en mate van atherosclerose. Deze resultaten suggereren het belang van ontstekingsprocessen bij het ontstaan van slagaderverstijving, hoewel een causaal verband uit dit cross-sectionele onderzoek niet kan worden afgeleid. De relatie tussen een afwijkend glucose metabolisme en slagaderverstijving werd onderzocht in de studie beschreven in hoofdstuk 3.2. In dit onderzoek, werd een verhoogd nuchter glucose, hetgeen een maat is voor een verstoord glucose metabolisme, gerelateerd aan de distensibiliteit van de arteria carotis communis. De

vaatwandstijfheid van individuen met een verhoogd nuchter glucose maar zonder diabetes mellitus is groter in vergelijking met de vaatwandstijfheid van individuen met een normaal nuchter glucose. De resultaten waren afhankelijk van de leeftijd. Onder de 75 jaar bleken individuen met een verhoogd nuchter glucose zonder diabetes mellitus een zelfde mate van vaatwandstijfheid te hebben als individuen met een normaal nuchter glucose, terwijl individuen met diabetes mellitus een stijvere vaatwand hadden. Boven de 75 jaar was de vaatwandstijfheid van individuen met een afwijkend nuchter glucose vergelijkbaar met de vaatwandstijfheid van individuen met diabetes mellitus. Hoofdstuk 2.4 beschrijft de associatie tussen alcohol consumptie en vaatwandstijfheid. Licht tot matige alcoholconsumptie was geassocieerd met een meer elastisch vaatstelsel. Een U-vormige associatie was aanwezig tussen alcoholconsumptie en vaatwandstijfheid bij vrouwen. Bij mannen werd dezelfde relatie gevonden, maar de resultaten waren niet statistisch significant. De associatie tussen alcoholconsumptie en vaatwandstijfheid was onafhankelijk van cardiovasculaire risicofactoren en mate van atherosclerose.

Hoofdstuk 3 beschrijft de relatie tussen slagaderverstijving en de bloeddruk.

Hoofdstuk 3.1 gaat in op de relatie tussen slagaderverstijving en orthostatische hypotensie. Leeftijd-gerelateerde vasculaire veranderingen kunnen een rol spelen bij het ontstaan van een verminderde hemodynamische respons bij het opstaan. De vaatwandstijfheid van individuen met orthostatische hypotensie was hoger dan de vaatwandstijfheid van individuen zonder orthostatische hypotensie. Een hogere vaatwandstijfheid ging gepaard met een grotere afname van de bloeddruk na het opstaan, terwijl een kleine verandering van de hartslag werd gezien. Deze bevindingen suggereren dat vaatwandstijfheid een rol kan spelen bij het ontstaan van een afwijkende baroreflex. In het onderzoek besproken in hoofdstuk 3.2 bestudeerden wij de voorspellende waarden van verschillende bloeddruk componenten voor het risico op hartinfarct, cerebrovasculaire accidenten en sterfte. Wij vonden dat de risicoschatters van de polsdruk even hoog waren als die van de systolische bloeddruk voor het optreden van een hartinfarct en voor totale sterfte. Voor cerebrovasculaire accidenten was de voorspellende waarde van de systolische bloeddruk hoger dan die van de polsdruk.

Of de meting van vaatwandstijfheid toevoegt aan de voorspelling van het optreden van coronaire hartziekte en cerebrovasculaire accidenten in de algemene populatie is onbekend. Het onderzoek beschreven in hoofdstuk 4.1 laat zien dat vaatwandstijfheid van de aorta een sterke voorspeller is van cardiovasculaire ziekte. De voorspellende waarde van de distensibiliteit van de arteria carotis communis was niet onafhankelijk van de traditionele risicofactoren. Individuen met stijvere vaatwand van de aorta hadden een twee maal hogere kans op het ontwikkelen van coronaire hartziekte en cerebrovasculaire accidenten in vergelijking met individuen met een lagere vaatwandstijfheid van de aorta. Bovendien had de meting van de vaatwandstijfheid van de aorta additionele voorspellende waarde naast de

traditionele risicofactoren en atherosclerose. De conclusie van dit hoofdstuk is dat slagaderverstijving geen onschuldig verouderingsproces is maar is gerelateerd aan een verhoogd risico op cardiovasculaire ziekten. Tot slot bevat hoodstuk 5 een algemene discussie over de onderzoeken die in dit proefschrift zijn beschreven.

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About the author

The author of this thesis was born on December 16th 1967 in Catanzaro, Italy. After his graduation at high school (Liceo Ginnasio Pitagora, Crotone, Italy) he attended the Medical School at the University of Roma and subsequently at the University of Messina (Italy), where he graduated cum laude in 1994. He followed the training in Geriatric Medicine, and subsequently he moved to Rotterdam, The Netherlands. Since September 1st 1999 he is Geriatrician within the section of Geriatric Medicine (head Dr. T.J.M. van der Cammen), at the Department of Internal Medicine (head Prof. dr. M.A.D.H. Schalekamp, from April 2000 head Prof. dr. H.A.P. Pols) of the Erasmus MC, Rotterdam. He started to work on this thesis in September 2000 at the Department of Epidemiology & Biostatistics (head Prof. dr. A. Hofman) of the Erasmus MC, Rotterdam. Since 2003 he is member of Supervisory Board of the Rheumaverpleeghuis, Rotterdam. Francesco and Annet have two children, Giuseppe (12) and Maarten (5).

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