Arterial stiffness in postmenopausal women: determinants of pulse wave velocity

Corinne E.I. Lebrun[a,b], Yvonne T. van der Schouw[a], Annette A.A. Bak[a], Frank H. de Jong[b], Huibert A.P. Pols[b], Diederick E. Grobbee[a], Steven W.J. Lamberts[b] and Michiel L. Bots[a]

Objective To investigate the degree and potential cardiovascular determinants of arterial stiffness, assessed by aortic pulse wave velocity (PWV) measurements, and to relate arterial stiffness to absolute 10–12-year risks of stroke, coronary heart disease and death, as estimated by available risk functions, in postmenopausal women.

Method We performed a cross-sectional study among 385 postmenopausal women, aged 50–74 years, sampled from the general population. Arterial stiffness was assessed non-invasively by measurement of aortic PWV using applanation tonometry. Information on health was obtained by medical history, registration of current medication, and physical examination. Height, weight, waist and hip circumferences, fasting glucose, total and high-density lipoprotein (HDL) cholesterol, triglycerides, resting blood pressure, and heart rate were measured. Three risk scores were used to estimate, for each individual, the absolute risk of stroke, coronary heart disease, and death within 10–12 years as a function of their cardiovascular risk factor profile. The relationship between PWV and these risk scores was subsequently determined.

Results Significant positive relationships with PWV were found for body mass index, fasting glucose, diabetes mellitus, and triglycerides in analyses adjusted for age, mean arterial blood pressure, and heart rate. Height and HDL cholesterol were inversely related to PWV. The risks of stroke, coronary heart disease, and death increased with increasing PWV in a linear graded manner.

Conclusions This cross-sectional study among postmenopausal women provides evidence that most of the established cardiovascular risk factors are determinants of aortic PWV. Increased PWV marks an increased risk of stroke, coronary heart disease, and death within 10–12 years. *J Hypertens* 20:2165–2172 © 2002 Lippincott Williams & Wilkins.

Introduction

Originally, increased arterial stiffness was considered to be intrinsic to the ageing process of the artery [1]. However, it has since been demonstrated that factors such as insulin resistance, hypertension, atherosclerosis, and end-stage renal disease (ESRD) [2–9] contribute to the stiffening of the arterial tree. The clearest consequences of arterial stiffening of large arteries, in particular the aorta, are increased pulsatile blood pressure as a result of a greater systolic (SBP) and a lower diastolic (DBP) pressure, which leads to a increased left ventricular workload in combination with a reduced perfusion of the coronary arteries in diastole [10]. Recent population-based studies have emphasized the increasing interest in markers of arterial stiffness, measurable in a non-invasive manner, each with it own characteristics: brachial pulse pressure, carotid distensibility, and aortic pulse wave velocity (PWV) [10,11].

There have been a number of studies indicating that increased incidences of cardiovascular disease are related to stiffer vessels [2–16]. The most prominent factor is age: with increasing age, arteries become stiffer, apparently in a similar manner for both men and women [17]. In addition, increased blood pressure and indicators of glucose intolerance are strongly related to increased arterial stiffness [18,19]. A number of studies have examined the relationship between arterial stiffness and the presence of atherosclerotic abnormalities elsewhere in the arterial tree [6–8]. An increased aortic PWV showed a linear graded relationship to carotid intima–media thickness, plaques in the carotid arteries,

lower extremity arterial disease, and plaques in the abdominal aorta [8]. In a group of patients with hypertension, aortic PWV was positively related to the absolute risk of cardiovascular disease, as estimated by available risk functions [20].

Most of the evidence that relates arterial stiffness to risk factors and the presence of atherosclerosis and prevalent cardiovascular disease comes from studies performed in specific groups of patients. Information on these issues for postmenopausal women is very limited. Some available data stem from studies of the influence of oestrogen; menopause seems to be associated with stiffer vessels [21–23]. However, studies that evaluated whether hormone replacement therapy (HRT) had a direct beneficial effect on arterial stiffness have yielded conflicting findings [24–26]. Nevertheless, studies of HRT and endothelial function [27], and of HRT and atherosclerosis [28] – both major determinants of arterial stiffness – showed positive results overall.

The aim of this study was to investigate the major determinants of aortic stiffness, measured by aortic PWV, in postmenopausal women. In addition, we related PWV to estimates of individual 10–12-year absolute risk of stroke, coronary heart disease, and death. Three risk scores were used to estimate the individual absolute risk of stroke, coronary heart disease, and death as a function of their cardiovascular risk factor profile [29–31].

Participants and methods
Study population
Participants were recruited from the PROSPECT study, one of the two Dutch cohorts participating in the European Prospective Investigation into Cancer and Nutrition (EPIC) [32]. In PROSPECT, a total of 17 395 healthy women presenting for breast cancer screening, who were aged 49–70 years and living in Utrecht and the surrounding area, were enrolled between 1993 and 1997. Using the baseline data from PROSPECT, we selected two groups of women who had experienced a natural menopause either between 1987 and 1989, or between 1969 and 1979. In addition, criteria for inclusion in the study were that the woman should have an intact uterus and at least one intact ovary, and should not have used sex steroids after the reported date of last menstruation. These criteria were used because the primary objective of the present study was to elucidate the role of endogenuous sex hormones on several markers of frailty. Of 1803 (1149 + 653) eligible women, 902 (451 + 451) were invited to participate and 553 (61%) agreed to do so. The aim of our study was to enrol 200 women in each group; 403 (207 + 196) participants were finally included in the study. Women were considered sufficiently healthy to participate when they were physically and mentally able to visit the study centre without assistance. Each participant underwent all tests and assessments during two visits to the study centre. The study was approved by the Institutional Review Board of the University Medical Center Utrecht and written informed consent was obtained from all participants. Data collection took place between September 1999 and March 2000.

Measurements
Information on health was obtained by medical history, registration of current medication and physical examination. A standardized questionnaire on use of oestrogen, alcohol consumption, and smoking was obtained from all women. Individuals were categorized as current smokers, former smokers, and those who had never smoked. For current and past smokers, the number of pack-years was calculated as the average number of packs of cigarettes smoked per day multiplied by the total years of smoking. Height, weight, and waist and hip circumferences were measured with the woman in a standing position wearing indoor clothes and no shoes. Body mass index (BMI) was calculated as the weight in kilograms divided by the square of the height in metres.

Blood pressure and heart rate were measured during the first visit using an oscillometric-automated device (Dinamap 8100, Critikon). Measurements were conducted before 1100 h after an overnight fast. After 5 min of rest, blood pressure was taken at the right brachial artery simultaneously with heart rate, twice with the participant lying down and three times with the participant in a standing position, with 1 min between each measurement. SBP and DBP were defined as the average of the two supine measurements. Mean arterial blood pressure (MAP) was calculated as $DBP + [(SBP - DBP)/3]$. Pulse pressure was defined as SBP minus DBP. Hypertension was defined as SBP at least 160 mmHg, DBP at least 95 mmHg, use of antihypertensive medication, or a combination thereof [33]. Isolated systolic hypertension was defined as SBP at least 160 mmHg and DBP 90 mmHg or less without medication. Fasting venous blood samples were obtained between 0800 and 1100 h. Total cholesterol, high-density lipoprotein (HDL) cholesterol, triglyceride, glucose and albumen were measured reflectometrically using commercial enzymatic kits with a Vitros 250 (dry chemistry; Johnson & Johnson, Rochester, Minnesota, USA). Glucose was measured by the glucose oxidase/peroxidase method. Total cholesterol was measured by adaptation of the cholesterol oxidase/peroxidase method (fixed time). Triglycerides were measured by the lipase/glycerolkinase/GPO/POD method (fixed time). HDL cholesterol was measured after precipitation with dextran sulphate/Mg^{2+}. The low-density lipoprotein (LDL) cholesterol concentration was estimated using the Friedewald formula [34].

Hypercholesterolaemia was defined as a total cholesterol concentration of 6.5 mmol/l or more, or use of cholesterol-decreasing drug treatment. Diabetes mellitus was defined as fasting serum glucose of 7.0 mmol/l or more, or the use of oral glucose-decreasing drugs or insulin [35].

Arterial stiffness

The Sphygmocor system was used for non-invasive measurement of the stiffness of the aorta [36] (PWV system, PWV Medical, Sydney, Australia). After the woman had rested for 5–10 min in the supine position, aortic PWV was measured by sequential recordings of the arterial pressure waveform at the carotid artery and the femoral artery, using a hand-held micromanometer-tipped probe on the skin at the site of maximal arterial pulsation. Gating the recordings at those two sites to the electrocardiogram (ECG) enabled PWV to be measured. Recordings were taken when a reproducible signal was obtained with high-amplitude excursion – usually 10 consecutive beats, to cover a complete ventilatory cycle. The wave transit time was calculated by the system software, using the R wave of a simultaneously recorded ECG as a reference frame. Distances from the carotid sampling site to the suprasternal notch and from the suprasternal notch to the femoral artery were measured using a compass [37]. The aortic PWV (m/s) was automatically calculated as the distance between the suprasternal notch and the femoral artery minus the distance between the carotid sampling site and the suprasternal notch, divided by the time interval between the systolic R wave and the femoral systolic up-stroke minus the time interval between the systolic R wave and the carotid systolic up-stroke. Aortic PWV was determined as the mean of at least three consecutive beats recorded during 10 s of data acquisition. All measurements were performed by the same observer (C.E.I.L.).

We performed a reproducibility study among 27 participants, who underwent a second PWV measurement within 2 weeks after the first examination. The mean (SD) PWV was 9.55 (2.36) m/s at the first visit and 9.56 (2.01) m/s at the second visit, with a mean difference of 0.01 (1.0) m/s. The within-class correlation coefficient was 89.6%, indicating that 89.6% of the variance in the PWV measurements was attributable to differences between patients, whereas 10.4% could be attributed to differences between visits (measurement error and intra-individual variability).

Risk functions for stroke, coronary heart disease, and all-cause mortality

A risk function for an individual estimates the probability of the occurrence of an event within a certain time span as a function of the individual's level of the risk indicators. Risk functions are derived from analyses on data from longitudinal (cohort) studies, in which the relative and independent contribution of certain risk factors in predicting the occurrence of the event are evaluated.

The risk function for stroke was based on information from 10 years of follow-up of participants in the Framingham Heart Study, who were aged 55–84 years and initially free from stroke, at biannual examinations 9 and 14 [29]. A Cox proportional hazards model was used to estimate the contribution of cardiovascular risk factors to the occurrence of stroke. The report provides detailed information on how an individual's absolute 10-year risk of stroke may be estimated. For women, this is:

$$\{1 - 0.9353^{\exp[0.0699(\text{age})+0.0161(\text{SBP})+0.00026(\text{HRXSBP})}$$
$$^{+0.4404(\text{CVD})+0.8055(\text{ECGLVH})+0.5219(\text{smoking})}$$
$$^{+1.1173(\text{atrial fibrillation})+0.5604(\text{diabetes})-7.5766)]}\},$$

where HRXSBP = 0 if systolic pressure <110 or >200 mmHg, else HRXSBP is HRX(SBP − 110)(200 − SBP) and HRX = 1 when individual is receiving antihypertensive treatment; CVD, cardiovascular disease present; ECGLVH, left ventricular hypertrophy on ECG.

The risk function predicting 10-year coronary heart disease risk was obtained from data of the Framingham Heart cohort aged 30–74 years and free from cardiovascular disease at baseline [30]. A logistic regression model was used for risk estimation. Risk indicators included in the final model were age, SBP, total : HDL cholesterol ratio, left ventricular hypertrophy (on ECG), smoking, and diabetes mellitus. In the report [30], an extensive and detailed description is given of how to arrive at an estimate of the absolute risk of coronary heart disease within 10 years for an individual, given a certain set of cardiovascular risk factors.

The risk function to predict an individual's probability of dying within 11.5 years as a function of the levels of cardiovascular risk factors was obtained from findings in a follow-up study among 6057 individuals aged 20 years or over, conducted in the Netherlands [31]. A Cox proportional hazards model was used to identify the most relevant cardiovascular factors for death. For each individual, an absolute risk of dying within 11.5 years may be calculated. For women, this is:

$$\{1 - 0.99945^{\exp[0.081(\text{age})+0.010(\text{SBP})+0.001(\text{pulse rate})+0.119(\text{smoking})}$$
$$^{+0.160(\text{antihypertensive drugs})+0.700(\text{diabetes mellitus})}$$
$$^{+0.436(\text{myocardial infarction})-0.036(\text{BMI})]}\}$$

Data analysis

Data on PWV were not available for 18 women, leaving results from 385 women for analysis. General characteristics of the study population were described by the mean and SD. The association between PWV measurements and cardiovascular risk factors was evaluated using linear regression analysis, and the associations are presented with the linear regression coefficient (β) and its SE. As it is known from the literature that age, mean arterial blood pressure, and heart rate are major determinants of PWV, we adjusted for these factors when evaluating the association with other determinants using multiple linear regression. We evaluated whether the associations differed between women with an early menopause (1969–1979) or a late menopause (1987–1989). Because all interaction terms had a P value > 0.40, no evidence for modification was found. Therefore, overall findings are reported. For each of the 385 individuals, we calculated the absolute risk of stroke, coronary heart disease, and death, using the formulae described above. Then, we evaluated the association between these risk estimates and PWV using linear regression analyses. Statistical analyses were performed using SPSS for Windows (version 9.0).
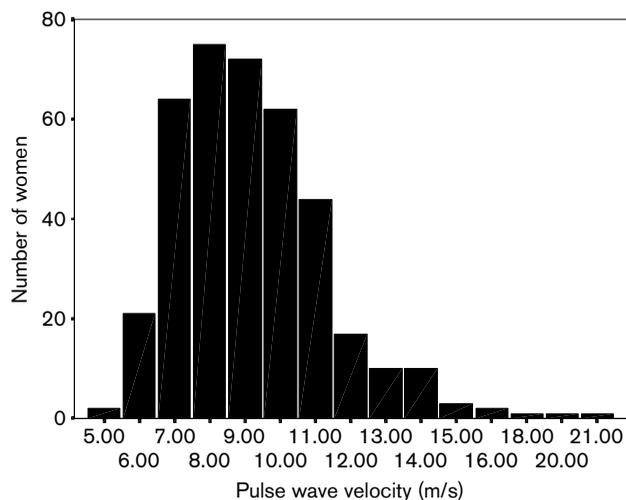
Results

The general characteristics of the study participants are given in Table 1. The 385 participants were aged 56–73 years. The PWV distribution is depicted in Figure 1, showing a mean (SD) PWV value of 9.2(2.2) m/s.

Fig. 1

Distribution of pulse wave velocity in a population of 385 postmenopausal women.

Determinants of PWV

The results of the analysis of risk factors and PWV are presented in Table 2. Increasing age, blood pressure (systolic, diastolic, pulse, and mean arterial), and heart rate were related to increased PWV. In analyses in

Table 1 General characteristics of the study population ($n = 385$)

Age (years)	66.2 (3.7)
Systolic blood pressure (mmHg)	147 (20.7)
Diastolic blood pressure (mmHg)	76 (13.6)
Mean arterial pressure (mmHg)	99.9 (14.9)
Pulse pressure (mmHg)	71.7 (14.2)
Heart rate (beats/min)	69.1 (10.1)
Hypertension (%)	37.4
Untreated isolated systolic hypertension (%)	12.5
Triglycerides (mmol/l)	1.45 (0.68)
Total cholesterol (mmol/l)	6.3 (1.0)
HDL cholesterol (mmol/l)	1.5 (0.40)
LDL cholesterol (mmol/l)	4.1 (1.0)
Hypercholesterolaemia (%)	52.7 (203)
Serum glucose (mmol/l)	5.2 (1.1)
Diabetes mellitus (%)	7.3
Body mass index (kg/m^2)	25.9 (4.1)
Waist : hip ratio	0.80 (0.06)
Smoking	
Current (%)	13
Past (%)	36.4
Pack-years	14.1 (18.2)
Cardiovascular disease (%)	11.2
Coronary heart disease (%)	9.9
Stroke (%)	1.6
Pulse wave velocity (m/s)	9.2 (2.1)

Values are expressed as mean (SD) in the case of continuous variables, and as percentages in case of categorical variables. HDL, LDL, high- and low-density lipoproteins. For definitions see text.

Table 2 Relationship between cardiovascular risk factors and pulse wave velocity

	β coefficient
Model I	
Age (years)	0.154 ± 0.028
Heart rate (beats/min)	0.045 ± 0.011
Systolic blood pressure (mmHg)	0.058 ± 0.004
Diastolic blood pressure (mmHg)	0.056 ± 0.007
Pulse pressure (mmHg)	0.070 ± 0.007
Mean arterial pressure (mmHg)	0.069 ± 0.006
Isolated systolic hypertension	1.554 ± 0.311
Model II	
Total cholesterol (mmol/l)	0.097 ± 0.090
LDL cholesterol (mmol/l)	0.156 ± 0.092
HDL cholesterol (mmol/l)	-0.863 ± 0.225
Triglycerides (mmol/l)	0.451 ± 0.135
Hypercholesterolaemia	0.189 ± 0.185
Glucose (mmol/l)*	0.237 ± 0.080
Insulin (mU/l)*	0.033 ± 0.016
Body mass index (kg/m^2)	0.079 ± 0.022
Waist : hip ratio	3.942 ± 1.359
Weight (kg)	0.017 ± 0.008
Height (m)	-3.522 ± 1.550
Smoking class	0.017 ± 0.132
Pack-years	0.018 ± 0.010

Values are mean $\pm$ SE. Model I: age, heart rate, blood pressure included in one model. Model II: age, heart rate, blood pressure included and one risk factor in one model. *In analyses with glucose and insulin, individuals with diabetes mellitus were excluded. The β coefficient reflects the change in PWV (m/s) when the risk factors increases by one unit. HDL, LDL, high- and low-density lipoproteins.

which age, mean arterial blood pressure, and heart rate were taken into account, graded associations were found for BMI, weight, height, LDL cholesterol, HDL cholesterol, triglycerides, glucose, diabetes mellitus, insulin, and pack-years of smoking. In a multivariate model, the main factors that remained independently related to an increased PWV were mean arterial pressure, pulse pressure, age, heart rate, the number of pack-years of cigarettes smoked, and HDL cholesterol (inverse relationship) (Table 3).

PWV and absolute risk of disease
The 10-year absolute risk of stroke increases gradually with increasing aortic PWV (Fig. 2). An increase in PWV of 1 m/s was associated with an increase in absolute risk of stroke of 0.9 [95% confidence interval (CI) 0.7 to 1.1]. The risk of dying within 11.5 years by quintile of aortic PWV is shown in Figure 3. An increase in PWV of 1 m/s was associated with increase in absolute risk of death of 2.2% (95% CI 1.8 to 2.6). The risk of coronary heart disease within 10 years by quintile of aortic PWV is given in Figure 4. A graded positive association was found, with an increase in PWV of 1 m/s associated with a 1.4% (95% CI 0.9 to 1.9) increased risk of coronary heart disease.

Discussion
This cross-sectional study provides evidence that, among postmenopausal women, increased arterial stiffness assessed non-invasively by PWV is significantly and positively associated with age, mean arterial pressure, pulse pressure, heart rate, and pack-years of cigarette smoking. Height and HDL cholesterol were found to be inversely related to PWV. In addition, this study suggests that PWV is a clear marker of an increased absolute risk of stroke, coronary heart disease, and death within 10–12 years.
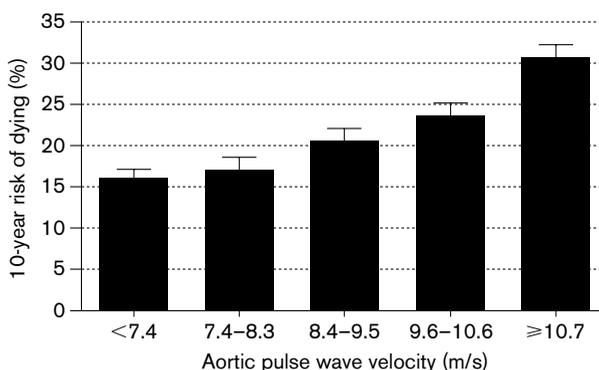
Before these results can be accepted, some aspects of this study need to be considered. The measurement of aortic PWV – distance divided by time – allows only an estimate of the actual distance travelled by the

Fig. 2
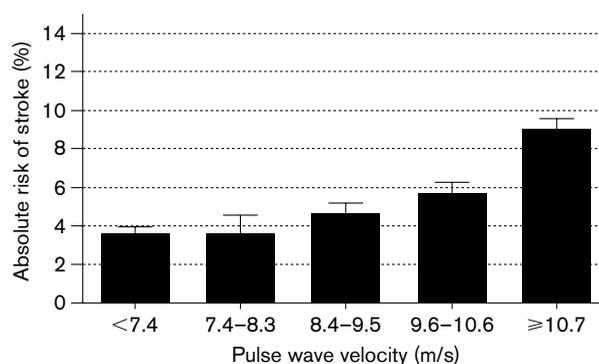
Absolute 10-year risk of stroke by aortic pulse wave velocity (quintiles) (assessed using Framingham Study risk function [29]).

Fig. 3

Absolute 11.5-year risk of death by aortic pulse wave velocity (quintiles) (assessed using a Netherlands follow-up study risk function [31]).

pulse, and not the exact distance. The latter can be assessed only with invasive procedures. We tried to approach the true distance by subtracting the distance between the suprasternal notch and the carotid location from the distance between the suprasternal notch and

Table 3 Associations of risk factors with pulse wave velocity in a multivariate model including the risk factors indicated

	β coefficient	SE	P
Age (years)	0.096	0.024	<0.001
Heart rate (beats/min)	0.025	0.009	0.006
Pulse pressure (mmHg)	0.047	0.007	<0.001
Mean arterial pressure (mmHg)	0.047	0.007	<0.001
Body mass index (kg/m$\leqslant$)	0.051	0.026	0.053
Waist : hip ratio	0.36	1.572	0.817
Glucose (mmol/l)	0.020	0.084	0.813
Insulin (mU/l)	−0.0038	0.014	0.786
HDL cholesterol (mmol/l)	−0.560	0.237	0.019
Smoking (pack-years)	0.025	0.010	0.015

HDL, high-density lipoprotein.

Fig. 4
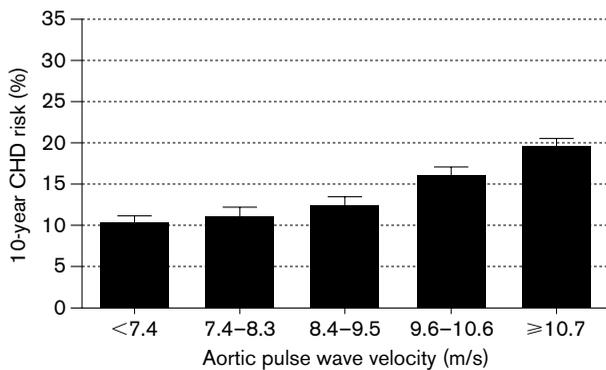
Absolute 10-year risk of coronary heart disease by aortic pulse wave velocity (quintiles) (assessed using Framingham Study risk function [30]).

the femoral sampling site, because the pulse travels in the opposite direction [10]. This might underestimate PWV, because arteries become longer and more tortuous with age. However, we consider the error in the distance to be relatively small and therefore not to affect our results seriously. If anything, it might bias our findings towards an underestimation of the associations.

Our findings confirm those from other studies indicating that age, blood pressure, and heart rate are related to increased aortic stiffness. The age-related increase in PWV may be explained by physiological degeneration of elastic fibres, with parallel increases in collagen and mucopolysaccharides within the media, and accumulation of vascular smooth muscle cells in the arterial wall. Those changes are known to be exacerbated by hypertension, arterial degeneration, and cardiac and arterial disease, and are most marked in central arteries such as the aorta [10,16,20,38,39]. The relationship to heart rate may be partly the result of an adrenergic influence on the aortic wall, increasing the smooth muscle tone and leading to increased stiffness of the arterial wall [40]. However, it has been shown that, in elastic arteries, the stiffening effect of tachycardia is exerted independently of sympathetic modulation of the vessel wall properties and that, in muscular arteries, removal of sympathetic influences unmasks the stiffening effect of tachycardia [41–43]. In addition to these three factors, an inverse relationship between body height and PWV has been reported in other studies. Short stature is associated with early systolic arrival of reflected waves, which stiffens the aorta and increases the pulsatile effort of the left ventricle [8,44,45]. Finally, in our analyses, indicators of the insulin resistance syndrome appeared to be related to an increased aortic stiffness – an observation in agreement with the findings of other studies [2,3].

Recently, attention has been focused on the effects of HRT in directly affecting vascular characteristics such as endothelial function and atherosclerosis, in an attempt to reduce the risk of future cardiovascular disease [27,28]. By influencing endothelial function and atherosclerosis, HRT may also affect arterial stiffness, as these are the two main determinants of arterial stiffness. At present, however, the advantages and disadvantages of HRT in reducing cardiovascular disease events in primary and secondary prevention continue to be strongly debated [27,46,47]. Our results points towards traditional risk factors as the main determinants of arterial stiffness at menopause, and provide a rationale for developing treatment strategies.

The present analyses suggest that an increased PWV is associated with an increased risk of stroke, coronary heart disease, and all-cause mortality, as estimated by available risk functions. This finding of an association of increased PWV and absolute risk of cardiovascular disease estimated using risk functions agrees with findings from a similar study performed in hypertensive individuals [16]. Although both studies suggest that PWV is a clear marker of risk, they had a cross-sectional design, which does not allow inferences as to cause and consequence. Furthermore, the contribution of measurement of arterial stiffness in predicting future disease, relative to that of other cardiovascular risk factors, cannot be determined using studies of that design, but needs to be addressed in a prospective follow-up study.

Direct evidence of the predictive value of measurements of arterial stiffness comes from a small number of studies, mostly among patients with ESRD. In a cohort of 241 patients with ESRD after a mean follow-up of 72 months, increased aortic PWV was associated with all-cause and cardiovascular mortality [15,48,49]. The importance of the prognostic value of aortic PWV measurements in patients with ESRD was further demonstrated in a cohort of 150 such patients [48]. In a recent study among 1980 patients with essential hypertension, who were followed for an average of 4.2 years [49], an increase in PWV of 5 m/s was associated with an increased risk of death of 2.14 (95% CI 1.71 to 2.67) and an increased risk of cardiovascular mortality of 2.35 (95% CI 1.76 to 3.14). These relationships remained statistically significant after adjustments for age, previous symptomatic cardiovascular disease, and diabetes mellitus. Information on the relation of arterial stiffness and risk of cardiovascular disease among other high-risk groups or for the population at large is still lacking.

The present study had a cross-sectional design and thus does not allow conclusions to be drawn that are based on longitudinal data. However, we believe that our conclusion that 'PWV is a marker of risk' may be substantiated by two observations. First, in our study,

an increased PWV was related to increased risk factors. Thus, if an increased PWV is found in a patient, this patient is likely to have increased risk factors and therefore is at increased risk of cardiovascular disease. This reasoning has been further substantiated by several follow-up studies relating an increased PWV to incident cardiovascular disease events. Second, in our study an increased PWV related to increased absolute risk of cardiovascular disease as assessed using the Framingham Heart Study risk function. This observation also indicates that, when an increased PWV is found in a patient, this patient is likely to be at increased risk of future cardiovascular disease events.

In conclusion, this cross-sectional study among postmenopausal women provides evidence that PWV of the aorta is associated with increases in most of the established cardiovascular risk factors. In addition, it supports the importance of assessment of arterial stiffness as a marker for cardiovascular disease risk that may be of use as an endpoint in observational and intervention studies.

Acknowledgements

References

1 O'Rourke MF. The arterial pulse in health and disease. *Am Heart J* 1971; **82**:687–702.
2 Emoto M, Nishizawa Y, Kawagishi T, Maekawa K, Hiura Y, Kanda H, *et al.* Stiffness indexes beta of the common carotid and femoral arteries are associated with insulin resistance in NIDDM. *Diabetes Care* 1998; **21**:1178–1182.
3 Lehmann ED, Gosling RG, Sonkensen PH. Arterial wall compliance in diabetes. *Diabet Med* 1992; **9**:114–119.
4 Benetos A, Lanvent S, Hoeks APG, Bontanyrie PH, Safow DE. Arterial alterations with ageing and high blood pressure. *Arterioscler Thromb* 1993; **13**:90–97.
5 Safar ME, Laurent S, Pannier BM, London GM. Structural and functional modifications of peripheral large arteries in hypertensive patients. *J Clin Hypertens* 1987; **3**:360–367.
6 Hirai T, Sasayama S, Kawasaki T, Yagi S. Stiffness of systemic arteries in patients with myocardial infarction. A noninvasive method to predict severity of coronary atherosclerosis. *Circulation* 1989; **80**:78–86.
7 Farrar DJ, Bond MG, Sawyer JK, Green HD. PWV and morphological changes associated with early atherosclerosis progression in the aorta of cynomolgus monkeys. *Cardiovasc Res* 1984; **18**:107–118.
8 van Popele NM, Grobbee DE, Bots ML, Asmar R, Topouchian J, Reneman RS, *et al.* Association between arterial stiffness and atherosclerosis: The Rotterdam Study. *Stroke* 2001; **32**:454–460.
9 London GM, Marchais SJ, Safar ME, Genest AF, Guerin AP, Metivier F, *et al.* Aortic and large artery compliance in end stage renal disease. *Kidney Int* 1990; **37**:137–142.
10 Nichols WW, O'Rourke MF, eds. *McDonald's blood flow in arteries. Theoretical, experimental and clinical principles.* Oxford: Oxford University Press, 1998.
11 Lehmann ED. Terminology for the definition of arterial elastic properties. *Pathol Biol* (*Paris*) 1999; **47**:656–664.
12 Demirovic J, Nabulsi A, Folsom AR, Carpenter MA, Szklo M, Sorlie PD, *et al.* Alcohol consumption and ultrasonographically assessed carotid artery wall thickness and distensibility. The Atherosclerosis Risk in Communities (ARIC) Study Investigators. *Circulation* 1993; **88**:2787–2793.
13 Reneman RS, Merode T van, Hick P, Muytjens AMM, Hoeks APG. Age-related changes in carotid artery wall properties in men. *Ultrasound Med Biol* 1986; **12**:465–471.
14 Simons PCG, Algra A, Bots ML, Grobbee DE, Graaf Y van der, for the SMART Study Group. Common carotid intima-media thickness and arterial stiffness. Indicators of cardiovascular risk in high-risk patients. The SMART study (Second Manifestations of ARTerial disease). *Circulation* 1999; **100**:951–957.
15 Blacher J, Guerin AP, Pannier B, Marchais SJ, Safar ME, London GM. Impact of aortic stiffness on survival in end stage renal disease. *Circulation* 1999; **99**:2434–2439.
16 Blacher J, Pannier B, Guerin AP, Marchais SJ, Safar ME, London GM. Carotid arterial stiffness as a predictor of cardiovascular and all-cause mortality in end stage renal disease. *Hypertension* 1998; **32**:570–574.
17 Smulyan H, Asmar RG, Rudnicki A, London GM, Safar ME. Comparative effects of ageing in men and women on the properties of the arterial tree. *J Am Coll Cardiol* 2001; **37**:1374–1380.
18 Liao D, Arnett DK, Tyroler HA, Riley WA, Chambless LE, Szklo M, *et al.* Arterial stiffness and the development of hypertension. The ARIC study. *Hypertension* 1999; **34**:201–206.
19 Amar J, Ruidavets JB, Chamontin B, Drouet L, Ferrieres J. Arterial stiffness and cardiovascular risk factors in a population-based study. *J Hypertens* 2001; **19**:381–387.
20 Blacher J, Asmar R, Djane S, London GM, Safar ME. Aortic pulse wave velocity as a marker of cardiovascular risk in hypertensive patients. *Hypertension* 1999; **33**:1111–1117.
21 Jonason T, Henriksen E, Kangro T, Vessby B, Ringqvist I. Menopause is associated with the stiffness of the common carotid artery in 50-year-old women. *Clin Physiol* 1998; **18**:149–155.
22 Westendorp IC, Bots ML, Grobbee DE, Reneman RS, Hoeks AP, Van Popele NM, *et al.* Menopausal status and distensibility of the common carotid artery. *Arterioscler Thromb Vasc Biol* 1999; **19**:713–717.
23 Nagai Y, Earley CJ, Kemper MK, Bacal CS, Metter EJ. Influence of age and postmenopausal estrogen replacement therapy on carotid arterial stiffness in women. *Cardiovasc Res* 1999; **41**:307–311.
24 Rodriguez-Macias KA, Naessen T, Bostrom A, Bergqvist D. Arterial stiffness is not improved in long-term use of estrogen. *Am J Obstet Gynecol* 2002; **186**:189–194.
25 Teede HJ, Liang YL, Kotsopoulos D, Zoungas S, Cravent R, McGrath BP. A placebo-controlled trial of long-term oral combined continuous hormone replacement therapy in postmenopausal women: effects on arterial compliance and endothelial function. *Clin Endocrinol* (*Oxf*) 2001; **55**:673–682.
26 Hayward CS, Samaras K, Campbell L, Kelly RP. Effect of combination hormone replacement therapy on ambulatory blood pressure and arterial stiffness in diabetic postmenopausal women. *Am J Hypertens* 2001; **14**:699–703.
27 Vita JA, Keaney JF Jr. Hormone replacement therapy and endothelial function: the exception that proves the rule? *Arterioscler Thromb Vasc Biol* 2001; **21**:1867–1869.
28 Hodis HN, Mack WJ, Lobo RA, Shoupe D, Sevanian A, Mahrer PR, *et al.* Estrogen in the prevention of atherosclerosis. A randomized, double-blind, placebo-controlled trial. *Ann Intern Med* 2001; **135**:939–953.
29 Wolf PA, D'Agostino RB, Belanger AJ, Kannel WB. Probability of stroke: a risk profile from the Framingham Study. *Stroke* 1991; **22**:312–318.
30 Anderson KM, Wilson PW, Odell PM, Kannel WB. An updated coronary risk profile. A statement for health professionals. *Circulation* 1991; **83**:356–362.
31 Hoes AW, Grobbee DE, Valkenburg HA, Lubsen J, Hofman A. Cardiovascular risk and all-cause mortality: a 12 year follow-up study in The Netherlands. *Eur J Epidemiol* 1993; **9**:285–292.
32 Peeters PH, Beckers CG, Hogervorst JM, Collette HJ. Effect on breast cancer screening response in The Netherlands of inviting women for an additional scientific investigation. *J Epidemiol Community Health* 1994; **48**:175–177.
33 Anonymous. The fifth report of the Joint National Committee on Detection, Evaluation, and Treatment of High Blood Pressure (JNC V). *Arch Intern Med* 1993; **153**:154–183.
34 Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem* 1972; **18**:499–502.
35 Stolk RP, de Vegt F, Heine RJ. What are the consequences of the new American guidelines for the diagnosis of diabetes mellitus in The Netherlands? *Ned Tijdschr Geneeskd* 1998; **142**:222–225.
36 O'Rourke MF, Gallagher DE. Pulse wave analysis. *J Hypertens* 1996; **14** (**suppl**):S147–S157.
37 Lehmann ED, Hopkins KD, Gosling RG. Assessment of arterial distensibility by automatic pulse wave velocity measurement. *Hypertension* 1996; **27**:1188–1191.

38 Mosti G, Iabichella ML, Picerni P. Pulse wave velocity. A new calculation method. *Minerva Cardioangiol* 2000; **48**:53–59.

39 Cockcroft JR, Wilkinson IB, Webb DJ. The Trevor Howell Lecture. Age, arterial stiffness and the endothelium. *Age Ageing* 1997; **26 (suppl 4)**:53–60.

40 Taquet A, Bonithon Kopp C, Simon A, Levenson J, Scarabin Y, Malmejac A, *et al.* Relations of cardiovascular risk factors to aortic pulse wave velocity in asymptomatic middle-aged women. *Eur J Epidemiol* 1993; **9**:298–306.

41 el Miedany YM, el Gaafary S, el Baddini MA. Osteoporosis in older adults with non-insulin-dependent diabetes mellitus: is it sex related? *Clin Exp Rheumatol* 1999; **17**:561–567.

42 Mircoli L, Mangoni AA, Giannattasio C, Mancia G, Ferrari AU. Heart rate-dependent stiffening of large arteries in intact and sympathectomized rats. *Hypertension* 1999; **34**:598–602.

43 London GM, Guerin AP, Pannier BM, Marchais SJ, Metivier F. Body height as a determinant of carotid pulse contour in humans. *J Hypertens* 1992; **10 (suppl)**:S93–S95.

44 Smulyan H, Marchais SJ, Pannier B, Guerin AP, Safar ME, London GM. Influence of body height on pulsatile arterial hemodynamic data. *J Am Coll Cardiol* 1998; **31**:1103–1109.

45 London GM, Guerin AP, Pannier B, Marchais SJ, Stimpel M. Influence of sex on arterial hemodynamics and blood pressure. Role of body height. *Hypertension* 1995; **26**:514–519.

46 Mendelsohn ME, Karas RH. The time has come to stop letting the HERS tale wag the dogma. *Circulation* 2001; **104**:2256–2259.

47 Barrett-Connor E. Looking for the pony in the HERS data. Heart and Estrogen/progestin Replacement Study. *Circulation* 2002; **105**:902–903.

48 Guerin AP, Blacher J, Pannier B, Marchais SJ, Safar ME, London GM. Impact of aortic stiffness attenuation on survival of patients in end stage renal disease. *Circulation* 2001; **103**:987–992.

49 Laurent S, Boutouyrie P, Asmar R, Gautire I, Laloux B, Guize L, *et al.* Aortic stiffness is an independent predictor of all-cause and cardio-vascular mortality in hypertensive patients. *Hypertension* 2001; **37**:1236–1241.