Causes and consequences of arterial stiffness

An epidemiological approach

The work for this thesis was conducted at the Department of Epidemiology & Biostatistics of the Erasmus Medical Centre, Rotterdam, the Netherlands. Financial support for this work by the Health research and development Council (ZON) and financial support for the Rotterdam Study by the Netherlands Organisation for Scientific Research (N.W.O.), and the Health research and development Council (ZON) is gratefully acknowledged.

The author gratefully acknowledges the collaboration with the Julius Centre for Patient Oriented Research, Utrecht (D.E. Grobbee, M.L. Bots); the Department of Fysiologie, Cardiovascular Research Institute Maastricht (CARIM), Maastricht (R.S. Reneman); the Department of Biofysica, Cardiovascular Research Institute Maastricht (CARIM), Maastricht (A.P.G. Hoeks); the Department of Internal Medicine, Academic Medical Centre, Amsterdam (W.J.W. Bos); TNO-Biomedical Instrumentation, Academic Medical Centre, Amsterdam (I. Guelen, B.E. Westerhof, K.H. Wesseling, N.A.M. de Beer); the Cardiovascular Institute, Paris, France (R. Asmar, J. Topouchian).

The printing of this thesis was supported by the Department of Epidemiology & Biostatistics, Erasmus Medical Centre, Rotterdam; the Julius Centre for Patient Oriented Research, Utrecht Medical Centre, Utrecht; Diabetes Fonds Nederland; Bayer BV; Bristol-Myers Squibb BV; Novartis Pharma BV; Servier Nederland BV; Sigma-Tau Ethifarma BV; Solvay Pharma BV; and Tramedico BV.

Cover design: Hanneke van Popele-Naterop, Harrow, England
Gerjan Konings, Utrecht
Layout: Bon Mot, Rotterdam
Printed by: Print Partners Ipskamp, Enschede

ISBN 90-9013855-2

Causes and consequences of arterial stiffness
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Oorzaken en gevolgen van vaatwandstijfheid
Een epidemiologische benadering

Proefschrift

ter verkrijging van de graad van doctor
aan de Erasmus Universiteit Rotterdam
op gezag van de Rector Magnificus
Prof.dr.ir. J.H. van Bemmel
en volgens besluit van het College voor Promoties.

De openbare verdediging zal plaatsvinden op
woensdag 6 september 2000 om 13:45 uur

door

Nicole Marie-Louise van Popele

geboren te Oss

Promotiecommissie

Promotoren	:	Prof.dr. D.E. Grobbee
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Overige leden	:	Prof. G.M. London
		Prof.dr. H.A.P. Pols
		Prof.dr. M.L. Simoons

Financial support by the Netherlands Heart Foundation for the publication of this thesis is gratefully acknowledged.

Voor mijn ouders

Papers and manuscripts based on the studies described in this thesis

Chapter 3

Van Popele NM, Westendorp ICD, Bots ML, Reneman RS, Hoeks APG, Hofman A, Grobbee DE , and Witteman JCM. Variables of the insulin resistance syndrome are associated with reduced arterial distensibility in healthy non-diabetic middle-aged women. Diabetologia 2000;43:665-672

Van Popele NM, Hak AE, Bots ML, Van der Kuip DAM, Reneman RS, Hoeks APG, Hofman A, Grobbee DE, and Witteman JCM. Impaired fasting glucose is associated with increased arterial stiffness in elderly subjects; The Rotterdam Study. (Submitted)

Chapter 4

Van Popele NM, Grobbee DE, Bots ML, Asmar R, Topouchian J, Reneman RS, Hoeks APG, Van der Kuip DAM, Hofman A, and Witteman JCM. The association between arterial stiffness and atherosclerosis; The Rotterdam Study. (Submitted)

Chapter 5

Van Popele NM, Hollander M, Bots ML, Van der Kuip DAM, Reneman RS, Hoeks APG, Asmar R, Hofman A, Breteler MMB, Grobbee DE, and Witteman JCM. Measures of arterial stiffness are associated with myocardial infarction and stroke; The Rotterdam Study. (Submitted)

Guelen I, Van Popele NM, Westerhof BE, Hofman A, Grobbee DE, Asmar R, Wesseling KH, Witteman JCM, and Bos WJW. Aortic stiffness and the balance between cardiac oxygen supply and demand in an elderly population; The Rotterdam Study. (Submitted)

Van Popele NM, Vliegenthart R, Grobbee DE, Asmar R, Van der Kuip DAM, Hofman A, De Feijter PJ, Oudkerk M, and Witteman JCM. Aortic stiffness is associated with increased severity of coronary atherosclerosis; The Rotterdam Study. (Submitted)

Chapter 6

Van Popele NM, Bos WJW, De Beer NAM, Van der Kuip DAM, Hofman A, Grobbee DE, and Witteman JCM. Arterial stiffness as underlying mechanism of disagreement between an oscillometric blood pressure monitor and a sphygmomanometer. Hypertension, in press

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Chapter 1

Introduction

Hypertension is a well-known cause of cardiovascular morbidity and mortality in Western countries and puts a heavy burden on the health care system. Among adults in a general population of Western countries, prevalence of hypertension is about 6%. The prevalence of hypertension increases with age. Besides increasing prevalence with age, the nature of hypertension changes with age.[1] Hypertension among a middle-aged population is predominantly a diastolic hypertension or a mixed systolic and diastolic hypertension.[2] Elderly subjects with hypertension, on the other hand, often only have elevated systolic blood pressure levels with normal or even low diastolic blood pressure levels.[1,3] This type of hypertension is known as isolated systolic hypertension and is accompanied by an elevated pulse pressure.[4]

Traditionally, isolated systolic hypertension was seen as a normal and innocent consequence of ageing and attention was primarily focused on the harmful effect of elevated diastolic blood pressure. Recently, arising awareness of the potential increased risk associated with isolated systolic hypertension and the accompanying elevated pulse pressure level[5,6] has led to an extensive number of prognostic studies on this topic.[7-12] These uniformly showed that isolated systolic hypertension and an elevated pulse pressure are associated with an increased cardiovascular risk. Recent trials on the effect of treatment of isolated systolic hypertension in elderly subjects showed a large benefit in terms of reduced cardiovascular risk associated with treatment.[13-15]

The aetiology of isolated systolic hypertension and elevated pulse pressure level lies in stiffening of the arterial tree.[4,16] A healthy arterial system comprises of arteries with elastic vessel walls, capable of dampening the pressure wave created by the beating heart. This feature of the arterial system is known as the Windkessel function and facilitates a steady blood flow throughout the body despite a pulsatile flow generated by the beating heart. When the elastic vessel wall properties of the arterial system decrease and the vessel wall becomes stiffer, the high pressure generated by the heart is no longer dampened. This results in an elevated systolic pressure and a decreased diastolic pressure and thus an elevated pulse pressure in the arterial system.

As improvement of health care in Western society has led to an enormous increase in life expectancy, resulting in an increasing number of elderly subjects, it is important to focus on age-related conditions that could be a cause of serious morbidity in elderly subjects. This includes stiffening of the arterial tree. Recently, accurate methods to non-invasively measure arterial stiffness have become available. Several relatively small studies, mostly in selected patient groups, have

explored determinants of arterial stiffness and the association of stiffening of the arteries with cardiovascular disease. The process of arterial stiffening has sparsely been evaluated in population-based studies.

The aim of this thesis is to study causes and consequences of stiffening of the arterial tree. The studies presented in this thesis are primarily based on the Rotterdam Study, a population-based cohort study among elderly subjects.

Chapter 2 describes two non-invasive methods used to assess arterial stiffness in the Rotterdam Study, and discusses their strengths and limitations. Insight is given in the distribution of arterial stiffness in elderly subjects according to age, gender and blood pressure. Finally, the association between both measures of arterial stiffness is evaluated. Chapter 3 focuses on abnormalities in glucose metabolism in relation to arterial stiffness. In chapter 3.1, components of the insulin resistance syndrome are related to arterial stiffness in healthy middle-aged women. This chapter is based on a population-based study among middle-aged women living in the town Zoetermeer in the Netherlands. Chapter 3.2 examines whether an impaired glucose metabolism is associated with increased arterial stiffness in elderly subjects. The association of arterial stiffness with atherosclerosis is described in chapter 4. The studies described in chapter 5 concern arterial stiffness in relation to cardiovascular disease. In chapter 5.1, the association of arterial stiffness with history of myocardial infarction or stroke is described. In chapter 5.2, stiffness of the aorta is related the calcifications of the coronary arteries. The consequences of aortic stiffness on cardiac oxygen supply and demand are described in chapter 5.3. Finally, chapter 6 focuses on the consequences of arterial stiffness for assessment of blood pressure. In chapter 7, the main results of the studies described in this thesis are reviewed and the strengths and limitations of the studies performed are discussed. This chapter concludes with discussing causes and consequences of arterial stiffness in light of the main findings and provides suggestions for future research on the topic.

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CHAPTER 2

Arterial stiffness in an elderly population

INTRODUCTION

Recently, accurate methods to non-invasively measure arterial stiffness have become available. Two of the most frequently used methods are the measurement of pulse wave velocity over a certain part of the arterial tree and the measurement of changes is arterial diameter due to changes in arterial pressure over the cardiac cycle at one specific point in the arterial tree. These methods to measure arterial stiffness were incorporated in the third examination phase of the Rotterdam study, a population-based cohort study in elderly subjects. The purpose of this chapter is to describe both measures of arterial stiffness in detail and to provide general epidemiological data. Values of arterial stiffness in the population of elderly subjects are shown according to age, gender and blood pressure. Finally, we present the association between the two different measures of arterial stiffness. Before addressing arterial stiffness in the Rotterdam Study, some background information on elastic properties of the arterial wall is given.

ELASTIC PROPERTIES OF THE ARTERIAL WALL

An artery is a viscoelastic tube, whose diameter varies with pulsating pressures due to contraction of the heart. The function of the arterial system is to deliver an adequate, constant amount of blood to tissues and organs. To ensure an adequate blood flow throughout the cardiac cycle, the pulsatile blood flow generated by the heart must be converted into a steady blood flow to the peripheral circulation. This function is sometimes described as the cushioning function of arteries and is enabled by the viscoelastic properties. For a full interpretation of the elastic behaviour of the arterial wall, knowledge about the composition of the elastic material is required.

The main elastic materials of the arterial wall are collagen and elastin. Another major constituent of the arterial wall is smooth muscle which, while contributing to the tension in the wall, cannot properly be regarded as elastic material. The arterial wall consists of three concentric regions: the tunicas intima, media, and adventitia. Demarcation between the tunicas intima and media is provided by the lamina elastica interna, a structure that consists of a fenestrated membrane of elastin lined on the intimal side by a coarse fibrous network. The lamina elastica externa demarks the tunica adventitia. The inner layer of an arterial wall, the tunica intima, consists of vascular endothelium. The outer layer of an arterial

wall, the tunica adventitia, is a region of collagen and some elastin tissue that merges with the surrounding connective tissue. The mid layer, the tunica media, forms the large part of the arterial wall and is the principal determinant of its mechanical properties. At physiological distending pressure, the tunica media consists of elastin and collagen fibers and smooth-muscle cells that are precisely oriented and form well-defined layers. The distribution of elastin and collagen in the arterial wall differs strikingly between the central and peripheral arteries.[1] In the proximal aorta elastin is the dominant component, in the distal aorta the content reverses, and in peripheral arteries collagen dominates. Elastin is much more elastic than collagen, so with increasing distance from the heart, the arteries become stiffer. The smooth muscle content increases with increasing distance from the heart.

With increasing age, certain histologic changes are seen in the arterial wall. The principal changes occur in the tunica intima and media. Endothelial cells of the intima become more irregular in size and shape. The subendothelial layer becomes thickened with an increase in connective tissue content. In the media, the elastic fibers and laminas lose the orderly arrangement seen in earlier live and display thinning, splitting, fraying and fragmentation. Degeneration of elastic fibers is associated with an increase in collagenous fibers and ground substance, and often with calcium deposition in degenerate elastic material. These changes in arterial wall structure lead to increased stiffness with age.[2]

The efficiency of the cushioning function of arteries depends on the viscoelastic properties of the arterial wall. These viscoelastic properties are frequently described in terms of compliance, distensibility or stiffness, which is the inverse of distensibility. Under normal conditions, about 40% of the stroke volume, generated by a heart beat, is forwarded directly to the peripheral arteries during systole and the remainder 60% is stored in the capacitive arteries, like the aorta and other large arteries. During this process, the arterial walls are distended and part of the energy is stored. During diastole, the stored energy recoils the capacitive arteries, squeezing the stored blood forward in the peripheral circulation. When the wall of arteries become stiffer, the cushioning function is altered and the energy needed for arterial distension during systole increases while the storage capacity decreases. This results in a higher proportion of the stroke volume that is forwarded directly to the peripheral circulation and thus an exaggerated peak systolic pressure. As less blood is stored in the capacitive arteries, the elastic recoil during diastole is decreased resulting in a decreased diastolic blood pressure. The increase in systolic blood pressure and the decrease in diastolic blood pressure both lead to an increased pulse pressure.

Increased pulse pressure as consequence of increased arterial stiffness can also be caused by another mechanism. Ventricular ejection generates a primary pulse wave travelling from the heart to the peripheral circulation with a given speed known as the pulse wave velocity. The primary pulse wave is reflected at any structural or geometric point in the arterial tree, resulting in a second pulse wave travelling backwards towards the heart. The actual pulse at a certain point in the arterial system is a summation of the forward travelling primary pulse wave and the backward travelling reflected pulse wave. In young subjects, the primary and reflected pulse waves meet during diastole resulting in an increase (upstroke) of the diastolic pressure (Figure 1, upper figure). This increase in diastolic pressure promotes coronary perfusion during diastole. As arteries become stiffer at older age, the velocity of the pulse wave increases, as a result of which the primary and reflected pulse waves meet during systole thereby increasing the systolic blood

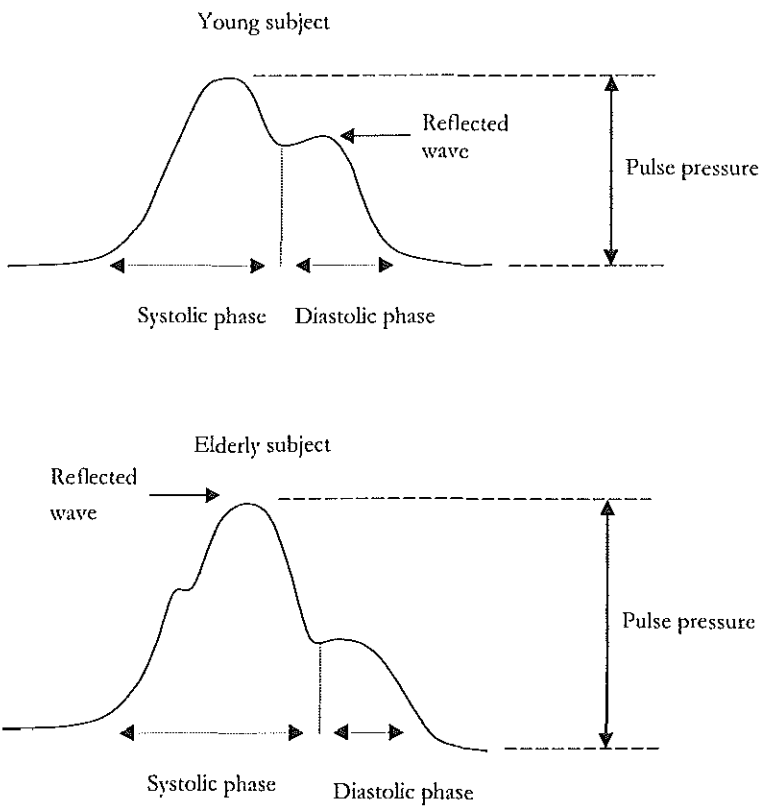


Figure 1
Schematic representations of the aortic pulse wave in a young subject (upper figure) and in an elderly subject (lower figure).

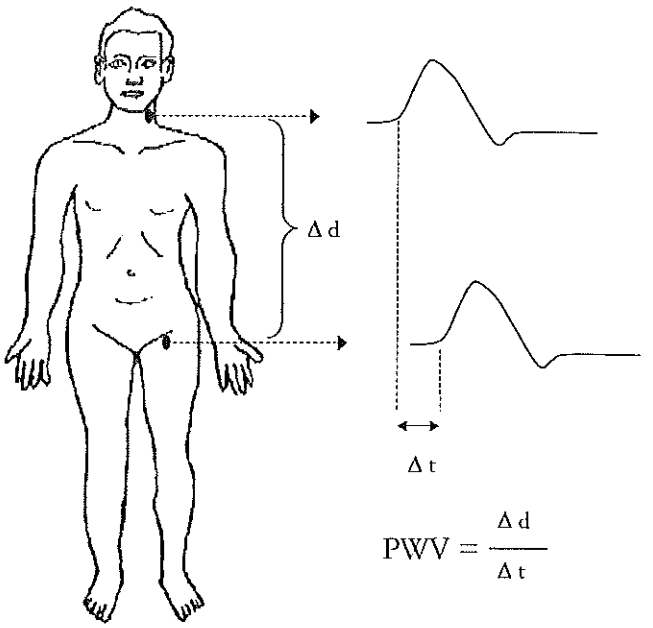
pressure. Due to the lack of an upstroke during diastole, the diastolic blood pressure is decreased (Figure 1, lower figure).

Arterial stiffness measurements in the Rotterdam Study

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 measurement. The two methods to measure arterial stiffness were performed on the same day in the same room by two different technicians. The sequence was always the same e.g., first a measurement of carotid-femoral pulse wave velocity and approximately ten minutes later, during which other measurements were performed, a measurement of distensibility of the common carotid artery. During the complete session, the subjects were in supine position.

Carotid-femoral pulse wave velocity

After five minutes of rest in supine position, blood pressure was measured twice with a conventional sphygmomanometer and the mean was taken as the subjects reading. Subsequently pulse wave velocity was measured using an automatic device (Complior, Colson, Garges-lès-Gonesse Cx, France).[3] Some technical aspects of this measurement are provided in the appendix of this chapter. A schematic representation carotid-femoral pulse wave velocity measurement is shown in Figure 2. The pulse wave in the carotid artery and the pulse wave in the femoral artery were simultaneously recorded and displayed on a computer screen. The time delay between the two simultaneously recorded pulse waves is assessed by determining the time delay between the rapid upstrokes of the simultaneously recorded pulse waves. The time delay is measured automatically by the computer software. The distance travelled by the pulse wave between the carotid artery and the femoral artery was measured over the surface of the body using a tape measure. Pulse wave velocity is then calculated as the ratio of the distance travelled by the pulse wave and the foot-to-foot time delay between the pulse waves and expressed in meters per second. Pulse wave velocity varies with respiration as respiration modifies the intra-thoracic pressure, the vessel wall tensions and thus the arterial blood pressure. For this reason, the average of at least 10 successive measurements, to cover a complete respiratory cycle, was used in the analyses.



Δ d = distance between the carotid artery and the femoral artery

Δ t = time delay between arrival of the pulse wave in the carotid artery and the femoral artery

Figure 2
Schematic representation of measurement of carotid-femoral pulse wave velocity (PWV).

Three observers performed all measurements. A reproducibility study was performed in 47 subjects who were invited twice exactly one week apart and had their pulse wave velocity measured by three observers at both occasions. Mean pulse wave velocity (standard deviation) over all measurements was 14.1 (2.8). The limits of agreement were ± 3.8 m/s, indicating that the difference in two measurements within one subject lies with 95% certainty within ± 3.8 m/s. The coefficient of variation was 9.9% and the intra-class correlation coefficient was 0.80.

Strengths of measuring carotid-femoral pulse wave velocity

An important strength of the carotid-femoral pulse wave velocity measurement is that it is a simple, accurate and reproducible method, which can be performed quickly with relatively inexpensive equipment. Carotid-femoral pulse wave velocity can be performed within ten minutes. In comparison with other methods

to measure arterial stiffness, which require ultrasound or even MRI, a measurement of carotid femoral pulse wave velocity requires a relatively simple computer with a software application and two connected tonometers. The measurement is easy to perform and does not require extensively trained personnel. Our study showed a good reproducibility, which is in agreement with other reproducibility data.[3]

Another advantage of carotid-femoral pulse wave velocity is that it measures stiffness over a large part of the arterial tree, thereby providing a measure of general stiffness of that part of the arterial tree. Other methods, which measure arterial stiffness at one specific point in the arterial tree, could have the disadvantage of that specific point not being representative of a larger arterial area. For example, presence of an atherosclerotic plaque might affect the elastic properties of the arterial wall at a specific point. This is also a problem when interest is specifically in measuring arterial stiffness at one specific point, for example when repeated measurements over time are performed to measure progression of arterial stiffness. The presence of an atherosclerotic plaque would then interfere with accurate measuring of progression of arterial stiffness, independent of progression of atherosclerosis.

Limitations of measuring carotid-femoral pulse wave velocity

The carotid-femoral pulse wave velocity method has several limitations as well. Firstly, carotid-femoral pulse wave velocity combines measurement of elastic arteries (proximal aorta) with more muscular arteries (distal aorta, the iliac artery and the femoral artery), making it impossible to evaluate differences in elastic properties or differences in determinants of the process of arterial stiffening between elastic arteries and more muscular arteries. The contribution of smooth muscle to the elastic properties of arteries has been a controversial topic. Historically, the wall tension due to the distending blood pressure was thought to be very high and it did not seem likely that tension generated by vascular smooth muscle would be sufficient to alter this.[1] It has been shown however, that the tension generated by vascular smooth muscle is much higher than originally calculated[4] and that substances that alter vessel wall tone also influence arterial elastic properties.[5] With increasing interest in drugs that alter vascular tone as possible treatment for increased arterial stiffness, evaluating differences in elastic properties between arteries with different amount of vascular smooth muscle may be of great interest.

Secondly, the pulse waves in the carotid artery and femoral artery travel in opposite directions, while measurement of carotid-femoral pulse wave velocity

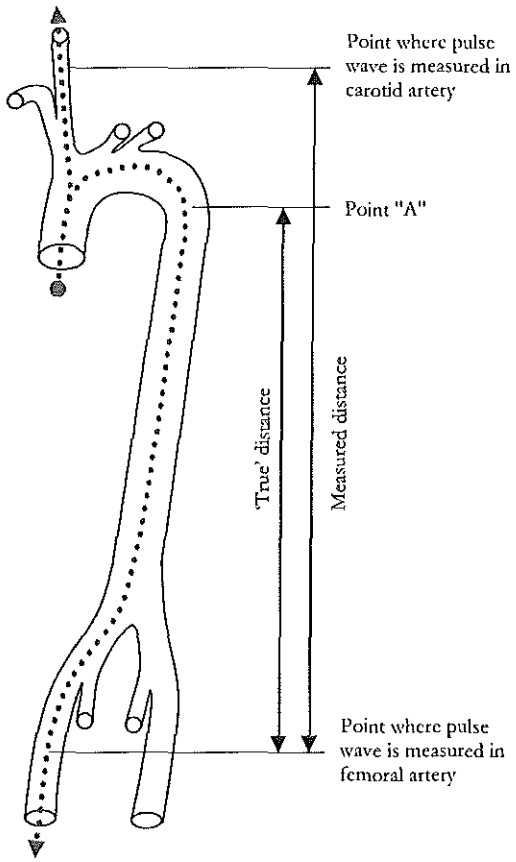


Figure 3
Schematic representation measuring the distance when assessing carotid-femoral pulse wave velocity. Point "A" indicates the position of the counterpart of the carotid pulse wave, at the moment the carotid pulse wave is measured.

is based on the assumption that the pulse wave travels from carotid artery to the femoral artery. The effect of this incorrect assumption is mainly an overestimation of the distance between the sites of the pulse waves belonging to the estimated time-delay, resulting in overestimation of the velocity of the pulse waves (Figure 3). From aortic valve to the origin of the carotid artery, the pulse wave travels in one direction. Then part of the pulse wave travels up the carotid artery and part of the pulse wave continuous down the descending aorta. The moment we measure the pulse wave in the carotid artery, its counterpart has descended in the thoracic aorta to a certain "point A". The pulse wave then continuous its way down the descending aorta until it is measured in the femoral artery. The correct distance, belonging to the measured time-delay between the pulse waves, is the distance from "point A" to the point in the femoral artery where the pulse wave is measured. This distance is shorter then the distance measured between the carotid artery and the femoral artery. Because variations in anatomy are limited,

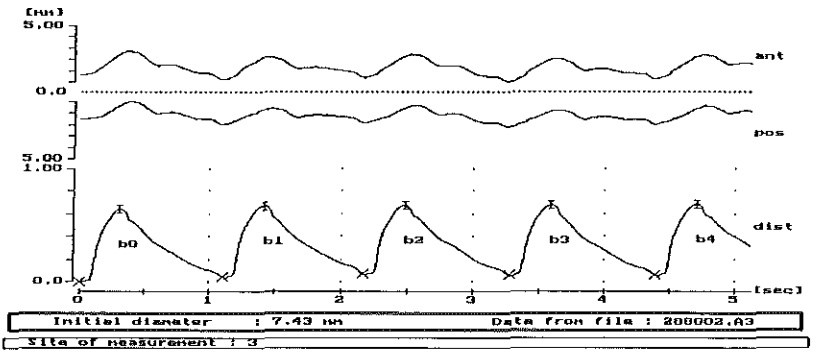
this error can be considered similar for all subjects and therefore will probably not have seriously biased our results. Another point concerning the measurement of the distance is that the distance is measured over the surface body, thereby being dependent on body build. The measured distance between the carotid artery and the femoral artery in two subjects with the same body height will be considerably shorter in a thin subject than in a more obese subject. This may lead to bias in the results. As far as we know, there are no studies relating body build to the course of the aorta. Therefore, it is very difficult to assess the consequences of measuring the distance over the body surface.

Distensibility of the common carotid artery

The vessel wall motion of the right common carotid artery was measured by means of a Duplex scanner (Ultramark IV, ATL, Bothell, Washington, USA) and a data-acquisition system connected to a personal computer (vessel wall movement detector system).[6,7] Some technical aspects of this measurement are provided in the appendix of this chapter. Shortly, the system enables the transcutaneous assessment of the displacement of 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. The procedure provides data on end-diastolic diameter (diam), the absolute change in lumen diameter during systole (dist), and the change in lumen diameter relative to its end-diastolic diameter (dist/diam) for each captured heart beat (Figure 4). In the analyses, we used mean values of maximal four cardiac cycles during a maximum of three successive recordings. Blood pressure was measured with a Dinamap automatic blood pressure recorder, and read 2 times at the right upper arm during the measurement session. The mean was taken as the subjects reading. Pulse pressure was defined as the difference between systolic and diastolic blood pressure. The cross-sectional arterial wall distensibility coefficient is calculated according to the following equation[7,8]:

$$\text{Distensibility coefficient} = (2\ \text{dist/diam})/\ \text{pulse pressure}\ (10^{-3}/\text{kPa})$$

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. Three observers performed all measurements. A reproducibility study was performed in 47 subjects who were invited twice exactly one



beat	dist (µm)	diam (mm)	dist (%)	dA/A (%)	RR-int (ms)	rise-time (ms)	ECG-10% (ms)
0	637	7.43	8.58	17.88	1100	143	97
1	634	7.47	8.49	17.71	1065	145	91
2	612	7.49	8.16	17.00	1110	142	96
3	624	7.48	8.34	17.38	1115	143	91
4	630	7.48	8.42	17.54	---	146	93
mean	627	7.47	8.40	17.50	1098	144	94
stdev	10	0.02	0.16	0.34	23	2	3

Figure 4
Example of common carotid arterial distensibility measurement.
The two upper lines show the movement of the anterior (ant) and posterior (post) arterial wall during the cardiac cycle. The bottom line shows the change in lumen diameter resulting from the movement of the arterial walls. The program automatically calculates several parameters for every heartbeat among which the absolute change in lumen diameter (dist in µm), the end-diastolic lumen diameter (diam in mm) and the change in lumen diameter relative to its end-diastolic diameter, which indicates the relative distension of the artery (dist/diam = dist in %).

week apart and had their common carotid distensibility coefficient measured by three observers at both occasions. Mean distensibility coefficient (standard deviation) over all measurements was 10.8 (4.9). The limits of agreement were ± 6.7 (10^{-3}/kPa), indicating that the difference in two measurements within one subject lies with 95% certainty within ± 6.7 (10^{-3}/kPa). The coefficient of variation was 22.5% and the intra-class correlation coefficient was 0.80. In the studies presented in this thesis, 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).

Strengths of measuring common carotid artery distensibility

The method of assessing common carotid artery distensibility with the vessel wall movement detector system has several strengths. Our reproducibility study showed that the end-diastolic diameter, the change in diameter, and the disten-

sibility coefficient can be assessed reliably, which is in agreement with another reproducibility study.[7] In comparison with other ultrasonographic methods, this method is rather insensitive to interaction of echo-signals from closely related objects as the vessel wall movement detector system is not based on only amplitude tracking, but on detecting phase differences in the radio-frequency (RF) signal, making the measurement of changes in arterial diameter more accurate.[6,7] The off-line analysis has the specific advantage of restricting recording time.[7] Another advantage of this method is that the method assesses arterial distensibility at one specific site in the arterial tree making it possible to compare structural vessel wall changes of different arteries in relation to determinants and disease.

Limitations of measuring common carotid artery distensibility

A disadvantage of measuring vessel wall properties at one specific point is that local changes might affect the elastic properties of that part of the vessel wall. This could result in that specific point not being representative of a larger arterial area, as discussed before.

There are some other limitations of measuring common carotid arterial distensibility. Firstly, assumptions are made about the circular cross-sectional nature of vessels, which may not always hold true.[9] Another assumption is that the increase in arterial volume during systole is caused by distension (increase in cross-sectional area) rather than elongation.[10] This assumption seems to be reasonable because under normal circumstances no lengthening of the artery is seen on B-mode images during systole.[8] 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 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.[10] 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.[1] On the other hand, non-invasive cuff-based measurement of blood pressure underestimates pulse pressure.[11] Several groups compared non-invasively measured elastic aortic properties or systemic vascular resistance, based on methods that use of brachial pulse pressure in stead of aortic pulse pressure, with invasive techniques or post-mortem measurements.[12-14] These studies support the validity of using of brachial pressures as a proxy of aortic pressures. To the best of our knowledge, there are no studies evaluating the validity of using the brachial pulse pressure in

stead of carotid pulse pressure. However, the aorta is a more central artery than the carotid artery. If using brachial pressures in stead of aortic pressures is reasonably valid, the bias introduced by using brachial pressures in stead of carotid pressures probably will be limited also. Finally, the distensibility coefficient indicates distension of an artery relative to its diameter. The diameter of an artery can change through the influence of various processes that are likely to be associated with arterial stiffness, like aging and atherosclerosis. This might introduce bias in the estimate of arterial stiffness.

ARTERIAL STIFFNESS IN AN ELDERLY POPULATION

Study population

A total of 4148 subjects was eligible for a measurement of arterial stiffness at the third follow-up examination phase of the Rotterdam Study. Of these subjects, 3550 subjects (86%) had a measurement of carotid-femoral pulse wave velocity and 3098 subjects (79%) had a measurement of distensibility of the common carotid artery. Missing information on arterial stiffness was almost entirely due to logistic reasons. Of the 3550 subjects with a measurement of pulse wave velocity, 69 subjects (1.9%) were excluded from the analyses because the variation between the successive pulse wave velocity measurements was more then 10% or a minimum of 10 successive measurements, to cover a complete respiratory cycle, was not reached. Thus, 3481 subjects with a measurement of carotid-femoral pulse wave velocity were available for analysis. None of the subjects with a measurement of common carotid distensibility were excluded from the analyses. Information on both measures of arterial stiffness was available for 2766 subjects. For evaluating the distribution and values of both measures of arterial stiffness, we included all subjects with information on both methods.

Statistical analysis

The distribution of both measures of arterial stiffness was assessed in strata of gender. Mean values were calculated per 5-years age categories in strata of gender and per 10 mmHg categories of systolic blood pressure, diastolic blood pressure, mean blood pressure and pulse pressure for the total cohort, using analysis of variance. Mean blood pressure was defined as diastolic blood pressure + 1/3 * (systolic blood pressure - diastolic blood pressure). Pulse pressure

was defined as systolic blood pressure - diastolic blood pressure. To avoid small numbers in a particular category, some subjects with values at the lower or upper end of the distribution were assigned to the nearest higher or lower category. Differences in mean pulse wave velocity and mean distensibility coefficient between men and women per 5-years age-strata were tested using analysis of variance.

Results

Characteristics of the subjects are shown in table 1. Levels of cardiovascular risk factors were in the high normal range, as expected in a general population of elderly subjects. In figure 5, the distribution of carotid-femoral pulse wave velocity and the distribution of the common carotid distensibility coefficient are shown for men and women separately. The mean value of both carotid-femoral pulse wave velocity and the common carotid distensibility coefficient was lower in females than in males, indicating less aortic stiffness and a stiffer common carotid artery in females as compared to males. When we adjusted the differences in arterial stiffness between women and men for age, mean blood pressure, heart rate, and body height, the differences became even more pronounced and

Table 1
Characteristics of study population (n = 2766).

Characteristic	Value
Age (years)	71.8 (59.7 - 101.0)
Men (%)	42
Systolic blood pressure (mmHg)	143 ± 21
Diastolic blood pressure (mmHg)	75 ± 11
Body mass index (kg/m^2)	26.7 ± 3.8
Waist-to-hip ratio	0.92 ± 0.1
Smoking	
current (%)	37
past (%)	50
Total cholesterol (mmol/l)	5.8 ± 1.0
HDL-cholesterol (mmol/l)	1.4 ± 0.4
Serum glucose (mmol/l)	5.9 ± 1.5
Distensibility coefficient (pa^{-1})	10.6 ± 4.4
Pulse wave velocity (m/s)	13.4 ± 3.0

Values are expressed as mean ± standard deviation or as percentage.
Age is given as mean (range).

were highly significant for both arterial sites. The difference (95% confidence interval) in pulse wave velocity was -0.99 (-0.81 to -1.18) (m/s) and the difference in distensibility coefficient was -1.62 (-1.35 to -1.82) (10^{-3}/kPa) for women as compared for men, after adjustment for age, mean blood pressure, heart rate, and body height. Figure 6 shows the mean values of carotid-femoral pulse wave velocity and common carotid distensibility per 5-years age-categories in strata of gender. Pulse wave velocity increased non-linearly with increasing age. The distensibility coefficient decreased non-linearly with increasing age. With advancing age, both aorta and carotid artery became stiffer but the increase in stiffness of both arteries attenuated at high age. Women under 80 years of age had a significantly less stiff aorta and a significantly stiffer common carotid artery as compared to men of the same age, except for common carotid arterial stiffness in

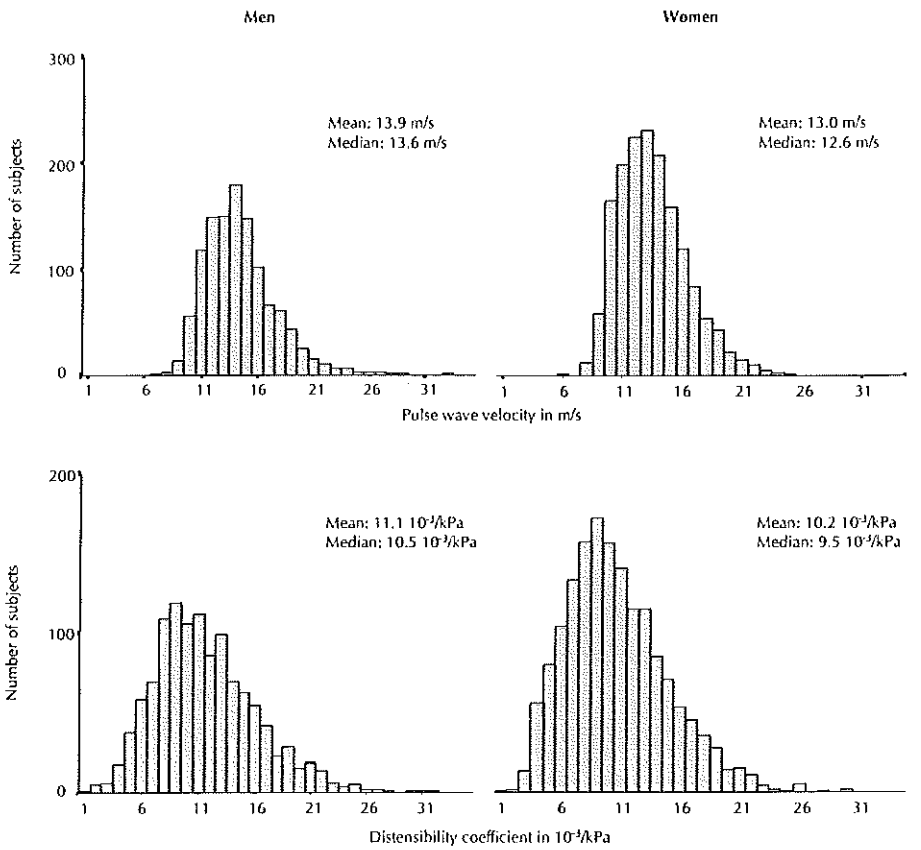


Figure 5
Distribution of carotid-femoral pulse wave velocity (PWV) and the distensibility coefficient (DC) of the common carotid artery in strata of gender.

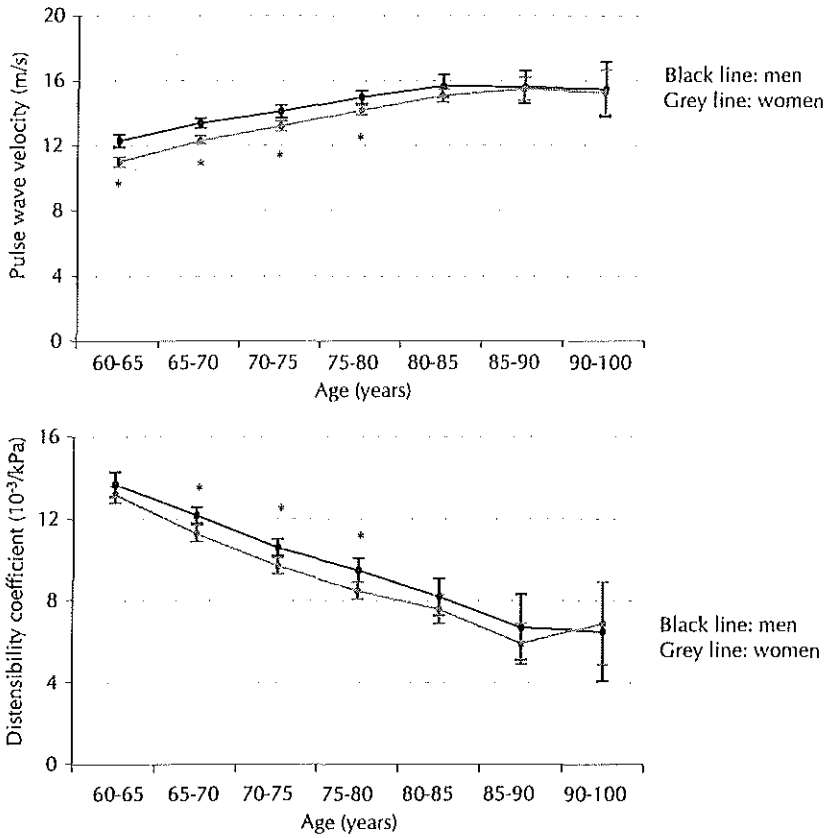


Figure 6
Mean (95% confidence interval) carotid-femoral pulse wave velocity (upper figure) and mean (95% confidence interval) common carotid distensibility coefficient (lower figure) per 5-years age category in strata of gender. * $p < 0.05$ for difference between men and women.

women of 60 to 65 years of age. Above 80 years of age, there was no significant difference in arterial stiffness of the aortic or common carotid artery between women and men. Mean values of arterial stiffness per 10 mmHg-categories of various blood pressure measures is shown in figure 7 for carotid-femoral pulse wave velocity and in figure 8 for common carotid distensibility. The relation of both measures of arterial stiffness with various blood pressure measures was in general the same for both measures but, as expected, in opposite directions. The relations of both measures of arterial stiffness with systolic blood pressure and pulse pressure were non-linear, flattening off at higher blood pressures. The association of arterial stiffness with mean arterial blood pressure in the total population was nearly linear and only levelling off when the mean arterial blood pressure was above 140 mmHg. The relation between both measures of arterial

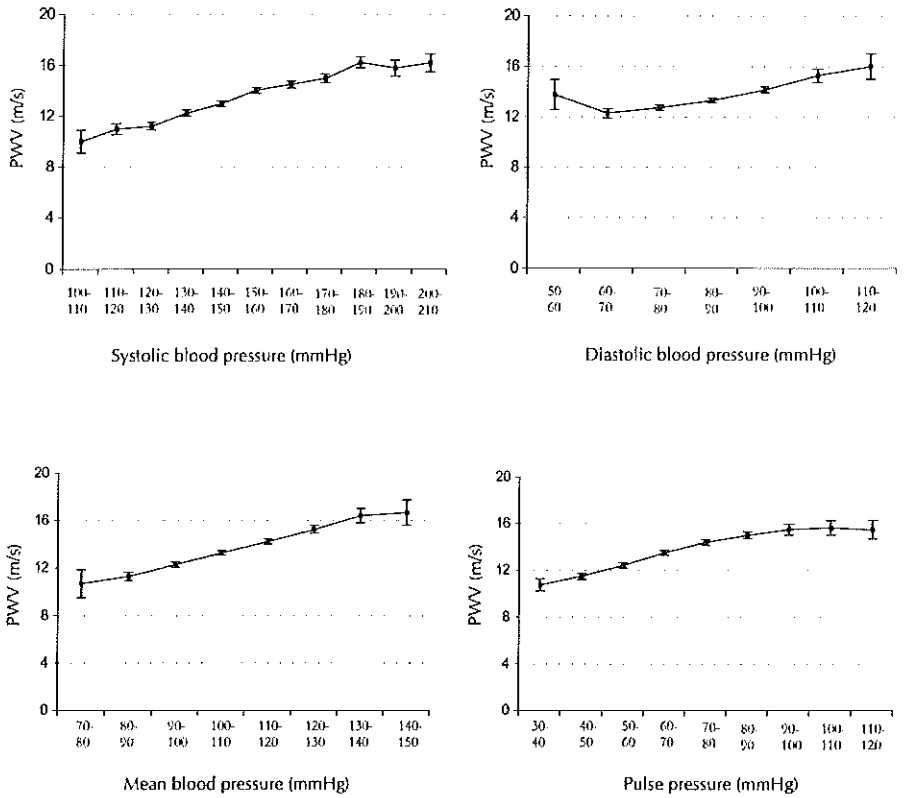


Figure 7
Mean (95% confidence interval) carotid femoral pulse wave velocity (PWV) per categories of 10 mmHg of systolic blood pressure (upper left figure), diastolic blood pressure (upper right figure), mean blood pressure (lower left figure), and pulse pressure (lower right figure).

stiffness with diastolic blood pressure was non-linear and resembled a J-shape relation, especially for pulse wave velocity. The first (lowest) diastolic blood pressure category showed slightly increased arterial stiffness as compared to the second diastolic blood pressure category (60-70 mmHg). From the second diastolic blood pressure category onwards, vessel wall stiffness increased with increasing diastolic blood pressure. The difference in mean pulse wave velocity between the first and second diastolic blood pressure category was significant (mean difference in m/s (95% confidence interval): 1.49 (0.24-2.74)).

Discussion

We found the aorta to be stiffer in men than in women but the common carotid

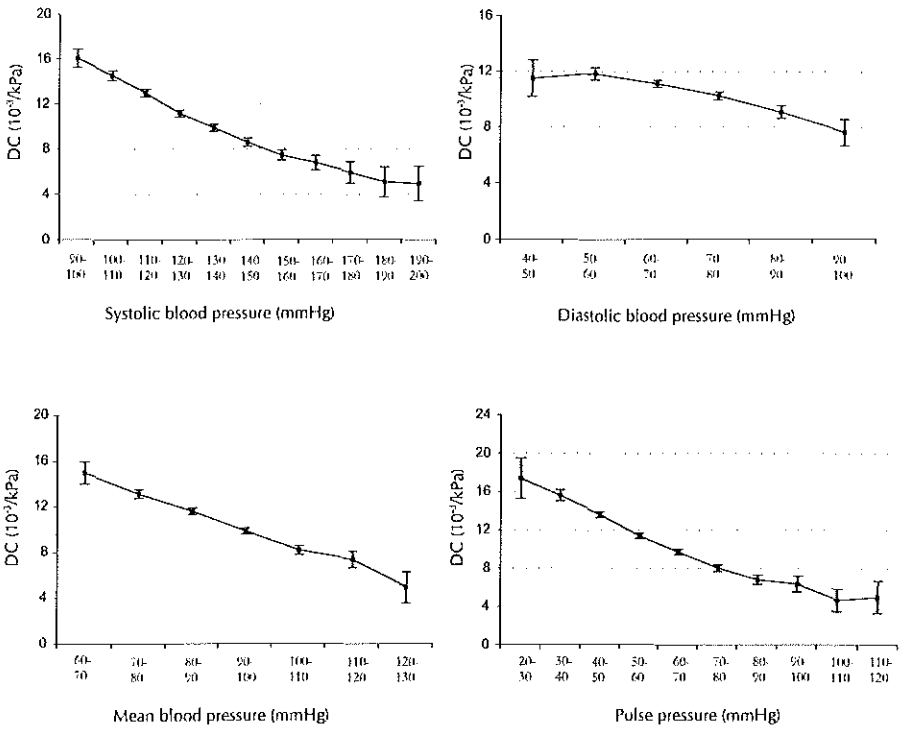


Figure 8
Mean (95% confidence interval) common carotid distensibility coefficient (DC) per categories of 10 mmHg of systolic blood pressure (upper left figure), diastolic blood pressure (upper right figure), mean blood pressure (lower left figure), and pulse pressure (lower right figure).

artery to be stiffer in women than in men in subjects under 80 years of age. In previous studies, differences in arterial stiffness between men and women have also been found and findings between studies were contradictory. One study in 600 subjects under 65 years of age found the aorta to be stiffer in men[15] whereas two other studies, in subjects aged 18 to 77 years, found no clear sex-differences in stiffness of the aorta between men and women in respectively 83 and 418 subjects.[3,16] In a study among young subjects, the carotid artery has been found to be stiffer in male subjects as compared to female subjects.[17] Another study in 119 subjects found the carotid artery to be stiffer in men as compared to women only in subjects younger than 15 years and subjects 70 years of age, while men and women with an age in between were comparable with respect to carotid arterial stiffness.[18] In 100 subjects aged 6 to 25 years, significantly larger diameter increase of the common carotid artery over the cardiac cycle was found in male subjects as compared to female subjects, suggesting a stiffer common carotid

artery in males.[19] However, in the same study no difference was found in the common carotid elastic modulus between males and females.[19] Another study in 109 subjects found a lower distensibility of the common carotid artery in females as compared to males, irrespective of age.[20]

There are possible explanations for the observed gender-differences between studies. Firstly, menopause is associated with an increase in arterial stiffness.[21] Therefore, observed gender-differences across studies might be explained by differences in inclusion of pre- or postmenopausal women between studies. The differences in age of women included in the previous studies, however, does not seem to fully explain the observed gender-differences between studies. One study explored whether female sex hormones could explain the differences but could not disclose an effect of the menstrual cycle on arterial wall properties.[22] This study, however, included only 12 subjects, and had little power to show an influence of hormones during the menstrual cycle on arterial stiffness. Secondly, the process of arterial stiffening could be heterogeneous across various parts of the arterial tree due to a different effect of gender specific factors, like hormones, on different parts of the arterial tree. Thirdly, methods used to assess arterial stiffness differed between some of the studies. When the different methods measure different elastic wall properties and these are differently related to gender, this could lead to gender-differences across studies. However, gender-differences in arterial stiffness have also been discrepant between studies that used the same method to assess arterial stiffness.[18,20] Moreover, even if different methods measure different elastic wall properties, these are likely to be highly correlated.

Most previous studies on the association between gender and arterial stiffness were performed on relatively small numbers of subjects (n≈100) and may have had not enough power to disclose an association. Our results in a large population of nearly 2800 subjects showed that in a general population of subjects aged 60 years and over, women have a stiffer common carotid artery as compared to men while the reverse is true for stiffening of the aorta. The finding of inconsistent gender-differences across arterial sites may indicate that stiffening of the arterial tree is not a generalised process. Large population-based studies in younger subjects on the association between gender and arterial stiffness at several sites of the arterial tree would help to elucidate the influence of gender on stiffening of various arterial sites. These studies should preferably include the perimenopausal period.

In agreement with previous studies[3,16,23,24] we found increasing stiffness of the aorta and increasing stiffness of the common carotid artery with increasing age.

The increase in stiffness of both arteries with advancing age levelled off at very old age, which could be due to the fact that at very old age nearly everyone has developed stiff arteries, thereby limiting a further linear increase in arterial stiffness with age. Another explanation could be selective loss of the subjects with the stiffest arteries in the higher age-categories due to fatal cardiovascular disease. Also in concordance with other studies[3,16,24-26], we found increasing stiffness of the aorta and the common carotid artery with increasing systolic and diastolic blood pressure, pulse pressure and mean arterial blood pressure. The increase in stiffness of both arteries with increasing blood pressure attenuated at high pressures. This probably reflects maximal dilatation and stretching of collagen fibers at high blood pressures and thus maximal stiffness of the arterial wall. The strong association between arterial stiffness and blood pressure may be explained by a self-perpetuating, reinforcing process that originates from high blood pressure being a determinant of arterial stiffening and arterial stiffening in its turn being a determinant of increased blood pressure.[27] We found a tendency towards increased arterial stiffness of both arteries at very low diastolic blood pressures, which was significant for carotid-femoral pulse wave velocity. This supports the view that arterial stiffness, besides leading to an increase in systolic blood pressure, also leads to a decrease in diastolic blood pressure.[27] An increase in diastolic blood pressure leads to an increase in mean arterial pressure. A slightly decreased diastolic blood pressure due to increased arterial stiffness apparently does not reverse a positive association between increased diastolic blood pressure, and thus increased mean arterial pressure, and arterial stiffening. When we adjusted the association between aortic stiffness and diastolic blood pressure for mean arterial blood pressure, the association became negative, and similarly the association between common carotid arterial stiffness and diastolic blood pressure was positive when adjusted for mean arterial blood pressure (data not shown).

Association between both measures of arterial stiffness

Statistical analysis

The association between both measures of arterial stiffness was evaluated using multiple linear regression analyses, with the common carotid distensibility coefficient as dependent and carotid-femoral pulse wave velocity as the independent variable and by calculating the correlation between pulse wave velocity and the

distensibility coefficient. We also calculated the relative risk of having increased common carotid arterial stiffness for subjects with increased aortic stiffness as compared to subjects with a more distensible aorta, using logistic regression analyses. For this analysis we re-coded aortic pulse wave velocity and distensibility of the common carotid arterial in variables indicating increased arterial stiffness, which will be referred to as presence of aortic stiffness and presence of common carotid artery stiffness, respectively. Presence of aortic stiffness was graded as 0 for all subjects in the lowest quartile of pulse wave velocity and 1 for all subjects in the highest quartile of pulse wave velocity. Presence of common carotid arterial stiffness was graded as 0 for all subjects in the highest quartile of the distensibility coefficient and 1 for all subjects in the lowest quartile of the distensibility coefficient.

Results

The relationship between the common carotid distensibility coefficient and carotid-femoral pulse wave velocity was found to be quadratic: distensibility coefficient = 27.4 - 1.9 * (pulse wave velocity) + 0.04 * (pulse wave velocity)^2 [p total model $\leq$ 0.001]. The relationship is shown in figure 9. The correlation between the distensibility coefficient and pulse wave velocity was -0.41 ($p < 0.001$).

Figure 10 shows the distribution of subjects over quartiles of carotid-femoral pulse wave velocity in strata of the common carotid distensibility coefficient. Subjects with a distensible common carotid artery (4th quartile of the distensibility coefficient) were most likely to also have a distensible aorta (1st quartile of pulse wave velocity). Subjects with increased stiffness of the common carotid artery (1st quartile of the distensibility coefficient) were most likely to also have increased aortic stiffness (4th quartile of pulse wave velocity). Logistic regression analyses showed that subjects with increased aortic stiffness had a 30-fold increased risk of having increased common carotid artery stiffness (relative risk and 95% confidence interval: 31.2 (20.9 - 46.4)).

Discussion

With increasing interest in measures of arterial stiffness, both the number of reports on arterial stiffness and the number of different methods to measure arterial stiffness have increased.[9] Only one previous study examined the agreement between two different measures of arterial stiffness e.g., the carotid artery pressure-strain elastic modulus and aortic pulse wave velocity, and found a moderate

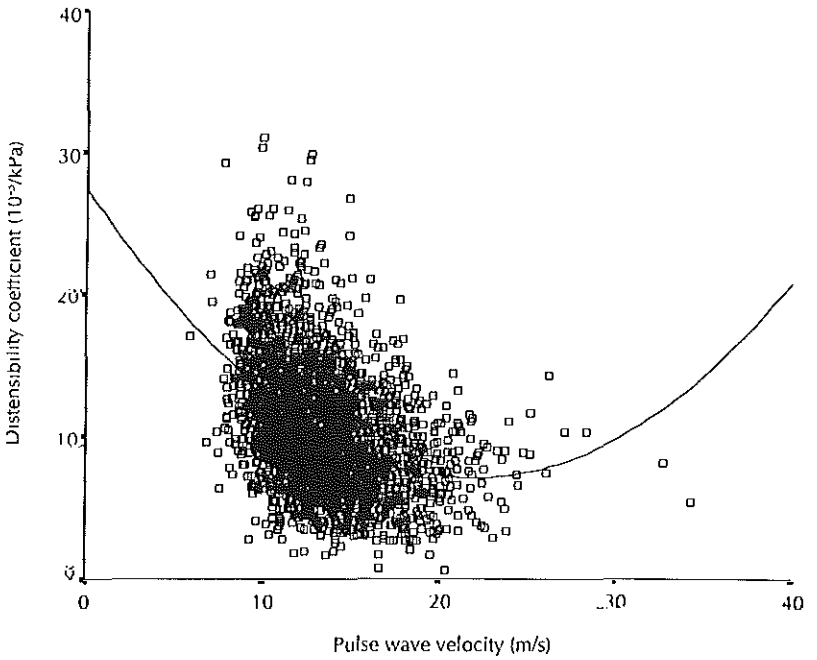


Figure 9
Relationship between the distensibility coefficient of the common carotid artery and carotid-femoral pulse wave velocity, The Rotterdam Study.

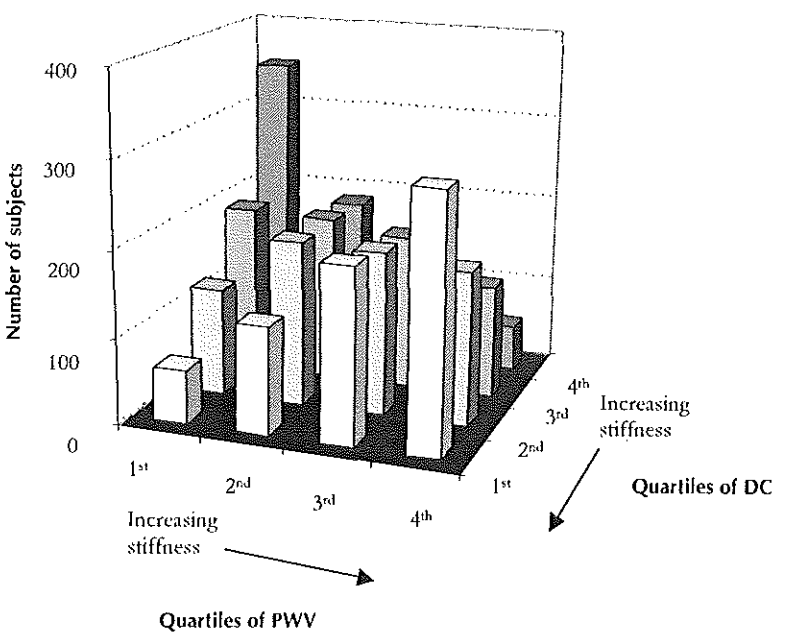


Figure 10
Distribution of subjects per quartile of aortic pulse wave velocity (PWV) in strata of quartiles of the common carotid distensibility coefficient (DC).

association between them.[24] In agreement with this study, we found a moderate association between two different measures of arterial stiffness. The correlation between the common carotid distensibility coefficient and carotid-femoral pulse wave velocity was 41%. There are several possible explanations for this moderate association. Firstly, arterial stiffness is not a generalised process throughout the arterial tree. The observed gender-difference between the aorta and the common carotid artery supports this hypothesis. Differences in arterial stiffness between different parts of the arterial tree may be due to a different composition of arterial walls with regard to amount and distribution of elastic and muscular fibres. Secondly, the methods used to assess arterial stiffness in the two different parts of the arterial tree were based on different principles that might measure different elastic wall properties, resulting in a moderate association. Thirdly, the moderate association may also partly be explained by measurement error in both methods. Finally, interpreting an association between two measurements of arterial stiffness at different moments in time is complex because blood pressure is an important determinant of arterial stiffness and is intertwined in the association. Despite the moderate linear association, however, when selecting on extremes of arterial stiffness in both arteries, agreement was remarkably high and subjects with a clearly stiffened aorta were at a high risk of also having a stiff common carotid artery.

We observed a non-linear, quadratic association between carotid-femoral pulse wave velocity and distensibility of the common carotid artery. It is conceivable that a maximal arterial stiffness as measured with one method means that arterial stiffness as measured with the other method also has reached its maximum. Also, a distensible artery as measured with one method may imply that the other method will also measure a high distensibility in that artery. This is likely to result in a quadratic relationship.

APPENDIX

Technical aspects of the Complior method

Carotid-femoral pulse wave velocity was measured with the Complior method[3], which uses a sampling acquisition frequency of 500 Hz to digitise the pressure waveforms that are subsequently stored in a re-circulating memory buffer. Preprocessing analyses automatically adjusts the gain of each waveform for an equality of the two signals. The time delay between the two simultaneously

recorded pulse waves is assessed by determining the time delay between the rapid upstrokes of the simultaneously recorded pulse waves. The rapid upstroke is chosen as the marking point of the beginning of the pulse wave because the rapid upstroke is not influenced by changes of the arterial pulse wave occurring when the pulse wave travels from heart to the periphery. First, spikes that may be present in the pulse waveform are removed, using a moving digital filtering algorithm, because they will interfere with later processing. The rapid upstroke of the pulse waves is assessed by differentiating the digitised pulse waves to determine the peak value. This occurs near the centre of the upstroke. Subsequently, the delay between the two pulse waves is determined by performing a correlation calculation between the data of the two parts of the pulse waveforms. The correlation algorithm is repeated several times after time-shifting the distal pressure upstroke by subtracting one sample period. This translation of the distal curve is performed in a working window of 588 data points per waveform, which covers a time period from 0.74 to 1.47 seconds. This is sufficient to always capture at least one complete cardiac pressure upstroke. The number of data point shifts needed to gain the best-fit correlation coefficient determines the time delay between the pulse waves.

Technical aspects of the vessel wall movement detector system

For measuring common carotid distensibility, a region at 1.5 cm proximal to the origin of the bulb of the carotid artery is identified with a 7.5 MHz transducer. An M-line perpendicular to the vessel is selected. After the echo-system is switched to M-mode storage of data starts. During 5-6 cardiac cycles radio-frequency (RF) signals are digitised and temporarily stored. The positions of the anterior and posterior vessels wall are marked by the observer with the setting of two data windows on the first RF signal stored. The data are transferred to a personal computer. The cumulative change in phase between the successive RF-lines is calculated for the anterior and posterior wall windows, where the position of the sample gates is continuously adjusted to the detected displacement. The procedure provides data on end-diastolic diameter (D), the absolute stroke change in diameter during systole (ΔD), and the relative stroke change in diameter ($(\Delta D)/D$) for each captured heart beat. With this system a wall displacement of a few micrometers can be resolved.[6] Pulse pressure is calculated from blood pressure measurement at the brachial artery and defined as systolic blood pressure - diastolic blood pressure. The cross-sectional arterial wall distensibility coefficient can be calculated with the following formula[7,8]:

distensibility coefficient = $(2\Delta D/D)/\Delta P$ (10^{-3}/kPa)

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Chapter 3

Arterial stiffness and glucose metabolism

3.1

Variables of the insulin resistance syndrome are associated with reduced arterial distensibility in healthy non-diabetic middle-aged women

Abstract

Background: *The insulin resistance syndrome is related to arterial stiffness in diabetic subjects. Whether the insulin resistance syndrome is also related to arterial stiffness in non-diabetic subjects is less clear. We studied the association between parameters of the insulin resistance syndrome in relation to arterial distensibility in healthy middle-aged non-diabetic women.*

Methods: *This study was performed in 180 non-diabetic women, aged 43-55, selected from the general population. Arterial distensibility was assessed in the carotid artery. The associations were evaluated using linear regression analyses.*

Results: *Strong associations were found between arterial distensibility and the parameters of the insulin resistance syndrome: body mass index, waist-to-hip ratio, high-density-lipoprotein-cholesterol, triglycerides, glucose, insulin, apolipoprotein A1, plasminogen activator inhibitor-1-antigen and tissue-type plasminogen activator-antigen. After additional adjustment for mean arterial pressure, common carotid arterial distensibility remained associated with body mass index: β-coefficient(95% confidence interval) per kg/m²: -0.24(-0.42;-0.06), waist-to-hip ratio: -26.62(-40.59;-12.65) per m/m, triglycerides: -1.42(-2.77;-0.08) per mmol/l, plasminogen activator inhibitor-1-antigen: -0.01(-0.02;-0.00) per ng/ml and borderline significant associated with high-density-lipoprotein-cholesterol: 1.93(-0.01;3.87; p=0.07) per mmol/l. Clustering of parameters of the insulin resistance syndrome was strongly related to decreased arterial distensibility which remained after adjustment for mean arterial pressure. No association was found between arterial distensibility and parameters that are not part of the insulin resistance syndrome: total cholesterol, LDL-cholesterol and apolipoprotein B.*

Conclusion: *The results of this study show that parameters of the insulin resistance syndrome are associated with decreased arterial distensibility of the common carotid artery in healthy non-diabetic subjects.*

INTRODUCTION

Clustering of risk factors for cardiovascular disease (hypertension, obesitas, dyslipidemia), resistance to insulin-stimulated glucose uptake, and cardiovascular disease in an individual is known as the insulin resistance syndrome.[1] The insulin resistance syndrome occurs in non-insulin-dependent diabetic patients but also in non-diabetic individuals with a normal glucose tolerance. In glucose-clamp studies in non-obese individuals with normal oral glucose tolerance resistance to insulin-stimulated glucose uptake was found in approximately 25% of these subjects.[2] These subjects secrete high levels of insulin to maintain normal glucose levels despite their insulin resistance, leading to hyperinsulinemia.

Hyperglycemic conditions lead to increased arterial stiffness by increased collagen cross linking due to non-enzymatic glycation.[3-5] Emoto and coworkers showed that insulin resistance is associated with decreased arterial distensibility in NIDDM subjects[6], but little is known about the role of insulin resistance on arterial distensibility in healthy non-diabetic subjects. As decreased arterial distensibility is associated with increased cardiovascular risk[7,8] it is of importance to know whether insulin resistance is associated with decreased arterial distensibility in healthy subjects.

The Atherosclerosis Risk in Communities (ARIC) study examined the association between fasting glucose and insulin concentrations with arterial stiffness in a large population-based setting and found these variables to be associated with increased arterial stiffness indexes. This study however, did not include all parameeters of the insulin resistance syndrome.[9] One study described an association of insulin resistance, as assessed by the euglycaemic hyperinsulinaemic clamp, with decreased arterial compliance and distensibility in healthy young subjects, which was more pronounced in women than in men.[10] This study was performed in a relatively small group of 17 men and 17 women. The objective of the present study was to examine the association between arterial distensibility and all parameters of the insulin resistance complex in 180 healthy non-diabetic women. Parameters of the insulin resistance complex included BMI, waist-to-hip ratio, HDL-cholesterol, triglycerides, apolipoprotein A1, PAI-1-antigen and tPA-antigen. Additionally, we examined whether clustering of the parameters of the insulin resistance syndrome within one subject is related to arterial distensibility.

METHODS

Study population

Our study population comprised women aged 43-55 years, selected from the general population and participating in a study on the cardiovascular effects of natural menopause. Women were selected from 6845 respondents to a mailed questionnaire about the menopause, which was sent to all women aged 40 to 60 years living in the town of Zoetermeer, The Netherlands (respons rate 54%). Exclusion criteria were diabetes mellitus, prevalent clinical cardiovascular disease, and use of antihypertensive medication or cholesterol-lowering drugs. Women reporting use of female hormones (hormone replacement therapy or oral contraceptives) within 6 months prior to the clinical examination were also excluded, as were women currently smoking 5 or more cigarettes per day. Eligible for the study were women with an early or late menopause. Of these subjects, 186 pre- and postmenopausal women, aged 43-55 years, were randomly selected. The study was approved by the medical ethics committee of the Erasmus University Medical School. All women gave informed consent.

Clinical and biochemical parameters

During an examination at the research center, a medical history was taken by a physician. Height, weight, and waist and hip circumference were measured with indoor clothes without shoes. BMI (weight in kg divided by height in m^2) and waist-to-hip ratio were computed. Alcohol drinking habits and cigarette smoking history were obtained by a standardized questionnaire. Blood pressure was assessed with a Dinamap automatic blood pressure recorder (Critikon Inc, Tampa, Florida, USA). After 5 minutes rest in supine position, blood pressure was read 4 times at the right upper arm, and the mean was used in the analyses. Hypertension was defined as a systolic blood pressure > = 160 mmHg and/or a diastolic blood pressure > = 95 mmHg. Pulse pressure (ΔP) was defined as systolic blood pressure minus diastolic blood pressure. Mean arterial pressure (MAP) was calculated by the following formula: diastolic blood pressure + 1/3 * pulse pressure.

Venous blood samples were drawn from each subject after a 12 hours fast. The samples were stored at -80°C, and subsequently serum parameters were determined using a Kone Specific Analyzer (Kone Instruments, Espoo, Finland).

Total cholesterol was measured with an automated enzymatic method[11], using the CHOD-PAP High Performance reagent kit from Boehringer Mannheim (Germany). HDL-cholesterol was measured by the phosphotungstate method according to Burstein[12] with a minor modification as described by Grove.[13] The overall coefficients of variation for total cholesterol and HDL-cholesterol were 2.9% and 3.7%, respectively. LDL-cholesterol was computed with the Friedewald formula.[14] Triglycerides were determined by using a reagent kit from Boehringer Mannheim (Germany) after enzymatic hydrolysis of the triglycerides with subsequent determination of liberated glycerol by colorimetry. No correction was made for serum free glycerol. The overall coefficient of variation of this method did not exceed 3.2%. Apolipoprotein A1 and B were measured by an automated turbidi-metric immuno-assay using the reagent kits of Orion Diagnostics (Espoo, Finland). Glucose was enzymatically determined by the Hexokinase method (Instruchemie, Hilversum, The Netherlands). Serum insulin was determined by Metric assay (Biosource Diagnostics, Fleuris, Belgium). This assay has no cross-reactivity with either pro-insulin or C-peptide. Plasminogen activator inhibitor-1 (PAI-1)-antigen and tissue-type plasminogen activator (tPA)-antigen were determined by enzyme-linked immunosorbent assays (ELISA) (Innotest PAI-1, Innogenetics NV, Zwijngaarde, Belgium and Imulyse™, Biopool, Umea, Sweden, respectively). Fasting insulin levels were used as a measure of insulin resistance.[15]

Arterial distensibility

Arterial distensibility is assessed by measuring the distensibility of the common carotid artery and expressed as the distensibility coefficient (DC). 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] 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 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. 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 ultrasonog-

raphy. Based on the B-mode recording an M-line perpendicular to the artery was selected, and the received radio frequency signals were recorded over 5 cardiac cycles and digitally stored. 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 successive values of 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 from the recording during five cardiac cycles. Blood pressure was measured with a Dinamap automatic blood pressure recorder, and read 4 times at the right upper arm during the measurement session. The mean was taken as the subjects reading. The cross-sectional arterial wall distensibility coefficient was calculated according to the following equation[18]: $DC = (2\Delta D/D)/\Delta P$ (10^{-3}/kPa).

With this system a wall displacement of a few micrometers can be resolved[16] and diameter (D), ΔD, ΔD/D, DC can be assessed reliably.[17] 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. All measurements were done by a single performer. A reproducibility study was performed in which 15 participants underwent a second examination within one month from the initial examination. The coefficients of variation for distension and the lumen diameter were 8.5% and 1.2%, respectively. 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 data).

Statistical analysis

Of the total study population of 186 subjects, data on distensibility of the common carotid artery were missing for 6 subjects; therefore the analyses were based on 180 subjects. The clinical and biochemical features of the population are presented as mean ± standard deviation, as median (interquartile range) for variables with a skewed distribution, or as percentages. Pearson's correlation coefficients or Spearman's correlation coefficients in case of variables with a skewed distribution were calculated of the clinical and biochemical parameters with MAP. The associations of arterial distensibility with the clinical and biochemical parameeters were studied using multiple linear regression analyses of the DC on each parameter separately, adjusted for age and menopausal status (model A), for age,

menopausal status, and MAP (model B) and for age, menopausal status, MAP, and insulin (model C). Model B was additionally extended with MAP-squared. Of parameters, which were still associated with arterial distensibility of the common carotid artery in model B, we calculated mean values per tertile of the DC adjusted for age, menopausal status and MAP using one way analyses of covariance. A test for trend was performed using multiple linear regression analysis with the tertiles of the DC as ordinal variable. To examine whether the strong associations of body composition variables with distensibility were mediated by arterial diameter, we additionally performed analyses with ($2\Delta D/\Delta P$) in stead of the DC as dependent variable in relation to BMI and waist-to-hip ratio, adjusted for age, MAP and D.

Finally, we evaluated whether clustering of parameters of the insulin resistance syndrome was associated with decreased arterial distensibility. For this purpose, we created a compound score referred to as clustering score. It was calculated as the sum of Z-scores of the parameters of the insulin resistance syndrome (BMI, waist-to-hip ratio, HDL-cholesterol, triglycerides, glucose, insulin, apolipoprotein A1, PAI-1-antigen and tPA-antigen). A Z-score indicates the position of an individual value of a parameter in the total distribution of that parameter in the population and is calculated as follows: (individual value - mean value)/standard deviation. The association between this variable and arterial distensibility was studied using linear regression adjusted for age and MAP. All analyses were performed using the BMDP statistical package.

Results

The clinical and biochemical characteristics of the study population are presented in table 1.

Table 2 shows the correlation between MAP and different clinical and biochemical parameters. All clinical and biochemical parameters were highly associated with MAP.

The association between clinical and biochemical parameters of the insulin resistance complex and arterial distensibility, adjusted for age and menopausal status (model A) and after additional adjustment for MAP (model B) are shown in table 3. In model A, associations were found of BMI, waist-to-hip ratio HDL-cholesterol, triglycerides, glucose, insulin, apolipoprotein A1, PAI-1-antigen, tPA-antigen with arterial distensibility of the common carotid artery. As expected,

Table 1
Baseline characteristics of 180 women aged 43 to 55 years from the Zoetermeer study population.

Variable	Subjects N = 180
Age (years)	50.9 ± 2.3
BMI (kg/m^2)	24.9 ± 4.0
Waist-to-hip ratio (m/m)	0.77 ± 0.05
Smoking status (%)	
Current[†]	6.5
Past	40.3
Systolic blood pressure (mmHg)	121 ± 14
Diastolic blood pressure (mmHg)	68 ± 10
Pulse pressure (mmHg)	52 ± 10
Hypertension (%)	2.2
Total cholesterol (mmol/l)	6.2 ± 1.0
HDL-cholesterol (mmol/l)	1.6 ± 0.4
LDL-cholesterol (mmol/l)	4.1 ± 0.9
Triglycerides (mmol/l)*	1.0 (0.8 - 1.3)
Glucose (mmol/l)	5.5 ± 0.6
Insulin (picomol/l)*	44 (32 - 59)
Apolipoprotein A1 (mg/dl)	154 ± 32
Apolipoprotein B (mg/ml)	102 ± 26
PAI-1-antigen (ng/ml)*	53 (34 - 85)
tPA-antigen (ng/ml)	6.3 ± 2.4
Diameter (mm)	6.7 ± 0.6
Distensibility (mm)	0.38 ± 0.10
Distensibility coefficient (10^{-3}/kPa)	16.7 ± 5.0

Data are given as mean ± standard deviation, as median (interquartile range) for variables with skewed distribution (*) or as percentages
[†] Subjects who smoked 5 or more cigarettes a day were excluded from the study population

BMI, waist-to-hip ratio, triglycerides, glucose, insulin, PAI-1-antigen and tPA-antigen were negatively and HDL-cholesterol and apolipoprotein A1 positively associated with arterial distensibility. We did not find associations of the parameters that are not part of the insulin resistance syndrome (total cholesterol, LDL-cholesterol and apolipoprotein B) with common carotid artery distensibility. After additional adjustment for MAP (model B), BMI, waist-to-hip ratio, triglycerides and PAI-1-antigen remained significantly associated with decreased common carotid artery distensibility ($p = 0.01$ for BMI, $p = <0.001$ for waist-

Table 2
Correlation of clinical and biochemical parameters with mean arterial pressure in 180 women aged 43 to 55 years from the Zoetermeer study population.

	R	p-value
BMI (kg/m^2)	0.19	<0.001
Waist-to-hip ratio (m/m)	0.19	<0.001
Total cholesterol (mmol/l)	0.18	0.01
HDL-cholesterol (mmol/l)	-0.17	0.02
LDL-cholesterol (mmol/l)	0.17	0.02
Triglycerides (mmol/l)*	0.32	<0.001
Glucose (mmol/l)	0.31	<0.001
Insulin (picomol/l)*	0.25	0.001
Apolipoprotein A1 (mg/dl)	-0.15	0.04
Apolipoprotein B (mg/ml)	0.23	0.001
PAI-1-antigen (ng/ml)*	0.22	0.003
tPA-antigen (ng/ml)	0.26	<0.001

Results are presented as Pearson's correlation-coefficients or as Spearman's correlation coefficients for variables with a skewed distribution (*) and p-values

to-hip ratio, p = 0.04 for triglycerids and p = 0.05 for PAI-1-antigen). HDL-cholesterol remained borderline significantly (p=0.07) associated with decreased common carotid artery distensibility. The clustering score had a strong negative association with common carotid artery distensibility, which remained after additional adjustment for MAP. Adding MAP-squared to model B did not alter the results (data not shown). The percentage explained variance of the distensibility coefficient by the various parameters of the insulin resistance complex in model B ranged from 10.8% (apolipoprotein B) to 18.7% (waist-to-hip ratio). Figure 1 shows mean values of those parameters of the insulin resistance complex which were still (borderline) associated to decreased arterial distensibility after additional adjustment for MAP (model B) per tertile of arterial distensibility of the common carotid artery, adjusted for age, menopausal status, and MAP. As expected, the highest values BMI, waist-to-hip ratio, triglycerids, and PAI-1-antigen and the lowest value of HDL cholesterol were found in the lowest tertile of the DC. In all cases, except for PAI-1-antigen, the test for trend was significant (p *trend*: 0.003 for BMI and waist-to-hip ratio, 0.009 for HDL-cholesterol, and 0.038 for triglycerids). In model C, with further adjustment for insulin, no attenuation of the associations of BMI and waist-to-hip ratio with common carotid arterial distensibility were found (β-coefficient (95% confidence inter-

Table 3
Association of clinical and biochemical parameters with the distensibility coefficient of the common carotid artery in 180 women aged 43 to 55 years from the Zoetermeer study population.

	Model A		Model B	
	ß-coeffi-cient	95% C.I.	ß-coeffi-cient	95% C.I.
BMI (kg/m²)	-0.34	-0.52 to -0.16	-0.24	-0.42 to -0.06
Waist-to-hip ratio (m/m)	-32.8	-46.6 to -19.1	-26.6	-40.6 to -12.6
Total cholesterol (mmol/l)	-0.01	-0.77 to 0.80	0.27	-0.48 to 1.03
HDL-cholesterol (mmol/l)	2.63	0.66 to 4.61	1.93	-0.01 to 3.87
LDL-cholesterol (mmol/l)	-0.03	-0.87 to 0.81	0.22	-0.60 to 1.03
Triglycerides (mmol/l)	-2.23	-3.51 to -0.95	-1.42	-2.77 to -0.08
Glucose (mmol/l)	-1.37	-2.70 to -0.04	-0.57	-1.92 to 0.78
Insulin (picomol/l)	-0.02	-0.05 to 0.00	-0.01	-0.04 to 0.01
Apolipoprotein A1 (mg/dl)	0.03	0.002 to 0.05	0.02	-0.01 to 0.04
Apolipoprotein B (mg/ml)	-0.02	-0.05 to 0.009	-0.01	-0.04 to 0.02
PAI-1-antigen (ng/ml)	-0.02	-0.03 to -0.006	-0.01	-0.02 to -0.00
tPA-antigen (ng/ml)	-0.33	-0.63 to -0.03	-0.18	-0.48 to 0.11
Clustering score	-0.27	-0.38 to -0.15	-0.20	-0.32 to -0.07

Results are presented as ß-coefficients (increase in carotid distensibility in 10^{-3}/kPa per unit increase of the parameter) and 95 % C.I. (confidence interval). Model A is adjusted for age and menopausal status, model B is adjusted for age, menopausal status, and mean arterial pressure.

val) per kg/m² increase for BMI: -0.24 (-0.44 ; -0.04) and per m/m increase for waist-to-hip ratio: -25.6 (-40.0 ; -11.3). The associations of triglycerides and PAI-1-antigen with common carotid artery distensibility was lost after additional adjustment for insulin (β-coefficient (95% confidence interval) per mmol/l increase for triglycerides: -1.31 (-2.80 ; 0.17), per ng/ml increase for PAI-1-antigen: -0.01 (-0.02 ; 0.002).

We next performed analyses with (2ΔD/ΔP) index as dependent variable in relation to BMI and waist-to-hip ratio. Waist-to-hip ratio was strongly associated to the (2ΔD/ΔP) index, adjusted for age, MAP and D: β-coefficient (95% confidence interval) per m/m: -10.3 (-17.28 ; -3,30), whereas BMI was not associated with the (2ΔD/ΔP) index: β-coefficient (95% confidence interval) per kg/m²: -0.06 (-0.15 ; 0,03).

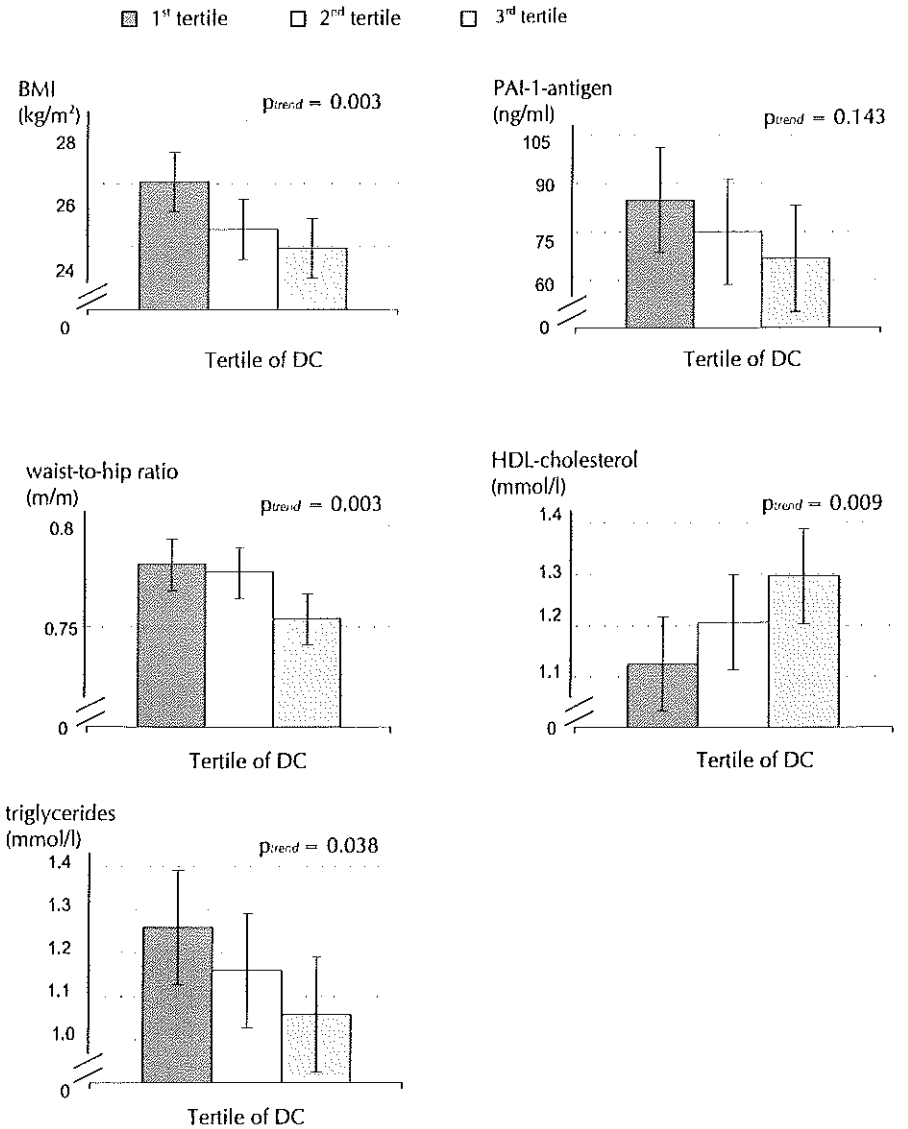

Figure 1
Estimated marginal means (95% confidence intervals) of various parameters of the insulin resistance complex per tertile of distensibility coefficient, adjusted age, menopausal status and mean arterial blood pressure.

DISCUSSION

The results of the present study indicate that in healthy non-diabetic middle-aged women, parameters of the insulin resistance syndrome are strongly associated with reduced distensibility of the common carotid artery. Furthermore, common carotid artery distensibility had a strong inverse association with clustering of parameters of the insulin resistance syndrome within one subject. Parameters which are not part of the insulin resistance syndrome (total cholesterol, LDL-cholesterol, and apolipoprotein B) were not related to arterial distensibility.

Some methodological issues need to be discussed before we can interpret our findings. 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.[19] 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.[20] On the other hand, non-invasive cuff-based measurement of blood pressure underestimates pulse pressure.[21] Several groups showed the validity of the use of brachial pressures.[22-24] Secondly, in analyses with arterial distensibility, a measure very 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 the pulse pressure. However, 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, we repeated the analyses after additional adjustment for MAP. This attenuated the associations, which in part could be due to over-correction because arterial stiffness is not only a consequence of high blood pressure but will by itself also result in a higher blood pressure. After adjustment for MAP, the variables BMI, waist-to-hip ratio, triglycerides and PAI-1 antigen remained associated and HDL-cholesterol borderline associated with decreased arterial distensibility. Clustering of the parameters of the insulin resistance syndrome remained strongly associated with a marked decrease of common carotid artery distensibility after additional adjustment for

MAP. On the other hand, adjustment for MAP in a linear model might not be adequate as the relation between arterial distension and MAP flattens off at higher blood pressure levels, making the overall relation non-linear. This could result in residual confounding. Although, in the normal pressure range, as in the present study, the flattening off barely occurs in elastic arteries as the common carotid artery, we additionally adjusted the models for MAP-squared. This term was not associated with arterial distensibility in any of the models and did not alter the results. Table 2 shows that MAP is related not only to the parameters of the insulin resistance syndrome, but also has a strong relation with total cholesterol, LDL cholesterol and apolipoprotein B. The absence of an association between these biochemical parameters and arterial distensibility (Table 3) makes it unlikely that the observed associations can be explained solely by residual confounding by blood pressure level.

The present study is part of a study designed to evaluate the effect of natural menopause. This study includes both premenopausal and postmenopausal women. As hormonal status is known to affect parameters of the insulin resistance complex[25] and hormonal status might also have a effect on arterial distensibility[26], hormonal status could act as a confounder in the association under study. We therefore adjusted all the models for menopausal status.

The insulin resistance syndrome is associated with arterial stiffness in non-insulin-dependent diabetic patients.[6] There are few reports concerning the association of insulin resistance syndrome with arterial stiffness in normal non-diabetic individuals. Results from a population-based study suggest that insulin concentrations affect arterial stiffness independent of its effects on atherosclerosis.[9] Some studies examined serum lipids, lipoprotein levels and serum glucose and insulin as determinants of arterial stiffness in various populations. These studies, however, report conflicting results. Some studies found no association between lipids and arterial distensibility.[27-29] Some studies found no association between apolipoprotein A1 and apolipoprotein B with arterial stiffness after adjustment for blood pressure.[29,30] Several studies did find an association between serum lipids and arterial distensibility although not always in the same direction.[30-34] Serum glucose and/or insulin have been found to be positively associated with arterial distensibility in several studies.[9,29-31,33] However, some of these studies did not adequately adjust for blood pressure. One study found no correlation between area under the glucose tolerance curve and arterial distensible after adjustment for age and blood pressure.[34] There are several possible explanations for conflicting results in these previous studies. First, most of these studies were performed in small groups of subjects. Second, the different studies were per-

formed on heterogeneous study populations, ranging from healthy cohorts to hypertensive, hypercholesterolemic or diabetic patients. Third, there is a large variation in measures of arterial distensibility used. It is still unclear whether locally measured arterial distensibility represents the same arterial wall characteristics as pulse wave velocity which determines arterial stiffness measured over a large part of the arterial tree. Furthermore, some studies presented results unadjusted for age or blood pressure. Finally, contradictory findings with respect to the direction of the association between arterial distensibility and serum lipids can be due to differences in age of study populations. There is compelling evidence that early nonsclerotic atheromatous changes of the arterial wall in relatively young subjects (age < 50 years) decrease rather than increase arterial stiffness.[35-37] One study concerned the association of insulin resistance with arterial stiffness in healthy subjects and found this association to be more pronounced in women then in men.[10] This association was independent of MAP, lipid concentrations and body composition.

The association of insulin with arterial distensibility was lost after adjustment for MAP. This in contrast with another study[10], in which an association was found of insulin resistance, assessed by the euglycaemic, hyperinsulinaemic clamp, with arterial distensibility independent of MAP. However, we used fasting insulin as marker of insulin resistance while it is known that fasting insulin is a reasonable though imperfect marker of insulin resistance in subjects with a normal glucose tolerance.[15]

Although BMI and waist-to-hip ratio are strongly related to insulin[38], the association of BMI and waist-to-hip ratio with arterial distensibility remained after further adjustment for insulin (model C). The associations of HDL-cholesterol, triglycerides and PAI-1-antigen with arterial distensibility did not remain after additional adjustment for insulin. Some authors suggested that obesity could lead to an increased arterial diameter and therefore performed analyses in which they used the ($\Delta D/\Delta P$) index as dependent variable.[10] They found no association of this index with variables of body composition and concluded that only arterial diameter, and not arterial distensibility, is related to variables of body composition. In our study, in agreement with the other study, BMI also was not associated to the ($2\Delta D/\Delta P$) index, independent of arterial diameter. However, waist-to-hip ratio had a strong inverse association with the ($2\Delta D/\Delta P$) index, independent of age, MAP and arterial diameter.

Hyperglycemic conditions can lead to increased arterial stiffness by increased collagen cross linking due to non-enzymatic glycation.[3-5] This may be the mechanism underlying our observation of an association between the insulin resist-

ance syndrome and decreased arterial distensibility. Arterial stiffness plays a role in the etiology of isolated systolic hypertension and increased cardiac load[39], and has been found to be associated with increased cardiovascular risk.[7,8] Therefore, knowledge of potential modifying determinants of arterial stiffness may be important in the prevention of cardiovascular disease. Recent evidence suggest that there is therapeutic potential to treat hyperglycemic induced arterial stiffness by breakers of advanced glycation end products.[40]

In summary, the results of our study show that parameters of the insulin resistance syndrome are associated with decreased arterial distensibility in healthy women. The present analysis only comprised middle-aged women. Future studies are needed to confirm this association in other age groups and in men.

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3.2

Impaired fasting glucose is associated with increased arterial stiffness in elderly non-diabetic subjects
The Rotterdam Study

Abstract

Background: *In young and middle-aged non-diabetic subjects, impaired glucose metabolism has been found to be related to arterial stiffness. Impaired fasting glucose frequently occurs in elderly non-diabetic subjects, but has not been examined in relation to arterial stiffness in the elderly. We studied the association between impaired fasting glucose and arterial stiffness in 2987 subjects, aged 60 years or over. The study was part of the Rotterdam Study, a population-based cohort study.*

Methods: *Arterial stiffness was assessed by measuring common carotid arterial distensibility. Mean distensibility was calculated for non-diabetic subjects, subjects with impaired fasting glucose and diabetic subjects adjusted for age, gender, and mean arterial pressure.*

Results: *In the total cohort, common carotid distensibility decreased with increasing impairment of glucose metabolism. Among subjects under 75 years of age, subjects with impaired fasting glucose were comparable to non-diabetic subjects with respect to arterial stiffness. Among subjects above 75 years of age, subjects with impaired fasting glucose had stiffer arteries than non-diabetic subjects, reaching the same arterial stiffness as diabetic subjects. Distensibility coefficients (10^{-3}/kPa) of non-diabetic subjects, subjects with impaired fasting glucose and diabetic subjects were 11.7, 11.6, and 10.5 for subjects under 75 years of age and 8.5, 7.8, 7.7 for subjects above 75 years of age.*

Conclusions: *Our findings indicate that impaired fasting glucose is related to increased arterial stiffness in non-diabetic men and women, but an advanced stage of arterial stiffness, comparable with that of diabetic subjects, is reached at high age.*

Introduction

Stiffening of the arteries is more pronounced in diabetic subjects than in non-

diabetic subjects.[1-5] Furthermore, in subjects with non-insulin-dependent 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. Giltay and colleagues found an association of insulin resistance with increased arterial stiffness in healthy young subjects.[7] In healthy non-diabetic middle-aged women, variables of the insulin resistance syndrome were found to be associated with reduced arterial distensibility.[8] The Atherosclerosis Risk in Communities study examined fasting blood glucose in relation to arterial stiffness indexes in a large population-based study of middle-aged subjects.[9] In this study, a significant association between fasting glucose and arterial stiffness was found in healthy non-diabetic participants.

Impairments in glucose metabolism frequently occur in elderly non-diabetic subjects[10,11], but never has been related to arterial stiffness in the elderly. Increased arterial stiffness is associated with increased cardiovascular risk.[12,13] Therefore, it is of importance to know whether an impaired glucose metabolism is accompanied by arterial stiffening in elderly non-diabetic subjects. The aim of the present study was to examine whether arterial stiffness is increased in elderly non-diabetic subjects with an impaired glucose metabolism, relative to subjects with a normal glucose metabolism.

METHODS

Study population

The Rotterdam Study 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.[14] 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.

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).

Assessment of the 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, 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.[15] 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.[15,16] Non-diabetic status 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.[15] 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.[15]

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 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.[17,18] 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:[19] distensibility coefficient = (2ΔD/D)/ ΔP (10^{-3}/kPa).

With this system a wall displacement of a few micrometers can be resolved[17] and diameter (D), ΔD, ΔD/D, and the distensibility coefficient can be assessed reliably.[18] 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. A reproducibility study in 47 subjects 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. This is allowed because in previous studies no differences could be detected between arterial wall properties of the right and left common carotid artery (unpublished results).

Population for analyses

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 non-diabetic subjects, 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 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 non-diabetic subjects, 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. The analyses were performed for the

Table 1
Characteristics of the study population by glucose status, The Rotterdam Study, 1997-1999.

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

Values are given as mean ± standard deviation except for age that is given as mean (range) and categorical variables[¶] that are given as percentage.
$^{*}p < 0.05$ diabetics versus non diabetics, $^{\dagger}p < 0.05$, diabetics versus impaired fasting glucose, $^{\ddagger}p < 0.05$ impaired fasting glucose versus non diabetics, adjusted for age, $^{\S}p < 0.05$, diabetics versus non diabetics, adjusted for age, $^{\#}p < 0.05$, diabetics versus impaired fasting glucose, adjusted for age.

total cohort and within age-strata (a prior 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

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

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

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), the Rotterdam Study, 1997-1999.

Variable	beta-coefficient* (95% C.I.)
Age (years)†	-0.28 (-0.30 ; -0.26)
Gender‡	-0.89 (-1.18 ; -0.60)
Mean arterial pressure (mmHg)§	-0.17 (-0.18 ; -0.16)
Fasting glucose (mmol/l)#	-0.29 (-0.39 ; -0.19)
Total cholesterol (mmol/l)#	-0.06 (-0.19 ; 0.07)
HDL-cholesterol (mmol/l)#	0.76 (0.44 ; 1.09)
Body mass index (kg/m^2)#	-0.09 (-0.12 ; -0.06)
Waist-to-hip ratio#	-2.58 (-3.99 ; -1.17)

* Increase in distensibility coefficient (10^{-3}/kPa) for every unit increase of the independent variable.
† Adjusted for gender; ‡ adjusted for age; § adjusted for age and gender; # adjusted for age, gender and mean arterial pressure.
HDL-cholesterol = high-density-lipoprotein cholesterol.
C.I. = confidence interval.

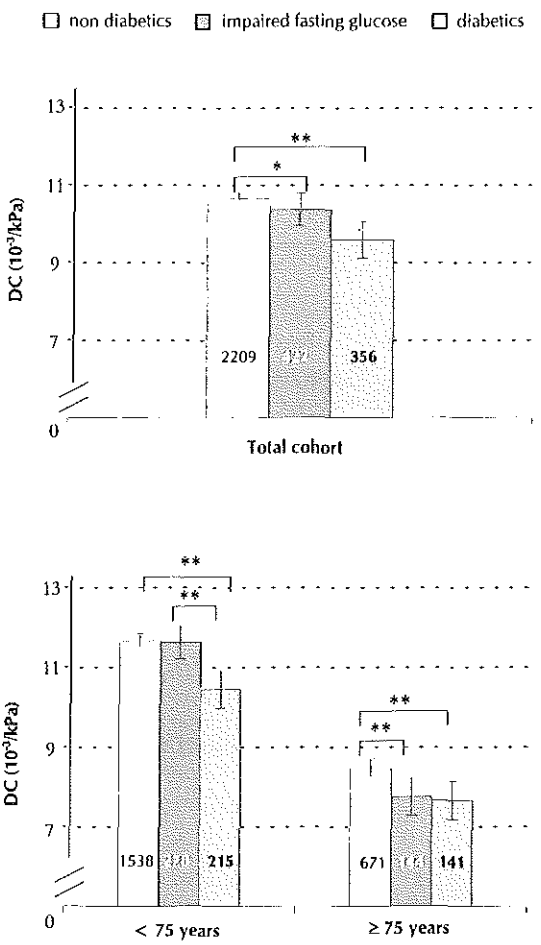

Figure 1
Mean distensibility coefficient (95% confidence intervals) in non-diabetic subjects, subjects with impaired fasting glucose and diabetic subjects of the Rotterdam Study in the total cohort (upper figure) an in strata of age (lower figure) adjusted for age, gender and mean arterial blood pressure. The number of subjects within each group is indicated at the lower end of the respective bar. DC = distensibility coefficient.
*** p = 0.12, ** p < 0.01.**

Figure 1 shows the mean distensibility coefficient of the common carotid artery for non-diabetic subjects, 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 non-diabetic subjects, 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 non-diabetics subjects 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 non-diabetic subjects 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 non-diabetic subjects, 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 non-diabetic subjects 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 non-diabetic subjects (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 non-diabetic subjects (mean difference between subjects with IFG and non-diabetic subjects: 0.7 (0.2 to 1.2; p=0.007) and between diabetic subjects and non-diabetic subjects: 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 non-diabetic subjects 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 non-diabetic subjects.

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.[19] 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.[20] On the other hand, non-invasive cuff-based measurement of blood pressure underestimates pulse pressure.[21] Several groups showed the validity of the use of brachial pressures.[22-24] 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.[25,26] IFG is a metabolic stage, intermediate between normal glucose homeostasis and diabetes and is associated with insulin resistance.[15,16] Studies concerning insulin resistance and an impaired glucose metabolism in relation to arterial stiffness are scarce and confined to diabetic subjects or young to middle-aged healthy subjects.[6,7,9] In subjects with non-insulin-dependent 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 Atherosclerosis Risk in Communities (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 fasting glucose level was above normal.[9]

In the present study, we compared arterial stiffness of subjects with IFG with that of non-diabetic and diabetic subjects. In contrast to the ARIC study, we only found increased arterial stiffness in subjects with IFG among subjects aged 75 years or over. 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 non-diabetic subjects, 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. 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 non-diabetic subjects, subjects with IFG to diabetic subjects in the total cohort (Table 1, Figure 1). Moreover, 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.[27-30] 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.[31,32]

An impaired glucose metabolism is a frequent condition in elderly non-diabetic subjects.[10,11] 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. 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[12,13] and recent evidence suggests opportunities to treat arterial stiffness induced by hyperglycaemia in the near future.[33] 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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CHAPTER 4

Association between arterial stiffness and atherosclerosis

The Rotterdam Study

Abstract

Background: *Studies on the association between arterial stiffness and atherosclerosis are contradictory. We studied several indicators of atherosclerosis in relation to stiffness of the aorta and the common carotid artery.*

Methods: *This study was conducted within the Rotterdam Study in over 3000 elderly subjects aged between 60 and 101 years. Aortic stiffness was assessed by measuring carotid-femoral pulse wave velocity and common carotid arterial stiffness by measuring common carotid distensibility. Atherosclerosis was assessed by common carotid intima-media thickness, plaques in the carotid artery and in the aorta and the presence of peripheral arterial disease. Data were analyzed by analyses of covariance with adjustment for age, gender, mean arterial pressure, and heart rate.*

Results: *Increasing common carotid intima-media thickness and increasing severity of plaques in the carotid artery and in the aorta were all strongly associated with increased aortic and common carotid arterial stiffness (p for trend < 0.01 for all associations). Subjects with peripheral arterial disease had significantly increased aortic stiffness (p=0.001) and borderline significantly increased common carotid arterial stiffness (p=0.078) as compared to subjects without peripheral arterial disease. Results were similar after additional adjustment for cardiovascular risk factors and after exclusion of subjects with prevalent cardiovascular disease.*

Conclusion: *This population-based study shows that arterial stiffness is strongly associated with atherosclerosis at various sites in the vascular tree.*

INTRODUCTION

Accurate non-invasive methods to measure arterial stiffness have recently become available and are relatively easy to perform.[1-4] Results from several small studies have suggested that subjects with cardiovascular disease have increased arterial stiffness as compared to subjects without cardiovascular disease.[5-8] Arterial stiffness has also been shown to be a predictor of all-cause and cardiovascular mortality in subjects with end-stage renal disease.[9,10] These results suggest an association between arterial stiffness and atherosclerosis. Studies on a direct association between indicators of arterial stiffness and indicators of atherosclerosis, however, show conflicting results. Some studies reported a relation between arterial stiffness and atherosclerosis[11-13], but others could not demonstrate such a relationship.[14-17] Most of the studies were performed in small groups of selected subjects and investigated the association between arterial stiffness and atherosclerosis in one vessel bed only. The objective of the present study was to examine

the association between arterial stiffness and atherosclerosis at different sites in the arterial tree in a large group of unselected, non-hospitalized subjects. Arterial stiffness was assessed in the aorta by measuring carotid-femoral pulse wave velocity (PWV) and in the common carotid artery by measuring the distensibility coefficient (DC). As indicators of atherosclerosis we used common carotid intima-media thickness, presence of plaques in the carotid artery and in the abdominal aorta, and the presence of peripheral arterial disease.

METHODS

Study population

The Rotterdam Study 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.[18] The baseline measurements were performed between 1990 and 1993. The third follow-up examination phase took place from 1997 until 1999. The Medical Ethics Committee of the Erasmus University approved the study and written informed consent was obtained from all participants.

Cardiovascular disease and risk factors

Information on cardiovascular risk factors was collected during the third follow-up examination. Information on current health status, medical history, drug use and smoking behavior was obtained using a computerized questionnaire during a home interview. At the research center, blood pressure was measured twice in sitting position at the right arm with a random zero sphygmomanometer. The average of the two measurements was used in the analyses. Height and weight were measured while the subject was wearing lightweight clothes and no shoes. Body mass index (weight/height^2) was calculated. Serum total cholesterol and high-density lipoprotein (HDL) cholesterol were determined using an automatic enzymatic procedure (Boehringer Mannheim Systems, Mannheim, Germany). Serum glucose was determined by the hexokinase method (Boehringer Mannheim Systems, Mannheim, Germany). 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 to or greater than 7.0 mmol/l.[19]

Prevalent cardiovascular disease was defined as a history of myocardial infarc-

tion or stroke. Information on cardiovascular disease at the baseline examination of the Rotterdam Study was assessed during a home interview. A history of myocardial infarction and stroke was confirmed by reviewing the medical records from the general practitioner (GP) and/or medical specialist or by ECG. From baseline onwards, occurrence of myocardial infarction or stroke was reported by general practitioners (GP) in the research area (85% of the cohort) by means of a computerized system. Research physicians verified all information by checking patient-records at the GP. The GPs outside the research area (15%) were visited once a year by research physicians to check patient-records. In addition, discharge reports and letters of medical specialists were obtained for hospitalized patients.

Indicators of atherosclerosis

The indicators of atherosclerosis used in this analyses were measured at the third examination phase, except the presence of calcified plaques in the abdominal aorta, which was determined during the second examination phase between 1993 and 1995.

Intima-media thickness was measured by recording of ultrasonographic images of both the left and right carotid artery, using a 7.5 MHz linear array transducer (ATL UltraMark IV, Advanced Technology Laboratories, Bethel, Washington, USA). The lumen-intima interface and the media-adventitia interface of the near and far wall of the distal common carotid artery were measured off-line. The protocol has been described in detail elsewhere.[20,21] The common carotid intima-media thickness was determined as the average of near and far wall measurements of both left and right side.

The presence of plaques in the carotid artery was assessed by evaluating the ultrasonographic images of the common, internal and bifurcation site of the carotid artery for the presence of atherosclerotic lesions. Plaques were defined as a focal widening relative to adjacent segments, with protrusion into the lumen composed of either only calcified deposits or a combination of calcification and noncalcified material. No attempt was made to quantify the size of the lesions. A total carotid plaque score was defined by summation of the presence of plaques at far and near wall of left and right side at three locations (maximum score of 12). Severity was graded as no plaques (score 0), mild plaques (score 1 to 4), moderate plaques (score 5 to 8) and severe plaques (score 9 to 12).

Atherosclerosis of the abdominal aorta was determined using a lateral X-ray of the lumbar spine (T12-S1), on which the presence of calcified deposits was deter-

mined. Calcified plaques were considered present when linear densities were clearly visible in an area parallel and anterior to the lumbar spine (L1-L4).[22] Severity was graded from 0 (no calcified plaques) to 5 (aorta outlined with calcified plaques) according to length of affected area. Subsequently, subjects were classified into having no (grade 0), mild (grade 1), moderate (grade 2 and 3) or severe (grade 4 and 5) atherosclerosis of the abdominal aorta.

The presence of peripheral arterial disease was assessed by the ankle-brachial pressure index, which is the ratio of the systolic blood pressure at the ankle to the average systolic blood pressure at the right arm. Systolic blood pressure of the posterior tibial artery at both left and right ankle was measured using an 8 MHz continuous wave doppler probe (Huntleigh 500 D, Huntleigh Technology, Bedfordshire, UK) and a random zero sphygmomanometer with the subject in supine position.[23] The ankle-brachial pressure index was calculated for both ankles. In agreement with the approach followed by Fowkes[24], we used the lowest ankle-brachial pressure index in either leg to determine presence of peripheral arterial disease. Peripheral arterial disease was considered present when ankle-brachial pressure index in either leg was lower than 0.9.

Arterial stiffness

Aortic and common carotid arterial stiffness were measured during the third examination phase. Carotid-femoral PWV was measured with subjects in supine position. Before measurement of PWV, blood pressure was measured twice with a sphygmomanometer after five minutes of rest and the mean was taken as the subjects reading. Mean arterial pressure (MAP) was calculated by the following formula: diastolic blood pressure + 1/3 * (systolic blood pressure - diastolic blood pressure). Carotid-femoral PWV was assessed using an automatic device (Complior, Colson, Garges-lès-Gonesse Cx, France)[4] that assessed the time delay between the rapid upstroke of the feet of simultaneously recorded pulse waves in the carotid artery and the femoral artery. The distance traveled by the pulse wave between the carotid artery and the femoral artery was measured over the surface of the body using a tape measure. PWV was calculated as the ratio between the distance traveled by the pulse wave and the foot-to-foot time delay and expressed in meters per second. The average of at least 10 successive measurements, to cover a complete respiratory cycle, was used in the analyses.

Common carotid distensibility was assessed with the subjects in supine position and the head tilted slightly to the contralateral side. The vessel wall motion of the right CCA was measured by means of a Duplex scanner (Ultramark IV,

ATL, Bothell, Washington, USA) connected to a vessel wall movement detector system. The details of this technique have been described elsewhere.[1,25] After five minutes rest, 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 with a Dinamap automatic blood pressure recorder and the mean was taken as the subjects reading. Pulse pressure (ΔP) was calculated as the difference between systolic and diastolic blood pressure. MAP was calculated with the same formula as described by measurement of PWV. The cross-sectional arterial wall DC was calculated according to the following equation[26]: $DC = (2\Delta D/D)/\Delta P$ (10^{-3}/kPa).

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). A reproducibility study in 47 subjects showed an intra-class correlation coefficient of 0.80 for carotid-femoral PWV and 0.80 for common carotid DC.

Population for analysis

Of 4024 subjects eligible for a physical examination in the third examination phase, carotid-femoral PWV was measured in 3550 subjects and common carotid distensibility was measured in 3098 subjects. Missing information on PWV or common carotid distensibility was almost entirely due to logistic reasons. Of 3550 subjects with a measurement of PWV, 69 subjects (1.9%) were excluded from the analyses because the variation between the successive PWV measurements was more than 10% or less than 10 successive measurements were made, leaving 3481 subjects for analyses. All subjects with a measurement of common carotid distensibility were included in the analyses. Of all subjects with a PWV measurement, 47% had information on carotid intima-media thickness, 87% had information on carotid plaques, 93% had information on plaques in the aorta and 96% had information on presence of peripheral arterial disease. Of all subjects with a measurement of the DC, 53% had information on carotid intima-media thickness, 91% had information on carotid plaques, 92% had information on plaques in the aorta and 96% had information on presence of peripheral

arterial disease. Missing information on indicators of atherosclerosis was due to logistic reasons. The large number of subjects with missing information on carotid intima-media thickness was due to leeway in the off-line analysis of ultrasonographic images. Missing information on other covariates for analyses was maximal 3.3% for subjects with information on PWV and maximal 4.0% for subjects with information on the DC and also due to logistic reasons.

Statistical analysis

The association between arterial stiffness and indicators of atherosclerosis was evaluated using one way analysis of covariance. Mean PWV adjusted for age, sex, MAP and heart rate was calculated per quartile of the continuous indicators of atherosclerosis or per category of the categorical indicators of atherosclerosis. Analogously, mean DC adjusted for age, sex, MAP and heart rate was calculated per quartile of the continuous indicators of atherosclerosis or per category of the categorical indicators of atherosclerosis. A test for trend was performed using multiple linear regression analysis with the quartiles or categories of the different indicators of atherosclerosis as ordinal variables. Analyses were repeated after exclusion of subjects with prevalent cardiovascular disease. Next, the associations were evaluated using multiple linear regression analysis with PWV or the DC as dependent variable and the different indicators of atherosclerosis as independent variables, adjusted for age, sex, MAP and heart rate and several cardiovascular risk factors (body mass index, total cholesterol, HDL cholesterol, serum glucose, smoking and diabetes mellitus). Finally, the association between both indicators of arterial stiffness was evaluated using multiple linear regression analyses, with the distensibility coefficient as dependent and pulse wave velocity as the independent variable and, additionally, by calculating the correlation between pulse wave velocity and the distensibility coefficient. All analyses were performed using SPSS 8.0 statistical package for Windows 95 (SPSS Inc., Chicago, Illinois, USA).

RESULTS

Table 1 presents the baseline characteristics of the study population. Levels of cardiovascular risk factors were in the high normal range, as expected in a general population of elderly subjects. Mean values of PWV per quartile or per category of the indicators of atherosclerosis, adjusted for age, sex, MAP and heart rate are

Table 1
Characteristics of the study population.

Characteristic	Value
Age (years)	72 (60 - 101)
Men†	58 %
Systolic blood pressure (mmHg)	143 (21)
Diastolic blood pressure (mmHg)	75 (11)
Heart rate (bpm)	70 (11)
Cholesterol:	
Total (mmol/l)	5.8 (1.0)
HDL (mmol/l)	1.4 (0.4)
Body mass index (kg/m^2)	26.8 (4.0)
Diabetes†	12 %
Smoking†:	
Current	16 %
Former	50 %
Past	34 %
Pulse wave velocity (m/s)	13.5 (3.0)
Distensibility coefficient (10^{-3}/kPa)	10.5 (4.4)
Intima-media thickness (μm)	0.88 (0.16)
Plaques in carotid artery	
No	30.8 %
Mild	41.8 %
Moderate	24.9 %
Severe	2.5 %
Calcified plaques in the aorta	
No	43.3 %
Mild	21.0 %
Moderate	30.7 %
Severe	5.0 %
Peripheral arterial disease	18.1 %

Values are given as mean ± standard deviation except for age that is given as mean (range) and categorical variables† that are given as percentage.

shown in figure 1. PWV consistently increased with increasing common carotid intima-media thickness, plaques in the carotid artery and plaques in the aorta (test for trend: $p<0.05$ for all associations). Presence of peripheral arterial disease was associated with a significantly increased PWV as compared to absence of peripheral arterial disease. As expected, the associations of indicators of atherosclerosis with PWV were positive, as a higher PWV indicates increased aortic stiffness. Mean values of the common carotid DC per quartile or per category of the indicators of atherosclerosis, adjusted for age, sex, MAP and heart rate are

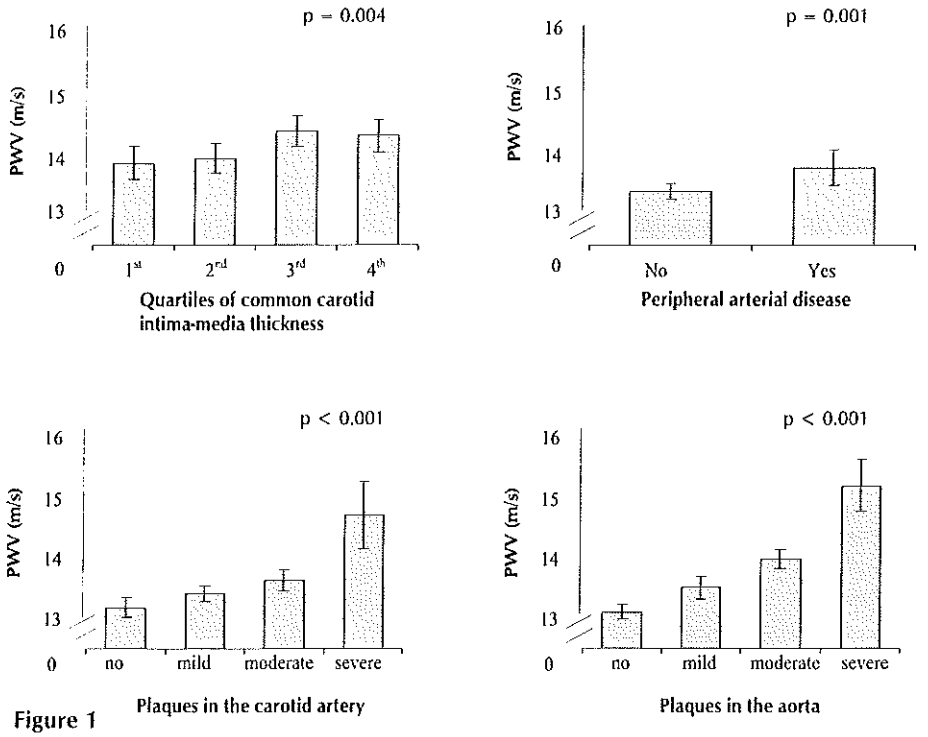

Figure 1

Mean pulse wave velocity (PWV) per quartile of common carotid intima-media thickness and per category of presence of peripheral arterial disease, plaques in the carotid artery and plaques in the aorta, adjusted for age, sex, mean arterial pressure and heart rate in elderly subjects of the Rotterdam Study. Bars indicate 95% confidence interval; p-values indicate p for trend, except for peripheral arterial disease in which the p-value indicates p for difference between the groups.

shown in figure 2. The DC consistently decreased with increasing intima-media thickness and plaques of the common carotid artery and plaques in the aorta (test for trend: $P<0.05$ for all three associations). Presence of peripheral arterial disease was associated with a borderline significantly decreased DC as compared to absence of peripheral arterial disease. As expected, the associations of indicators of atherosclerosis with the DC were negative, as a lower DC indicates increased common carotid arterial stiffness. Results were the same after excluding subjects with prevalent cardiovascular disease (n=503) (data not shown).

The results of the multiple linear regression analysis are shown in table 2. Significant associations between PWV and all indicators of atherosclerosis were observed, adjusted for age, sex, MAP and heart rate and cardiovascular risk factors. The DC was significantly associated with common carotid intima-media thickness, plaques in the carotid artery and plaques in the aorta, and borderline

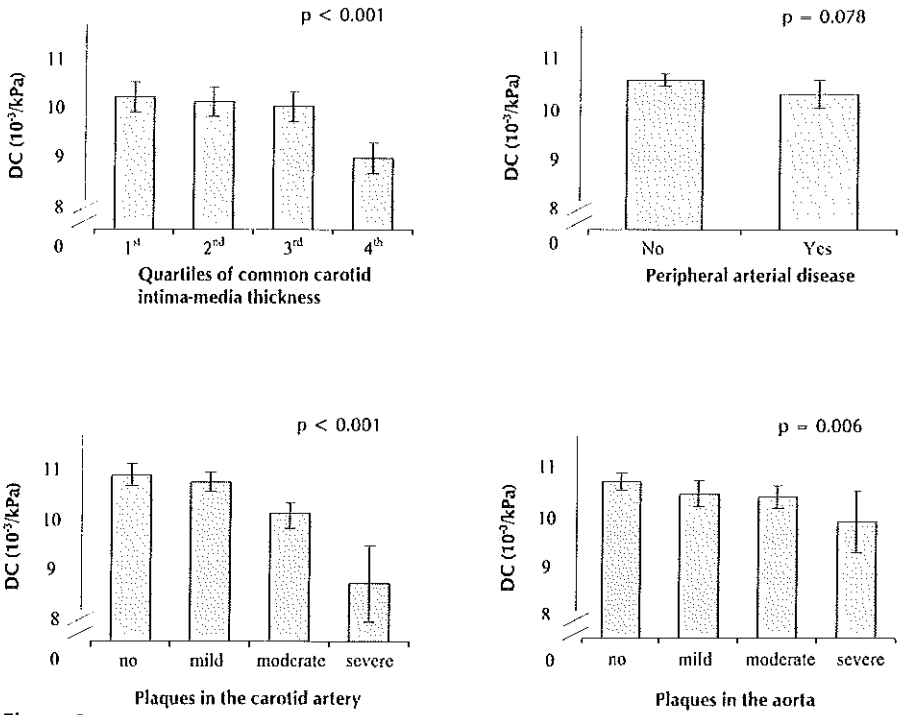

Figure 2
Mean distensibility coefficient (DC) per quartile of common carotid intima-media thickness and per category of presence of peripheral arterial disease, plaques in the carotid artery and plaques in the aorta, adjusted for age, sex, mean arterial pressure and heart rate in elderly subjects of the Rotterdam Study. Bars indicate 95% confidence interval; p-values indicate p for trend, except for peripheral arterial disease in which the p-value indicates p for difference between the groups.

significantly associated with presence of peripheral arterial disease (p=0.09), after adjustment for age, sex, MAP and heart rate and cardiovascular risk factors.
A quadratic relationship was found between the distensibility coefficient and pulse wave velocity: distensibility coefficient = 27.4 - 1.9 * (pulse wave velocity) + 0.04 * (pulse wave velocity)^2 [p total model $\leq$ 0.001]. The correlation between the distensibility coefficient and pulse wave velocity was -0.41 ($p < 0.001$).

DISCUSSION

The objective of this population-based study was to examine arterial stiffness in relation to atherosclerosis at different sites in the arterial tree. We found aortic stiffness to be significantly associated with common carotid intima-media thick-

Table 2
Multiple linear regression (ß) coefficients and their 95% confidence intervals (CI) describing the association* between aortic stiffness and common carotid stiffness and different indicators of atherosclerosis in elderly subjects from the Rotterdam Study.

Indicator of atherosclerosis	Aortic stiffness (m/s)	Common carotid stiffness (10^{-3}/kPa)
	β-coefficient (95% C.I.)	β-coefficient (95% C.I.)
Common carotid intima-media thickness (µm)	0.96 (0.01 ; 1.91)	-3.12 (-4.17 ; -2.08)
Plaques in the carotid artery:		
Mild (versus none)	0.20 (-0.02 ; 0.66)	-0.10 (-0.40 ; 0.21)
Moderate (versus none)	0.40 (0.15 ; 0.66)	-0.68 (-1.05 ; -.032)
Severe (versus none)	1.30 (0.70 ; 1.90)	-2.29 (-3.15 ; -1.43)
Calcified plaques in the aorta:		
Mild (versus none)	0.39 (0.17 ; 0.61)	-0.22 (-0.55 ; 0.11)
Moderate (versus none)	0.80 (0.60 ; 1.02)	-0.25 (-0.56 ; 0.06)
Severe (versus none)	2.06 (1.59 ; 2.52)	-0.80 (-1.49 ; -0.11)
Presence of peripheral arterial disease (versus absence)	0.29 (0.05 ; 0.52)	-0.30 (-0.64 ; 0.04)

*All models adjusted for age, gender, mean arterial pressure, heart rate, total cholesterol, HDL-cholesterol, serum glucose, smoking, body mass index and presence of diabetes mellitus.

ness, plaques in the carotid artery and in the aorta, and presence of peripheral arterial disease. Common carotid arterial stiffness was significantly associated with all indicators of atherosclerosis except for a borderline significant association with the presence of peripheral arterial disease. Results were similar after additional adjustment for cardiovascular risk factors and after exclusion of subjects with prevalent cardiovascular disease.

Some aspects of this study need to be discussed. Firstly, we use several non-invasive measures as indicators of atherosclerosis. Intima-media thickness and plaques in the common carotid artery have shown to be adequate indicators of atherosclerosis of the carotid artery.[27-29] Radiographically detected calcifications in the aorta correlate well with atherosclerotic plaques observed at autopsy and in most cases visible calcification represented advanced atherosclerosis.[30] Yao and colleagues compared the ankle-brachial pressure index with arteriography of the distal aorta and arteries of the lower extremities and demonstrated that the pressure index is a valuable and sensitive method of assessment of occlusive arterial disease.[31] Secondly, information on the different indicators of atherosclerosis was not available for every subject with a PWV measurement. This was

mainly due to logistic reasons, and missing information, therefore, is likely to be randomly distributed over categories of severity of arterial stiffness and thus will not have introduced bias in the estimates. Thirdly, for determining the presence of plaques in the abdominal aorta, we used x-rays from the second examination phase, which took place on average four years before the third examination phase in which arterial stiffness was measured. The reason for this was that x-rays made in the third examination phase were not yet evaluated for the presence of aortic plaques at the time of the present analyses. Using x-rays from the second follow-up examination phase may have led to some misclassification in severity of plaques in the aorta, but this misclassification is non-differential with respect to arterial stiffness and thus, if present, will have led to an underestimation of the associations.

Previous studies on the association between arterial stiffness and atherosclerosis reported conflicting results. Non-invasive measurement of distensibility of the carotid artery has been shown to be closely related to post-mortem established atherosclerosis of the carotid artery.[12] The presence of atheromatous plaques in the aorta has been found to be strongly correlated with decreased aortic distensibility in subjects with various pathologies.[13] Among hypertensive patients, those with high aortic PWV as compared to those with low aortic PWV, had a higher frequency of carotid artery stenosis and tended to have a higher frequency of aortic and lower limb atherosclerotic lesions.[11] In contrast to the above, other studies found no relation between arterial stiffness and atherosclerosis. One study found the severity of aortic atherosclerosis to be unrelated to the loss of aortic distensibility and observed a steadily progress of loss of aortic distensibility with increasing age regardless of the atherosclerotic severity.[14] In an ecological study, Avolio and colleagues found similar changes in PWV with age in populations with different prevalence of atherosclerosis and concluded that arterial distensibility is not associated with atherosclerosis.[15] Megnien and colleagues found no association between aortic stiffness as determined by PWV and coronary and extracoronary atherosclerosis in a cross-sectional study of 190 asymptomatic men at risk for coronary heart disease.[17] This study, however, comprised only a small number of subjects. The Atherosclerosis Risk in Communities (ARIC) study examined the relation between distensibility and intima-media thickness of the common carotid artery. They did not observe an association between arterial wall thickness and increased arterial stiffness, except for the thickest 10% of the artery walls only.[16] We found increased common carotid stiffness only in the highest quartile of intima-media thickness of the common carotid artery (Figure 2), which resembles the findings of the ARIC study, but

observed increased aortic stiffness in the two upper two quartiles of aortic stiffness (Figure 1). The absence of a clear association between arterial stiffness and intima-media thickness in the lower two quartiles of intima-media thickness is in agreement with recent evidence that suggests that intima-media thickness may only reflect atherosclerosis beyond a certain level.[32]

Several possibilities for the observed association between arterial stiffness and atherosclerosis can be hypothesized. One possibility is that presence of atherosclerosis leads to stiffening of the arteries. In favor of this hypothesis is the study of Farrar and colleagues showing an increase in PWV in cynomolgus monkeys fed an atherogenic diet and a decrease in PWV in cynomolgus monkeys fed an atherosclerosis regression diet.[33] An alternative possibility is that increased arterial stiffness leads to vessel wall damage and atherosclerosis. Without the shock-absorbing capacity, the stiff arterial wall may be subjected to increased intraluminal stress on impact of increased pulsatile pressure.[34] A third possibility is that both mechanisms apply and that atherosclerosis is not only a consequence of arterial stiffness, but may by itself in advanced stages also increase arterial stiffness. This would result in a self-perpetuating, reinforcing process. A final possibility is that arterial stiffness and atherosclerosis are independent processes that frequently occur at similar sites in the arterial tree without the existence of a causal relationship. Future long-term longitudinal studies, preferably starting in young subjects, will be needed to elucidate the temporal relationship between arterial stiffness and atherosclerosis.

The strong association of aortic stiffness with atherosclerosis at various sites of the arterial tree suggests that aortic stiffness can be used as an indicator of generalized atherosclerosis. Whether this also holds for common carotid arterial stiffness is less clear as common carotid arterial stiffness was associated with carotid and aortic atherosclerosis but not clearly with the presence of peripheral arterial disease. Possibly, assessment of atherosclerosis in the abdominal aorta was more accurate than assessment of atherosclerosis of the peripheral arteries, which was assessed by a proxy.

Stiffening of the arterial tree leads to an increase in systolic blood pressure and simultaneously a decrease in diastolic blood pressure resulting in a wide pulse pressure.[35] The increased systolic blood pressure has a negative effect on the heart due to an increased workload, while the reduced diastolic blood pressure may limit coronary perfusion. These effects of increased pulse pressure may explain the association between arterial stiffness and myocardial infarction.[36,37] Recent evidence shows that the combination of a high systolic blood pressure and a low diastolic blood pressure resulting in a wide pulse pressure is also a

strong risk factor for stroke.[38,39] The strong association between arterial stiffness and atherosclerosis observed in our study provides an additional explanation for the association between arterial stiffness and cardiovascular disease.

In conclusion, the results of this population-based study in elderly subjects suggest that arterial stiffness is associated with atherosclerosis at various sites in the arterial tree.

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Chapter 5

Arterial stiffness and cardiovascular disease

5.1

Measures of arterial stiffness are associated with myocardial infarction and stroke *The Rotterdam Study*

Abstract

Background: *Functional and structural arterial abnormalities have been associated with risk of cardiovascular disease. In the present study, the association between arterial stiffness and prevalent cardiovascular disease was evaluated in a large population-based study and compared with that between non-invasively measured atherosclerosis and prevalent cardiovascular disease.*
Methods: *The study included 3818 elderly participants of the third examination phase of the Rotterdam Study. Ninety-five subjects had a history of myocardial infarction and seventy-eight subjects had a history of stroke. Arterial stiffness was determined by carotid-femoral pulse wave velocity and common carotid distensibility. Measures of atherosclerosis were the ankle-brachial pressure index and plaques in the carotid artery. Analyses were performed using logistic regression analyses, adjusted for age, sex, mean arterial pressure, and heart rate.*
Results: *Subjects with severe aortic stiffness had four-times as often a previous myocardial infarction, compared to the reference category (odds ratio (95% confidence interval): 4.0 (1.8-9.2)). Aortic stiffness was not clearly associated with stroke. Subjects with severe carotid stiffness had two-times as often a previous myocardial infarction (2.3 (1.1-5.1)) and twelve-times as often a previous stroke (12.6 (2.7 - 58.1)), compared to their reference category. The association of arterial stiffness with cardiovascular disease was comparable in magnitude with the association of atherosclerosis with cardiovascular disease.*
Conclusion: *The results of this study show that arterial stiffness is related to cardiovascular disease in a general population of elderly subjects.*

Introduction

Measures of arterial stiffness have been found to be related with myocardial infarction in several cross-sectional studies among various populations[1-7] and in

two longitudinal studies in subjects with end-stage renal disease.[8,9] The relation of arterial stiffness with stroke has been scarcely addressed. One small cross-sectional study reported increased aortic stiffness in patients with stroke.[10] Previous studies were all performed in small groups of selected subjects. The aim of the present study was to determine the association of arterial stiffness with myocardial infarction and stroke, in a large population-based study among elderly subjects. We compared the strength of an association between arterial stiffness and prevalent cardiovascular disease with the strength of an association between non-invasively measured atherosclerosis and prevalent cardiovascular disease. As measures of arterial stiffness we used carotid-femoral pulse wave velocity (PWV) and common carotid arterial (CCA) distensibility. Measures of atherosclerosis were the ankle-brachial pressure index (ABPI) and plaques in the carotid artery. We related both measures of arterial stiffness and both measures of atherosclerosis to history of myocardial infarction or stroke in a cross-sectional population-based study among 3818 elderly subjects participating in the third examination phase of the Rotterdam Study.

Methods

Study population

The Rotterdam Study 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.[11] Shortly, 7983 subjects aged 55 years or over were included in the first (baseline) examination phase that took place between 1990-1993. From 1997 until 1999 the third examination phase took place for which 5901 subjects of the original cohort were eligible. The remaining subjects of the cohort died in the meantime (n=1992), were lost to follow-up (n=35), or were not invited to participate because they were living in nursing homes outside the study area (n=55). Of all subjects invited for the third examination phase, 4148 subjects attended the physical examinations. The Medical Ethics Committee of the Erasmus University approved the study and written informed consent was obtained from all participants.

Measures of arterial stiffness

Arterial stiffness was measured by two different methods e.g., the carotid-femoral 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. The order of measurements was fixed with first a PWV measurement and approximately 10 minutes later a measurement of common carotid arterial distensibility. Subjects were instructed to refrain from smoking and from taking coffee, tea, alcohol or pain-medication on the day of the measurements, and from taking alcohol on the day of the measurements and the day before.

Carotid-femoral PWV was measured with subjects in supine position. After five minutes of rest blood pressure was measured twice with a conventional sphygmomanometer and the mean was taken as the subjects reading. Subsequently carotid-femoral PWV was measured. The time delay between the rapid upstroke of the feet of simultaneously recorded pulse waves was measured using an automatic device (Complior, Colson, Garges-lès-Gonesse Cx, France).[12] The distance traveled by the pulse wave between the carotid artery and the femoral artery was measured over the surface of the body using a tape measure. PWV was calculated as the ratio between the distance traveled by the pulse wave and the foot-to-foot time delay and expressed in meters per second. The average of at least 10 successive measurements, to cover a complete respiratory cycle, was used in the analyses.

Common carotid distensibility was assessed with the subjects in supine position, with the head tilted slightly to the contralateral side. The vessel wall motion of the right CCA was measured by means of a Duplex scanner (Ultramark IV, ATL, Bothell, Washington, USA) connected to a vessel wall movement detector system. The details of this technique have been described elsewhere.[13,14] After five minutes rest, 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 ($(\Delta D)/D$) were computed as the mean of four cardiac cycles of three successive recordings. Blood pressure was measured twice with a Dinamap automatic blood pressure recorder and the mean was taken as the subjects reading. Pulse pressure (ΔP) was calculated as the difference between systolic and diastolic blood pressure. The

cross-sectional arterial wall DC was calculated according to the following equation[15]: DC = (2ΔD/D)/ ΔP (10^{-3}/kPa).

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 CCA (unpublished results). A reproducibility study in 47 subjects showed an intra-class correlation coefficient of 0.80 for carotid-femoral PWV and 0.80 for the common carotid DC. Mean arterial pressure (MAP) was calculated from blood pressure readings during both measurements of arterial stiffness by the formula: MAP = diastolic blood pressure + 1/3 * ΔP.

Measures of atherosclerosis

The presence of atherosclerosis was assessed by two different methods e.g., the ABPI as measure of the presence of peripheral artery disease (PAD) and the presence of plaques in the carotid arteries.

Ankle systolic blood pressure was measured at the posterior tibial artery at both left and right ankle using an 8 MHz continuous wave doppler probe (Huntleigh 500 D, Huntleigh Technology, Bedfordshire, UK) and a random zero sphygmomanometer with the subject in supine position.[16] The ratio of the systolic blood pressure at the ankle to the systolic blood pressure at the right arm was measured for both ankles and the lowest ABPI in either leg was used.[17]

Ultrasonography of both left and right common and internal carotid arteries and left and right carotid artery bifurcation was performed with a 7.5 MHz linear-array transducer (Ultramark IV, ATL, Bothell, Washington, USA). Plaques were defined as a focal widening of the wall relative to adjacent segments, with protrusion into the lumen 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. A total carotid plaque score was obtained by summation of absence (score 0) or presence (score 1) of plaques on both sides at the three locations at the far and near wall of the carotid artery (maximum score of 12).

Vascular disease

A history of myocardial infarction or stroke was defined as a first or recurrent myocardial infarction or a first or recurrent stroke occurring between the baseline examination and the third examination phase (mean duration of follow-up: 6.6 years (range: 5.3 - 10.2 years)). Events were reported by general practition-

ers (GP) in the research area (85% of the cohort) by means of a computerized system. Research assistants subsequently collected all available information on the reported events. The GPs outside the research area (15%) were visited once a year by research assistant to check patient-records. In addition, discharge reports and letters of medical specialists were obtained for hospitalized patients. Two research physicians independently coded events according to the International Classification of Diseases, 10th edition[18], using all available information of an event. Myocardial infarction was coded as I21 and stroke was coded as I61 - I64. If there was disagreement, consensus was reached in a separate session by the same two research physicians. Subsequently, a medical expert in the field reviewed all events coded by the research physicians and checked whether all coding rules had been applied correctly. In case of discrepancies between the coding of the medical expert and the research physicians, the judgement by the expert was considered definite. A myocardial infarction was considered definite when a cardiologist or ECG confirmed the diagnosis and considered probable when a diagnosis of myocardial infarction was considered certain by the general practitioner in the absence of information from a cardiologist or ECG. A myocardial infarction was considered possible in all other cases. In the analysis, we only included definite and probable myocardial infarctions. A stroke was considered definite if the diagnosis was based on both clinical symptoms and neuro-imaging. A probable stroke was considered if no computer tomography (CT) or magnetic resonance imaging (MRI) was made but when symptoms were suggestive for stroke according to the GP or neurologist. In case of a fatal stroke reported by the GP, a cardiac cause of death should have been excluded to reach a diagnosis of probable stroke. The stroke was considered possible if a neurologist diagnosed a 'possible stroke' without neuro-imaging or if a GP recorded a fatal stroke and could not exclude a cardiac cause of death. When CT or MRI was performed and showed a hemorrhage or infarct the type of stroke was coded accordingly. A stroke was coded as unspecified when no CT or MRI was available to judge the subtype. In the analysis we included only definite and probable strokes. Of the 78 strokes included, 64 strokes were cerebral infarctions, 3 strokes were primary intracerebral haemorrhages, and 11 strokes were unspecified.

Vascular risk factors

Information on smoking was obtained during a home interview using a computerized questionnaire. Antropometric measures were obtained at the research center

while the subject was wearing lightweight clothes and no shoes, and included height, weight, waist and hip circumference. Body mass index (weight/height^2) and the waist-to-hip ratio were calculated. After an overnight of fasting, blood was obtained at the research center by venapuncture with minimal stasis using a 12 gauge Butterfly needle. 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).

Population for analyses

Of all participants who were eligible for a measurement of arterial stiffness (n=4148), a measurement of carotid-femoral PWV or a measurement of common carotid distensibility or both was available for 3818 subjects. From the 3818 eligible subjects, 339 subjects had a previous myocardial infarction or stroke before baseline examination but did not have a myocardial infarction or stroke after the baseline examination. These were excluded from the analyses, leaving 3474 subjects for analyses. In these subjects, information on PWV was available for 3175 subjects, information on common carotid distensibility was available for 2825 subjects, information on the ankle-brachial pressure index was available for 3302 subjects, and information on common carotid plaques was available for 3285 subjects. Missing information on measures of arterial stiffness or atherosclerosis was almost entirely due to logistic reasons.

Statistical analysis

Characteristics of subjects without myocardial infarction or stroke, subjects with myocardial infarction and subjects with stroke were tested for differences between groups after adjustment for age using analyses of covariance for continuous variables and logistic regression analyses for dichotomous variables. The odds ratios for previous myocardial infarction or stroke per quartile of measures of arterial stiffness or atherosclerosis were calculated using logistic regression analyses adjusted for age, sex, MAP and heart rate. For this purpose, we re-coded the carotid plaque score in four categories in such a way that each category comprised approximately 25% of the subjects. Increasing quartiles of both PWV and carotid plaques represent increasing severity, while increasing quartiles of both the common carotid DC and ABPI represent decreasing severity. Accordingly,

we chose the lowest or the highest quartile as the reference category. Analyses were repeated with additional adjustment for several cardiovascular risk factors (total cholesterol, HDL-cholesterol, glucose, body mass index, waist-to-hip ratio, and smoking). Separate models were used for PWV, the common carotid DC, the ABPI, and CCA plaques. Next, we examined whether the associations of both arterial stiffness and atherosclerosis with previous myocardial infarction

Table 1
Characteristics of the study population, The Rotterdam Study.

Characteristic	Subjects without myocardial infarction or stroke	Subjects with myocardial infarction	Subjects with stroke
Number	3310	95	78
Age (years)	72 (6.8)	74 (6.7)*	76 (7.6)†
Men (%)	39	67‡	48§
Systolic blood pressure (mmHg)	143 (21)	138 (21)‡	146 (20)
Diastolic blood pressure (mmHg)	76 (11)	70 (11)‡	75 (12)
Heart rate (bpm)	74 (12)	66 (12)‡	71 (13)
Smoking			
current (%)	15.8	17.9	13.0
past (%)	48.4	60.0‡	57.1
Body mass index (kg/m^2)	26.8 (4.0)	26.1 (4.1)	26.9 (3.6)
Waist-to-hip ratio (cm/cm)	0.92 (0.10)	0.95 (0.08)‡	0.95 (0.08)§
Total cholesterol (mmol/l)	5.9 (1.0)	5.4 (1.0)‡	5.9 (1.3)
HDL-cholesterol (mmol/l)	1.4 (0.4)	1.2 (0.3)‡	1.3 (0.3)§
Serum glucose (mmol/l)	5.9 (1.4)	6.4 (2.3)‡	6.2 (2.1)
Peripheral arterial disease (%)	15.6	32.2‡	40.9§
Presence of plaques in carotid artery (%)	14.4	30.4‡	29.0§
Distensibility coefficient (10^{-3}/kPa)	10.6 (4.4)	10.5 (4.8)	8.0 (3.3)§
Pulse wave velocity (m/s)	13.4 (3.0)	14.8 (3.3)‡	14.6 (3.1)

Values are expressed as mean (standard deviation) in case of continuous variables and as percentages in case of categorical variables.
* $p<0.05$ subjects with myocardial infarction versus subjects without myocardial infarction or stroke; † $p<0.05$ subjects with stroke versus subjects without myocardial infarction or stroke; ‡ $p<0.05$ subjects with myocardial infarction versus subjects without myocardial infarction or stroke, adjusted for age; § $p<0.05$ subjects with stroke versus subjects without myocardial infarction or stroke, adjusted for age.

and stroke were independent of each other by additional adjusting for the other. All analyses were performed using the SPSS 9.0 statistical package for Windows 98 (SPSS Inc., Chicago, Illinois, USA).

RESULTS

Characteristics of the study population are presented in table 1. Subjects without a previous myocardial infarction or stroke were younger, less often male, had fewer vascular risk factors and less often atherosclerotic disease as compared to subjects with previous myocardial infarction or stroke. Subjects with previous myocardial infarction had lower systolic and diastolic blood pressures and a lower heart rate as compared to subjects without previous myocardial infarction.

PWV and carotid plaques had the strongest association with previous myocar-

Table 2
Risk (OR) and 95% confidence interval (CI) of myocardial infarction per quartile of risk indicator.

	Model 1*			Model 2†		
	n	events	OR (95% CI)	n	events	OR (95% CI)
PWV (m/s)	3175	79		2858	70	
1st (≤ 11.3)	795	11	1.0 (reference)	652	10	1.0 (reference)
2nd (11.3-13.1)	794	15	1.7 (0.7 - 3.8)	747	13	1.5 (0.6 – 3.6)
3rd (13.1-15.1)	795	23	2.6 (1.2 - 5.6)	736	21	2.4 (1.0 – 5.4)
4th (>15.1)	791	30	4.0 (1.8 - 9.2)	723	26	3.5 (1.5 – 8.5)
DC of CCA (10^{-3}/kPa)	2825	77		2663	70	
1st (≤ 7.4)	706	23	2.3 (1.1 - 5.1)	572	23	2.0 (0.9 – 4.5)
2nd (7.4-10.0)	706	20	1.8 (0.9 - 3.8)	586	17	1.4 (0.6 – 2.9)
3rd (10.0-13.2)	707	15	1.1 (0.5 - 2.3)	604	11	0.8 (0.4 - 1.7)
4th (> 13.2)	706	19	1.0 (reference)	628	19	1.0 (reference)
ABPI	3302	87		2428	57	
1st (≤ 0.95)	787	36	3.2 (1.6 - 6.4)	586	19	2.2 (1.0 - 5.1)
2nd (0.95-1.05)	848	24	2.2 (1.1 - 4.7)	595	18	2.2 (1.0 - 5.0)
3rd (1.05-1.14)	832	16	1.6 (0.7 - 3.5)	613	11	1.3 (0.5 - 3.3)
4th (> 1.14)	835	11	1.0 (reference)	634	9	1.0 (reference)
Plaques in CA	3285	90		2485	61	
1st (0)	1074	11	1.0 (reference)	814	8	1.0 (reference)
2nd (1)	601	8	1.3 (0.5 - 3.2)	453	8	1.6 (0.6 - 4.3)
3rd (2-3)	815	25	2.7 (1.3 - 5.6)	633	18	2.4 (1.0 - 5.6)
4th (4-12)	795	46	4.3 (2.4 - 8.6)	585	27	3.3 (1.4 - 7.6)

n = number of subjects; PWV = pulse wave velocity; DC = distensibility coefficient; CCA = common carotid artery; ABPI = ankle-brachial pressure index; CA carotid artery. * Model 1: adjusted for age, sex, mean arterial pressure and heart rate. † Model 2: as model 1, except models with PWV and common carotid DC additionally adjusted for ABPI and CA plaques and models with ABPI and CA plaques additionally adjusted for PWV and common carotid DC.

dial infarction (Table 2, Model 1). Subjects in the highest quartile of both PWV and carotid plaques had four-times more often a myocardial infarction as compared to subjects in the respective reference categories. Subjects in the lowest quartile of common carotid DC had two-times more often a myocardial infarction and subjects in the lowest quartile of the ABPI had three-times more often a myocardial infarction as compared to their reference categories. Results were similar after additional adjustment for vascular risk factors (data not shown). The odds ratios for previous myocardial infarction associated with arterial stiffness and atherosclerosis, respectively, decreased slightly when adjusted for the presence of the other, though results generally remained significant (Table 2, Model 2).

Previous stroke was most strongly associated with the common carotid DC (Table 3, Model 1). Subjects in the lowest quartile of the common carotid DC had twelve-times more often a stroke as compared to the reference category.

Table 3
Risk (OR) and 95% confidence interval (CI) of stroke per quartile of risk indicator.

	Model 1*			Model 2†		
	n	events	OR (95% CI)	n	events	OR (95% CI)
PWV (m/s)	3175	72		2858	60	
1st (≤ 11.3)	795	8	1.0 (reference)	744	6	1.0 (reference)
2nd (11.3-13.1)	794	11	1.0 (0.4 - 2.6)	722	7	0.9 (0.3 - 2.7)
3rd (13.1-15.1)	795	30	2.4 (1.0 - 5.5)	704	27	3.1 (1.2 - 7.9)
4th (>15.1)	791	23	1.4 (0.5 - 3.4)	688	20	1.8 (0.6 - 5.1)
DC of CCA (10^{-3}/kPa)	2825	62		2663	53	
1st (≤ 7.4)	706	32	12.6 (2.7 - 58.1)	648	28	9.9 (2.1 - 46.6)
2nd (7.4-10.0)	706	13	5.9 (1.3 - 27.3)	664	9	3.7 (0.8 - 18.1)
3rd (10.0-13.2)	707	15	7.2 (1.6 - 32.0)	669	14	6.7 (1.5 - 30.0)
4th (> 13.2)	706	2	1.0 (reference)	682	2	1.0 (reference)
ABPI	3302	69		2428	49	
1st (≤ 0.95)	787	29	2.0 (1.0 - 3.9)	544	18	1.7 (0.8 - 3.8)
2nd (0.95-1.05)	848	17	1.3 (0.6 - 2.7)	634	12	1.2 (0.5 - 2.9)
3rd (1.05-1.14)	832	10	0.8 (0.3 - 1.8)	628	9	1.0 (0.4 - 2.5)
4th (> 1.14)	835	13	1.0 (reference)	622	10	1.0 (reference)
Plaques in CA	3285	76		2485	55	
1st (0)	1074	11	1.0 (reference)	814	8	1.0 (reference)
2nd (1)	601	17	3.0 (1.3 - 6.7)	453	11	2.3 (0.9 - 5.7)
3rd (2-3)	815	17	2.0 (0.9 - 4.9)	633	13	1.5 (0.6 - 3.7)
4th (4-12)	795	31	3.0 (1.4 - 6.5)	585	23	2.5 (1.1 - 5.8)

n = number of subjects; PWV = pulse wave velocity; DC = distensibility coefficient; CCA = common carotid artery; ABPI = ankle-brachial pressure index; CA carotid artery. * Model 1: adjusted for age, sex, mean arterial pressure and heart rate. † Model 2: as model 1, except models with PWV and common carotid DC additionally adjusted for ABPI and CA plaques and models with ABPI and CA plaques additionally adjusted for PWV and common carotid DC.

Subjects in the highest quartile of carotid plaques had three times more often a stroke and subjects in the lowest quartile of the ABPI had two-times more often a stroke as compared to the respective reference categories. PWV was not clearly associated with stroke. Results were similar after additional adjustment for vascular risk factors (data not shown). The odds ratios for previous myocardial infarction associated with arterial stiffness and atherosclerosis, respectively, decreased slightly when adjusted for the presence of the other, though results generally remained significant (Table 3, Model 2). Subjects in the highest category of the common carotid DC had still ten-times more often a stroke as compared to subjects in the reference category, independent of the presence of atherosclerosis.

Discussion

Our results show that aortic stiffness is increased in the presence of a myocardial infarction. The strength of the association between aortic stiffness and myocardial infarction is comparable with that of atherosclerosis. CCA stiffness is also associated with previous myocardial infarction, though less strong. CCA stiffness is strongly associated with previous stroke. The association is stronger than that of measures of atherosclerosis with previous stroke. All observed associations of arterial stiffness with cardiovascular disease were independent of generalized atherosclerosis.

Some methodological aspects need to be discussed. Firstly, assessment of vascular events from baseline examination onwards was complete for both myocardial infarction and stroke until January 1998. Because arterial stiffness was measured from September 1997 until July 1999, it is possible that at the time of the present analyses recent occurrences of a myocardial infarction or stroke were unknown. This will have resulted in incorrectly classifying some subjects as free of myocardial infarction or stroke. This misclassification of disease, however, can be considered to be independent of arterial stiffness and thus, if present, will have led to an underestimation of the observed association. Secondly, changes in life-style are likely to be induced by a cardiovascular event. This could diminish long-term contrast in arterial stiffness and atherosclerosis between subjects with and without a history of an event. To minimize the effect of changes in lifestyle without losing too many events we included only myocardial infarctions and strokes that occurred after baseline, resulting in a history of five to ten years. Thirdly, some subjects could not be included for various reasons. Evaluating the association of risk indicators with disease in subjects with prevalent disease

means only including survivors of myocardial infarction and stroke. Additionally, survivors of a myocardial infarction or stroke with considerable physical impairment might not be willing to visit the research center. Further, not for all subjects attending the third examination phase was information available on measures of arterial stiffness and measures of atherosclerosis due to logistic reasons. We do not think that missing these subjects has seriously altered our results. Missing subjects with severe disease will probably have led to an underestimation of the association, while random loss of subjects due to logistic reasons will not have biased our results.

We found both aortic and CCA stiffness to be associated with previous myocardial infarction. This is in agreement with previous studies but these studies included only a small number of subjects.[1-7] We found CCA stiffness to be associated with previous stroke, while no such association was found for aortic stiffness. Previous studies on the association between measures of arterial stiffness and stroke are limited to one cross-sectional study on aortic stiffness. This study found, in contrast with our results, a strong association.[10]

The association of arterial stiffness with cardiovascular disease may be explained partly through an association of arterial stiffness with atherosclerosis. Our results indicate, however, that the association between measures of arterial stiffness and previous myocardial infarction and stroke was only slightly attenuated after including measures of atherosclerosis in the model. This suggests that additional mechanisms play a role. Arterial stiffness leads to an increase in systolic blood pressure and simultaneously a decrease in diastolic blood pressure and thus an increase in pulse pressure. This may negatively affect the myocardium as an increase in systolic blood pressure leads to an increased workload of the heart, while a decrease in diastolic blood pressure may limit coronary perfusion. The stronger association of aortic stiffness as compared to CCA stiffness with myocardial infarction can probably be explained by the larger influence of the thoracic aorta than the CCA on load of the heart. Recent evidence shows that the combination of a high systolic blood pressure and a low diastolic blood pressure, resulting in an increased pulse pressure, is also a strong risk factor for stroke.[19,20] Our results show that CCA stiffness was strongly associated with previous stroke, comparable with the association of measures of atherosclerosis with previous stroke. Some authors suggest that the risk of embolisms due to rupture of plaques is increased in stiff arteries.[21] Especially when inhomogeneities in stiffness in and around the plaque are present, this is likely to result in increased shear stress and subsequent rupture. Besides enlargement of the pulse pressure, this mechanism could add to an association between CCA stiffness and

previous stroke. This mechanism would imply an interaction between common carotid artery plaques and stiffness in which the presence of one increases the risk associated with the other and vice versa. Unfortunately, we could not evaluate a possible interaction because of too small numbers of events.

We could not demonstrate an association between aortic stiffness and stroke, though subjects in the third quartile of PWV had a slightly increased risk of stroke as compared to subjects in the reference category. The absence of a clear association is difficult to explain, as one would expect to find an association between aortic stiffness and previous stroke when arterial stiffening is a generalized process throughout the arterial tree. Also, aortic stiffness probably contributes to an increased carotid pulse pressure. As mentioned before, one cross-sectional study found a strong association between aortic stiffness and stroke.[10] More studies are needed to elucidate the association between aortic stiffness and stroke.

This is the first large population-based study showing an association between arterial stiffness and cardiovascular disease. The strength of the association of arterial stiffness with cardiovascular disease is comparable with the strength of non-invasive measures of atherosclerosis with cardiovascular disease, which are established indicators of cardiovascular disease. Finding the association of arterial stiffness with myocardial infarction and stroke to be independent of atherosclerosis suggests that arterial stiffness is not only a risk indicator through an association with atherosclerosis but, possibly, also is a risk factor for cardiovascular disease in a general population of elderly subjects.

In conclusion, our results showed that arterial stiffness is associated with cardiovascular disease in a general population of elderly subjects.

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5.2

Aortic stiffness and the balance between cardiac oxygen supply and demand in an elderly population
The Rotterdam Study

Abstract

Background: *Aortic stiffness is an independent predictor of mortality. This has been explained in part by an increased cardiac oxygen demand in subjects with an increased aortic stiffness. We determined whether an increased aortic stiffness, as measured with aortic pulse wave velocity (PWV), may also decrease the perfusion pressure and thus cardiac oxygen supply potential.*

Methods: *PWV and aortic pressure waves, reconstructed from finger pressure waves, were obtained in 2490 elderly subjects, as part of the Rotterdam Study, a population based study in the elderly. Cardiac oxygen supply and demand were estimated using pulse wave analysis techniques, and related to PWV in multiple linear regression analyses after adjustment for sex, age, mean blood pressure and heart rate.*

Results: *Cardiac oxygen demand: both the systolic pressure time index (SPTI) and the rate pressure product increased with increasing PWV, 0.089 mmHg.s (95%CI: 0.049-0.128) and 42.2 mmHg/min (95%CI: 34.1-50.3) per 1 m/s increase in PWV respectively. Cardiac oxygen supply potential: the diastolic pressure time index (DPTI) decreased 0.098 mmHg.s per 1 m/s increase in PWV (95%CI: 0.052-0.145); the diastolic time fraction did not change with increasing PWV. In concordance the supply:demand ratio DPTI/SPTI decreased 0.006 for every 1 m/s increase in PWV (95%CI: 0.003-0.008).*

Conclusion: *Increments in aortic stiffness, as estimated with PWV, are not only related to increased cardiac oxygen demand, but also, due to a decreased diastolic perfusion pressure, to a decrease in cardiac oxygen supply potential. This offers an additional explanation for the relation between aortic stiffness and cardiovascular mortality.*

INTRODUCTION

Pulse wave velocity (PWV) in the aorta, which is related to aortic stiffness[1], is a potent predictor of both cardiovascular and all-cause mortality in a high risk population of patients with end-stage renal disease.[2] Pulse pressure, which is also largely determined by aortic stiffness[3], has also been shown to be an independent predictor of cardiovascular events and mortality both in the general population[4-6], and in subjects with hypertension[6,7], renal failure[8], or left ventricular dysfunction.[9] Since both PWV and pulse pressure are closely related to aortic stiffness, it is likely that aortic stiffness is the underlying cause of both risk factors for cardiovascular and non-cardiovascular mortality.

Apart from a possible correlation with atherosclerosis and coronary artery disease[10-13], the relation between aortic stiffness and cardiovascular mortality has been explained by an increase in cardiac oxygen demand: the cardiac afterload increases when the left ventricle has to pump into a less distensible aorta.[6] Decreased coronary perfusion, due to decreasing diastolic perfusion pressure and/or diastolic perfusion time, may, however, add to this risk, as demonstrated in animal experiments.[14]

We set out to determine whether increased aortic stiffness is associated with increased cardiac oxygen demand, and also with decreased cardiac oxygen supply potential, by a decreased cardiac perfusion pressure and/or relative perfusion time. Aortic stiffness was estimated with PWV measurements, whereas cardiac oxygen supply potential and cardiac oxygen demand were estimated using pulse wave analysis techniques.

METHODS

Subjects

This study was conducted within the Rotterdam study, an ongoing 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.[15] Measurements for the present study took place during a follow-up examination, between March 1997 and December 1999. The Medical Ethics Committee of the Erasmus University approved the study and written informed consent was obtained from all participants. The

analysis was performed on data of the first 3082 subjects, in whom both a PWV measurement and a finger pressure measurement were present. No further in- or exclusion criteria were applied.

Measurements

As a measure of aortic stiffness we measured the carotid-femoral pulse wave velocity. We used PWV, rather than pulse pressure, as a measure for aortic stiffness, since the indices of cardiac oxygen supply potential and cardiac oxygen demand are obtained independently of PWV but not of pulse pressure. With the subjects in supine position, the time delay between the feet of simultaneously recorded pulse waves was measured using an automatic device (Complior™, Colson, Garges-lès-Gonesse Cx, France).[1] The distance between the pulse recording sites at the carotid and femoral arteries was measured over the surface of the body using a tape-measure. PWV was calculated as the ratio between this distance and the foot-to-foot time delay and expressed in meters per second. The average of at least 10 measurements was used in the analyses, to cover a complete respiratory cycle. In a reproducibility study within 47 subjects of the Rotterdam study, PWV measurements showed an intraclass correlation coefficient of 0.80.

Finger pressure was measured noninvasively with FinapresTM (Ohmeda 2300e, Ohmeda, Louisville, Colorado)[16] at the middle finger of the left hand during the PWV measurements. An upper arm cuff was positioned on the same arm. It was inflated after the PWV measurements, to measure the Riva-Rocci systolic brachial artery pressure to correct for a possible pressure gradient between upper arm and finger using a return-to-flow algorithm.[17] Finger pressure and upper arm cuff pressure were digitized at 100 Hz and stored.

Pulse wave analysis

Aortic pressure waves were reconstructed from finger pressure by correcting both for pulse wave distortions and for a possible pressure gradient. We used aortic pressure, rather than finger pressure, for calculation of the indices of cardiac oxygen demand and supply potential, since wave reflections may cause substantial differences between the aortic pulse and peripheral pulses.[18] Such differences could affect the validity of the indices. A generalized transfer function[19,20] was used to correct for the physiological pulse wave distortions, mainly due to these reflections, occurring between aorta and finger. This transfer

function was developed with data from simultaneous measurements of finger pressure with Finapres and ascending aortic pressure with catheter-mounted micromanometers (unpublished data) and looks similar to the transfer function published by Karamanoglu and Feneley.[20] It compensates for the pulse wave distortion by slightly amplifying frequencies below 1 Hz, while attenuating higher frequency components with a peak attenuation around 4 to 5 Hz. Generalized waveform filters for the upper limb show little inter-individual variation, particularly at low frequencies.[20,21] Individual correction for a possible pressure gradient was based on a return-to-flow measurement, i.e. the pressure in a deflating upper arm cuff at the moment that the first pressure pulse is detected at the finger.[17] This provides level correction to upper arm pressure levels, which is considered acceptable since the difference between mean aortic pressure and mean brachial artery pressure is usually negligible.[22] The BeatScope software package (TNO-BMI, Amsterdam, the Netherlands) was used to detect return-to-flow and to calculate beat to beat values of heart rate and systolic, diastolic and mean reconstructed aortic pressure, with mean pressure defined as the true integrated mean pressure. Measurements with more than ten artifacts in finger pressure recordings, as determined by BeatScope, were excluded. Only recordings of at least one minute with a stable finger pressure signal and a successful return-to-flow measurement were used for further analysis.

To estimate cardiac oxygen supply potential the Diastolic Pressure Time Index (DPTI in mmHg.s)[23,24] was calculated as the area under the diastolic part of the aortic pressure wave (Figure 1). Because of the non-invasive nature of the measurements, we did not, as originally described by Buckberg et al.[23] subtract left ventricular pressure from aortic pressure before calculating DPTI. Diastole was defined as the period between the incisura in the aortic pressure wave and the start of the next systolic upstroke. Diastole determined from calculated aortic presssure waves differed 6.5±1.8% (mean ± standard deviation) from diastole determined from measured aortic waves in unpublished data. Cardiac oxygen supply potential was also assessed by calculating the Diastolic Time Fraction (DTF)[23,25] as the ratio of diastolic time and the interbeat interval (Figure 1). Both DPTI and DTF have been shown to correlate with subendocardial and mid-myocardial blood flow[23,24] when vasodilatory reserve is exhausted.[25-27] To estimate cardiac oxygen demand we calculated the Systolic Pressure Time Index (SPTI, in mmHg.s)[23,24] as the area under the systolic part of the aortic pressure curve (Figure 1), and the Rate Pressure Product (RPP in mmHg/min)[28] as the product of heart rate and systolic aortic pressure. Both SPTI and RPP have been shown to correlate with cardiac oxygen consumption.[23,24,28] DPTI/SPTI, or the

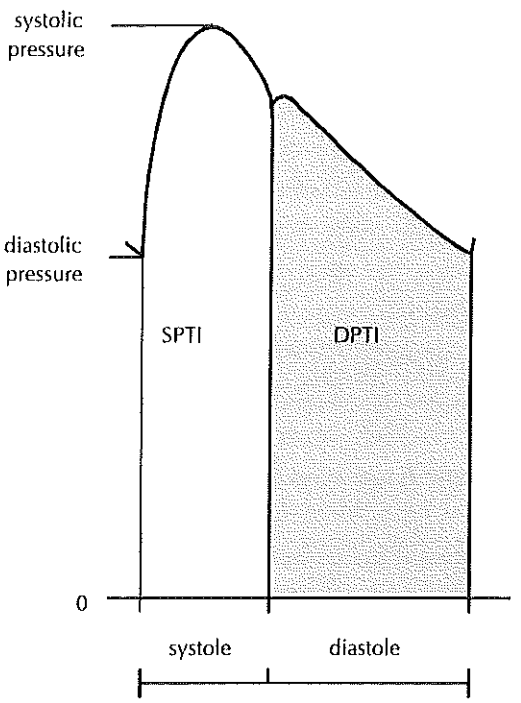


Figure 1
Calculation of the indices used to estimate oxygen supply and demand.
The Systolic Pressure Time Index (SPTI) and the Rate Pressure Product (RPP, the product of heart rate and systolic pressure) are estimates of cardiac oxygen demand. The Diastolic Pressure Time Index (DPTI), and the Diastolic Time Fraction (DTF, the ratio of the diastolic time and the interbeat interval) are estimates of the cardiac oxygen supply potential. DPTI/SPTI estimates the balance between cardiac oxygen supply and demand.

"supply:demand ratio" estimates the balance between cardiac oxygen supply and demand.[23,24] A decrease of this ratio below a critical level has been shown to be related to the occurrence of myocardial ischemia.[24,29]

Population for Analysis

A total of 2490 subjects was eventually used for the analysis, since 592 subjects were excluded. In 89 of them return-to-flow was not detected by BeatScope, eighter because the upper arm cuff was inflated to a level that was too low or because the deflation rate was too high. 308 more subjects were excluded because the finger pressure measurement was not stable for at least one minute before inflation of the upper arm cuff and 166 subjects were excluded because their finger pressure measurement contained more than ten artifacts. Twenty subjects were excluded because the number of repeated PWV measurements was smaller than ten and nine more subjects were excluded because the variation between the repeated PWV measurements was more than 10%.

Data analysis

After testing for a normal distribution, mean and standard deviation were calculated for all continuous parameters. The association of PWV with DPTI, DTF, SPTI, RPP and DPTI/SPTI was evaluated in five separate multiple linear regression analyses. In these analyses DPTI, DTF, SPTI, RPP and DPTI/SPTI were used as dependent variable and PWV, age, sex, mean aortic pressure and heart rate as independent variables. The regression analyses were performed for the total cohort and for men and women separately. DPTI, DTF, SPTI, RPP and DPTI/SPTI were also calculated per quartile of PWV, adjusted for age, sex, mean aortic blood pressure and heart rate, using analyses of covariance. A test for trend was performed using multiple linear regression with the indicators for oxygen supply and demand (DPTI, DTF, SPTI, RPP and DPTI/SPTI) as dependent variables in separate models and the quartiles of PWV, adjusted for age, sex, mean aortic pressure and heart rate, as ordinal independent variable. The correlation between PWV and heart rate was calculated in a multivariate model adjusting for age, gender and mean aortic pressure. The correlations between PWV and systolic and diastolic pressure were calculated in multivariate models adjusting for age, gender and mean aortic pressure and heart rate. A difference was considered to be statistically significant when the two-sided P-value was below 0.05. All analyses were performed with the SPSS 8.0 for Windows 95 statistical package (SPSS Inc., Chicago, Illinois, USA).

RESULTS

Baseline characteristics of the study population and average values of blood pressure, heart rate, PWV, and the supply and demand parameters are presented in Table 1.

Cardiac oxygen supply potential

DPTI decreased with 0.098 mmHg.s for every 1 m/s increase in PWV after adjustment for age, gender, mean aortic blood pressure, and interbeat interval (Table 2). In this model we adjusted for interbeat interval, rather than HR, since the relation between HR and DPTI was not linear, whereas that between interbeat interval and DPTI was. DPTI in the highest PWV quartile differed significantly from that in the other quartiles (Figure 2). Other differences between

Table 1
Characteristics of the study population.

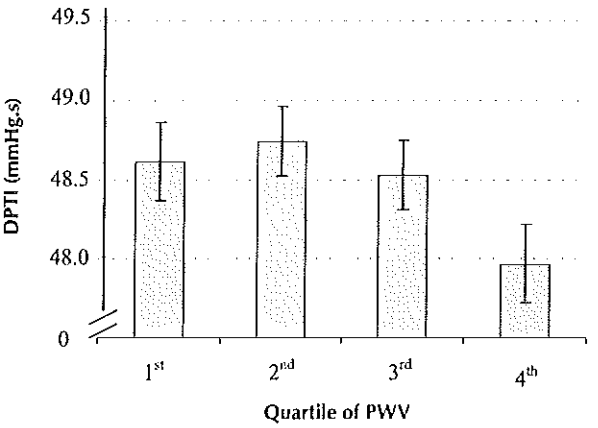
Characteristic	Value
Age (years)	71.6 ± 6.6
Female (%)	58.5
Diabetes Mellitus (%)	12.1
Weight (kg)	74.6 ± 12.4
Body Mass Index (kg/m^2)	26.8 ± 3.8
Blood Pressure (mmHg)	
Systolic	151.3 ± 21.1
Diastolic	84.6 ± 10.5
Heart Rate (min^{-1})	71.7 ± 11.0
Pulse Wave Velocity (m/s)	13.5 ± 3.0
Oxygen Supply Parameters	
DPTI (mmHg.s)	48.5 ± 11.0
DTF	0.616 ± 0.041
Oxygen Demand Parameters	
SPTI (mmHg.s)	37.7 ± 5.8
RPP (mmHg/min)	9426 ± 1999
Supply:Demand Ratio	
DPTI/SPTI	1.29 ± 0.23

DPTI: Diastolic Pressure Time Index, DTF: Diastolic Time Fraction, SPTI: Systolic Pressure Time Index, RPP: Rate Pressure Product.
Values are expressed as mean ± standard deviation for continuous parameters, or as percentage in case of categorical parameters.

Table 2
Beta coefficient and its 95% Confidence Interval (CI) describing the association of pulse wave velocity (PWV) with indices of cardiac oxygen supply potential and cardiac oxygen demand.

Indices	Beta - coefficient	95 % C.I.	P-value
Oxygen Supply Parameters			
DPTI (mmHg.s)	-0.098	-0.145 - -0.052	< 0.001
DTF	0.0003	0.0 - 0.0006	0.132
Oxygen Demand Parameters			
SPTI (mmHg.s)	0.089	0.049 - 0.128	< 0.001
RPP (mmHg/min)	42.2	34.1 - 50.3	< 0.001
Supply:Demand Ratio			
DPTI/SPTI	-0.006	-0.008 - -0.003	< 0.001

DPTI: Diastolic Pressure Time Index, DTF: Diastolic Time Fraction, SPTI: Systolic Pressure Time Index, RPP: Rate Pressure Product. All models were adjusted for age, sex, mean aortic pressure, and heart rate except the model with DPTI, which was adjusted for age, sex, mean aortic pressure, and interbeat interval.



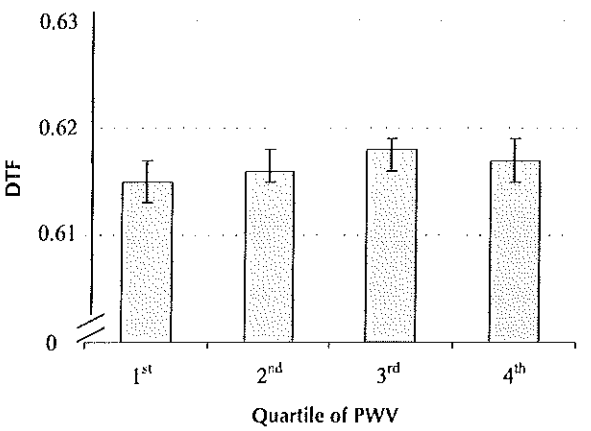


Figure 2
Diastolic Pressure Time Index* (DPTI) and the Diastolic Time Fraction† (DTF), two estimates of cardiac oxygen supply potential, per quartile of pulse wave velocity (PWV).

* Association adjusted for age, sex, mean aortic blood pressure and heart rate
† Association adjusted for age, sex, mean aortic blood pressure and interbeat interval

Bars indicate standard deviation.

quartiles PWV did not reach significance, but the test for trend was significant ($P = 0.003$). DTF did not change with PWV, when adjusted for age, gender, mean aortic blood pressure, and HR (Table 2). DTF in the third PWV quartile differed significantly from that in the lowest quartile (Figure 2). Other differences between quartiles PWV were not significant, neither was the test for trend ($P = 0.141$).

Cardiac oxygen demand

SPTI increased 0.089 mmHg.s for every 1 m/s increase in PWV, after adjustment for age, gender, mean aortic blood pressure, and HR (Table 2). SPTI differed between most quartiles of PWV (Figure 3), only the difference between

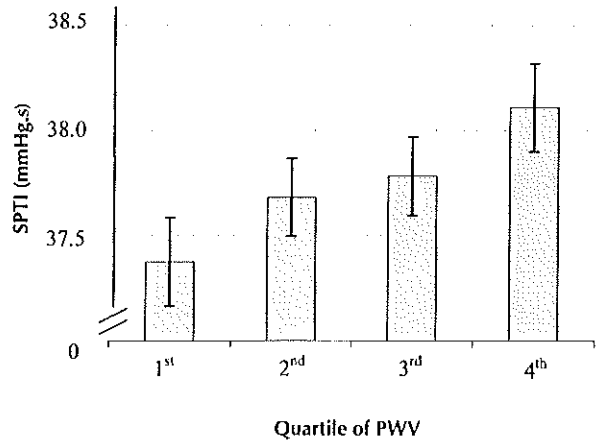
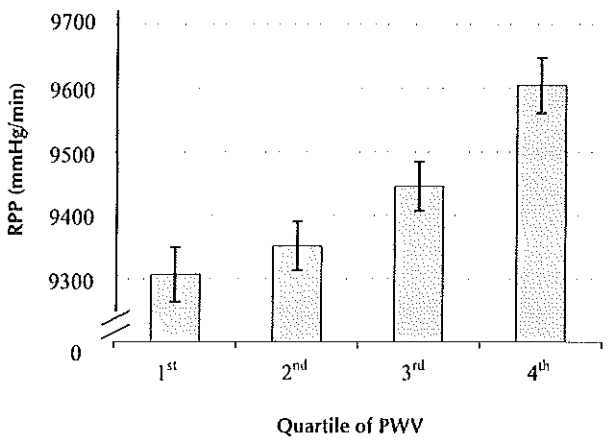


Figure 3
Mean Systolic Pressure Time Index* (SPTI), and the mean Rate Pressure Product* (RPP), two estimates of cardiac oxygen demand, per quartile of pulse wave velocity (PWV).

* Associations adjusted for age, sex, mean aortic blood pressure and heart rate.

Bars indicate standard deviation.



the second and the third quartile was not significant. The test for trend was significant ($P < 0.001$).

RPP was also positively associated with PWV after adjustment for age, gender, mean aortic blood pressure, and HR. RPP increased with 42.2 mmHg/min per 1 m/s increase in PWV (Table 2). The results of the model were similar when RPP was calculated with Riva-Rocci/Korotkoff systolic upper arm pressure, rather than with systolic aortic pressure (data not shown). RPP differed between most quartiles of PWV (Figure 3), only the difference between the first and the second quartile was not significant. The test for trend was significant ($P < 0.001$).

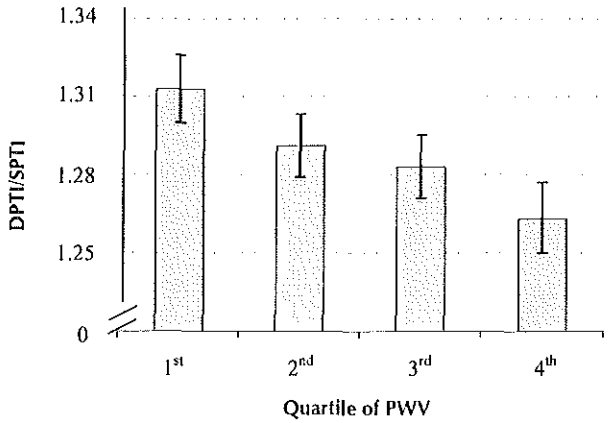


Figure 4
The balance between cardiac oxygen supply and demand* (DPTI/SPTI) per quartile of pulse wave velocity (PWV).

* Association adjusted for age, sex, mean aortic blood pressure and heart rate.

Bars indicate standard deviation.

Supply:demand ratio

The cardiac oxygen supply:demand ratio DPTI/SPTI decreased with 0.006 for every 1 m/s increase in PWV, after adjustment for age, gender, mean aortic blood pressure, and heart rate (Table 2). The supply:demand ratio differed significantly between all quartiles PWV (Figure 4). The test for trend was also significant ($P < 0.001$).

All models gave similar results in males and females (data not shown). PWV correlated positively to heart rate ($r = 0.18$, $P < 0.001$) in a multivariate model adjusting for age, gender and mean aortic pressure. PWV also correlated positively to systolic blood pressure ($r = 0.22$, $P < 0.001$), and negatively with diastolic aortic pressure ($r = -0.20$, $P < 0.001$) in multivariate models adjusting for age, gender, mean aortic pressure and heart rate.

Discussion

An increased cardiac oxygen demand has been suggested as an explanation for the relation between arterial stiffness and cardiovascular mortality.[6] In the present study we show that PWV, a measure of aortic stiffness, is not only related to an increased oxygen demand, but also to a decreased cardiac oxygen supply potential.

Some limitations of the study need to be discussed. Firstly, resting heart rate and carotid and aortic stiffness have, as in this study, been shown to be related to

each other.[30,31] Heart rate is a confounder in the association of PWV and the indices. We therefore adjusted for heart rate when studying the effect of PWV. The correlation between PWV and the 5 parameters studied was probably also weakened by the fact that we adjusted for age, gender and mean aortic pressure, three important physiological determinants of aortic stiffness.[32] We did, conservatively, correct for all these factors in order to be certain to study the effect of aortic stiffness itself on the estimates of cardiac oxygen supply and demand, rather than merely the combined effect of age, gender, mean aortic pressure and heart rate. However, by doing so we might have reduced the correlation between PWV and the indices. Secondly, the parameters shown in this study were calculated from aortic pressure waves reconstructed from noninvasively measured finger artery pressure recordings, using a generalized transfer function.[19,20] This transfer function mainly corrects for the amplification of the systolic pressure.[17,20,21] The use of it, therefore, mainly affects calculation of SPTI and RPP. However, results were similar when RPP was calculated from sphygmomanometrically obtained systolic blood pressure. For the calculation of pressure in diastole, and thus for calculation of DPTI, the lower frequency components of the transfer function are of importance. Since generalized waveform filters in the upper limb show little inter-individual variation at these low frequencies[20,21], calculation of DPTI with respect to pressure is hardly affected by the use of a generalized transfer function. The cardiac incisura, however, is a high frequency phenomenon. Because of larger inter-individual variation at high frequencies, the use of a generalized transfer function might hamper the identification of the cardiac incisura in reconstructed waves. Such identification is important for calculation of DPTI, DFT, SPTI and DPTI/SPTI. However, in agreement with others[20,21], we observed only minor differences between diastolic periods determined from measured aortic pressure waves and diastolic periods from reconstructed aortic pressure waves. Therefore, we feel that the associations of PWV with the estimates of cardiac oxygen demand and supply potential were not due to error introduced by the use of the transfer function.

Any decrease in the supply:demand ratio, DPTI/SPTI, may cause ischemia if the coronary flow reserve is exhausted.[24] This ratio has been shown to correlate with subendocardial flow in animal studies.[23] In healthy subjects subendocardial perfusion is hampered if DPTI/SPTI falls below 0.4 to 0.6.[24,29] The ratio's observed in this study, measured while subjects were resting, remained well above this limit in nearly all subjects. However, the ratio is known to decrease during exercise[29] and the critical limit increases when the vasodilatory reserve is exhausted, e.g. by the presence of coronary artery stenoses or left ventricular

hypertrophy.[24] Thus any decrease in DPTI/SPTI ratio may not cause myocardial ischemia in healthy subjects, but may explain coronary ischemia in subjects with existing coronary artery disease. DPTI and DTF have both been shown to correlate with subendocardial and midmyocardial blood flow[23,24], especially when vasodilatory reserve is exhausted.[25-27] SPTI and RPP have both been shown to correlate with cardiac oxygen consumption.[23,24,28]

The supply:demand ratio DPTI/SPTI decreased with increasing PWV. As hypothesized, the decrease in DPTI/SPTI with increasing PWV could both be attributed to a decrease in DPTI and to an increase in SPTI with increasing PWV. We might have underestimated the effect of PWV on DPTI and DPTI/SPTI, since we did not subtract the diastolic ventricular pressure before calculating DPTI, as originally described.[23] Subjects with higher PWV might be expected to have more left ventricular hypertrophy[33] and thus higher end diastolic ventricular pressures. Therefore it is very well possible that the decrease of DPTI and of DPTI/SPTI with increasing PWV was underestimated. DTF, a measure for the relative length of the diastolic time, did not change with increasing PWV. The decrease in DPTI with increasing PWV should therefore be attributed to a decrease in diastolic pressure in subjects with stiffer aortas. The lower diastolic pressure in subjects with stiffer aortas can be explained in two ways. It can either be due to a decreased windkessel function, or to a shift of reflected pressure waves into systole. In young subjects reflected pressure waves reach the ascending aorta during diastole, adding to the diastolic perfusion pressure. Aortic stiffness causes an increase in PWV. When PWV increases, reflected pressure waves arrive in the ascending aorta during systole, thus adding to the ventricular load, and cardiac oxygen demand.[18] Furthermore, these early reflected waves no longer add to the diastolic pressure, explaining the decrease in diastolic pressure and DPTI with increasing PWV observed in this study. The decrease in DPTI with increasing PWV indicates that the cardiac oxygen supply potential decreases with increasing aortic stiffness.

Even the stable DTF points to a decreased oxygen supply potential. The coronary oxygen supply potential is known to decrease in the presence of a stable DTF, when the left ventricular afterload increases.[34] The latter is certainly the case, given the increase in SPTI with PWV.

In healthy subjects, a decrease in diastolic perfusion pressure and/or time can be compensated by coronary dilatation. A decrease in oxygen supply potential results in a decreased coronary perfusion, only when the vasodilatory reserve is deminished or even exhausted.[25-27] The latter can be the case if coronary artery disease is present, or in case of left ventricular hypertrophy.[24] Aortic stiffness,

which results in part from atherosclerosis[35], has indeed been shown to correlate with intima media thickness in the common carotid artery[11], and with the presence of coronary artery disease.[12,13] Aortic stiffness, and the resulting systolic pressure augmentation, are also involved in the pathogenesis of left ventricular hypertrophy, by increasing the left ventricular afterload.[13,18,33] Thus an elevated PWV might not only cause a decrease in cardiac oxygen supply potential, it is also linked to those clinical conditions in which this decrease in oxygen supply potential is most relevant and potentially damaging.

Both the increase in SPTI and the increase in RPP indicate an increase in cardiac oxygen demand with increasing PWV. The increase in SPTI could be attributed to an increase in systolic pressure. This can in turn either be due to a decreased windkessel function, or to the afore-mentioned shift of reflected pressure waves into systole in subjects with increased aortic stiffness.[18] The increase in RPP results both from the increase in systolic pressure and heart rate with increasing PWV.

In conclusion, our findings support the view that the relationship between mortality and aortic stiffness or pulse pressure might not only be explained by an increase of oxygen demand, but also by a decrease of oxygen supply potential with increasing aortic stiffness.

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5.3

Aortic stiffness is associated with atherosclerosis of the coronary arteries *The Rotterdam Study*

Abstract

Background: *Aortic stiffness can lead to low diastolic blood pressure, thereby possibly limiting coronary perfusion. This can be compensated by coronary vasodilatation in healthy subjects but this reactive vasodilatation is limited in the presence of coronary atherosclerosis, leading to subendocardial ischemia. Therefore, simultaneous occurrence of both coronary atherosclerosis and aortic stiffness can lead to increased risk of subendocardial ischaemia. We studied the combined occurrence of both phenomena in a large, unhospitalized older population.*

Methods: *The study was performed in 763 subjects of the Rotterdam Study, a population-based study of elderly subjects. Coronary atherosclerosis was assessed by measuring coronary calcification with electron beam tomography and expressed as a total calcium score. The total calcium score was log-transformed because of its highly skewed distribution. Aortic stiffness was assessed by measuring carotid-femoral pulse wave velocity. The association between pulse wave velocity and coronary calcification was evaluated after adjustment for age, sex, mean arterial pressure and heart rate.*

Results: *Linear regression analyses showed that increased pulse wave velocity was associated with a higher log total coronary calcium score (β-coefficient (95% confidence interval): 0.12 (0.05-0.18), p<0.001). Logistic regression analyses showed that for each standard deviation increase in pulse wave velocity, the risk on advanced coronary calcification increased with 47% (odds ratio (95% confidence interval): 1.47 (1.05-2.06)).*

Conclusions: *The results of this population-based study in elderly subjects showed a strong association between aortic stiffness and coronary atherosclerosis. The occurrence of both processes simultaneously may indicate a high-risk group for subendocardial ischaemia.*

Introduction

Several small studies showed that subjects with cardiovascular disease have increased aortic stiffness as compared to subjects without cardiovascular disease.[1-3] Aortic stiffness has also been shown to be a predictor of all-cause and cardiovascular mortality in subjects with end-stage renal disease.[4] These results suggest an association between arterial stiffness and atherosclerosis. However, one study has evaluated the relation between aortic stiffness and calcification of the coronary arteries assessed with electron beam tomography, and could not demonstrate an association.[5] Aortic stiffness can have a negative effect on the myocardium as increased aortic stiffness leads to an increase in systolic blood pressure, thereby increasing the workload and oxygen-consumption of the heart. Furthermore, the diastolic blood pressure is decreased by aortic stiffness, thereby limiting the coronary perfusion.[6] In healthy subjects, a decreased coronary perfusion pressure can be compensated by coronary vasodilatation.[7] However, in the presence of coronary atherosclerosis, the vasodilatory reserve is limited and a decreased perfusion pressure, in these circumstances, can lead to decreased oxygen supply, especially subendocardial supply.[8-11] From this follows that the negative effect of aortic stiffness on the heart can be expected to be more pronounced when aortic stiffness is associated with coronary atherosclerosis. If these two phenomena indeed occur together more frequently than expected by chance, this would indicate the presence of a high-risk group at increased risk for subendocardial ischemia. The objective of the present study was to study the association between aortic stiffness and atherosclerosis of the coronary arteries, assessed by measuring coronary calcification with electron beam tomography, in a large population-based study. The study was performed in the Rotterdam Study, a cohort study among elderly subjects.

Methods

Study Population

The Rotterdam Study 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.[12] In March 1997, the third examination phase started. The Medical Ethics Commit-

tee of the Erasmus University approved the study and written informed consent was obtained from all participants. For the present study, the first 780 subjects who attended the follow-up examination and had both a measurement of aortic stiffness and coronary calcification were eligible.

Aortic stiffness

Aortic stiffness was assessed by measuring carotid-femoral pulse wave velocity (PWV). PWV was not measured in 12% of all subjects who attended the third examination phase, which was almost entirely due to logistic reasons. 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 measurement. Carotid-femoral PWV was measured with the subject in supine position. After five minutes rest, blood pressure was measured twice with a conventional sphygmomanometer and the mean was taken as the subjects reading. Mean arterial blood pressure (MAP) was calculated by the following formula: diastolic blood pressure + 1/3 * (systolic blood pressure - diastolic blood pressure). Subsequently PWV was measured. The time delay between the feet of simultaneously recorded pulse waves was measured using an automatic device (Complior, Colson, Garges-lès-Gonesse Cx, France).[13] The distance traveled by the pulse wave between the carotid artery and the femoral artery was measured over the surface of the body using a tape measure. PWV was calculated as the ratio of the distance traveled by the pulse wave and the foot-to-foot time delay and expressed in meters per second. The average of at least 10 successive measurements, to cover a complete respiratory cycle, was used in the analyses. All measurements were performed by three observers. A reproducibility study in 47 subjects showed an intra-class correlation coefficient of 0.80 for carotid-femoral PWV.

Coronary atherosclerosis

Coronary atherosclerosis was assessed by measuring coronary calcification with an Imatron C-150 EBT scanner (Imatron, South San Fransisco, California). All subjects under 85 years of age were invited for measurement of coronary calcification by electron beam tomography. Subjects were placed in supine position. The scan was obtained using a neutral, transverse position of the subject with the single slice mode (SSM), with 3mm slice thickness, 100ms exposure time at 130kV and 630 mA, during ECG gating at 80% of the R-R interval in suspended

inspiration. In this mode 38 adjacent slices were obtained from the level of the root of the aorta through the entire heart. Coronary calcification was quantified off-line by encircling each area with high density in the course of an epicardial coronary artery thus indicating a region of interest around the presumed lesion. Software, provided by AccuImage Diagnostics Corperation displays within this region of interest, all pixels having Hounsfield units higher than 130. The calcium score is then obtained by multiplying the area of interest, when it was larger than 0.65 mm^2, with a factor indicating maximum density within that area, as proposed by Agatston.[14] As the distribution of the calcium score is highly skewed, a log-transformation of the calcium score is used in the analyses, according to the following formula: log calcium score = ln (total calcium score + 1). The value of 1 was added to the total calcium score as many subjects had a total calcium score of zero. Subjects in the highest quartile of the calcium score were considered to have advanced calcification. Subjects without calcifications of the coronary arteries (12.7%) were used as reference group.

Cardiovascular risk factors and atherosclerosis

Information on smoking behavior was obtained during a home interview using a computerized questionnaire. Anthropometric measures were obtained at the research center while the subject was wearing lightweight clothes and no shoes, and included height, weight, waist and hip circumference. Body mass index (weight/$height^2$) and the waist-to-hip ratio were calculated. After an overnight of fasting, blood was obtained at the research center by venapuncture with minimal stasis using a 12 gauge Butterfly needle. Serum total cholesterol and high-density lipoprotein (HDL) cholesterol were determined using an automatic enzymatic procedure (Boehringer Mannheim Systems, Mannheim, Germany). Glucose was enzymatically determined by the Hexokinase method (Boehringer Mannheim Systems, Mannheim, Germany).

The presence of plaques in the common carotid artery was assessed by on-line evaluation of ultrasonographic images of the common carotid artery. Plaques in the common carotid artery was coded as 0 (no plaques) and 1 (presence of plaques at the far or near wall of the left or right common carotid artery). Systolic blood pressure of the posterior tibial artery was measured at both left and right ankle using an 8 MHz continuous wave doppler probe (Huntleigh 500 D, Huntleigh Technology, Bedfordshire, UK) and a random zero sphygmomanometer with the subject in supine position.[15] The ratio of the systolic blood pressure at the ankle to the average systolic blood pressure at the right arm was computed.

This ankle-brachial pressure index was calculated for both ankles. In agreement with the approach followed by Fowkes[16], we used the lowest ankle-brachial pressure index in either leg in the analyses. A low ankle-brachial pressure index indicates the presence of atherosclerosis of the lower extremities.

Population for analyses

The first 780 subjects who attended the third examination phase of the Rotterdam Study and in whom both aortic stiffness and coronary calcification was measured were eligible for the present analyses. Nine subjects were excluded because the total calcium score was higher than 5000. Additionally, five subjects were excluded because the number of repeated PWV measurements was smaller than ten and five more subjects were excluded because the variation between the repeated PWV measurements was more than 10%. In total 19 subjects were excluded, leaving 763 subjects for analyses. In a sub-analysis in which we adjusted for cardiovascular risk factors, we included all subjects with complete information on all cardiovascular risk factors (n=739). In a sub-analysis in which we adjusted for the presence of atherosclerosis we included all subjects with complete information on presence of atherosclerosis in the carotid artery and peripheral artery disease (n=715).

Statistical analysis

The association between PWV and the total calcium score was examined in three ways. Firstly, the mean log calcium score was calculated per quartile of PWV. Differences between the groups were tested using one way analyses of covariance, adjusted for age, sex, MAP and heart rate. Secondly, we determined whether PWV as a continuous variable was associated with the log calcium score, using multiple linear regression analysis with log calcium score as the dependent variable and PWV as the independent variable, adjusted for age, sex, MAP, and heart rate (model A). Model A was extended with several cardiovascular risk factors being body mass index, total cholesterol, HDL cholesterol, diabetes mellitus, and smoking (model B). Model A was also extended with measures of atherosclerosis being the presence of atherosclerosis in the arteries of the lower extremities and presence of plaques in the common carotid artery (model C). Finally, logistic regression analyses was used to calculate the odds ratio and its 95% confidence interval for presence of advanced coronary calcification per standard deviation increase in PWV, adjusted for age, sex, MAP, and heart rate. All analyses were

performed using the SPSS 8.0 statistical package for Windows 95 (SPSS Inc., Chicago, Illinois, USA).

RESULTS

Characteristics of the study population are presented in table 1. The levels of cardiovascular risk factors and presence of atherosclerosis were in the high normal range, as expected for a general population of elderly subjects. As distribution of the total calcium score was highly skewed, both the median and inter-quartile range of the total calcium score and the mean and standard deviation of its log-transformation are given.

Values of log total calcium score per quartile of PWV, adjusted for age, sex, MAP and heart rate are shown in figure 1. Mean values of log total calcium score

Table 1
Characteristics of the study population, The Rotterdam Study.

Characteristic	Value
Age (years)	70 ± 5.6
Male (%)	48
Systolic blood pressure (mmHg)	150 ± 20
Diastolic blood pressure (mmHg)	84 ± 10
Heart rate (min^{-1})	73 ± 12
Body mass index (kg/m^2)	26.8 ± 3.6
Total cholesterol (mmol/l)	5.8 ± 0.9
HDL-cholesterol (mmol/l)	1.4 ± 0.4
Smoking	
current (%)	17
past (%)	52
Presence of diabetes mellitus (%)	11
Presence of plaques in common carotid artery (%)	15
Presence of peripheral artery disease (%)	14
Total calcium score*	114.5 (8.25 - 542.8)
Log total calcium score	4.2 ± 2.5
Pulse wave velocity	13.07 ± 2.77

Values of continuous variables are expressed as mean ± standard in case of a normal distribution or as median (inter-quartile range) in case of a non-normal distribution*. Categorical variables are expressed as percentage.

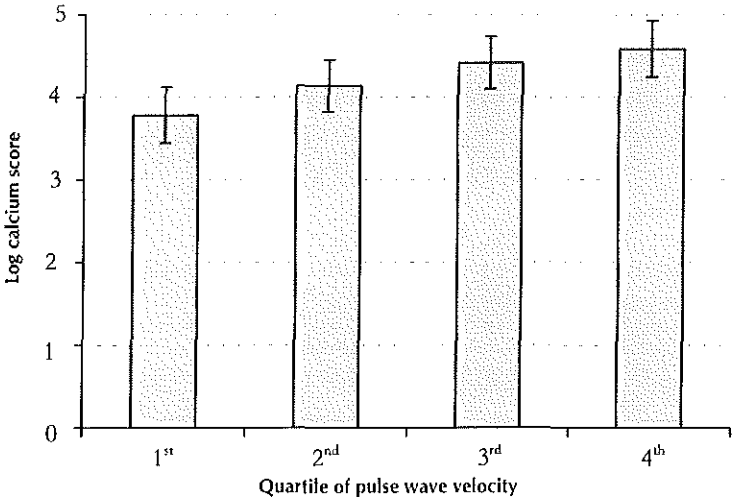

Figure 1
Mean log calcium score (95% confidence intervals) per quartile of pulse wave velocity, adjusted age, sex, mean arterial blood pressure, and heart rate.

were 3.78, 4.14, 4.41, and 4.59 for the first, second, thirth and fourth quartile of PWV, respectively. Subjects in the third and fourth quartile of PWV had a significantly higher log calcium score as compared to subjects in the first quartile of PWV ($p = 0.009$ and $p = 0.002$, respectively). The results of the regression analysis are presented in table 2. Pulse wave velocity was highly significantly associated with the log calcium score, after adjustment for age, sex, MAP and heart rate. Additional adjustment for several cardiovascular risk factors did hardly affect the strength of the association while additional adjustment for the presence

Table 2
Linear regression coefficients describing the increase in log total calcium score per 1 m/s increase in pulse wave velocity, The Rotterdam Study.

Model	N	B-coefficient*	95 % confidence interval	p-value
Model A†	763	0.116	0.051 to 0.181	< 0.001
Model B‡	739	0.113	0.048 to 0.178	0.001
Model C§	715	0.081	0.015 to 0.147	0.017

* Model A: adjusted for age, sex, mean arterial blood pressure and heart rate.
‡ Model B: as model A with additional adjustment for body mass index, total cholesterol, HDL-cholesterol, smoking and diabetes mellitus.
§ Model C: as model A with additional adjustment for peripheral artery diseases and plaques in the common carotid artery.

of carotid and peripheral atherosclerosis attenuated the association, though the association remained significant (Table 2). For each standard deviation increase in PWV, the risk on advanced coronary calcification increased with 47% (OR and its 95% confidence interval: 1.47 (1.05-2.06)), after adjustment for age, sex, MAP and heart rate.

Discussion

The results of our population-based study in elderly subjects show that aortic stiffness is associated with atherosclerosis of the coronary arteries.

Some aspects of the study need to be discussed. Firstly, we use coronary calcification, as detected by electron beam tomography, as a measure of coronary atherosclerosis. Blankenhorn summarized the evidence that coronary artery calcification occurs only at sites involved with atherosclerosis.[17] Several studies showed that calcification is more often present in nonstenotic disease than in stenotic disease.[18] A total calcium score assessed by electron beam tomography has been shown to correlate well with the histomorphometric calcium area[19] and with histopathologic established coronary atherosclerotic plaque area.[20] The total coronary calcium score, assessed by electron beam tomography, is strongly related to angiographically established coronary artery disease.[21] Secondly, we excluded some subjects (1%) with total calcium scores above 5000 from our analyses. We consider these calcium scores to be out of the physiological plausible range. These high calcium scores might have been induced by partly overprojection of the aorta and thus including calcification of the aorta in the total calcium score. Repeating the analyses including these subjects, however, did not alter the results. Thirdly, the calcium score as constructed by Agatson et al.[14] uses both the area of calcification and the density, as weighing factor of the calcified area. The density of a calcified lesion is coded as 1 to 4 depending on the maximum density in the area. This can result in an inaccurate score as only the maximum density is used for the total calcified area. The reproducibility of calcium scoring using Agatsons formula has been shown to be limited.[18,22] At this moment, however, the calcium score based on Agatsons formula is still widely used as a quantitative measure of coronary calcification and is the only widely accepted and available scoring system. Any misclassification of the total calcium score induced by using the Agatson formula is likely to be independent of the arterial stiffness and will therefore have lead to an underestimation of the association.

We found a strong association between aortic stiffness and atherosclerosis of the coronary arteries, which is in accordance with some previous small studies that showed increased aortic stiffness in subjects with coronary artery disease assessed by angiography.[1,23,24] However, one previous study examined the association of aortic stiffness and coronary calcification measured by electron beam tomography in 190 asymptomatic men at risk for cardiovascular disease but found no association.[5] The relatively small number of subjects in the study, resulting in little power to disclose an association, might explain the discrepancy with our results.

Common determinants of arterial stiffening and atherosclerosis might partly explain the observed association. If this hypothesis is correct, adjustment for common determinants of both processes is expected to attenuate the association between aortic stiffness and coronary atherosclerosis. However, when additional adjustment for the presence of cardiovascular risk factors as common determinants was performed, the strength of the association did hardly change. Besides common determinants, other mechanisms may explain the association between aortic stiffness and atherosclerosis of the coronary arteries. There is evidence that the presence of atherosclerosis leads to stiffening of the arteries.[25] Conversely, increased arterial stiffness may lead to atherosclerosis by vessel wall damage. Without the shock-absorbing capacity, the stiff arterial wall may be subjected to greater shear and intraluminal stresses due to an increased pulsatile pressure.[26] Both atherosclerosis and arterial stiffness are likely to be generalized processes, occurring throughout the arterial system when present. If this is true and if one process is a causal factor in the pathogenesis of the other and vice versa, one would expect to find a strong, synergistic, association between arterial stiffness and atherosclerosis independent of the vessel beds studied. Additional adjustment for the presence of atherosclerosis at other sites of the arterial tree attenuated the observed association, which is consistent with this view.

Increased aortic stiffness can lead to an increase in systolic blood pressure, thereby increasing the workload and oxygen-consumption of the heart, and simultaneously to a decrease in diastolic blood pressure, thereby possibly limiting the coronary perfusion.[6] A heart with a normal coronary circulation is capable of regulating coronary blood flow by means of vasodilatation to secure metabolic needs of the myocardium even when diastolic perfusion pressure falls.[7] In the presence of coronary artery disease, however, this regulation mechanism can be exhausted.[8-11] In these circumstances, a fall in aortic diastolic blood pressure and a subsequent decrease of coronary perfusion pressure can lead to myocardial ischemia, especially subendocardial ischemia. An experimental study in

dogs showed that decreased aortic compliance greatly increased the risk of subendocardial ischemia in the presence of coronary stenosis.[27] Our results show that subjects with increased aortic stiffness have a 47% higher risk of having advanced calcifications of the coronary arteries. This may indicate a group at risk for subendocardial ischemia and subsequent cardiac events when the mechanism as observed in animals also applies to humans. This could imply that antihypertensive therapy in subjects with isolated systolic hypertension and increased pulse pressure due to increased arterial stiffness could be hazardous because of further lowering the diastolic blood pressure. However, large clinical trials showed that blood pressure lowering drugs in subjects with isolated systolic hypertension and a high pulse pressure greatly decrease cardiovascular risk.[28-30] A recent meta-analysis that showed a large benefit of treating isolated systolic hypertension in the elderly, however, also showed that for every level of systolic blood pressure, the diastolic blood pressure was inversely associated with cardiovascular mortality.[31] This paradox might be explained by a greater favorable effect of antihypertensive therapy on cardiac oxygen demand by lowering the systolic blood pressure as compared to the hazardous effect of antihypertensive therapy on cardiac oxygen supply by lowering the diastolic blood pressure. Furthermore, several blood pressure lowering drugs may also decrease stiffness of the arteries[32-36], which by itself may lead to an increase in diastolic blood pressure. In subjects with an increased pulse pressure due to increased arterial stiffness it may be indicated to selectively lower the systolic blood pressure without altering diastolic blood pressure.[31,37]

In conclusion, the results of this population-based study in elderly subjects show that aortic stiffness frequently occurs in combination with atherosclerosis of the coronary arteries. As subjects with coronary atherosclerosis have may lost their ability to compensate for a decreased coronary perfusion following increased aortic stiffness, it is important to recognize the frequent simultaneous concurrence of these conditions.

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Chapter 6

Arterial stiffness as underlying mechanism of disagreement between an oscillometric blood pressure monitor and a sphygmomanometer

Abstract

Background: *Oscillometric blood pressure devices tend to overestimate systolic blood pressure and underestimate diastolic blood pressure as compared to sphygmomanometers. Recent studies indicate that discrepancies in performance between these devices may differ between healthy and diabetic subjects. Arterial stiffness in diabetics could be the underlying factor explaining these differences. We studied differences between a Dinamap oscillometric blood pressure monitor and a random-zero sphygmomanometer in relation to arterial stiffness in 1808 healthy elderly subjects.*

Methods: *The study was conducted within the Rotterdam Study, a population based cohort study of subjects aged 55 years and over. Systolic and diastolic blood pressure differences between a Dinamap and a random-zero sphygmomanometer were related to arterial stiffness, as measured by carotid-femoral pulse wave velocity.*

Results: *Increased arterial stiffness was associated with higher systolic and diastolic blood pressure readings by the Dinamap as compared to the random-zero sphygmomanometer, independent of age, sex and average mean blood pressure level of both devices. The beta-coefficient (95% confidence interval) was 0.25 (0.00-0.50) mmHg/(m/s) for the systolic blood pressure difference and 0.35 (0.20-0.50) mmHg/(m/s) for the diastolic blood pressure difference.*

Conclusion: *The results indicate that a Dinamap oscillometric blood pressure device, in comparison to a random-zero sphygmomanometer, overestimates systolic and diastolic blood pressure readings in subjects with stiff arteries.*

INTRODUCTION

Automatic oscillometric blood pressure devices are frequently used to measure blood pressure. Several studies, evaluating their performance in comparison with a Hawksley random-zero or conventional sphygmomanometer, showed that oscillometric devices tend to overestimate SBP and underestimate DBP as compared to sphygmomanometers.[1-4] Recent studies indicate that differences in performance between these devices may differ between healthy and diabetic subjects.[5-7] One study, comparing a Dinamap 8100 oscillometric device to a Hawksley random-zero sphygmomanometer in diabetic subjects, found that the Dinamap overestimated SBP below 118 mmHg and underestimated SBP above 152 mmHg, while DBP was underestimated over the whole range of pressures.[5] Another study compared a SpaceLabs 90207 oscillometric device with a sphygmomanometer in diabetic subjects and healthy controls. The SpaceLabs device overestimated SBP both in diabetic subjects and in controls, but the overestimation

was more pronounced in the diabetic subjects. DBP was underestimated in both groups, but less pronounced in diabetic subjects.[6,7]

An oscillometric blood pressure device determines blood pressure by detecting a sequence of oscillations in cuff pressure while the pressure is reduced.[8] As diabetic patients have stiffer arteries than non-diabetic subjects[9], arterial stiffness could be the underlying mechanism of the more pronounced differences between oscillometric devices and sphygmomanometers in this group. We evaluated determinants of differences between an oscillometric blood pressure device and a sphygmomanometer in a large population-based cohort of elderly subjects.

Methods

Study Design

This study was conducted within the Rotterdam study. The Rotterdam Study 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.[10] For the present analyses, all measurements took place during a follow-up examination between March 1997 and January 1999. Blood pressure measurements taken by a Dinamap and a random-zero blood pressure monitor were compared in the first 1808 subjects, which participated at the follow-up examination. The Medical Ethics Committee of the Erasmus University approved the study and written informed consent was obtained from all participants.

Measurement of Blood Pressure

Blood pressure was measured in a fixed order, first with a Dinamap xl vital signs monitor (Critikon Inc, Tampa, Florida, USA) and approximately 15 minutes later with a Hawksley MKII random-zero sphygmomanometer (Hawksley and Sons Ltd). A medical doctor took all Dinamap readings with the subject in supine position. An experienced research nurse, who was not aware of Dinamap recordings, took all random-zero readings while the subject was sitting. Blood pressure was measured twice at the right arm after five minutes of rest and cuff size as recommended by the manufacturer was used on all occasions. For random-zero recordings, Korotkoff sounds phase one and five were taken for SBP and DBP,

respectively. Readings were recorded to the nearest 2 mmHg.

Measurement of Arterial Stiffness

Arterial stiffness was assessed by carotid-femoral PWV. The time delay between the rapid upstroke of the feet of simultaneously recorded pulse waves in the carotid artery and the femoral artery was measured using an automatic device (Complior, Colson, Garges-lès-Gonesse Cx, France).[11] The distance traveled by the pulse wave between carotid and femoral artery was measured over the surface of the body using a tape measure. PWV was calculated as the ratio of the distance traveled by the pulse wave and the foot-to-foot time delay and expressed in meters per second. To cover a complete respiratory cycle, the average of at least 10 successive measurements was used in the analyses.

Control-study

The population-based study was performed on a large number of subjects, thereby optimizing the opportunity to study determinants of differences between blood pressure measuring devices. However, several aspects in the design of the population-based study may create differences between blood pressure devices. We conducted a second study to examine whether observed differences between the two monitors in the population-based study were due to these non-optimal design-aspects. To optimize conditions, this control-study was performed according to the British Hypertension Society protocol Part II, validation procedures in elderly subjects.[12] Both devices were compared in two groups of 28 subjects, selected from the 1808 subjects of the population-based study. Selection was based on their SBP and DBP differences, age and arterial stiffness status, as defined by PWV, observed in the population-based study. One group comprised subjects with SBP and DBP differences between devices, age and PWV all below the mean of the respective distributions in the population-based study (non-stiff group), the other group comprised subjects with these characteristics all above the mean of the respective distributions in the population-based study (stiff group). This selection resulted in assigning subjects with lower or slightly higher Dinamap readings than random-zero readings to the group referred to as non-stiff group and assigning subjects with considerably higher Dinamap readings than random-zero readings to the group referred to as stiff group. Thus, selection was made on both arterial stiffness status and blood pressure differences between devices. Under the assumption that there are no unknown alterna-

tive explanations for the association between arterial stiffness and differences between the devices, observing the same difference between the devices in a new study, in which non-optimal design aspects are removed, indicates that the difference can be truly ascribed to arterial stiffness. A sequential comparison was performed on the right arm with a single cuff. The length of the cuff was chosen to be sufficient to encircle 80% of the subject's arm circumference. A conventional sphygmomanometer was included in the comparison. The three different devices were used alternately. A total of three blood pressure measurements, two minutes apart, were performed with each device while the subject was sitting, without prior rest. The order of the device was determined by randomization with a dice. One experienced research assistant, who was unaware of the research question, performed all measurements. For readings with a sphygmomanometer, Korotkoff sounds phase one and five were taken for SBP and DBP, respectively. Readings were recorded to the nearest 2 mmHg. The same equipment was used throughout the study period.

Statistical Analysis

In the population-based study, blood pressure values are based on the mean of two successive readings. Differences are presented as Dinamap minus random-zero values. A paired T-test was used to evaluate whether differences between random-zero and Dinamap were significantly different from zero. Determinants of the SBP and DBP difference were evaluated using multiple linear regression analyses with SBP or DBP difference as dependent variable, adjusted for average mean blood pressure level of both devices (Dinamap + random-zero/2). This analysis was done for the total cohort and in strata of sex. Subsequently, mean SBP and DBP differences were calculated per quartile of PWV, adjusted for age, sex, and average mean blood pressure level of both devices, using analyses of covariance. A test for trend was performed using multiple linear regression analyses with quartiles of PWV as ordinal variable.

In the control-study, blood pressure values were based on the mean of three readings with each device. Differences are presented as Dinamap minus random-zero values, Dinamap minus conventional sphygmomanometer values, and random-zero minus conventional sphygmomanometer values. A paired T-test was used to evaluate whether observed differences were significantly different from zero. A two-sample T-test was used to evaluate blood pressure differences between the non-stiff group and the stiff group within and between devices.

A difference was considered to be statistically significant when the two-sided

p-value was below 0.05. All analyses were performed with the statistical package SPSS 8.0 for Windows 95 (SPSS Inc., Chicago, Illinois, USA).

RESULTS

Population-based Study

Characteristics and blood pressure values of the study population of the population-based study are shown in Table 1. Mean SBP difference (95% confidence interval) between the Dinamap and random zero was 10.9 (10.2-11.6) mmHg and mean DBP difference was 4.8 (4.3-5.1) mmHg. A positive difference indicates that the Dinamap reading was higher than the random-zero reading. Age was a significant determinant for both the SBP and DBP difference. The β-coefficient (95% confidence interval) was 0.105 (0.003 to 0.207) mmHg per year increase in age for the SBP difference and 0.183 (0.120 to 0.246) mmHg per year increase in age for the DBP difference, adjusted for sex, and average mean blood pressure level of both devices. Subsequent analyses showed that arterial stiffness was a significant determinant for both the SBP and DBP difference, adjusted for age, sex, and average mean blood pressure level of both devices. The β-coefficient (95% confidence interval) was 0.25 (0.00 to 0.50) mmHg per 1 m/s increase in PWV for the SBP difference and 0.35 (0.20 to 0.50) mmHg per 1 m/s increase in PWV for the DBP difference. The positive regression coefficients indicate

Table 1
Characteristics and blood pressure values of the study population of the population-based study, the Rotterdam Study, 1997-1999.

Characteristic	Population-based study N = 1808
Age in years (range)	73 (61-95)
Males (%)	39
	mean (standard deviation)
Pulse wave velocity (m/s)	14.0 (3.2)
SBP random zero (mmHg)	141 (20)
DBP random zero (mmHg)	74 (11)
SBP Dinamap (mmHg)	152 (23)
DBP Dinamap (mmHg)	78 (11)

SBP = systolic blood pressure, DBP = diastolic blood pressure

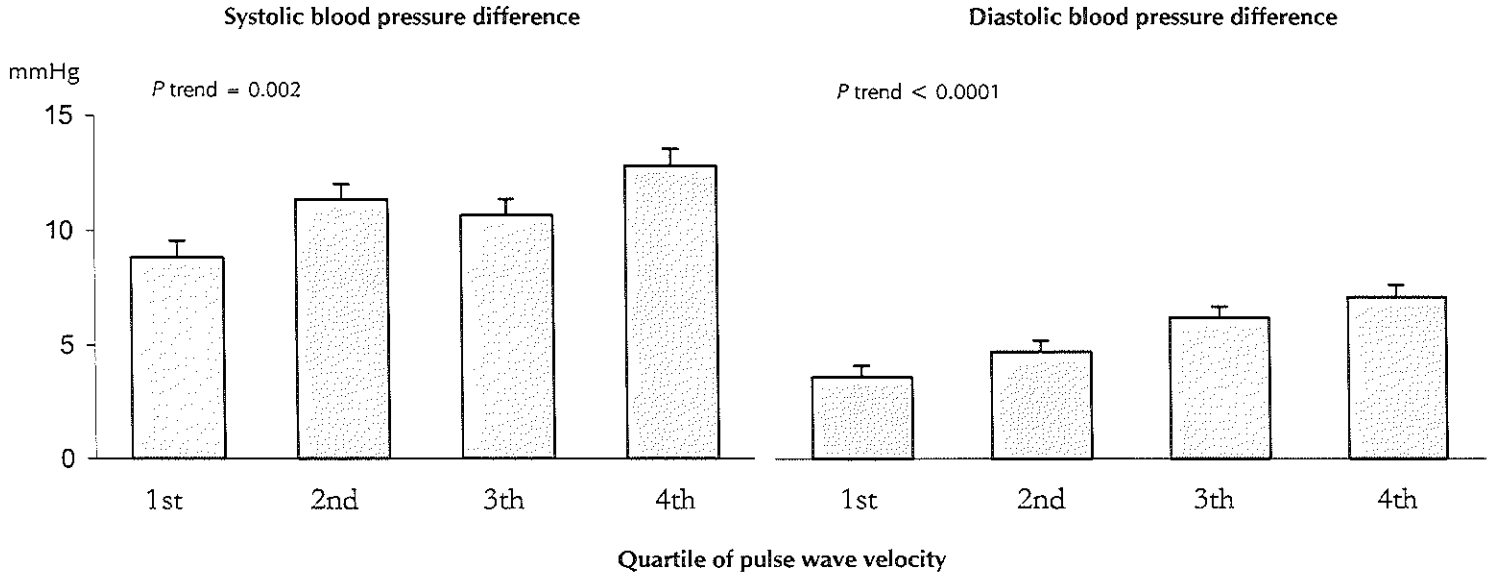

Figure 1
Mean blood pressure difference per quartile of pulse wave velocity (m/s) (Dinamap minus random-zero), adjusted for age, sex, and average blood pressure level of both devices, in 1808 subjects from the population-based study, The Rotterdam Study, 1997 - 1999. Bars indicate standard errors of the mean.

higher SBP and DBP readings by Dinamap as compared to random-zero with increasing age and increasing arterial stiffness. Results were the same for men and women separately (data not shown). In Figure 1, the association between arterial stiffness and blood pressure differences is shown in quartiles of the PWV distribution.

Control-study

The characteristics of the study population of the control-study are shown in Table 2. Observed differences in all comparisons, for both the stiff and non-stiff group, were significantly different from zero, except the DBP difference between Dinamap and random-zero in the stiff group and the SBP difference between Dinamap and a conventional sphygmomanometer in the stiff group (Table 3). The direction of SBP and DBP differences varied and agreement between monitors was sometimes better in the stiff group than in the non-stiff group. However, in agreement with the population-based study, their was a general trend towards more positive SBP and DBP readings in the stiff group than in the non-stiff group by the Dinamap as compared to both sphygmomanometers. In the comparison of Dinamap with random-zero, the SBP and DBP differences were significantly more positive in the stiff group than in the non-stiff group. In the

Table 2
Characteristics and blood pressure values of the study population of the control-study, the Rotterdam Study, 1997-1999.

Characteristic	non-stiff group n = 28	stiff group n = 28
Age in years (range)	68 (62-76)	83 (77-91)
Males (%)	14	46
	mean (standard deviation)	
Pulse wave velocity (m/s)	12.1 (1.1)	18.0 (2.7)
SBP random zero (mmHg)	121 (14)	131 (17)*
DBP random zero (mmHg)	74 (9)	72 (12)
SBP Dinamap (mmHg)	129 (17)	143 (20)*
DBP Dinamap (mmHg)	70 (10)	73 (12)
SBP conventional sphygmomanometer (mmHg)	131 (14)	143 (20)*
DBP conventional sphygmomanometer (mmHg)	80 (8)	76 (13)

SBP = systolic blood pressure, DBP = diastolic blood pressure.
* $P < 0.05$, stiff group versus non-stiff group.

Table 3
Student's T-test for equality of mean SBP and DBP differences between the Dinamap and the random zero, the Dinamap and conventional sphygmomanometer, and the random zero and conventional sphygmomanometer in the non-stiff versus the stiff group of the control-study, the Rotterdam Study, 1997-1999.

Blood pressure difference	Group		Difference	P-value
	non-stiff	stiff		
SBP Dinamap - random zero (mmHg)	8.0	12.7	-4.7	0.01
DBP Dinamap - random zero (mmHg)	-4.2	1.3	-5.5	0.002
SBP Dinamap - conventional sphygmomanometer (mmHg)	-2.5	0.3	-2.8	0.077
DBP Dinamap - conventional sphygmomanometer (mmHg)	-9.8	-3.1	-6.7	<0.001
SBP random zero - conventional sphygmomanometer (mmHg)	-10.5	-12.4	1.9	0.22
DBP random zero - conventional sphygmomanometer (mmHg)	-5.5	-4.4	-1.1	0.36

SBP = systolic blood pressure, DBP = diastolic blood pressure

comparison of Dinamap with a conventional sphygmomanometer, the SBP difference was borderline significantly more positive and the DBP difference was significantly more positive in the stiff group as compared to the non-stiff group. In the comparison of random-zero with a conventional sphygmomanometer, the SBP and DBP differences were not significantly different between the non-stiff and stiff group.

Discussion

Our results show that arterial stiffness is associated with an overestimation of SBP and DBP by a Dinamap oscillometric blood pressure device as compared to a Hawksley random-zero sphygmomanometer. The control-study, conducted according to the British Hypertension Society protocol, confirms that arterial stiffness is a determinant of overestimation of SBP and DBP by the Dinamap as compared to a random-zero sphygmomanometer in subjects with stiff arteries.

Some aspects of the study need to be discussed. Firstly, we adjusted all analyses for mean blood pressure level (average of both devices) because PWV is highly dependent on blood pressure and the difference between the devices increased with increasing blood pressure level (data not shown). Secondly, PWV

was calculated using the distance between carotid and femoral artery as distance belonging to the time-delay between the pulse waves. This distance is longer than the 'true' distance resulting in overestimation of the pulse wave velocity. Because variations in anatomy are limited, this overestimation can be considered similar for all subjects and therefore will not have seriously affected our results. Thirdly, we related carotid-femoral PWV to blood pressure difference between devices measured at the brachial artery. We thereby assumed that vessel wall stiffness of the carotid-femoral vessel bed is representative of brachial arterial stiffness. It is known, however, that there is a reasonable heterogeneity among vessel wall properties of different arterial regions.[13,14] To our knowledge, there are no studies comparing vessel wall properties of brachial artery with that of the carotid-femoral vessel bed, making it difficult to accurately assess the validity of our assumption. However, we assume that misclassification in brachial arterial stiffness status is non-differential and thus, if present, will have resulted in an underestimated of the association.

Design-aspects of the population-based study may have created the observed differences between devices. Examples of such design-aspects are the fixed order in which the device were used, the subjects body-position during measurement (sitting versus supine) and the observer (nurse versus doctor). The expected effect of these aspects on differences between the devices, however, does not always correspond with observed differences. For example, it is known that blood pressure measured in sitting position is generally higher than blood pressure measured in supine position.[15,16] The difference in body-position in the population-based study can not explain our results as Dinamap blood pressures were higher than random-zero blood pressures and subjects were in supine position during Dinamap recordings while sitting during random-zero recordings. The design-aspects of the population-based study can also not explain that the blood pressure differences between devices are dependent on arterial stiffness status, which is confirmed in the control-study.

An alternative interpretation of our findings is that increased arterial stiffness leads to underestimation of SBP and DBP by the random-zero sphygmomanometer as compared to the Dinamap. Previous studies indicate that the random-zero underestimates SBP and DBP as compared to a conventional sphygmomanometer.[17] In agreement with this, we found an underestimation of SBP and DBP measured by the random-zero in comparison with the conventional sphygmomanometer in both the stiff and non-stiff group of the control-study. However, between the stiff and non-stiff group, no significant difference in blood pressure differences between devices was observed, indicating that the underestimation

by random-zero was not related to arterial stiffness. The underlying mechanism by which blood pressure is measured by a sphygmomanometer is also not compatible with this alternative interpretation. Current thinking on the origin of Korotkoff sounds during sphygmomanometry is that they might be generated by movement of the vessel wall.[18] Increased arterial stiffness could diminish vessel wall movements resulting in decreased loudness of Korotkoff sounds.[18] This would lead to lower SBP but higher DBP readings with a sphygmomanometer in subjects with stiff arteries. As we found both lower SBP and DBP by the random-zero as compared to the Dinamap, this alternative explanation is only compatible with the observed difference in SBP, and therefore unlikely.

Previous studies showed that oscillometric devices tend to overestimate SBP and underestimate DBP as compared to conventional sphygmomanometers.[1-4] In the population-based study, Dinamap overestimated both SBP and DBP as compared to random-zero. The relatively old age of this study population could be the reason for finding an overestimation of DBP, as increasing age was an determinant of overestimation of SBP and DBP by Dinamap. In line with the previous studies, we did find an overestimation of SBP and an underestimation of DBP by Dinamap as compared to random-zero in younger subjects with distensible arteries (non-stiff group) in the control-study. In comparison with a conventional sphygmomanometer, Dinamap underestimated both SBP and DBP in the non-stiff group. This underestimation became less in the stiff group. Although not in line with previous studies, it does support our hypothesis that a Dinamap uniformly gives more positive blood pressure readings in subjects with stiff arteries.

Our results with respect to stiffness are in line with a previous study that found more overestimation of SBP and less underestimation of DBP in diabetic subjects as compared to healthy controls by a SpaceLabs device compared to a conventional sphygmomanometer.[7] It is well known that diabetic subjects have stiffer arteries than non-diabetic subjects.[9] Increased arterial stiffness in diabetic subjects might be the underlying mechanism of the observed difference between the devices. Another study evaluated differences between a Dinamap 8100 and random-zero sphygmomanometer in diabetic subjects and found an overestimation of SBP at low SBP values, an underestimation of SBP at higher SBP levels and an underestimation of DBP at all DBP values by Dinamap compared to the random-zero. This is discordant with our results. We observed increasing difference between the Dinamap and the random-zero with increasing blood pressure level. When arterial stiffness is indeed a determinant of SBP difference, this is what one would expect to find as SBP rises when arteries become stiffer.

The mechanism by which arterial stiffness leads to higher SBP and DBP readings by Dinamap is not clear. It might be explained by changes in oscillograms of subjects with stiff arteries. The algorithm by which Dinamap determines SBP and DBP from an oscillogram is not publicly known. Therefore, it is unfeasible to speculate about the effect of changes, observed in oscillograms of subjects with stiff arteries, on blood pressure determination by Dinamap.

Accurate blood pressure determination and diagnosis of hypertension is essential in subjects with a compromised cardiovascular system. Therefore, more studies are needed to elucidate the consequences that arterial characteristics entail on blood pressure measurement by oscillometric devices. Most subjects participating in validation studies are healthy volunteers. Elderly volunteers might have relative young arterial systems. We suggest including special subgroups like subjects with arterial stiffness, advanced atherosclerosis, hypertension, cardiovascular disease or diabetes, in validation studies of blood pressure measuring devices.

In conclusion, the results of our study suggest that arterial stiffness is a determinant of higher systolic and diastolic blood pressure readings by a Dinamap oscillometric blood pressure device as compared to sphygmomanometers.

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Chapter 7

General discussion

The aim of the studies described in this thesis was to provide epidemiological insight on causes and consequences of stiffening of the arterial tree. Each previous chapter includes a discussion on the merits and limitations of the study described in that chapter. This chapter will provide more general views on the methodological considerations when performing epidemiological studies on arterial stiffness. Before these are addressed, some background on the studies and an overview of the main findings is given. Next, the pathogenesis, clinical implications, and possibilities for treatment of arterial stiffness are reviewed. This discussion finishes with suggestions for further epidemiological research on stiffening of the arterial tree.

BACKGROUND

The number of studies on stiffness of the arterial tree has markedly increased in the past decade. Reasons for this increase are the recent availability of accurate non-invasive measures to measure arterial stiffness and the awareness of increased cardiovascular risk associated with increased arterial stiffness when arteries become stiffer. Most studies on causes and consequences of stiffening of the arterial tree have been performed in small groups of selected subjects. Population-based studies evaluating the aetiology and prognosis of arterial stiffening are lacking, with the exception of a population-based study conducted in middle-aged subjects.[1]

The Rotterdam Study is a population-based cohort study that started in 1990 and included 7983 elderly subjects, aged 55 years or over at baseline. In 1997, the third examination phase started, in which two frequently used methods to measure arterial stiffness were incorporated. These methods are measurement of carotid-femoral pulse wave velocity, which is a measure of aortic stiffness and measurement of distensibility of the common carotid artery, which is a measure of common carotid arterial stiffness. Of the 4148 subjects who were eligible for physical examination in the third examination phase, carotid-femoral pulse wave velocity was measured in 3550 subjects and distensibility of the common carotid artery was measured in 3098 subjects. The studies described in this thesis were mainly performed in this group of elderly subjects. One study on determinants of arterial stiffness was performed among a separate group of healthy middle-aged women.

MAIN FINDINGS

Determinants of arterial stiffness

Two studies described in this thesis addressed determinants of arterial stiffness. One study was performed in the elderly participants of the Rotterdam Study and the other was performed in healthy middle-aged women. Determinants examined were various biochemical variables including lipids, glucose, insulin, and body composition measures. Both studies examined the association of these variables with distensibility of the common carotid artery. In a study in healthy non-diabetic middle-aged women we found variables that are part of the insulin-resistance syndrome to be related with decreased distensibility of the common carotid artery, whereas variables that are not part of the insulin-resistance syndrome were not (chapter 3.1). The degree of clustering of variables of the insulin-resistance syndrome within one subject was assessed by a summation score of all variables of the insulin-resistance syndrome. This clustering score was strongly related to decreased distensibility (every unit increase in clustering score was associated with a decrease in common carotid distensibility of 0.27 10^{-3}/kPa (95% confidence interval: 0.15 to 0.38)). In the elderly subjects of the Rotterdam Study we found glucose, HDL-cholesterol, body mass index and waist hip ratio, which are all variables of the insulin resistance syndrome, to be related to decreased distensibility of the common carotid artery (chapter 3.2). Elevated total cholesterol, which forms no part of the insulin-resistance syndrome, was not related to decreased distensibility. In addition, we observed that subjects under 75 years of age with impaired fasting glucose were comparable in arterial stiffness with healthy non-diabetic subjects while above 75 years of age, arterial stiffness of subjects with impaired fasting glucose reaches that of diabetic subjects. The results of these two studies are in accordance with previous studies that showed a relation between the insulin-resistance syndrome and arterial stiffness in diabetic subjects[2] as well as in non-diabetic subjects.[1,3]

Arterial stiffness and atherosclerosis

The terms atherosclerosis and arteriosclerosis are frequently used indiscriminately to refer to alterations and lesions in large arteries. From an etymological point of view, the term arteriosclerosis identifies arterial stiffness[4], while atherosclerosis refers to atheromatic lesions of the arterial wall that frequently are calcified. Sev-

eral small studies showed that subjects with cardiovascular disease have increased arterial stiffness as compared to subjects without cardiovascular disease.[5-12] Arterial stiffness has also been shown to be a predictor of all-cause and cardiovascular mortality in subjects with end-stage renal disease.[13,14] An association between arterial stiffness and cardiovascular disease suggest an association between arterial stiffness and atherosclerosis. Studies on a direct association between measures of arterial stiffness and measures of atherosclerosis, however, report conflicting results. Some studies reported a positive relation between arterial stiffness and atherosclerosis[15-18], but others could not demonstrate such a relationship.[19-22] We evaluated the association of both aortic stiffness and common carotid stiffness with several measures of atherosclerosis (chapter 4) and found strong associations of both measures of arterial stiffness with atherosclerosis at several sites in the arterial tree. Common carotid intima-media thickness, plaques in the carotid artery, and plaques in the aorta were all highly associated with both aortic and common carotid arterial stiffness (p for trend ≤ 0.01 for all associations). Subjects with peripheral arterial disease had significantly increased aortic stiffness ($p = 0.001$) and borderline significant increased common carotid arterial stiffness ($p = 0.078$) as compared to subjects without peripheral arterial disease.

Arterial stiffness and cardiovascular disease

Arterial stiffness and previous myocardial infarction and stroke

Several small cross-sectional studies have addressed the relation between measures of arterial stiffness and cardiovascular disease. These studies uniformly showed increased arterial stiffness in subjects with a previous myocardial infarction.[5-9,11,12] Only one cross-sectional study examined the relation between arterial stiffness and stroke and found increased arterial stiffness in subjects with stroke.[10] We examined aortic stiffness and common carotid arterial stiffness in relation to previous myocardial infarction and stroke (chapter 5.1). Subjects with severe aortic stiffness had four-times as often a previous myocardial infarction, as compared to the reference category (relative risk and 95% confidence interval: 4.0 (1.8-9.2)). Aortic stiffness was not clearly associated with previous stroke. Subjects with severe carotid stiffness had two times as often a previous myocardial infarction (2.3 (1.1-5.1)) and twelve times as often a previous stroke (12.6 (2.7 - 58.1)), as compared their reference category. The associations persisted after adjustment for the presence of atherosclerosis suggesting that other mechanism than an association between arterial stiffness and atherosclerosis may explain the association between arterial stiffness and cardiovascular disease.

Aortic stiffness and the balance between cardiac oxygen supply and demand

Increased arterial stiffness leads to an increase in pulse pressure. Pulse pressure has been shown to be an independent predictor of cardiovascular events and mortality in general populations[23,24] as well as in selected patient groups.[25-27] This can partly be explained by an increase in cardiac oxygen demand due to an increased afterload following increased pulse pressure.[28] However, a decrease in diastolic blood pressure, following increased arterial stiffness, can add to increased cardiovascular risk by decreasing coronary perfusion pressure and thereby decreasing cardiac oxygen supply. In healthy subjects, a decreased coronary perfusion pressure can be compensated by coronary vasodilatation.[29] But in the presence of coronary atherosclerosis, the vasodilatory reserve is limited and a decreased perfusion pressure, in these circumstances, can lead to decreased oxygen supply, especially subendocardial supply.[30-33] An experimental study in dogs showed that decreased aortic compliance greatly increased the risk of subendocardial ischaemia in the presence of coronary stenosis due to a decreased diastolic perfusion pressure following decreased diastolic blood pressure.[34] We evaluated aortic stiffness in relation to both cardiac oxygen demand and cardiac oxygen supply (chapter 5.2). Increased aortic stiffness was associated to increased cardiac oxygen demand but also to decreased cardiac oxygen supply. In accordance, increased aortic stiffness was strongly associated with a decrease in the cardiac oxygen supply/demand ratio. An increase of pulse wave velocity of 1 m/s was associated with a decrease in cardiac oxygen supply/oxygen demand ratio of 0.006 (95% confidence interval: 0.003 - 0.008).

Aortic stiffness and calcification of the coronary arteries

In chapter 5.3, we related aortic stiffness to calcifications of the coronary arteries, as assessed by electron beam tomography, in the elderly subjects of the Rotterdam Study. We found a strong association between aortic stiffness and calcifications of the coronary arteries. Subjects with increased aortic stiffness had a 47% higher risk of having advanced calcification of the coronary arteries as compared subjects without increased aortic stiffness. These results in combination with the results of chapter 5.2 indicates that subjects with increased aortic stiffness are likely to have both a decreased cardiac oxygen supply as well as an increased risk of advanced coronary atherosclerosis. This may identify a group at high risk of subendocardial ischaemia as their coronary arteries can not compensate for the decreased cardiac oxygen supply.

Consequences of arterial stiffness for blood pressure measurement

Automatic oscillometric blood pressure devices are frequently used to measure blood pressure non-invasively. Studies have evaluated their performance in comparison with a conventional sphygmomanometer or the Hawksley random-zero sphygmomanometer and showed that oscillometric devices tend to overestimate systolic blood pressure and underestimate diastolic blood pressure as compared to sphygmomanometers.[35-38] An oscillometric blood pressure device determines blood pressure by detecting a sequence of oscillations in cuff pressure as the cuff pressure is reduced from above systolic to below diastolic pressure.[39] Changes in arterial elastic properties might lead to differences in oscillations and as a consequence false blood pressure readings by oscillometric devices. Some studies indicate that differences in performance between these devices may differ between healthy and diabetic subjects.[40-42] As diabetic patients have stiffer arteries as compared to non-diabetic subjects[43], stiffening of the arteries of diabetic subjects could be the underlying mechanism of the more pronounced differences between oscillometric devices and sphygmomanometers in this group. In the elderly subjects of the Rotterdam Study, we found that increasing stiffness of the aorta was associated with higher systolic blood pressure readings (p *trend* = 0.002) and higher diastolic blood pressure readings (p *trend* < 0.0001) by a Dinamap oscillometric blood pressure device as compared to a Hawksley random-zero sphygmomanometer (chapter 6.1).

METHODOLOGICAL CONSIDERATIONS

Study design

There are three main types of non-experimental epidemiological studies e.g., longitudinal studies, case-control studies and cross-sectional studies. All studies described in this thesis are cross-sectional in design. In contrast to a longitudinal study, in a cross-sectional study all measurements are made at the same point in time. When the determinant changes over time and particularly when the determinant is affected by the outcome, differential misclassification can occur, which can bias the results of a cross-sectional study. For example, blood pressure may decrease after a myocardial infarction due to forward failure of the heart. When the association between blood pressure and myocardial infarction is assessed in a cross-sectional study after the myocardial infarction has occurred, the associa-

tion will be underestimated as the high blood pressure that caused the myocardial infarction is no longer present.

In this thesis, two studies are described that concern the aetiology of arterial stiffness, one of which was performed in the Rotterdam Study. The measures of arterial stiffness were incorporated in the third examination phase of the Rotterdam Study from 1997 until 1999. Possible determinants of arterial stiffness were measured at both the first and the third examination phase of the Rotterdam Study. We chose a cross-sectional approach to study determinants of arterial stiffness in the Rotterdam Study. In general, when evaluating determinants of an outcome, longitudinal studies are preferred to measure the determinant before occurrence of the outcome. However, it is more difficult to measure a determinant before occurrence of the outcome when the outcome is a process developing throughout life, like stiffening of the arterial tree. The two studies described in this thesis that concerned determinants of arterial stiffness, addressed body composition measures and several biochemical variables like lipids and serum glucose as determinants of arterial stiffness. These variables are unlikely to change due to increased stiffening of the arterial tree. Thus evaluating the association between these determinants and arterial stiffness in a cross-sectional study will not introduce bias to the effect estimate. When evaluating these determinants, the relevant exposure to the determinant has to be determined. It is difficult to appraise whether the determinant at baseline or the current determinant better reflects the relevant exposure. Exposure at baseline does not take changes between baseline and current level into account. We therefore chose to relate current determinants to arterial stiffness.

A cross-sectional study design is an adequate design when an association between two variables is evaluated and interest in whether two variables are related to each other rather than in the etiological coherence between the two variables. Two studies described in this thesis concerned the association of arterial stiffness with extra-coronary and coronary atherosclerosis in chapter 4 and chapter 5.3, respectively. For both studies a cross-sectional study design was chosen.

One cross-sectional study described in this thesis concerned arterial stiffness as risk indicator for cardiovascular disease. We evaluated the association between arterial stiffness and previous myocardial infarction and stroke. Preferably, the value of a risk indicator is evaluated in a longitudinal follow-up study, in which the risk indicator is measured before the occurrence of the outcome. However, in the Rotterdam Study, arterial stiffness was measured between 1997 and 1999 resulting in a too short a follow-up time for adequate longitudinal analyses. As it

is possible that arterial stiffness changes after the occurrence of a cardiovascular event by changes in life-style, blood pressure or therapy, misclassification of arterial stiffness might have occurred. This would be differential misclassification as it is dependent on the outcome and could result in information-bias. Also, it is likely that arterial stiffness changed because of passage of time, as stiffening of the arteries is a process developing throughout life. To minimise these effects on arterial stiffness, we only included recent histories of myocardial infarction or stroke.

Selection bias

Selection bias occurs when the relation between the determinant and the outcome is different for those who participated and those who would be theoretically eligible but did not participate. Selection bias can lead to either an underestimation or an overestimation of an association and can not be corrected for in the analyses.

The studies presented in this thesis were mainly performed as part of the Rotterdam Study. Selection in the Rotterdam Study can have occurred both at baseline due to non-response or at the time of the third examination phase due to non-response or due to missing subjects who died (survival bias) or were lost to follow-up. At baseline, all inhabitants of the suburb Ommoord in Rotterdam, The Netherlands who were aged 55 years or over were invited to participate. A total of 7983 subjects (response rate of 78%) agreed to participate. Of the 7983 subjects who participated at the baseline examination phase, 1992 subjects (25%) died between the first and third examination phase, 55 subjects (0.7%) were living in nursing homes outside the study area and were not invited to participate and 35 subjects (0.4%) were lost to follow up. Thus, a total of 5901 subjects were eligible for the third examination phase of whom 4730 subjects (80%) participated at a home-interview and 4148 subjects (70%) at physical examinations at the research centre.

Non-responders may differ with respect to attitude towards health, risk factor status, socio-economic status, and health status from responders to a study. Therefore, it is likely that non-responders are older, have higher levels of risk factors, increased arterial stiffness and more disease as compared to subjects participating in the study. However, for evaluating associations in terms of relative risk, bias due to non-response only occurs when non-response is related to the determinant independent of its relation to the outcome. This is less likely to occur and therefore non-response will probably not have seriously biased the

results of our study.

Subjects who died before the third examination phase were also likely to have had a higher risk factor and disease status. This could lead to survival-bias if subjects who were still alive and were able to participate in the third examination phase are healthy survivors who are not susceptible to the effects of determinants under study. In these subjects the relation between the determinant and the outcome is different than in subjects who died. When evaluating associations in elderly subjects, it is likely that those most susceptible to the determinants under study already died at younger ages, therefore, some survival-bias is likely to have occurred and inevitable.

Of the 4148 subjects participating at the physical examinations of the third examination phase, a measurement of aortic stiffness was available for 3550 (86%) and a measurement of common carotid distensibility was available for 3098 (75%) Missing information on most determinants of arterial stiffness was less frequent. Missing information on any variable was mainly due to logistic reasons, therefore non-differential with respect to status of variable related to arterial stiffness. The relation between determinant and outcome is therefore unlikely to be different for subjects who were examined and subjects who missed part of the examination due to logistic reasons and thus will not have led to biased results.

Confounding

Bias due to confounding occurs in etiologic analyses when a third factor is related to both the determinant and the outcome and is not an intermediate in the causal pathway between determinant and outcome. There are several ways to deal with confounding. One of the most applied means is statistical adjustment by including the confounder as a covariate in the analyses. To be able to statistically adjust for a confounder, knowledge is acquired concerning which variables might be confounders in the association of interest and information on those variables must be available. When a confounder is present but not measured and thus can not be controlled for in the analyses, the effect estimate will be biased. This is called residual confounding.[44] Another problem with handling of confounding is misclassification of the confounder. When misclassification of the confounder occurs, statistical methods used for control of confounding do not adequately deal with the bias introduced to the effect estimate.[45] This type of confounding is known as resonance confounding.[46]

Important confounders in studies on the relation between potential determi-

nants and arterial stiffness or between arterial stiffness and cardiovascular disease are age, gender, blood pressure, and heart rate. These are all strongly correlated with arterial stiffness and are likely to be correlated with determinants of arterial stiffness or consequences of arterial stiffness independent of their association with arterial stiffness. Therefore, all analyses in the present thesis were statistically adjusted for age, gender, blood pressure, and heart rate. When evaluating the association of arterial stiffness with atherosclerosis or with cardiovascular disease it is not clear whether cardiovascular risk factors are true confounders or precursors in the associations under study. Adjusting for a precursor will unjustly diminish the association. However, as we were also interested in an association of arterial stiffness with atherosclerosis or with cardiovascular disease independent of cardiovascular risk factors, we chose to evaluate these associations both with and without additional adjustment for cardiovascular risk factors.

In studies concerning associations with measures of arterial stiffness, resonance confounding can occur due to misclassification of blood pressure. There are two different reasons for misclassification of blood pressure. Firstly, blood pressure is measured at the brachial artery and used to adjust aortic stiffness or common carotid arterial stiffness, thereby assuming that brachial blood pressure is representative of blood pressure in the aorta and common carotid artery. Mean arterial pressure can be considered to be relatively constant between central arteries and peripheral arteries. Several studies showed that mean arterial pressure declines only 1-3 mmHg from the central arterial system to the peripheral arteries.[47-49] Therefore, adjusting analyses with pulse wave velocity using brachial mean arterial pressure in stead of aortic mean arterial pressure probably will not have seriously affected our results. The same holds for using brachial mean pressure in stead of carotid mean arterial pressure when adjusting analyses with common carotid distensibility. Another issue concerns pulse pressure. 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. However, pulse pressure is known to be higher in the brachial artery than in more central vessels like the carotid artery because arterial pressure waves undergo transformation in the arterial tree.[50] This issue is discussed in detail in chapter 2 when discussing the limitations of measuring common carotid distensibility as method to assess arterial stiffness.

Another way whereby resonance confounding can occur is by incorrect statistical adjustment for blood pressure. Theoretically, the relation of mean arterial pressure with both carotid-femoral pulse wave velocity and common carotid dis-

tensibility is not linear, levelling off at higher pressures as a higher mean arterial pressure in the artery stretches the elastin and collagen fibres in the arterial wall, making the artery less distensible. Adjustment for mean arterial pressure in a linear model might therefore not be adequate. However, it is likely that the association between mean arterial pressure and distensibility of an artery is unique for each individual. Adequately adjustment for mean arterial pressure would then ask for separate models for each individual, which is unfeasible. Most of the studies described in this thesis were conducted in an elderly population with relatively high blood pressures. Subjects in our studies are therefore likely to have a mean arterial pressure in the pressure range where the relation between mean arterial pressure with arterial stiffness is levelled off. However, we found the association of arterial stiffness with mean arterial pressure in the total population to be linear for almost the entire pressure range. The association only attenuated when the mean arterial pressure was above 140 mmHg (Chapter 2). Only 1.1% of the subjects had a mean arterial pressure above 140 mmHg. Furthermore, when evaluating the associations in strata of high and low mean arterial pressure, the associations were in general the same. This argues against a major effect of resonance confounding by mean arterial pressure.

Pathogenesis of arterial stiffness

The most common factors leading to increased stiffening of the arterial tree are age and hypertension. The effect of age on the arterial wall is mainly due to tissue fatigue, resulting in diameter enlargement and elastin degradation.[50,51] These alterations are more pronounced in central arteries like the thoracic aorta and carotid artery then in more peripheral arteries such as the femoral and radial artery.[52] Hypertension induces several structural changes in the arterial wall including vessel wall hypertrophy and changes in the extracellular matrix.[50,53] Moreover, local hormonal changes in hypertensive subjects can have pressure-independent effects on the arterial wall, mainly caused through modification of cell growth or syntheses of extracellular matrix.[54,55]

Other factors besides age and hypertension can alter the elastic properties of the arterial wall. The endothelium is known to release several vasoactive substances including nitric oxide and endothelin-1, which may influence arterial stiffness, by two different mechanisms. The first functional mechanism is by directly influencing the vascular tone. The other mechanism is by changing the vessel wall structure. Nitric oxide has an antimitogenic effect while endothelin-1 has a

mitogenic effect. Changes in the balance between nitric oxide and endothelin-1 may promote vascular proliferation and atheroma formation.[56] Plasma endothelin-1 concentration has been shown to have a significant positive correlation with aortic stiffness in patients with coronary artery disease.[57] Important determinants of endothelial dysfunction are age and gender, with endothelial dysfunction occurring earlier in men.[58] Part of the effect of age and gender on arterial stiffness could be trough endothelial dysfunction. Homocysteine has also been related to endothelial dysfunction.[59-61] However, studies on the association between homocysteine and arterial stiffness to date reported conflicting results. Two studies in high-risk patients observed an association between plasma homocysteine levels and increased arterial stiffness[62,63] while another study in subjects with mild hyperhomocysteinaemie could not demonstrate an association.[64] Decreased bioavailability of endogenous nitric oxide has been found to be associated with a number of risk factors for arterial stiffening, such as hyperlipidaemia and diabetes.[65] In the studies described in chapter 4.1 and 4.2, we found diabetes mellitus but also components of the insulin-resistance syndrome, like dyslipidaemia, to be associated with decreased distensibility. Endothelial dysfunction might partly explain the association of diabetes mellitus and insulin-resistance with decreased distensibility. However, hyperglycaemia by itself may also lead to increased arterial stiffness by increased collagen cross-linking due to non-enzymatic glycation.[66-68]

Recently, research has included genetic factors of the renin-angiotensin system as potential determinants of arterial stiffness.[4] The AT_1-receptor plays an important role in actions that angiotensin II entails on the cardiovascular system and an AT_1-receptor gene A/C polymorphism is recently identified. The presence of the AT_1 C allele in hypertensive subjects has been shown to be associated with increased aortic stiffness, independent of blood pressure levels.[69,70] In these studies, several interaction between this genetic polymorphism and other risk factors for stiffening of the arterial tree, like age and the total cholesterol:high-density cholesterol ratio have been observed. These results suggest that the AT_1-receptor polymorphism might modulate the effects of hypertension, ageing and lipids on large arteries.

Finally, another process that might play a role in the pathogenesis of arterial stiffness is described in chapter 3.1 and concerns a reinforcing, self-perpetuating process due to the fact that arterial stiffness and atherosclerosis are both determinants and consequences of the other. Calcified atherosclerotic plaques are likely to stiffen the arterial wall. On the other hand, without the shock-absorbing capacity, the stiff arterial wall may be subjected to greater intraluminal stresses due to

an increased pulsatile pressure and therefore is likely to be at increased risk of atherosclerotic changes.[71]

TREATMENT OF ARTERIAL STIFFNESS

Our results and results from previous studies suggest that arterial stiffness is causally related to cardiovascular risk. Treatment of arterial stiffness may decrease cardiovascular risk and the possibility of treating arterial stiffness increasingly gains attention. Several anti-hypertensive drugs have been evaluated in their potential to improve arterial compliance. A marked increase in arterial distensibility has been shown with ACE inhibitors, calcium channel blockers and nitrates.[72-76] It has been suggested that the combination of drugs, for example a calcium channel blocker and a ACE inhibitor, could be particularly useful in patients with arterial stiffness, since both compounds have shown a high efficacy in improving large artery compliance, and provide synergistic effects when given together.[4] Drugs that selectively decrease systolic blood pressure without altering diastolic blood pressure[77,78], could be an advantage in subjects with increased arterial stiffness.

Recent evidence suggests that there is therapeutic potential to treat hyperglycaemia induced arterial stiffness by breakers of advanced glycation end products.[79] Although still in the animal-experimental phase, this provides a promising possibility to treat increased arterial stiffness in diabetic subjects and subjects with impaired glucose tolerance. Also, inhibition of the production of glycation end products has been shown to prevent arterial stiffening with age in rats.[80]

Drugs that increase the endothelial nitric oxide release are also potential beneficial drugs to increase arterial compliance. Oestrogen increases the basal endothelial nitric oxide release in post-menopausal women[81], and also has been shown to decrease aortic stiffness.[82] In healthy subjects, an elevation of the homocysteine concentration induced by oral methionine has been shown to be associated with an impairment of vascular endothelial function, which could be prevented when pre-treatment with vitamin C was given.[60] Vitamin C has been shown to reduce arterial stiffness in healthy subjects.[83] Cholesterol reduction increases the bioavailability of nitric oxide and lipid-lowering therapy might therefore have potential as arterial stiffness reducing drug.[65] Also, endothelin receptor antagonists have been suggested as potential arterial stiffness-reducing drugs.[65]

CLINICAL RELEVANCE

It is well known that diabetic subjects have stiffer arteries as compared to non-diabetic subjects.[43,84-87] Insulin resistance has been found to be positively associated with arterial stiffness in patients with non-insulin-dependent diabetes mellitus[2] and in healthy subjects.[1,3] We found variables of the insulin resistance syndrome and impaired levels of fasting glucose, which is an indicator of an impaired glucose metabolism, to be associated with decreased distensibility in healthy subjects (chapter 3.1 and 3.2, respectively). In chapter 5.1, we have shown that increased arterial stiffness is associated with cardiovascular risk. Therefore, healthy subjects with high fasting glucose levels and insulin-resistance may be at increased cardiovascular risk. In these subjects, advise to change an unhealthy lifestyle or antihypertensive and lipid-lowering therapy is indicated. Some classical antihypertensive drugs have been shown to decrease arterial stiffness. On the basis of our results, antihypertensive drugs that also improve arterial distensibility may be the drugs of first choice to treat high blood pressure in subjects with high fasting glucose levels and insulin-resistance.

The strong associations observed in chapter 4 of aortic stiffness and common carotid arterial stiffness with measures of atherosclerosis at several sites in the arterial tree suggest that measures of arterial stiffness may be used as indicators of generalised atherosclerosis. In chapter 5.1 we found that both measures of arterial stiffness are indicators of previous myocardial infarction while distensibility of the common carotid artery was also a strong indicator of previous stroke. The results showed that the magnitude of the associations between myocardial infarction and stroke with measures of arterial stiffness were comparable with the magnitude of the associations of measures of atherosclerosis with myocardial infarction and stroke. Moreover, the association of arterial stiffness with myocardial infarction and stroke was independent of the presence of atherosclerosis. This suggests that the association of arterial stiffness with cardiovascular disease does not only exist through an association of arterial stiffness with atherosclerosis. These results and results from previous studies[5-14] strongly suggest that the process of arterial stiffness is associated with increased cardiovascular risk. Therefore, increased stiffening of the arterial tree is not merely an innocent consequence of advancing age but rather can be viewed as a new cardiovascular risk factor.

In chapter 5.2 we showed that increased aortic stiffness is associated with an increase in cardiac oxygen demand. Increased aortic stiffness was also associated

with a decrease in cardiac oxygen supply. This is probably due to a decrease in coronary perfusion due to a decrease in aortic diastolic blood pressure. Healthy subjects are capable of coronary vasodilatation to compensate for a decreased coronary perfusion.[29] However, in the presence of coronary atherosclerosis, the vasodilatory reserve is limited and a decreased perfusion pressure, in these circumstances, can lead to decreased oxygen supply, especially subendocardial supply.[30-33] In chapter 5.3 we showed that subjects with increased aortic stiffness are at high risk of also having severe coronary calcifications. Thus, increased aortic stiffness leads to a decreased coronary perfusion but is also associated with a loss of the ability to compensate for that decreased coronary perfusion through its association with coronary calcifications. Therefore, it can be expected that subjects with increased aortic stiffness are at high risk of developing cardiac ischaemia, especially subendocardial ischaemia. From this it can be hypothesised that antihypertensive therapy in subjects with isolated systolic hypertension and increased pulse pressure due to increased arterial stiffness may be hazardous when further lowering the diastolic blood pressure. However, large clinical trials showed that blood pressure lowering drugs in subjects with isolated systolic hypertension and a high pulse pressure greatly decrease cardiovascular risk.[88-90] A recent meta-analysis that showed a large benefit of treating isolated systolic hypertension in the elderly, however, also showed that for every level of systolic blood pressure, diastolic blood pressure was inversely associated with cardiovascular mortality.[78] The paradox might be explained by a greater favourable effect on cardiac oxygen demand of lowering the systolic blood pressure as compared to the hazardous effect on cardiac oxygen supply of lowering the diastolic blood pressure. Furthermore, classical blood pressure lowering drugs might also decrease stiffness of the arteries[72-76], which by itself may lead to an increase in diastolic blood pressure. Therefore, in subjects with increased arterial stiffness and an increased pulse pressure, it may be indicated to focus on selectively lowering systolic blood pressure without altering diastolic blood pressure.[77,78]

In chapter 6.1 we show that increased arterial stiffness can lead to an overestimation of systolic and diastolic blood pressure by automatic oscillometric blood pressure monitors, varying from only 1 mmHg in some subjects to nearly 10 mmHg in other subjects. Oscillometric blood pressure devices are frequently used. Our findings indicated that caution is warranted with interpreting blood pressure readings from automatic devices in subjects who may have stiff arteries, like elderly, hypertensive subjects, and diabetic subjects.

FUTURE RESEARCH

Atherosclerosis is thought to be a generalised process throughout the arterial tree. Whether arterial stiffening is also a generalised process throughout the arterial tree is less clear. Our results suggest that this may not be the case. We found the aorta to be stiffer in men and the common carotid artery to be stiffer in women. Furthermore, whereas common carotid arterial stiffness was strongly related to previous stroke, no clear relation was found between aortic stiffness and previous stroke. Differences in elastic and muscular content of the arterial wall could lead to differences in arterial stiffening between different arterial sites. The common carotid artery is mainly an elastic artery. The ascending aorta is also predominantly elastic but the abdominal artery is more muscular. Also different functions of different arteries could mean that for example hormonal influences on the arterial wall differ between arterial sites with different functions. This could lead to differences in arterial stiffening between these different arterial sites. The function of the common carotid artery is to distribute the amount of blood flow to the brain while the aorta has mainly a transporting function. Differences between both arterial sites in our studies, however, may also be explained by the different methods used to assess aortic stiffness and common carotid arterial stiffness. Studies on the association between arterial stiffness and cardiovascular disease suggest that arterial stiffness is a cardiovascular risk factor and more research on stiffening of the arterial tree is warranted. However, when various studies are based on measurement of arterial stiffness at various sites in the arterial tree assessed with various methods, results can only be combined when stiffening of the arterial tree is a generalised process. To address this issue, studies are needed that use the same method for assessing stiffness in different parts of the arterial tree. Another issue is the issue of the numerous definitions of arterial stiffness. In a recent review, 24 definitions of arterial elastic properties were identified.[91] As the author of the review states, the ever-increasing variety of new terminology may have ramifications for the widespread application of measurements of arterial elastic properties. One of the goals for future research in the field of arterial stiffness is to reach consensus over terminology used.

Age and blood pressure are well known determinants of stiffening of the arterial tree. Studies on the association between gender and arterial stiffness in various age groups report conflicting results. However, most of these studies were performed on small numbers of subjects. We found that women have a stiffer common carotid artery as compared to men while men have a stiffer aorta as

compared to women. Finding different gender-differences across arterial sites may indicate that stiffening of the arterial tree is not a generalised process. Large population-based studies in younger subjects on the association between gender and arterial stiffness at several sites in the arterial tree would help to elucidate the influence of gender on stiffening of various arterial sites. These studies should preferably include the perimenopausal period. We studied the association between fasting glucose and variables of the insulin-resistance syndrome with decreased arterial distensibility in a large group of healthy subjects. Studies evaluating other determinants of arterial stiffening are sparse and mainly performed in a small number of subjects. To date, a large part of the variability in arterial stiffness remains unexplained. Therefore, more studies on possible determinants of arterial stiffness are needed. Candidates for further evaluation as determinant of arterial stiffening in future studies are homocysteine, nutritional parameters, alcohol intake, physical activity and genetic factors. Future studies would preferably be population-based to limit the possibility of selection-bias and include a large number of subjects to enhance power to show small associations or associations despite measurement error.

Several cross-sectional studies, including studies presented in this thesis, suggested that arterial stiffness is a risk factor for cardiovascular disease. Assumptions as derived from these studies need verification by relating arterial stiffness to incident cardiovascular events and mortality in longitudinal studies. Recently, two longitudinal studies in subjects with end-stage renal disease were published.[13,14] These studies showed that arterial stiffness is a strong predictor of cardiovascular mortality. Longitudinal studies in high-risk subjects, like end-stage renal disease, have the advantage of a high event rate and therefore probably need shorter follow-up time to show an association. However, it is also interesting to know whether arterial stiffness is a risk factor for cardiovascular disease in the general population, which awaits the results of population-based follow-up studies like the Rotterdam Study.

Several studies have focussed on treatment of arterial stiffness. Most attention has been paid to the classical antihypertensive drugs. As mentioned before, a marked decrease in arterial stiffness can be reached with ACE-inhibitors, calcium channel blockers and nitrates.[72-76] Selectively decreasing systolic blood pressure without altering diastolic blood pressure may be an advantage in subjects with increased arterial stiffness. Future studies on treatment of arterial stiffness should focus on comparisons in efficacy of drugs that have been shown to decrease arterial stiffness in randomised placebo-controlled double-blind trials.

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Chapter 8

Summary
Samenvatting

8.1

Summary

With an increasing number of elderly subjects in modern Western Society it is important to address age-related conditions that may have adverse effects on health. Stiffening of the arterial tree is an age-related condition and studies on arterial stiffness so far suggest that increased arterial stiffness may increase the risk on cardiovascular disease. To date, stiffening of the arterial tree is mainly evaluated in studies with a relatively small group of selected subjects. The aim of this thesis was to study causes and consequences of arterial stiffness in a large population-based cohort of elderly subjects. The studies described in this thesis were mainly performed within the Rotterdam Study, a population-based cohort study of subjects aged 55 years and over.

Diabetes mellitus has frequently found to be associated with arterial stiffness. Diabetes related conditions, like the insulin resistance syndrome, are less frequently evaluated in relation to arterial stiffness. With regard to causes of stiffening of the arterial tree, the focus of studies described in this thesis is therefore on insulin resistance and variables associated with the insulin-resistance syndrome. The association of atherosclerosis and arterial stiffness is also addressed in the light of causes of stiffening of the arterial tree. With regard to consequences of stiffening of the arterial tree, focus is on cardiac and neurological consequences. Finally, we addressed consequences for non-invasive measurement of blood pressure.

Chapter 1 gives a general introduction to this thesis. **Chapter 2** introduces the concept of stiffening of the arterial tree and starts with some background on elastic properties of the arterial wall. Subsequently, two methods to measure arterial stiffness that are used in the Rotterdam Study are described in detail. The extent of arterial stiffness, as assessed by these methods, is shown according to age, gender and blood pressure among subjects from the Rotterdam Study. Finally, the association between these measures of arterial stiffness is given.

Chapter 3.1 addresses the association between variables of the insulin resistance syndrome and common carotid distensibility as measure of arterial stiffness in healthy middle-aged non-diabetic women. We found variables of the insulin-resistance syndrome (body mass index, waist-to-hip ratio, HDL-cholesterol, triglycerides, glucose, insulin, apolipoprotein A1, plasminogen activator inhibitor-1-antigen and tissue-type plasminogen activator-antigen) to be associated with increased arterial stiffness. No association was found between arterial stiffness and variables not part of the insulin resistance syndrome (total cholesterol, LDL-cholesterol and apolipoprotein B).

We further address the association between impairment of glucose metabolism and arterial stiffness in **chapter 3.2.** In this chapter, impaired fasting glucose, a measure of impaired glucose metabolism, was examined in relation to common carotid distensibility in elderly subjects. 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. Results were the same in men and women. In this chapter we additionally evaluated the association between several variables, including variables of the insulin-resistance syndrome, and arterial stiffness. In accordance with chapter 3.1, variables of the insulin-resistance syndrome were associated with increased arterial stiffness.

In **chapter 4** we address the association between arterial stiffness and atherosclerosis, two processes both affecting the arterial wall. Several small studies found a relation between arterial stiffness and cardiovascular disease, suggesting an association between arterial stiffness and atherosclerosis. Studies on a direct association between measures of arterial stiffness and measures of atherosclerosis, however, reported conflicting results. We examined the association of both aortic stiffness and common carotid arterial stiffness with measures of atherosclerosis at several sites of the arterial tree in elderly subjects. Aortic stiffness was assessed by measuring carotid-femoral pulse wave velocity. Common carotid arterial stiffness was assessed by measuring the distensibility coefficient of the common carotid artery. Measures of atherosclerosis were common carotid intima-media thickness, plaques in the carotid artery and in the aorta, and presence of peripheral arterial disease (assessed by the ankle-brachial pressure index). Our results showed that increasing common carotid intima-media thickness and

increasing severity of plaques in the carotid artery and in the aorta were all strongly associated with increased aortic and common carotid arterial stiffness. Subjects with peripheral arterial disease had significantly increased aortic stiffness and borderline significantly increased common carotid arterial stiffness as compared to subjects without peripheral arterial disease.

The studies described in **chapter 5** focus on consequences of arterial stiffness. In chapter **5.1,** the association between arterial stiffness and prevalent myocardial infarction and stroke is examined and compared with the association of atherosclerosis and prevalent myocardial infarction and stroke. Measures of arterial stiffness were carotid-femoral pulse wave velocity as a measure of aortic stiffness and common carotid distensibility as a measure of common carotid arterial stiffness. Measures of atherosclerosis were the ankle-brachial pressure index and plaques in the carotid artery. We found aortic stiffness to be strongly associated with myocardial infarction. The strength of the association between aortic stiffness and myocardial infarction was comparable with that between atherosclerosis and myocardial infarction. Common carotid artery stiffness was also associated with myocardial infarction, though less strongly. Common carotid artery stiffness was strongly associated with stroke, the strength of the association was comparable with that between atherosclerosis and stroke. All observed associations of arterial stiffness with cardiovascular disease were independent of generalised atherosclerosis.

Pulse pressure, which is largely determined by arterial stiffness, has been shown to be an independent predictor of cardiovascular events and mortality in a general population as well as in selected patient groups. This can partly be explained by an increase in cardiac oxygen demand due to an increased afterload due to an elevated pulse pressure. However, a decrease in diastolic blood pressure, following increased arterial stiffness, could add to increased cardiovascular risk by decreasing coronary perfusion pressure and thereby decreasing cardiac oxygen supply. In chapter **5.2** we examined the relation between aortic stiffness and both cardiac oxygen demand and cardiac oxygen supply. Aortic stiffness was assessed by measuring carotid-femoral pulse wave velocity. Cardiac oxygen demand and supply were estimated using pulse wave analysis of the aortic blood pressure waves, which were reconstructed from finger blood pressure waves. As expected, we found increased aortic stiffness to be associated with increased cardiac oxygen demand. However, we also found an association between aortic stiffness and decreased cardiac oxygen supply. In accordance, increased aortic stiffness was strongly associated with a decrease in the cardiac oxygen supply/demand ratio. In healthy subjects, a decreased coronary perfusion pressure can

be compensated by coronary vasodilatation. However, in the presence of coronary atherosclerosis, the vasodilatory reserve is limited and a decreased perfusion pressure, in these circumstances, may lead to decreased oxygen supply, especially subendocardial supply. Therefore, simultaneous occurrence of both coronary atherosclerosis and aortic stiffness can lead to increased risk of subendocardial ischaemia. This issue is addressed in **chapter 5.3,** in which the association between aortic stiffness and coronary atherosclerosis is examined. Coronary atherosclerosis was assessed by measuring coronary calcification with electron beam tomography. Aortic stiffness was assessed by measuring carotid-femoral pulse wave velocity. We found increased aortic stiffness to be associated with the presence of severe calcifications of the coronary arteries. Subjects with increased aortic stiffness had a 47% higher risk of having severe calcifications of the coronary arteries compared to subjects without stiff arteries.

In **chapter 6** we examine the consequences of increased arterial stiffness on te accuracy of non-invasive measurement of blood pressure. Automatic oscillometric blood pressure devices are frequently used to measure blood pressure. Recent studies indicate that differences in performance between automatic oscillometric blood pressure devices and sphygmomanometers may differ between healthy and diabetic subjects. An oscillometric blood pressure device determines blood pressure by detecting a sequence of oscillations in cuff pressure while the pressure is gradually reduced. As diabetic subjects have stiffer arteries than non-diabetic subjects have, arterial stiffness could be the underlying mechanism of the more pronounced differences between blood pressure values attained with oscillometric devices and sphygmomanometers in diabetic subjects. We examined whether arterial stiffness, as assessed by measuring carotid-femoral pulse wave velocity, is a determinant of the difference between blood pressure measured with a Dinamap automatic blood pressure device and a Hawksley random zero sphygmomanometer. We found that the Dinamap overestimated both systolic and diastolic blood pressure as compared to the random-zero sphygmomanometer in subjects with stiff arteries when compared to subjects without stiff arteries.

In **chapter 7** we discuss the results of the studies presented in this thesis and place the findings in a broader perspective. We conclude that arterial stiffness is not a benign condition. Arterial stiffness is associated with the presence of cardiovascular diseases like myocardial infarction and stroke. The studies described in this thesis had a cross-sectional design. Longitudinal studies are needed to examine the consequences of increased stiffening of the arterial tree on future health status.

8.2

Samenvatting

De vergrijzing van de moderne westerse samenleving maakt het belangrijk aandacht te besteden aan aandoeningen die gerelateerd zijn aan veroudering, met name wanneer deze een negatief effect op de gezondheid hebben. Verstijving van slagaders is een aan veroudering gerelateerd proces. Studies naar de gevolgen van slagaderverstijving suggereren dat slagaderverstijving tot een verhoogd risico op cardiovasculaire ziekte zoals een hartinfarct leidt. De tot op heden gepubliceerde studies met betrekking tot slagaderverstijving gebruikten echter kleine geselecteerde patiëntengroepen. Het doel van dit proefschrift was om oorzaken en gevolgen van verstijving van slagaders te onderzoeken in een grote groep deelnemers afkomstig uit de algehele bevolking. De studies beschreven in dit proefschrift zijn grotendeels uitgevoerd binnen het Erasmus Rotterdam Gezondheid en Ouderen (ERGO) onderzoek, een populatie-studie onder deelnemers van 55 jaar en ouder.

Veroudering is een belangrijke oorzaak van toegenomen slagaderverstijving. Bij bepaalde aandoeningen echter, verloopt het proces van slagaderverstijving versneld, hetgeen leidt tot stijve arteriën op jongere leeftijd. Eén van deze aandoeningen is suikerziekte, welke frequent gerelateerd is aan slagaderverstijving. Voor de aan suikerziekte gerelateerde aandoeningen zoals insuline resistentie en variabelen geassocieerd met het insuline-resistentie syndroom is de relatie met slagaderverstijving echter minder duidelijk. Met betrekking tot oorzaken van verstijving van het slagaders ligt het accent van de studies beschreven in dit proefschrift dan ook vooral op aandoeningen die aan diabetes mellitus gerelateerd zijn. De relatie tussen slagaderverkalking en slagaderverstijving is eveneens geëvalueerd in het licht van mogelijke oorzaken van verstijving van slagaders. Met betrekking tot de gevolgen van verstijving van slagaders ligt het accent op cardiale en neurologische gevolgen en gevolgen voor niet-invasieve meetmethoden van bloeddruk.

Hoofdstuk 1 is een algemene inleiding tot dit proefschrift. **Hoofdstuk 2** is een algemene introductie over het proces van slagaderverstijving en geeft achtergrondinformatie over de elastische eigenschappen van slagaders. Vervolgens worden twee veel gebruikte methoden om slagaderverstijving te meten in detail beschreven. Beide methoden zijn gebruikt in het ERGO onderzoek. De mate van slagaderverstijving die gemeten is met beide methoden in de populatie van oudere deelnemers wordt getoond naar leeftijd, geslacht en bloeddruk. Tenslotte wordt ingegaan op de associatie tussen de twee verschillende methoden om vaatwandstijfheid te meten.

Hoofdstuk 3.1 gaat in op de associatie tussen variabelen die geassocieerd zijn met het insuline-resistentie syndroom en distensibiliteit van de halsslagader. Distensibiliteit van de halsslagader geeft de uitzetting van de halsslagader tijdens het passeren van de polsgolf weer en is een maat voor slagaderverstijving. Het onderzoek werd uitgevoerd in een algemene populatie van gezonde vrouwen van middelbare leeftijd zonder suikerziekte. Van enkele andere variabelen die niet geassocieerd zijn met het insuline-resistentie syndroom werd eveneens de relatie met distensibiliteit van de halsslagader onderzocht. Onze resultaten lieten zien dat variabelen die geassocieerd zijn met het insuline-resistentie syndroom (quetelet index, taille-heup ratio, HDL-cholesterol, triglyceriden, glucose, insuline, apolipoproteïne A1, plasminogeen activator inhibitor-1-antigeen en weefsel-type plasminogeen activator-antigeen) geassocieerd waren met afgenomen distensibiliteit van de halsslagader. Er werd geen relatie gevonden tussen variabelen niet geassocieerd met het insuline-resistentie syndroom (totaal cholesterol, LDL-cholesterol en apolipoproteïne B) en distensibiliteit van halsslagader.

De relatie tussen een afwijkend glucose metabolisme en slagaderverstijving werd verder onderzocht in **hoofdstuk 3.2.** In dit hoofdstuk is een verhoogd nuchter glucose, hetgeen een maat is voor een afwijkend glucose metabolisme, gerelateerd aan distensibiliteit van de halsslagader in de oudere populatie van het ERGO onderzoek. De vaatwandstijfheid van individuen met een verhoogd nuchter glucose maar zonder suikerziekte is vergeleken met zowel de vaatwandstijfheid van individuen met een normaal nuchter glucose maar zonder suikerziekte en met de vaatwandstijfheid van individuen met suikerziekte. Onder de 75 jaar bleken individuen met een verhoogd nuchter glucose zonder suikerziekte een zelfde mate van vaatwandstijfheid te hebben als individuen met een normaal nuchter glucose zonder suikerziekte, terwijl individuen met suikerziekte 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 suikerziekte. Beide groepen hadden een stijvere vaatwand dan

individuen met een normaal nuchter glucose en zonder suikerziekte. De resultaten waren eenduidig voor mannen en vrouwen. In dit hoofdstuk hebben we additioneel de associatie tussen diverse variabelen, waaronder diegene die geassocieerd zijn met het insuline-resistentie syndroom, en slagaderverstijving bekeken. Variabelen die geassocieerd zijn met het insuline-resistentie syndroom bleken gerelateerd te zijn met toegenomen vaatwandstijfheid hetgeen in overeenstemming is met de resultaten van hoofdstuk 3.1.

In **hoofdstuk 4** beschrijven wij de relatie tussen slagaderverstijving en slagaderverkalking, beide processen die de vaatwand beïnvloeden. Eerdere kleine studies hebben een relatie laten zien tussen slagaderverstijving en cardiovasculaire ziekten. Dit suggereert het bestaan van een relatie tussen slagaderverstijving en slagaderverkalking. Echter, studies naar een directe relatie tussen slagaderverstijving en slagaderverkalking rapporteerden tegenstrijdige resultaten. Wij hebben de relatie bestudeerd tussen slagaderverstijving van zowel de aorta als de halsslagader en maten van slagaderverkalking op verschillende plaatsen in het vaatbed in een grote groep ouderen. Als maat voor vaatwandstijfheid van de aorta is de polsgolfsnelheid over het traject tussen de halsslagader en liesslagader gemeten. Als maat voor vaatwandstijfheid van de halsslagader is de distensibiliteit van de halsslagader gemeten. De verschillende maten van atherosclerose waren intima-media dikte van de halsslagader, de aanwezigheid van plaques in de halsslagader en in de aorta en de aanwezigheid van perifeer vaatlijden, vastgesteld met behulp van de enkel-arm index. De resultaten lieten zien dat toegenomen intima-media dikte van de halsslagader en toegenomen hoeveelheid plaques in zowel de halsslagader als in de aorta allen sterk geassocieerd waren met toegenomen vaatwandstijfheid van zowel de aorta als de halsslagader. Individuen met perifeer vaatlijden hadden een significant stijvere aorta en een bijna significant stijvere halsslagader, waneer vergeleken met individuen zonder perifeer vaatlijden.

In de studies beschreven in **hoofdstuk 5** ligt het accent op gevolgen van slagaderverstijving. **Hoofdstuk 5.1** beschrijft de relatie tussen slagaderverstijving en prevalente cardiovasculaire ziekten (hartinfarct en herseninfarct) en vergelijkt de sterkte van deze relatie met die van slagaderverkalking en prevalente cardiovasculaire ziekten. Als maat voor slagaderverstijving gebruikten wij polsgolfsnelheid over het traject tussen de halsslagader en liesslagader (maat voor vaatwandstijfheid van de aorta) en distensibiliteit van de halsslagader (maat voor vaatwandstijfheid van de halsslagader). Als maat voor slagaderverkalking gebruikten wij de enkel-arm index en de aanwezigheid van plaques in de halsslagader. Wij vonden dat vaatwandstijfheid van de aorta geassocieerd was met de aanwezigheid van een hartinfarct en dat de sterkte van deze associatie vergelijkbaar was met de

associatie van slagaderverkalking en de aanwezigheid van een hartinfarct. Vaatwandstijfheid van de halsslagader bleek ook geassocieerd te zijn met een de aanwezigheid van een hartinfarct, echter minder sterk. Vaatwandstijfheid van de aorta was niet geassocieerd met de aanwezigheid van een herseninfarct, terwijl vaatwandstijfheid van de halsslagader sterk geassocieerd was met de aanwezigheid van een herseninfarct. De sterkte was vergelijkbaar met de sterkte van een associatie tussen slagaderverkalking en de aanwezigheid van een herseninfarct. Alle geobserveerde associaties tussen vaatwandstijfheid en cardiovasculaire ziekten waren onafhankelijk van de aanwezigheid van slagaderverkalking.

Een verhoogde polsdruk wordt voor een groot deel veroorzaakt door slagaderverstijving. Eerder onderzoek laat zien dat polsdruk een onafhankelijke voorspeller is van cardiovasculaire ziekten en sterfte, zowel in de algehele populatie als in geselecteerde patiënten groepen. Dit kan voor een deel verklaard worden door een toegenomen cardiale zuurstof behoefte als gevolg van een toegenomen drukbelasting van het hart bij een verhoogde polsdruk. Echter, een verminderde diastolische bloeddruk, ten gevolge van toegenomen slagaderverstijving, zou kunnen bijdragen aan het verhoogde cardiovasculaire risico door een vermindering van de coronaire perfusie en daarbij een verminderde cardiale zuurstof toevoer. In **hoofdstuk 5.2** relateren wij vaatwandstijfheid van de aorta aan zowel cardiale zuurstof behoefte als cardiale zuurstof toevoer. Als maat voor vaatwandstijfheid van de aorta is de polsgolfsnelheid over het traject tussen de halsslagader en de liesslagader gemeten. Cardiale zuurstof behoefte en toevoer zijn bepaald door middel van analyse van de polsgolf in de aorta die is gereconstrueerd met behulp van drukmetingen in de vinger. Zoals verwacht vonden we dat toegenomen vaatwandstijfheid van de aorta geassocieerd was met een toegenomen cardiale zuurstof behoefte. We vonden echter ook dat toegenomen vaatwandstijfheid van de aorta geassocieerd was met een afgenomen cardiale zuurstof toevoer. Overeenstemmend was vaatwandstijfheid van de aorta sterk geassocieerd met een afgenomen ratio van cardiale zuurstof toevoer/behoefte. Gezonde individuen zijn in staat om een verminderde cardiale zuurstof toevoer te compenseren door middel van coronaire vasodilatatie. Echter, als de coronair vaten zijn verkalkt dan is de vasodilatoire reserve capaciteit beperkt en kan een verminderde coronair perfusie leiden tot een verminderde cardiale zuurstof toevoer, met name een verminderde subendocardiale zuurstof toevoer. Het tegelijkertijd voorkomen van zowel verkalking van coronair vaten als vaatwandstijfheid van de aorta zou dus kunnen leiden tot een verhoogd risico op subendocardiale ischaemie. **Hoofdstuk 5.3** beschrijft de associatie tussen vaatwandstijfheid van de aorta en verkalking van de coronair vaten. Vaatwandstijfheid van de aorta is gemeten door het

meten van de polsgolfsnelheid over het traject van de halsslagader tot de liesslagader. Verkalking van de coronair vaten is gemeten door het meten van coronaire calcificaties gebruik makend van electronenbundel tomografie. De resultaten van dit onderzoek tonen dat toegenomen vaatwandstijfheid van de aorta sterk geassocieerd was met de aanwezigheid van calcificaties in de coronair vaten. Individuen met een toegenomen vaatwandstijfheid van de aorta hadden een 47% verhoogt risico op het hebben van ernstige calcificaties van de coronair vaten.

In **hoofdstuk 6** evalueren wij of toegenomen slagaderverstijving consequenties heeft voor het niet-invasief meten van de bloeddruk. Automatische oscillometrische bloeddrukmeters worden frequent gebruikt voor het meten van de bloeddruk. Recente studies laten zien dat verschillen tussen een automatische oscillometrische bloeddrukmeter en een niet-automatische bloeddrukmeter (sphygmomanometer) anders zijn wanneer geëvalueerd bij gezonden individuen dan bij individuen met suikerziekte. Een oscillometrische bloeddrukmeter bepaalt de hoogte van de bloeddruk door middel van het detecteren van een serie oscillaties in de druk van de bloeddrukmeterband terwijl de druk in de band geleidelijk verminderd wordt. Individuen met suikerziekte hebben stijvere slagaders dan individuen zonder suikerziekte. Slagaderverstijving van individuen met suikerziekte kan de onderliggende oorzaak zijn van de meer uitgesproken verschillen tussen oscillometrische bloeddrukmeters en sphygmomanometers in deze groep. Wij hebben onderzocht of slagaderverstijving, gemeten door middel van het meten van de polsgolfsnelheid over het traject van de halsslagader tot de liesslagader, een oorzaak is van verschillen tussen een Dinamap oscillometrische automatische bloeddrukmeter en een Hawksley 'random-zero' sphygmomanometer. De resultaten lieten zien dat de Dinamap automatische bloeddrukmeter een hogere systolische bloeddruk en diastolische bloeddruk mat dan de 'random-zero' sphygmomanometer in individuen met stijve slagaders, vergeleken met individuen zonder stijve slagaders.

In **hoofdstuk 7** worden de belangrijkste resultaten van de onderzoeken die in dit proefschrift zijn beschreven geëvalueerd, rekening houdend met de restricties van deze onderzoeken. Geconcludeerd kan worden dat slagaderverstijving geen onschuldig verouderingsfenomeen is. Het stijver worden van de slagaders is gerelateerd aan een verhoogd risico op cardiovasculaire ziekten. De studies beschreven in dit proefschrift waren cross-sectioneel. Toekomstige longitudinale bevolkingsonderzoeken zijn nodig om het negatieve effect van slagaderverstijving op de gezondheid nader te evalueren.

Dankwoord

Het gezegde 'vele handen maken licht werk' is zeker van toepassing geweest bij het tot stand komen van dit proefschrift. Vele mensen hebben meegeholpen, meegedacht, toegekeken of op een andere wijze bijgedragen bij de voltooiing van dit proefschrift.

Bijzonder belangrijk voor de totstandkoming van dit proefschrift is mijn co-promotor Jacqueline Witteman geweest. Beste Jacqueline, ik prijs mij zelf gelukkig dat ik door jou ben begeleid. Je wist de juiste balans te vinden tussen het stimuleren van zelfstandig werken en toch altijd klaar te staan wanneer ik je nodig had. Je grote epidemiologische expertise en je analytische manier van denken zijn zeer belangrijk geweest bij mijn vorming als onderzoeker. Bedankt voor deze leerzame en gezellige tijd, ik hoop dat we elkaar nog regelmatig tegenkomen.

Voorts wil ik mijn beide promotoren professor Grobbee en professor Hofman bedanken. Beste Rick, de dag dat ik begon met dit onderzoek verliet jij net de afdeling om naar Utrecht te gaan. Ondanks de afstand en het inherent spaarzaam overleg heb ik veel van je geleerd. Je wist altijd direct je vinger op zwakke plekken te leggen en helder en scherp uit te leggen waar ik nog het een en ander kon bijschaven. Bedankt dat ik ook op persoonlijk vlak op je kon rekenen. Beste Bert, je hield altijd een oogje in het zeil aangaande de vorderingen van dit cardiologisch gerichte project. Daarnaast heb je mij enthousiast gemaakt voor epidemiologisch onderzoek. Ik heb met groot plezier onderzoek gedaan aan 'jouw' instituut.

Michiel Bots van het Julius Centrum speelde een belangrijke rol in dit onderzoek. Beste Michiel, bedankt dat je altijd klaarstond voor vragen en voor beoordeling van de manuscripten, zelfs al was je geen coauteur. Je expertise, je nuchtere en relativerende opmerkingen en je opbeurende woorden, ook op persoonlijk vlak, waren vaak meer dan welkom. Onze tripjes naar Parijs en San Diego had ik niet graag willen missen.

Willem Jan Bos van het AMC dank ik voor zijn kennisoverdracht aangaande

fysiologische processen. Beste Willem Jan, ik ben blij met je samengewerkt te hebben. Je was altijd te vinden voor een vruchtbare discussie en je klinische manier van benaderen was een welkome aanvulling op mijn epidemiologische aanpak. Berend Westerhof en Ilja Guelen van BMI-TNO dank ik voor hun hulp op het ERGO centrum. Voor de meest complexe apparatuuropstellingen verzonnen ze ingenieuze oplossingen.

I would like to thank professor Asmar en doctor Topouchian from Paris, France for their time and training regarding the pulse wave velocity measurement. Whenever I had a question or a technical problem with the Complior, I could always count on their immediate support. In Maastricht waren professor Reneman en professor Hoeks altijd bereid om mee te kijken en mee te denken met de bevindingen. Hun snelle en deskundige beoordeling van de manuscripten was zeer waardevol tijdens mijn eindsprint.

Het meten van vaatwandstijfheid op twee plaatsen bij ruim 3000 mensen was nooit mogelijk geweest zonder het enthousiasme van Inge Haumersen en Toos Stehmann. Toos en Inge, dit proefschrift berust deels op jullie vakkundigheid en inspanningen. Daarvoor wil ik jullie heel erg bedanken. De andere medewerkers van het ERGO centrum wil ik bedanken voor het verzamelen van talloze andere data en de gezellige uurtjes op het centrum. Het draaien van een ERGO dienst was altijd een welkome afwisseling op het werk achter de computer. Niet in de laatste plaats ben ik veel dank verschuldigd aan de deelnemers van het ERGO onderzoek voor hun enorme toewijding om mee te doen aan onderzoek dat met de jaren steeds uitgebreider wordt.

Deirdre van der Kuip neemt een bijzondere plaats in binnen het ERGO onderzoek. Deirdre, jouw extensieve reorganisatie op verschillende vlakken heeft ervoor gezorgd dat de dataverzameling van ERGO 3 secuur en vlot is verlopen. Daardoor kon dit proefschrift snel volgen. Ook je bijdrage aan de manuscripten was onmisbaar.

Vier jaar werken aan dit proefschrift zijn omgevlogen en niet in de laatste plaats vanwege de vele gezellige collegae. Mijn kamergenoten, Iris, Liesbeth en later Irene, zorgden voor de benodigde afleiding en gezelligheid en dat ik niet al te lang stond weg te dromen bij het uitzicht over de Maas. Mijn nieuwe kamergenoten, Margo, Saskia en Rogier, ben ik zeer dankbaar voor het feit dat ze gedurende de laatste maanden vol begrip hebben gezorgd voor veel rust op de kamer. Saskia, bedankt voor de vele kopjes koffie die je voor me hebt gezet. Het eetclubje van weleer, Iris, Paul, Carolien, Raan, Liesbeth, Sandra en natuurlijk Jan Cees, maakte dat ik nog regelmatig een warme maaltijd nuttigde in tijden dat het leven iets minder georganiseerd was. Antonio, Paul, Tommy en Sandra bleken

prima verhuizers. Bedankt dat we op jullie konden rekenen. Bas, samen bezig zijn met de laatste loodjes gaf me veel steun.

Paul Mulder dank ik voor zijn statistische expertise. Bij Bettina, Lidia en Maria kon ik altijd binnenlopen voor een 'klein' statistisch vraagje. Het secretariaat wil ik bedanken voor de secretariële ondersteuning, met name Marga die de agenda van Jacqueline uitstekend hanteerde. De jongens van de automatisering, met name Frank en Nano, wil ik bedanken voor alle IT-hulp. Annelies van Ruitenbeek van het Julius Centrum wil ik bedanken omdat ze ervoor zorgde dat de afstand tussen Utrecht en Rotterdam zo klein mogelijk bleef.

Gerjan Konings dank ik voor de artistieke manier waarop hij de omslag digitaal heeft vormgegeven. Anna Bosselaar dank ik voor de opmaak van dit boekje en omdat ze me op het laatste moment uit de brand hielp door voor een finishing touch van de omslag te zorgen.

Mijn vriendinnen Monique en Wietske wil ik bedanken voor het begrip als ik het weer eens af liet weten omdat ik het te druk had. Monique, je steun in moeilijke tijden is onvergetelijk.

Sandra, als buuf en collega moet ik je al enige tijd missen, hetgeen ik zeer jammer vind. Een goede conversatie onder het genot van een biertje in de Witte Aap is toch echt leuker dan via e-mail. Ik ben heel blij dat je straks naast me staat, daarmee ben je officieel onze huis-paranimf.

Ronald, jou wil ik bedanken omdat jouw vertrouwen in mijn kunnen maakte dat ik een promotieonderzoek durfde aan te vangen.

Mijn familie wil ik bedanken voor hun belangrijke rol in mijn leven. Mijn ouders hebben mij altijd mijn eigen weg laten kiezen. Ze hadden er vertrouwen in dat het wel goed met me zou komen. Lieve pa en ma, dat enorme vertrouwen heeft me gebracht tot waar ik nu ben. Daarom draag ik dit boekje aan jullie op. Mijn broer Kees is al mijn hele leven een belangrijke steun en toeverlaat. Kees, ik ben heel blij dat jij straks tijdens de verdediging aan mijn zijde zal staan. Mijn broer Ed heeft reeds op de middelbare school mijn ambitie geprikkeld. Ed, jij hebt mij altijd weten te stimuleren en daarmee heb je de basis voor dit boekje gelegd. Ook wil ik je bedanken voor het model staan voor de omslag. Hanneke en Petra wil ik bedanken omdat ze net als echte zussen er altijd voor me zijn. Hanneke, je hebt reeds eerder een kunstwerk voor mij gemaakt maar een medische illustratie was geheel nieuw voor je. Desalniettemin heb je het idee prachtig uitgewerkt. Heel erg bedankt daarvoor.

De beste ontdekking die ik heb gedaan op de afdeling Epidemiologie & Biostatistiek is Jan Cees. Lieve, lieve Jan Cees, je maakt me zo gelukkig dat alles de moeite waard is, hoe ingewikkeld dat soms ook is. Ik hou van je.

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About the author

Nicole van Popele was born on May 28th, 1969 in Oss, the Netherlands, where she attended secondary school at the Maasland College. In 1988 she started her medical study at the Erasmus University in Rotterdam, the Netherlands. During this period she performed a research project at the Neonatal Intensive Care Unit (Dr. J.N. van den Anker, Department of Neonatology, Sophia Children's Hospital, Rotterdam, the Netherlands) on the efficacy of erythropoëtin in premature born neonates. She graduated from medical school in 1995 after which she worked as a resident in Cardiology at the Zuiderziekenhuis in Rotterdam. In September 1996 she started the work described in this thesis at the Department of Epidemiology & Biostatistics (head: Prof. dr. A. Hofman) of the Erasmus Medical Centre in Rotterdam, in close collaboration with the Julius Centre of Patient Oriented Research (head: Prof. dr. D.E. Grobbee) of the Utrecht Medical Centre in Utrecht. During this period she obtained a Master of Science in Clinical Epidemiology at the Netherlands Institute for Health Sciences in Rotterdam. In October 2000 she will start as a resident at the Department of Internal Medicine of the Academic Medical Centre in Amsterdam, the Netherlands (head: Prof. dr. E. Briët) as part of her training as a cardiologist. She will continue her training as a cardiologist at the Department of Cardiology of the Academic Medical Centre in Amsterdam (head: Prof. dr. K.I. Lie).

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