Atherosclerotic plaques in the carotid arteries represent an important cause of cerebral ischemia. The composition of an atherosclerotic plaque is an important predictor for plaque rupture and subsequent thromboembolic events. Intraplaque hemorrhage (IPH) is considered as a high-risk component of the vulnerable plaque through contribution of cholesterol to the necrotic core of the plaque and by increasing macrophage infiltration, making the plaque more unstable.1–3 Several studies have indicated a strong association of IPH with cerebrovascular events.3–5 Furthermore, even in atherosclerotic lesions of asymptomatic subjects, IPH was shown to contribute to plaque progression and destabilization.6,7 Magnetic resonance imaging (MRI) has emerged as a reliable and accurate tool for discriminating plaque components in vivo and for detecting IPH.7,8 However, only few studies have investigated determinants of plaque composition in the carotid artery9,10 and, specifically, studies relating risk factors to IPH are scarce. We recently reported that sex, age, smoking, and hypertension are associated with IPH in the general population.11 Hypertension is a highly prevalent condition and a major contributor to atherosclerotic cardiovascular disease. However, the pathophysiology of the contribution of high blood pressure to atherosclerotic plaques is still not fully elucidated.

For a long time, hemodynamic forces have been linked to atherosclerosis formation and plaque destabilization.12 Plaque instability is, in part, determined by local factors, but it has been suggested that systemic factors are also important.13 Atherosclerotic plaques form at positions of disturbed blood flow and concomitant low and oscillating wall shear stress,14 whereas all structures of the arterial wall are influenced by blood pressure fluctuations.

As a continuation of our previous study,11 the current study was designed to determine the association of various blood pressure parameters with the presence of carotid IPH in a large population-based study.

Abstract—Intraplaque hemorrhage (IPH) is a characteristic of the vulnerable atherosclerotic plaque that has been associated with ischemic stroke. Not much is known about determinants of IPH. We studied whether blood pressure parameters are associated with presence of IPH. Within the framework of a prospective population-based cohort study, The Rotterdam Study, the carotid arteries of 1006 healthy participants ≥45 years and with intima-media thickening (≥2.5 mm) on ultrasound were imaged with a 1.5-T magnetic resonance imaging scanner. IPH was defined as a hyperintense signal on a 3D-T1w-GRE magnetic resonance sequence. Generalized estimation equation analysis, adjusted for age, sex, carotid wall thickness, and cardiovascular risk factors, was used to assess the association between blood pressure parameters and IPH. Magnetic resonance imaging of the carotid arteries revealed presence of IPH in 444 of 1860 plaques (24%). Systolic blood pressure and pulse pressure (PP) were significantly associated with IPH after adjustment for age and sex. In multivariate analysis, PP yielded the strongest association, with an odds ratio per SD increase in PP of 1.22 (95% CI, 1.07–1.40). The odds ratio per SD for systolic blood pressure was 1.13 (0.99–1.28). Only PP remained significant after additional adjustment for other blood pressure components. The combination of smoking and isolated systolic hypertension was associated with 2.5 times increased risk of IPH (1.2–5.2). In conclusion, PP was the strongest determinant of IPH independent of cardiovascular risk factors and other blood pressure components. The association between pulsatile flow and IPH may provide novel insights in the development of the vulnerable plaque. (Hypertension. 2013;61:76-81.)

Key Words: blood pressure ■ carotid artery ■ intraplaque hemorrhage ■ MRI ■ pulse pressure
Materials and Methods

Study Population
The study was performed within the framework of the Rotterdam Study, a population-based cohort study in The Netherlands, aimed at investigating determinants of various chronic diseases among the elderly. The study design and objectives of the Rotterdam Study were described elsewhere. Briefly, the baseline visit started between 1990 and 1993. All inhabitants of Ommoord, a suburb of Rotterdam, aged ≥45 years were invited to participate. All participants were subsequently invited every 3 to 4 years to the research center for follow-up examinations, including carotid ultrasonography. The ultrasonography protocol and reading has been described in detail previously. For each individual, carotid wall thickening was determined as the maximum near and far wall measurements.

From October 2007 onward, carotid MRI scanning was performed in all subjects with a maximum intima-media thickness ≥2.5 mm on carotid ultrasound. Until October 2009, ultrasonography in 1417 participants of the Rotterdam Study revealed intima-media thickness ≥2.5 mm in left, right, or both carotid arteries. These subjects were selected for MRI. Participants with contraindications for MRI, prior carotid endarterectomy, or poor image quality by MRI scan were excluded. As described previously, a total of 1006 complete MRI examinations were performed. This resulted in 1866 carotid arteries with plaques that were available for further analyses. Information on blood pressure levels, cardiovascular risk factors, and history of cardiovascular disease was obtained from the visit previous to MRI scanning. The Medical Ethics Committee of Erasmus Medical Center approved the study protocol, and written informed consent was obtained from all participants. The study procedures were in accordance with institutional guidelines.

Blood Pressure Parameters
Two blood pressure measurements were taken with a random-zero sphygmomanometer after 5 minutes of rest with the subject in a sitting position. The mean of the 2 blood pressure values was used in the analyses. Pulse pressure (PP) was calculated as PP = systolic blood pressure (SBP)−diastolic blood pressure (DBP); mean arterial pressure (MAP) was calculated as MAP = DBP+1/3 PP. To quantify the relative magnitude of PP to mean artery pressure, we normalized the PP to the MAP and referred to this value as the fractional PP: PP/MAP. Hypertension at baseline was defined as a minimal level of 140/100 mm Hg (according to European Society of Cardiology criteria) and the use of antihypertensive medication. The latter was assessed through automated linkage to pharmacies with computerized records. Assessment of antihypertensive drug usage included usage of diuretics, β blockers, calcium channel blockers, or angiotensin-converting enzyme inhibitors at date of MRI scanning.

Covariates
Information on medical history and smoking behavior was collected through home interviews, and covariates were measured at a study center visit as described previously. Smoking status was classified as current, past, and never. Body mass index (weight [kg]/height [m]^2) was calculated. Serum total cholesterol and high-density lipid-protein cholesterol values were measured using standard laboratory techniques. Diabetes mellitus was considered to be present when fasting blood glucose was >7.0 mmol/L, when nonfasting glucose was >11.1 mmol/L, or when antidiabetic medication was used. A history of cardiovascular disease was defined as a history of coronary heart disease or stroke at baseline.

MRI Acquisition and Image Analysis
All scans were obtained with a 1.5 T scanner with a bilateral phased-array surface coil. A standard scanning protocol was used with a total scanning time of about 30 minutes. Sequence parameters have been described in detail elsewhere. Two-dimensional time-of-flight magnetic resonance angiography was performed to cover the carotid bifurcation at both sides ranging from 15 mm caudal to 30 mm cranial from the point of bifurcation. Presence of IPH was determined using the 3-dimensional-T1w-GRE MRI sequence. Before the evaluation of plaque composition, the quality of all sequences in each MRI scan was rated on a 5-point scale (1=worst; 5=best). Scans were included in the analyses if the image quality was scored ≥3 on all MRI sequences. We assessed plaque characteristics in all plaques with a minimum carotid wall thickness of ≥2.0 mm on MRI.

Data Analysis
Data are expressed as mean±SD for quantitative variables and percentages for discrete variables. All analyses were carried out using SAS version 9.2 (Cary, NC). In primary analyses, we assessed the association of single blood pressure parameters, as continuous variables, with IPH. To adjust for the correlation between plaques in the 2 carotid arteries of the same participant, we performed Generalized Estimation Equation, with an independent or unstructured working correlation matrix including 2 levels per participant, namely the left and right carotid artery. To compare the associations between different blood pressure parameters and presence of IPH, odds ratios (ORs) with corresponding 95% CIs were estimated for a 1-SD difference in each component. Adjustments were made for age, sex, carotid wall thickness on MRI, smoking status (current, past, and never), body mass index, total cholesterol, and diabetes mellitus. Missing values in the covariates (6.1%) were imputed using the Expectation Maximization method. Probability values <0.05 were considered statistically significant.

The close correlation between SBP and PP hinders efforts to distinguish between these 2 components. Therefore, secondary analyses were performed in which the SBP and PP were additionally adjusted for the other blood pressure parameters. Probabilities of joint influences of SBP and DBP were estimated using the β-coefficients obtained from the generalized estimation equation model, for 55-year-old males. We previously found an interaction between smoking and hypertension in relation to risk of IPH. We here evaluate the interaction of smoking with types of hypertension reflecting different levels of blood pressure components. The following clinical classification was used: combined systolic/diastolic hypertension (SDH, SBP ≥140 and DBP ≥90 mm Hg), isolated systolic hypertension (ISH, SBP ≥140 and DBP <90 mm Hg), and isolated diastolic hypertension (SBP <140 and DBP ≥90 mm Hg). Normal blood pressure was defined as SBP <140 and DBP <90 mm Hg. The combinations of types of hypertension and smoking status were evaluated by adding a product term to the multivariate model, using never smoking and normal blood pressure as reference. Finally, to examine whether blood pressure components are associated with severity of the outcome (ie, bilateral IPH), we made categorical variables of blood pressure components based on quartiles. Hereby, the unilateral and bilateral IPH frequency was counted. Unadjusted analysis was conducted using Pearson χ² test.

Results
Of the 2012 carotid arteries scanned (n=1006 subjects), 1866 contained an atherosclerotic plaque >2.0 mm on MRI. Three participants were excluded because information of blood pressure level was unavailable. This resulted in 1860 carotid arteries for further analyses. Table 1 describes baseline characteristics of the study population. The mean age was 70.4±10.2 years and 52.1% was male. In 444 (24%) carotid arteries, IPH was revealed on MRI whereas 1422 (76%) carotid arteries did not have IPH. In comparison with carotid arteries without IPH, plaques with IPH had a thicker carotid wall (3.9±1.2 versus 3.1±0.8 mm) and 5.9% of the plaques caused moderate or severe stenosis (>30% stenosis) compared with 0.8% for plaques without IPH. Figure 1 shows magnetic resonance images of 3 carotid atherosclerotic plaques with intraplaque hemorrhage of subjects with high pulse pressure and low systolic blood pressure.
Table 1. Baseline Characteristics of the Study Population

<table>
<thead>
<tr>
<th>Variable</th>
<th>MRI Population (n=1006)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (male), n</td>
<td>524 (52.1%)</td>
</tr>
<tr>
<td>Age, y</td>
<td>70.4±10.2</td>
</tr>
<tr>
<td>Smoking</td>
<td></td>
</tr>
<tr>
<td>Never, n</td>
<td>248 (24.7%)</td>
</tr>
<tr>
<td>Past, n</td>
<td>440 (43.7%)</td>
</tr>
<tr>
<td>Current, n</td>
<td>318 (31.6%)</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>27.4±3.8</td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>5.6±1.1</td>
</tr>
<tr>
<td>HDL, mmol/L</td>
<td>1.4±0.4</td>
</tr>
<tr>
<td>Diabetes mellitus, n</td>
<td>162 (16.1%)</td>
</tr>
<tr>
<td>Medical history</td>
<td></td>
</tr>
<tr>
<td>Stroke, n</td>
<td>42 (4.2%)</td>
</tr>
<tr>
<td>CHD, n</td>
<td>155 (15.4%)</td>
</tr>
<tr>
<td>Hypertension, n</td>
<td>726 (72.2%)</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>143±20</td>
</tr>
<tr>
<td>Diastolic blood pressure, mm Hg</td>
<td>80±11</td>
</tr>
<tr>
<td>Mean arterial pressure, mm Hg</td>
<td>101.2±12.6</td>
</tr>
<tr>
<td>Pulse pressure, mm Hg</td>
<td>63.3±17.3</td>
</tr>
<tr>
<td>Fractional pulse pressure</td>
<td>0.6±0.2</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>70.4±13.7</td>
</tr>
<tr>
<td>Antihypertensive drug use, n (%)</td>
<td>478 (54.4%)</td>
</tr>
<tr>
<td>Carotid plaque on MRI scan</td>
<td></td>
</tr>
<tr>
<td>Unilateral plaque, n</td>
<td>146 (14.5%)</td>
</tr>
<tr>
<td>Bilateral plaque, n</td>
<td>860 (85.5%)</td>
</tr>
</tbody>
</table>

Categorical variables are presented as numbers (%). Continuous values are expressed as mean±SD.

MRI indicates magnetic resonance imaging; BMI, body mass index; HDL, high-density lipoprotein; and CHD, coronary artery disease.

Table 2 presents ORs per SD increase for each blood pressure parameter adjusted for age and sex (model 1) and adjusted for age, sex, carotid wall thickness, and cardiovascular risk factors (model 2). SBP, PP, and fractional PP were significantly associated with IPH after adjustment for age and sex. In multivariable analysis, PP yielded the strongest association with an OR per 1 SD in PP of 1.22 (1.06–1.39) compared with the reference category (normal blood pressure, never smoking). Because of the low number of subjects with isolated diastolic hypertension (n=24), we were unable to evaluate the modifying effect of smoking for this type.

Figure 2 shows the joint influence of SBP and DBP on IPH. Probabilities were determined from generalized estimation equation analysis for a male aged 55 years. The probability of IPH increased with increasing SBP level. However, at any given value of SBP, the probability of IPH is higher with lower DBP and thus higher PP.

Table 3 presents associations of SBP and PP with IPH, where these main variables were successively adjusted for each other as well as for the other blood pressure components. Adding any of the other blood pressure components to the model did not affect the association of PP with IPH, whereas the association of SBP with IPH was attenuated after adjustment for PP. Furthermore, risk estimates for SBP were increased after adjustment for DBP and MAP.
Finally, we examined the relation between PP and unilateral and bilateral presence of IPH. Figure 3 shows that the frequencies of unilateral and bilateral IPH increased with increasing quartile of PP (Pearson $\chi^2$ test $P<0.001$). The fourth quartile of PP harbors the highest percentage of bilateral IPH (16.7%).

**Discussion**

In a large population-based study, we evaluated the association between various blood pressure parameters and the presence of IPH in carotid atherosclerotic plaques of subjects from the general population. The pulsatile component of blood pressure, as indicated by PP and fractional PP, was the strongest determinant of IPH independent of carotid wall thickness and cardiovascular risk factors. Furthermore, the association of PP with IPH was independent of the other blood pressure parameters. These findings add to our previous work showing that hypertension is an important risk factor for IPH.11

PP and SBP are highly correlated because both parameters rise with increases in vascular resistance and large-artery stiffness. Our results show that not all subjects with the same level of SBP have the same IPH risk. Subjects with lower DBP and therefore higher PP, have a higher probability of IPH. In middle-aged and elderly subjects, an increase in PP with fixed SBP occurs solely as a function of declining DBP and is a consequence of a rise in large-artery stiffness.23

In a previous study, we found that current smoking and hypertension combined increased the risk of IPH almost 3-fold.11 In the current study, we extended the analysis by using different subtypes of hypertension, each type characterized by different values of MAP and PP. The combination of current smoking and ISH was associated with a 2.5 times increased risk of IPH. The strong and significant interaction between smoking and ISH compared with the nonsignificant interaction between smoking and SDH reflects the somewhat stronger effect of PP compared with SBP on IPH. However, our findings do not exclude an interaction of smoking and SDH in relation to risk of IPH. The smaller risk estimate for the combination of smoking and SDH in the current study as compared with the combination of smoking and hypertension, as reported by us previously,11 may be explained by the fact that SDH was based on blood pressure measurements only. We used lower threshold values (140/90 mm Hg versus 160/100 mm Hg), and subjects with ISH and isolated diastolic hypertension were naturally excluded from this subgroup.

Our findings are in agreement with previous studies in which PP has been reported as a predictor of acute coronary events and as an independent risk factor for stroke.21–26 The randomized multicenter clinical trial Systolic Hypertension in the Elderly Program found that in patients with ISH a decrease of the DBP and concomitant increase in PP increased the risk for stroke, coronary heart disease, and cardiovascular disease.26 This study also showed that the occurrence of both
stroke and total mortality was related to PP level at baseline independent of mean pressure.25

Plaque components, such as IPH and lipid with or without a necrotic core, are presumed to be important determinants of progression and destabilization of the plaque.2,24 That cyclic hemodynamic factors affect atherosclerosis progression and plaque instability is supported by several experimental models in which pulsatile strain was shown to affect the endothelium, vascular smooth muscle cells, and the production of elastin, collagen, and glycosaminoglycans, resulting in increased atherosclerotic plaque volume.37–39 However, rupture of a plaque occurs when the stresses within the plaque exceed the strength of the plaque. Accordingly one would expect a greater contribution of SBP than PP causing rupture of the neovessels. However, our findings suggest that the pulsatile mechanical load that acts on the arterial wall, irrespectively of the absolute value of blood pressure, is at least as important in the development of IPH.

IPH is believed to develop from the disruption of thin-walled microvessels that are lined by discontinuous endothelium without supporting–muscle cells.35 An underlying pathophysiological mechanism linking PP to IPH could include the involvement of arterial stiffness. Large elastic arteries, such as the aorta and the carotid arteries, work predominantly as cushions, but with progressive arterial stiffening, the pulsations are not completely absorbed and may extend to the microcirculation,32 as was already shown in the brains33 and the kidneys.34 Likewise, enhanced pulsatile flow in the vasa vasorum of the carotid arteries may occur and cause hemorrhage of the neovessels in the plaque. Another possible explanation includes the involvement of enhanced microvessel formation and hereby increasing the risk of IPH. IPH as well as rupture of the fibrous cap are associated with an increased density of microvessels in the plaque.35 When subjecting the vessel wall, and in specific vascular smooth muscle cells in vitro to cyclic strain, it was shown that vascular endothelial growth factor, which is an indicator of angiogenesis, is upregulated.36 Also nicotine has been found to accelerate plaque growth and vascularization, and it has been suggested that vascular endothelial growth factor has a role in this process.37,38 That both cyclic strain and nicotine are associated with enhanced angiogenesis may explain the interaction we found between smoking and ISH. Another theory involves the observations of Takaya et al7 who reported that IPH remained detectable in the same plaque even after 18 months, and therefore he suggested that either IPH does not resolve very rapidly or that IPH recurs in the same plaque repetitively. Recurrent rupture of the neovessels wall represents structural failure of a component of the diseased vessel.39 The wall continuously interacts with hemodynamic forces,40 and pulsatile pressure could possibly be the main mechanical trigger for such failure.

A major strength of this study is the large sample size and the high response rate. All 1860 carotid arteries were from 1006 individuals from the general population who had early signs of atherosclerosis on ultrasound. A limitation of this study is the cross-sectional design, which restricts our interpretation of the data with respect to cause and consequence. Although it is theoretically possible that IPH, or more in general presence of atherosclerosis, lead to an elevation in PP, a causal link is more likely to be in the opposite direction. In addition, blood pressure measurements can be variable, therefore we tried to minimize this phenomenon by using the mean of 2 blood pressure measurements. Furthermore, effect of modification by smoking was examined using types of hypertension based on single blood pressure measurements, irrespectively of the clinical diagnosis and usage of antihypertensive medication of the subjects. Also, plaques with wall thickness <2.0 mm were excluded because MRI differentiation between plaque components was not feasible in these small plaques.

Perspectives

The present study is the first study that investigated the association between different blood pressure parameters and IPH in a large population-based study. We found that the pulsatile component, as indicated by PP, had the strongest association with IPH. The association was independent of traditional cardiovascular risk factors and other blood pressure components. We found that the combination of current smoking and ISH strongly increased the risk of IPH. Repetitive deformations in the carotid artery induced by pulsatile flow may play an important role in atherosclerosis progression and plaque destabilization. The observed association between pulsatile flow and IPH and, additionally, the modifying effect of smoking provide novel insights in the pathology of the vulnerable plaque. However, further investigations are necessary to elucidate the pathophysiological mechanism.

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Disclosures

None.

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Blood Pressure Parameters and Carotid Intraplaque Hemorrhage as Measured by Magnetic Resonance Imaging: The Rotterdam Study

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