

Echogenic Carotid Plaques Are Associated With Aortic Arterial Stiffness in Subjects With Subclinical Carotid Atherosclerosis

Mahmoud Zureik, Jeanne-Marie Bureau, Mohammed Temmar, Chris Adamopoulos, Dominique Courbon, Kathryn Bean, Pierre-Jean Touboul, Athanase Benetos, Pierre Ducimetière

Abstract—A better understanding of the interrelationships between the structure and function of the large arteries would lead to optimize cardiovascular disease prevention strategies. In this study, we investigated the relationships of aortic arterial stiffness assessed by carotid-femoral pulse-wave velocity (PWV), with carotid plaque echogenicity assessed by B-mode ultrasound. We analyzed 561 subjects (without coronary heart disease or stroke) who were volunteers for free health examinations (age, 58.3 ± 10.8 years; 32.6% women). Extracranial carotid plaque echogenicity was graded from 1 (plaque appearing black or almost black) to 4 (plaque appearing white or almost white) according to the Gray-Weale classification. Plaques of grades 1 and 2 were defined as echolucent plaques, and plaques of grades 3 and 4 were defined as echogenic plaques. Fifty-one subjects (9.1%) had echolucent carotid plaques, 109 (19.4%) had echogenic plaques, and 401 (71.5%) had no plaques. Subjects with echogenic plaques had higher PWV mean (12.9 ± 2.8 m/s) compared with those without plaques (11.1 ± 2.3 m/s, $P < 0.001$) and compared with those with echolucent plaques (11.3 ± 2.3 m/s, $P < 0.01$). The PWV means in subjects without plaques and those with echolucent plaques were similar and not statistically different ($P = 0.55$). When multivariate adjustment for major known cardiovascular risk factors was performed, these results were not markedly modified. Similar patterns of results were also observed in many subgroups according to age, gender, and hypertensive status. This study provides the first evidence that echogenic but not echolucent carotid plaques are associated with aortic arterial stiffness. This association applies to individuals with normal blood pressure and those with elevated blood pressure. Assessment of the joint and interaction effects of plaque morphology and arterial stiffness on the occurrence of cardiovascular events would permit a better identification of high-risk subjects. (*Hypertension*. 2003;41:519-527.)

Key Words: atherosclerosis ■ carotid arteries ■ epidemiology ■ hypertension, arterial ■ ultrasonography

Studying the possible interrelationships between the structure and function of the large arteries would lead to a better understanding of the pathophysiology of vascular diseases, to a better evaluation of stroke and cardiovascular risks, and thus to the development of more adequate disease prevention strategies. In a recent study, we reported that the presence of carotid atherosclerotic plaques detected by B-mode ultrasound but not diffuse intima-media thickening was determinant of aortic arterial stiffness assessed by carotid-femoral pulse-wave velocity (PWV), independent of major known cardiovascular risk factors.¹ These findings obtained from a large study of hypertensive and normotensive subjects corroborate the recent results of the Rotterdam Study, showing that arterial stiffness was related to abdominal aortic and carotid atherosclerotic plaques.²

The mechanisms linking arterial stiffness to atherosclerosis are not known at present. Atherosclerotic changes in the arterial wall could include smooth muscle cell proliferation, deposition of lipid and accumulation of collagen, elastin,

and/or proteoglycans.^{3,4} However, it is not known whether plaques with different contents are differentially related to arterial stiffness. Plaque echogenicity as assessed by B-mode ultrasound has been found to reliably predict the content of soft tissue and the amount of calcification.⁵⁻⁸ Plaques that appear echolucent on B-mode ultrasound are lipid-rich, whereas echogenic plaques have a higher content of fibrous tissue and calcification.⁹⁻¹¹

In our above-mentioned study,¹ plaque echogenicity was assessed; in the current article, we report the cross-sectional associations of aortic PWV with carotid echolucent and echogenic plaques in 561 hypertensive and nonhypertensive subjects.

Methods

Details of this study have been reported previously.¹ Subjects were examined at the Center d'Investigations Préventives et Cliniques (the IPC Center). This medical center, which is subsidized by the French national health care system, provides all working and retired persons and their families with a free medical examination (a standard health checkup) every 5 years. In 1992 to 1993, the first subject, among

Received August 22, 2002; first decision September 12, 2002; revision accepted December 18, 2002.

From the National Institutes of Health and Medical Research (INSERM), Unit 258 (M.Z., M.T., C.A., D.C., A.B., P.D.), Villejuif; Centre d'Investigations Préventives et Cliniques (J.M.B., K.B., A.B.) and Centre de Diagnostic et de Prévention Neurovasculaire (P.J.T.), Paris, France.

Correspondence to Mahmoud Zureik, MD, PhD, INSERM Unit 258, Hôpital Paul Brousse, 16 av. Paul Vaillant Couturier, 94807 Villejuif Cedex, France. E-mail zureik@vjf.inserm.fr

© 2003 American Heart Association, Inc.

Hypertension is available at <http://www.hypertensionaha.org>

DOI: 10.1161/01.HYP.0000054978.86286.92

those examined daily, with antihypertensive drug treatment ($n=265$), the first subject with high systolic blood pressure (≥ 140 mm Hg) or with high diastolic blood pressure (≥ 90 mm Hg) ($n=272$), and the first 2 normotensive subjects ($n=543$), ≥ 18 years of age, were invited to participate in a study for further clinical and biological investigations of cardiovascular risk factors. Six years later, these 1080 subjects were invited from November 1998 to October 1999 and 672 subjects (62.6%) underwent clinical, biological, carotid ultrasound, and wave pulse velocity examinations. However, ultrasound assessment of the carotid arteries could not be performed in the last 79 examined subjects for logistic reasons. A further 29 subjects who reported at the second examination a history of angina, myocardial infarction, or stroke were excluded from the analysis. At the time of the first examination, there were no significant differences of cardiovascular risk factors between subjects who participated and those who did not participate in the second survey. All data shown in this report were obtained from the second examination. However, changes in hypertensive status between the first and the second examinations were also used in the analyses. The study protocol was approved by the Comité d'éthique du Center Hospitalier Universitaire de Cochin, and written informed consent was obtained from all study participants.

Medical History and Standard Biological Procedures

All participants were administered a standardized questionnaire that provided information about demographic background, occupation, medical history, drug use, and personal habits such as cigarette consumption. The body mass index (BMI) was computed as weight (in kilograms) divided by height (in meters) squared. Supine blood pressure was measured before the B-mode ultrasound examination in the right arm with the use of a manual sphygmomanometer. After a 10-minute rest period, systolic and diastolic blood pressures were measured 3 times with a 5-minute interval, and the average of the last 2 measurements was used in the statistical analyses. Subjects who reported having a medical history of using antihypertensive drugs or subjects who had systolic blood pressure ≥ 160 mm Hg or diastolic blood pressure ≥ 95 mm Hg were considered to be hypertensive. The changes in hypertensive status between the first and the second examinations were as follows: 225 subjects (40.1%) were normotensive at both examinations (normotensive₁-normotensive₂), 36 (6.4%) were hypertensive at the first examination but not at the second (hypertensive₁-normotensive₂), 51 subjects (9.1%) were normotensive at the first examination and hypertensive at the second (normotensive₁-hypertensive₂), and 249 subjects (44.4%) were hypertensive at both examinations (hypertensive₁-hypertensive₂). Nine subjects interrupted their antihypertension treatment in the hypertensive₁-normotensive₂ group, 26 initiated antihypertension treatment in the normotensive₁-hypertensive₂ group, and 150 continued their treatment in the hypertensive₁-hypertensive₂ group.

Fasting total serum cholesterol, HDL cholesterol, LDL cholesterol, and fasting plasma glucose were also measured. Subjects who reported a medical history of diabetes, use of antidiabetic drugs, or a fasting plasma glucose level ≥ 126 mg/dL were considered to be diabetic.

Pulse Wave Velocity

Carotid-femoral PWV was evaluated by one physician (who did not perform the ultrasound examination) before the B-mode ultrasound examination, with 2 pressure probes. This method with an automatic device (Complior, Colson) has been extensively analyzed.¹⁴ Briefly, 2 pressure waves were recorded transcutaneously at the base of the neck for the right common carotid artery and over the right femoral artery. PWV was determined as the foot-to-foot velocity. Pulse transit time was determined as the average of 10 consecutive beats. The distance traveled by the pulse wave was measured over the body surface as the distance between the 2 recording sites. Aortic PWV was calculated as the ratio of distance to transit time. The validation of this automatic method and its reproducibility have been previously

published, with an intraobserver repeatability coefficient of 0.93 and an interobserver reproducibility coefficient of 0.89.¹²

Ultrasonography

Ultrasound examinations were performed by 2 trained ultrasonographers using the Aloka SSD-650, with a transducer frequency of 7.5 MHz. Acquisition, processing, and storage of B-mode images were computer-assisted with the new version of a software previously described (M'ATHS).¹³ The new version uses a Windows environment (instead of DOS) and is compatible with a PC system. The sonographers were not aware of the PWV examination values.

The protocol, which was similar to that applied in the Aging Vascular Study (EVA Study),¹⁴⁻¹⁶ involved scanning of the common carotid arteries (CCAs), the carotid bifurcations (CBs), and the origins (first 2 cm) of the internal carotid arteries (ICAs). Both the near and far walls of these arterial segments were scanned longitudinally and transversely to assess the presence of plaques. The presence of plaques was defined as localized echo structures encroaching into the vessel lumen for which the distance between the media-adventitia interface and the internal side of the lesion was ≥ 1 mm (verified by measurement at the time of examination). The quantification of plaque thickness was made at the site of the maximal encroachment perpendicularly to the vessel wall by measuring the distance between the media-adventitia interface and the lesion surface facing the lumen. The sonographer could be computer-assisted in the identification of interfaces and placement of electronic calipers by examining the inflections of the density profile curve taken at the site of plaque. In the CB and ICA segments, most of the plaques were straddling both segments. Thus, only combined data from CB and ICA were recorded (CB-ICA).

In subjects with plaques, the total number of sites with plaques was calculated and ranged from 1 to 4 (left and right CCAs and CB-ICAs). A semiquantitative scale score was also used to assess the severity of plaques and graded as follows: 1 site (left or right) with plaques having thickness ≤ 2 mm (score=1), 2 sites with plaques having both thickness ≤ 2 mm or one site with plaques having thickness > 2 mm (score=2), 2 sites with plaques including at least 1 plaque with thickness > 2 mm (score=3), 2 sites with plaques having both thickness > 2 mm or annular plaque (score=4).

Plaque morphology in terms of ultrasound echogenicity was recorded at the time of examination and was graded from 1 to 4 according to the Gray-Weale classification.¹⁷ Grade 1 denotes low echogenicity, or echolucency (defined as a plaque appearing black or almost black), and grade 4 denotes strong echogenicity (defined as a plaque appearing white or almost white). The vessel lumen was used as the reference structure for defining echolucency, and the bright echo zone produced by the media-adventitia interface in the far wall was used as the reference for defining echogenicity. In the analysis, plaques of echogenicity 1 and 2 were defined as echolucent plaques, and plaques of grades 3 and 4 were defined as echogenic plaques. Figure 1 shows 1 echogenic plaque and 1 echolucent plaque. Of the 564 subjects with B-ultrasound examination, 3 were excluded because their plaques could not be classified for echogenicity. Thus, a total of 561 subjects were included in the final analyses.

When several plaques were detected in the same arterial segment (ie, CCA or CB-ICA), only the echogenicity of that showing the greatest encroachment into the lumen was recorded. The presence of plaque in each segment (left and right CCAs and CB-ICAs) is shown in Table 1. Among the 116 subjects with carotid plaques in the right side, 11 had plaques in the CCA-segment only, 94 in the CB-ICA segment only, and 11 in both segments. In the latter 11 subjects, the plaques in the 2 segments for each subject had similar echogenicity in 10 subjects. For the subject with different plaque echogenicity in the 2 segments, the echogenicity of the CB-ICA plaque was arbitrary used in the analyses. Among the 105 subjects with carotid plaques in the left side, 8 had plaques in the CCA-segment only, 90 in the CB-ICA segment only, and 7 in both segments (Table 1). In the latter 7 subjects, the plaques in the 2 segments for each subject had similar echogenicity in all cases. Overall, 55 subjects had plaques in the right side only, 44 in the left side only, and 61 in both sides. In the latter 61 subjects, the plaques in the 2 sides for each subject had similar

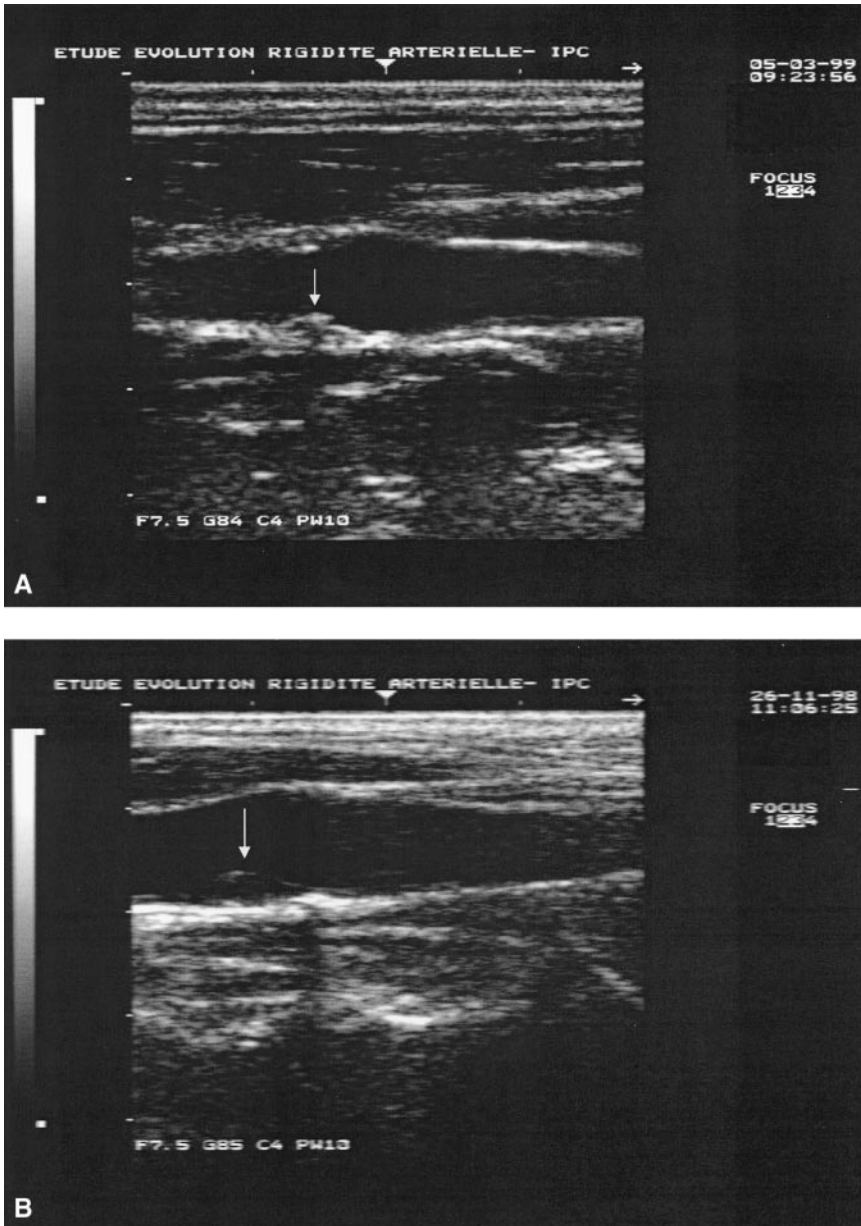


Figure 1. Ultrasound B-mode images of carotid plaques. A, Echogenic plaque; B, echolucent plaque.

echogenicity in 49 subjects and different echogenicity in 12 subjects. Since the PWV was measured in the right side, the echogenicity of the plaque located in the right side was used in the analysis for the 12 subjects with different plaque echogenicity in the left and right sides. All analyses were also repeated after the exclusion of these 12 subjects.

TABLE 1. Segment-Specific Carotid Plaques in the Studied Population

Carotid Segment	Right		Left	
	n	%	n	%
CCA segment only	11	2.0	8	1.4
CB-ICA segment only	94	16.9	90	16.1
Both segments	11	2.0	7	1.3
At least one segment	116	20.8	105	18.8

CCA indicates common carotid artery; CB-ICA, bifurcation-origin of the internal carotid artery. Differences of carotid plaque occurrence between right and left sites were not statistically significant.

The reproducibility of carotid plaque detection (presence or absence) has been reported elsewhere with a κ coefficient of 0.92 for agreement between readers.¹ For agreement on classification of plaque echogenicity, 70 consecutive images of CB-ICA segments with plaques as defined by the sonographers were reexamined off-line by a third sonographer to blindly assess the echogenicity of plaques. The κ coefficient for agreement between the 2 readings on classification of plaque echogenicity in the 2 categories was 0.69. Only the classification of plaque echogenicity assessed at the time of the ultrasound examination was used in the statistical analyses.

Intima-media thickness (IMT) was measured at a site free of any discrete plaques along a 10-mm-long segment of the far wall of the CCA, and the mean of the right and left CCA-IMT measurements was used in the analysis.

Data Analysis

Standard procedures from the Statistical Analysis System (SAS) were used for statistical analyses. The associations of PWV, used as a continuous variable, with carotid plaque (absence, echolucent, echogenic) were assessed by ANOVA for univariate analysis. ANCOVA and multiple logistic regression models were used for

TABLE 2. Population Characteristics and Pulse Wave Velocity According to Carotid Plaques

Parameters	Carotid Plaques			P (For Overall Difference)
	No (n=401)	Echolucent (n=51)	Echogenic (n=109)	
Women, %	36.9	17.7	23.9	0.002
Age, years	56.4±10.7	60.9±9.2	63.8±9.0	0.001
BMI, kg/m ²	26.5±4.0	27.1±3.9	27.5±3.9	0.03
Smoking habits, %				0.03
Never	51.9	35.3	43.1	
Ex-smokers	18.0	21.6	13.8	
Smokers	30.7	43.1	43.1	
Systolic BP, mm Hg	137.6±19.8	145.0±21.4	147.0±17.4	0.001
Diastolic BP, mm Hg	85.8±10.7	85.8±12.1	87.5±10.2	0.53
Antihypertension treatment, %	37.2	51.0	57.4	0.001
Hypertension, %	48.1	68.6	66.1	0.001
Change in hypertensive status, %				0.001
Normotensive ₁ -normotensive ₂	46.1	25.5	24.8	
Hypertensive ₁ -normotensive ₂	5.7	5.9	9.2	
Normotensive ₁ -hypertensive ₂	8.5	15.7	8.3	
Hypertensive ₁ -hypertensive ₂	39.7	52.9	57.8	
Diabetes, %	6.5	17.7	12.8	0.007
Total cholesterol, mg/dL	223.3±38.5	234.3±43.6	246.6±42.3	0.001
Antilipid treatment, %	15.3	21.6	31.8	0.001
HDL cholesterol, mg/dL	58.7±16.5	55.8±13.0	58.6±17.4	0.45
LDL cholesterol, mg/dL	141.6±37.7	155.0±38.1	162.6±43.1	0.001
Triglycerides, mg/dL	106.5±80.4	117.2±77.3	128.5±175.8	0.02*
CCA-IMT, mm	0.72±0.10	0.79±0.12	0.79±0.10	0.001
PWV, m/s	11.1±2.3	11.3±2.0	12.9±2.8	0.001

Data are mean±SD or %, as indicated. All data shown were obtained from the second examination, except changes in hypertensive status between the first and the second examinations. BMI indicates body mass index; BP, blood pressure; CCA-IMT, common carotid intima-media thickness; PWV, pulse wave velocity.

*Based on log-transformed values.

multivariate analysis. Potential confounding cardiovascular risk factors considered in the analyses were age, gender, BMI, smoking habits, systolic blood pressure, and antihypertension treatment (or hypertension), diabetes, total cholesterol and antilipid treatment, triglycerides, and CCA-IMT.

Results

Of the 561 subjects included in this study (mean age, 58.3±10.8 years; 32.6% women; 53.5% hypertensive), 51 (9.1%) had echolucent carotid plaques, 109 (19.4%) had echogenic plaques, and 401 (71.5%) had no plaques. Only 4 subjects had carotid stenosis >40% (2 echolucent and 2 echogenic carotid plaques). The mean number of sites with plaques was 1.5±1.6 in subjects with echogenic plaques and 1.5±1.7 in those with echolucent plaques ($P=0.69$). The mean thickness of plaques in the 2 groups were not statistically different ($P=0.82$). The mean thickness of echogenic plaques was 1.9±0.7 mm (25th percentile, 1.3 mm; median, 1.8 mm; 75th percentile, 2.4 mm; range, 1.1 to 5.1 mm). For echolucent plaques, the mean thickness was 2.0±0.7 mm (25th percentile, 1.4 mm; median, 1.9 mm; 75th percentile, 2.2 mm; range, 1.0 to 4.7 mm). The mean score of the severity of plaques was 2.1±1.0 in subjects with echogenic

plaques and 2.1±0.9 in those with echolucent plaques ($P=0.78$).

The main characteristics of the study population according to carotid plaques are shown in Table 2. As expected, subjects without carotid plaques had lower means of age, BMI, systolic blood pressure, total and LDL cholesterol, serum triglycerides, and CCA-IMT. They also had higher proportions of women and never-smokers and lower proportions of hypertensive and diabetic individuals. When analyses were limited to subjects with carotid plaques, those with echogenic plaques tended to be older ($P=0.05$) and to have higher mean values of total cholesterol ($P=0.05$). The other cardiovascular risk factors were not statistically different between subjects with echogenic plaques and those with echolucent plaques. In subjects with carotid plaques, the associations of age with number of sites with plaques, plaque thickness, and score of the severity were weak and not statistically significant (correlation coefficients were, respectively, 0.13, 0.11, and 0.08).

PWV was positively associated with carotid plaques (Table 2). Subjects with echogenic plaques had a higher mean PWV compared with those without plaques ($P<0.001$) and com-

TABLE 3. OR (95 % CI) for Echogenic vs Echolucent Carotid Plaques Associated With Cardiovascular Risk Factors and Pulse Wave Velocity

Parameters	Carotid Plaques: Echogenic vs Echolucent			
	Age- and Gender-Adjusted OR (95 % CI)	P	Multivariate Adjusted OR (95 % CI)	P
BMI per 4 kg/m ² increase*	1.15 (0.81–1.64)	0.45	1.06 (0.70–1.63)	0.77
Smoking habits				
Ex-smokers vs never smokers	0.63 (0.25–1.61)	0.28	0.63 (0.21–1.91)	0.24
Smokers vs never smokers	1.05 (0.46–2.32)	0.85	1.29 (0.50–3.35)	0.27
Systolic BP per 20 mm Hg increase*	1.06 (0.73–1.54)	0.75	1.05 (0.68–1.61)	0.81
Antihypertension treatment, yes vs no	1.23 (0.62–2.54)	0.56	1.27 (0.55–2.95)	0.58
Diabetes, yes vs no	0.76 (0.30–1.91)	0.55	0.51 (0.17–1.48)	0.22
Cholesterol per 40 mg/dL increase*	1.34 (0.97–1.85)	0.08	1.29 (0.89–1.87)	0.22
Antilipid treatment, yes vs no	1.83 (0.84–4.03)	0.13	1.82 (0.75–4.41)	0.19
Triglycerides per 1 log increase	1.49 (0.62–3.61)	0.37	0.94 (0.59–1.48)	0.78
CCA-IMT per 0.10 mm increase*	0.88 (0.61–1.26)	0.48	0.93 (0.63–1.38)	0.62
PWV (per 2.5 m/s increase)*	2.16 (1.33–3.50)	0.002	2.34 (1.36–4.00)	0.002

OR indicates odds ratio; CI, confidence interval; BMI, body mass index; BP, blood pressure; CCA-IMT, common carotid intima-media thickness; PWV, pulse wave velocity.

*Approximately one standard deviation.

pared with those with echolucent plaques ($P<0.01$). The means of PWV in subjects without plaques and those with echolucent plaques were similar and not statistically different ($P=0.55$). When multivariate adjustment for gender, age, BMI, smoking habits, systolic blood pressure, antihypertension treatment, diabetes, total cholesterol, antilipid treatment, serum triglycerides, and CCA-IMT was performed, these results were not markedly modified. The multivariate-adjusted mean (\pm SEM) PWV was 11.5 m/s (\pm 0.10) in subjects without plaques, 11.2 m/s (\pm 0.29) in subjects with echolucent plaques, and 12.4 m/s (\pm 0.21) in subjects with echogenic plaques (P for overall difference <0.001). In the multivariate analysis, substitution of change in hypertensive status between the 2 examinations for systolic blood pressure and antihypertension treatment did not alter the results. The multivariate-adjusted means of PWV according to carotid plaques groups were then, respectively, 11.4 m/s, 11.2 m/s, and 12.3 m/s (P for overall differences <0.001). When multivariate analyses were limited to subjects with carotid

plaques, PWV was the only factor significantly related to plaque echogenicity (Table 3). The odds ratio of echogenic plaque versus echolucent plaque associated with a 2.5-m/s increase of PWV was 2.34 [95% confidence interval, 1.36 to 4.00, $P<0.01$] (Table 3).

Figures 2 through 5 show the associations of PWV with carotid plaque echogenicity according to gender, age categories, hypertension status, and change in hypertensive status between the 2 examinations. Within each subgroup, subjects with echogenic plaques had higher mean PWV compared with those without plaques and compared with those with echolucent plaques, although statistical significance was not always reached. Similar patterns of results were also observed after the exclusion of the 12 subjects in whom plaque echogenicity was different in the left and right sides (data available from authors).

In subjects with echogenic plaques, the PWV mean was higher in those having more than one site with plaques than in those with only one site with plaques (13.6 ± 3.0 versus

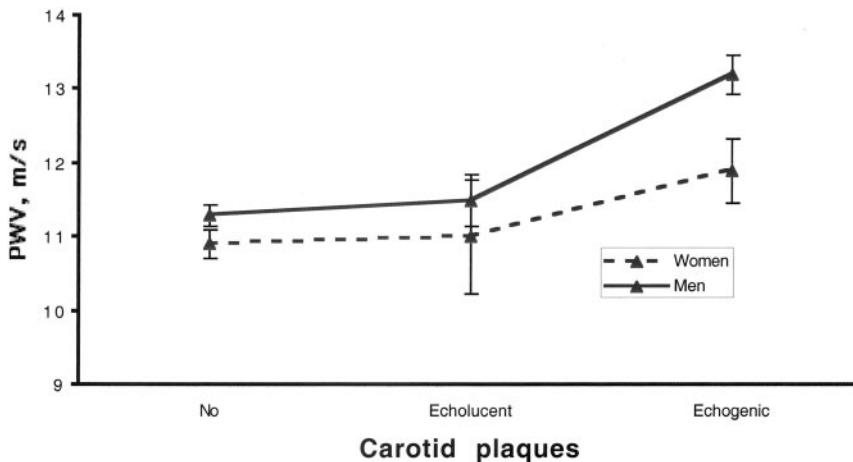


Figure 2. Pulse wave velocity and carotid plaque echogenicity in men and in women.

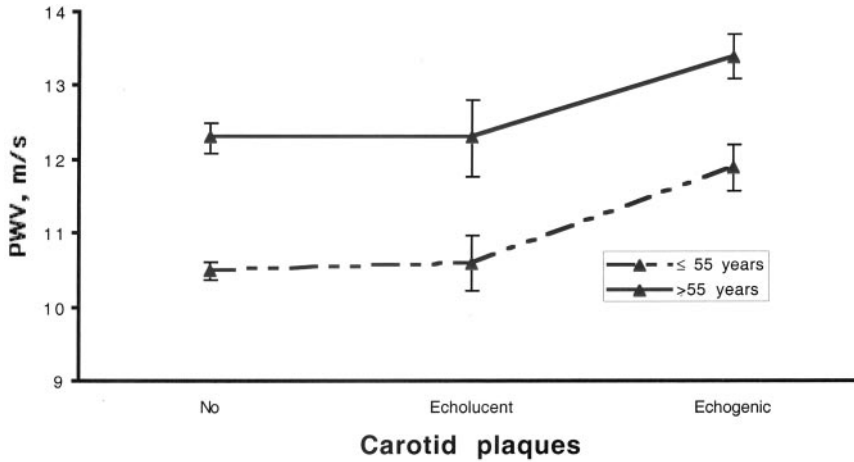


Figure 3. Pulse wave velocity carotid plaque echogenicity in subjects ≤ 55 years of age and in subjects >55 years of age.

12.4 \pm 2.7 m/s, $P < 0.03$). Similar trends were also observed according to score of the plaque severity (correlation coefficient=0.25, $P = 0.07$). In contrast, in subjects with echolucent plaques, the PWV mean was not different in those having more than one site with plaques and in those with only one site with plaques (11.5 \pm 2.5 versus 11.3 \pm 2.3 m/s, $P = 0.75$). In addition, the score of the plaque severity was not related to PWV in these subjects (correlation coefficient=0.06, $P = 0.70$).

Discussion

In this large-scale study in subjects free of coronary heart disease and stroke, we found that aortic PWV is associated with carotid echogenic plaques but not with echolucent plaques. The multivariate analyses taking into account the major conventional cardiovascular risk factors did not alter these findings. Similar patterns of results were observed in many subgroups according to age, gender, and hypertensive status.

Only one study conducted in subjects with end-stage renal disease has previously reported the relationships of arterial stiffness with plaque calcification.¹⁸ Guerin et al¹⁸ found that carotid and aortic stiffness were related to the presence and the extent of arterial calcified plaques in 3 arterial sites (carotid, aorta, femoral). One possible explanation of the associations between arterial stiffness and echogenic plaques

may that both alterations are dependent, in part, on the same systemic pathophysiological process leading to connective tissue extracellular matrix accumulation (including collagen, proteoglycans, and fibronectin elastic fibers).^{3,4} Changes in the ratio of collagen to elastin rather than deposition of lipid have been known to affect the elastic behavior and function of the arterial wall.^{19,20} Thus, one could expect that arterial stiffness would be more strongly related to plaques rich in fibrous tissue than to lipid-rich plaques. Another explanation is that the presence of calcified plaques in the arterial segment may lead to stiffen the arterial wall. Although the fact that the extent and severity of echogenic carotid plaques were gradually related to aortic PWV may tend to support this hypothesis, the cross-sectional design of our study does not allow us to determine the temporal sequence and the direction of the relationships between arterial stiffness and echogenic plaques.

An increased arterial stiffness had previously been reported in subjects with stroke, myocardial infarction, or congestive heart failure in cross-sectional studies.²¹⁻²³ In addition, aortic arterial stiffness has recently been shown to be a predictor of all-cause mortality in very old subjects²⁴ and in those with end-stage renal disease²⁵ and a predictor of cardiovascular morbidity and mortality in hypertensive subjects.^{26,27} However, all results should be interpreted with caution since they were based on a low number of events, and no population-

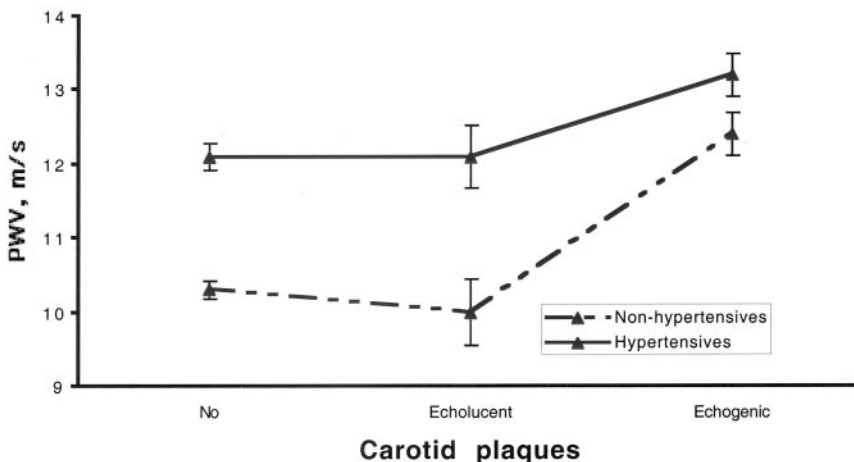


Figure 4. Pulse wave velocity and carotid plaque echogenicity in nonhypertensive and hypertensive subjects.

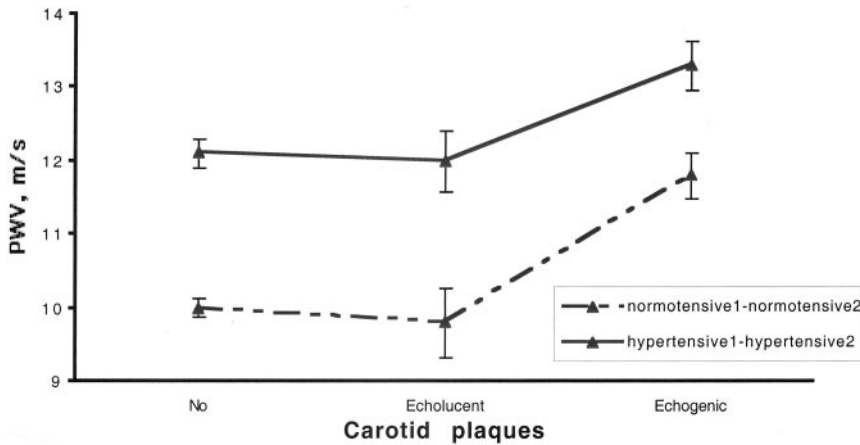


Figure 5. Pulse wave velocity and carotid plaque echogenicity according to hypertensive status during the 2 examinations.

based study has yet reported the prediction of cardiovascular events by arterial stiffness.

The importance of plaque morphology in the pathophysiology of stroke and other cerebrovascular events is now well accepted. Carotid echolucent plaques (rich in lipid) are considered to be markers of high-risk unstable plaques.¹¹ In fact, echolucency of carotid atherosclerotic plaques was associated with the presence and incidence of cerebrovascular events in symptomatic patients and asymptomatic patients with or without carotid stenosis.^{28–32} In contrast, prospective studies have failed to show that echogenic plaques are independently associated with cerebrovascular clinical events.^{30,33–35} In addition, in subjects with advanced carotid stenosis, asymptomatic patients had more echogenic plaques and patients with symptomatic cerebrovascular disease had more echolucent plaques.⁸ Taking into account the differential associations of arterial stiffness with echolucent and echogenic plaques and the fact that there is probably a differential association of these 2 sorts of plaques with vascular events, our results emphasize the need to assess the joint and interaction effects of plaque morphology and arterial stiffness on the occurrence of cardiovascular events.

In a recent report of this study, we reported that gender, age, and hypertension were the strongest determinants of arterial stiffness.¹ These results confirmed those of many other studies on this topic.^{36–37} In addition, major known cardiovascular risk factors were related to the presence of carotid plaques (echolucent or echogenic). Subjects with carotid echolucent plaques had lower mean of age compared with those with echogenic plaques, in accordance with the results of the Tromso Study.³⁸ This may be due to earlier-in-life constitution of echolucent plaques and/or to a survival selection bias causing overrepresentation of subjects with echogenic plaques. Although total cholesterol was higher in subjects with echogenic plaques in univariate analysis, this difference disappeared as soon as age and gender were taken into account in the statistical analysis (Table 3). The lack of associations between echolucent plaques with low HDL cholesterol and/or high triglycerides in our study does not support the results of previous investigations.^{39–41} An explanation of this discordance is that these studies have used color Doppler imaging to detect echolucent plaque.^{39–41} It is possible that these studies compared with ours may have

more sensitivity for the assessment of echolucent plaques. Furthermore, these latter investigations were conducted in subjects with carotid stenosis.^{39–41} Distinct pathological processes and risk factors of early and advanced atherosclerosis have been shown, and this may provide another explanation of the discrepancy of the observed results.⁴²

Several limits to our study should be noted. Because subjects were free of diagnosed cardiovascular diseases (angina, myocardial infarction, or stroke), our results could not be generalized to diseased subjects. We used carotid-femoral PWV as a marker of aortic stiffness. The carotid-femoral PWV determined from foot-to-foot transit time in the aorta (which eliminates the influence of wave reflections) provides an easy, safe, and reproducible method of assessing the aortic arterial stiffness.^{12,43} Our subjects had relatively minor degrees of carotid atherosclerosis, and few subjects had carotid stenosis. In the literature, plaque morphology was usually assessed in subjects with carotid stenosis. However, other epidemiological studies including the Cardiovascular Health Study have reported, with an acceptable reproducibility, the echogenicity of less severe plaques.^{30,38} One could argue that echogenic plaques are more easily assessed by B-mode ultrasound methods than echolucent plaques and that only severe echolucent plaques might have been detected. However, we do not think that this was markedly the case here. The ultrasound examinations have been performed by 2 experienced sonographers (5 and 18 years of experience), and the ratio of subjects with echolucent plaques to subjects with echogenic plaques (approximately 1:2) was globally comparable to those reported in the literature.^{30,38} Furthermore, the number of sites with plaques, plaque thickness, and plaque severity score were not different between subjects with echogenic plaques and those with echolucent plaques. In addition, both echolucent and echogenic plaques were related to major conventional cardiovascular risk factors (Table 2).

A consensus on ultrasonographic criteria for morphological characterization of carotid plaques has been previously published.⁴⁴ For plaque morphology assessment, we used the Gray-Weale classification,¹⁷ which is slightly different from the classification recommended in this consensus (4 grades instead of 5). We have paid particular attention to standardize echogenicity classification against defined and well-recognized reference structures located adjacent to the

plaque. In our study, the 2 ultrasound reference structures used as extremes on a gray scale were the vessel lumen on the one side and the media-adventitia interface on the other. These 2 reference structures are close to plaque and thus easier to be included in the same projection with the most echocally informative plaque and probably prone to lower interobserver variability.⁴⁵ The lack of adequate reference structures is probably a major source of error in studies evaluating ultrasound morphology.⁴⁵ In the present study, we obtained an acceptable reproducibility on the classification of carotid plaque morphology, in agreement with the results of other studies using the Gray-Weale classification.^{46,47} However, computer-assisted ultrasound quantitative evaluation of plaque morphology may probably improve the ultrasound assessment of plaque echogenicity.^{32,48}

Perspectives

This study provides the first evidence that aortic PWV was, independent of conventional cardiovascular risk factors, associated with carotid atherosclerotic echogenic plaques but not with echolucent plaques. Our study shows that the 2 types of plaque lesions share many risk factors, but some factors could be specifically associated with echogenic plaques alone or with echolucent plaques alone. Our results thus reinforce the concept that echogenic and echolucent plaques should be differentiated from each other and analyzed separately. Identification of other specific factors related to echogenic and echolucent plaques are necessary and may lead to a better understanding of the pathophysiology of atherosclerosis and vascular diseases. Recently, arterial stiffness and plaque echogenicity have been shown to predict clinical end points. The differential associations of PWV with echolucent and echogenic plaques observed in our study emphasize the need to assess the joint and interaction effects of plaque morphology and arterial stiffness on the occurrence of cardiovascular events. This would permit a better identification of cardiovascular high-risk subjects.

Acknowledgments

This study was performed with the help of the Fondation de France. We also thank the Caisse Nationale d'Assurance Maladie for supporting this work. We thank Pierre Iaria, MD, for performing the rereading study of plaque echogenicity.

References

- Zureik M, Temmar M, Adamopoulos C, Bureau JM, Courbon D, Thomas F, Bean K, Touboul PJ, Ducimetiere P, Benetos A. Carotid plaques, but not common carotid intima-media thickness, are independently associated with aortic stiffness. *J Hypertens*. 2002;20:85-93.
- van Poele NM N, Grobbee DE, Bots ML, Asmar R, Topouchian J, Reneman RS, Hoeks AP, van Der Kuip DA D, Hofman A, Witteman JC. Association between arterial stiffness and atherosclerosis: the Rotterdam Study. *Stroke*. 2001;32:454-460.
- Stary HC. Evolution and progression of atherosclerotic lesions in coronary arteries of children and young adults. *Atherosclerosis*. 1989;9(suppl 1):I-19-I-32.
- Stary HC, Chandler AB, Dinsmore RE, Fuster V, Glagov S, Insull W Jr, Rosenfeld ME, Schwartz CJ, Wagner WD, Wissler RW. A definition of advanced types of atherosclerotic lesions and a histological classification of atherosclerosis: a report from the Committee on Vascular Lesions of the Council on Arteriosclerosis, American Heart Association. *Circulation*. 1995;92:1355-1374.
- Wolverson MK, Bashiti HM, Peterson GJ. Ultrasonic tissue characterization of atheromatous plaques using a high resolution real time scanner. *Ultrasound Med Biol*. 1983;9:599-609.
- O'Donnell TF, Erdoes L, Mackey WC, McCullough J, Shepard A, Heggerick P, Isner J, Callow AD. Correlation of B-mode ultrasound image and arteriography with pathologic findings at carotid endarterectomy. *Arch Surg*. 1985;120:443-449.
- Feeley TM, Leen EJ, Colgan MP, Moore DJ, Hourihane DO, Shanik GD. Histologic characteristics of carotid artery plaque. *J Vasc Surg*. 1991;13:719-724.
- European Carotid Plaque Study Group. Carotid artery plaque composition: relationship to clinical presentation and ultrasound B-mode imaging. *Eur J Vasc Endovasc Surg*. 1995;10:23-30.
- El-Barghouty NM, Levine T, Ladva S, Flanagan A, Nicolaidis A. Histological verification of computerised carotid plaque characterisation. *Eur J Vasc Endovasc Surg*. 1996;11:414-416.
- Gronholdt M-LM, Wiebe BM, Laursen H, Nielsen TG, Schroeder TV, Sillesen H. Lipid-rich carotid artery plaques appear echolucent on ultrasound B-mode images and may be associated with intraplaque haemorrhage. *Eur J Vasc Surg*. 1997;14:439-445.
- Gronholdt ML. Ultrasound and lipoproteins as predictors of lipid-rich, rupture-prone plaques in the carotid artery. *Arterioscler Thromb Vasc Biol*. 1999;19:2-13.
- Asmar R, Benetos A, Topouchian J, Laurent P, Pannier B, Brisac AM, Target R, Levy BI. Assessment of arterial distensibility by automatic pulse wave velocity measurement: validation and clinical application studies. *Hypertension*. 1995;26:485-490.
- Touboul PJ, Prati P, Scarabin PY, Adrai V, Thibout E, Ducimetiere P. Use of monitoring software to improve the measurement of carotid wall thickness by B-mode imaging. *J Hypertens*. 1992;10(suppl 5):S37-S41.
- Zureik M, Ducimetiere P, Touboul PJ, Courbon D, Bonithon-Kopp C, Berr C, Magne C. Common carotid intima-media thickness predicts occurrence of carotid atherosclerotic plaques: longitudinal results from the Aging Vascular Study (EVA) study. *Arterioscler Thromb Vasc Biol*. 2000;20:1622-1629.
- Zureik M, Kauffmann F, Touboul PJ, Courbon D, Ducimetiere P. Association between peak expiratory flow and the development of carotid atherosclerotic plaques. *Arch Intern Med*. 2001;161:1669-1676.
- Zureik M, Robert L, Courbon D, Touboul PJ, Bizbiz L, Ducimetiere P. Serum elastase activity, serum elastase inhibitors, and occurrence of carotid atherosclerotic plaques. *Circulation*. 2002;105:2638-2645.
- Gray-Weale AC, Graham JC, Burnett JR, Byrne K, Lusby RJ. Carotid artery atheroma: comparison of preoperative B-mode ultrasound appearance with carotid endarterectomy specimen pathology. *J Cardiovasc Surg*. 1988;29:676-681.
- Guerin AP, London GM, Marchais SJ, Metivier F. Arterial stiffening and vascular calcifications in end-stage renal disease. *Nephrol Dial Transplant*. 2000;15:1014-1021.
- Safar ME, London GM, Asmar R, Frohlich ED. Recent advances on large arteries in hypertension. *Hypertension*. 1998;32:156-161.
- Intengan HD, Schiffrin EL. Vascular remodeling in hypertension: roles of apoptosis, inflammation, and fibrosis. *Hypertension*. 2001;38:581-587.
- Lehmann ED, Hopkins KD, Jones RL, Rudd AG, Gosling RG. Aortic distensibility in patients with cerebrovascular disease. *Clin Sci*. 1995;89:247-253.
- Hiari T, Sasayama S, Kawasaki T, Yagi SI. Stiffness of systemic arteries in patients with myocardial infarction. *Circulation*. 1989;80:78-86.
- Mitchell GF, Tardif JC, Arnold JM, Marchiori G, O'Brien TX, Dunlap ME, Pfeffer MA. Pulsatile hemodynamics in congestive heart failure. *Hypertension*. 2001;38:1433-1439.
- Meaume S, Benetos A, Henry OF, Rudnicki A, Safar ME. Aortic pulse wave velocity predicts cardiovascular mortality in subjects >70 years of age. *Arterioscler Thromb Vasc Biol*. 2001;21:2046-2050.
- 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.
- Boutouyrie P, Tropeano AI, Asmar R, Gautier I, Benetos A, Lacolley P, Laurent S. Aortic stiffness is an independent predictor of primary coronary events in hypertensive patients: a longitudinal study. *Hypertension*. 2002;39:10-15.
- Laurent S, Boutouyrie P, Asmar R, Gautier I, Laloux B, Guize L, Ducimetiere P, Benetos A. Aortic stiffness is an independent predictor of all-cause mortality in hypertensive patients. *Hypertension*. 2000;37:1236-1241.

28. Belcaro G, Laurora G, Cesarone MR, De Sanctis MT, Incandela L, Fascetti E, Geroulakos G, Ramaswami G, Pierangeli A, Nicolaides AN. Ultrasonic classification of carotid plaques causing less than 60% stenosis according to ultrasound morphology and events. *J Cardiovasc Surg.* 1993;34:287–294.
29. Iannuzzi A, Wilcosky T, Mercuri M, Rubba P, Bryan FA, Bond MG. Ultrasonic correlates of carotid atherosclerosis in transient ischemic attack and stroke. *Stroke.* 1995;26:614–619.
30. Polak JF, Shemanski L, O'Leary DH, Lefkowitz D, Price TR, Savage PJ, Brant WE, Reid C. Hypochoic plaque at US of the carotid artery: an independent risk factor for incident stroke in adults aged 65 years or older: Cardiovascular Health Study. *Radiology.* 1998;208:649–654.
31. Mathiesen EB, Bønaa KH, Joakimsen O. Echolucent plaques are associated with high risk of ischemic cerebrovascular events in carotid stenosis: the Tromsø Study. *Circulation.* 2001;103:2171–2175.
32. Gronholdt ML, Nordestgaard BG, Schroeder TV, Vorstrup S, Sillesen H. Ultrasonic echolucent carotid plaques predict future strokes. *Circulation.* 2001;104:68–73.
33. Johnson JM, Kennelly MM, Decesare D, Morgan S, Sparrow A. Natural history of asymptomatic carotid plaque. *Arch Surg.* 1985;120:1010–1012.
34. O'Holleran LW, Kennelly MM, McClurken M, Johnson JM. Natural history of asymptomatic carotid plaque. *Am J Surg.* 1987;154:659–661.
35. Bock RW, Gray-Weale AC, Mock PA, Robinson DA, Irwig L, Lusby RJ. The natural history of asymptomatic carotid artery disease. *J Vasc Surg.* 1993;17:160–169.
36. Benetos A, Laurent S, Hoeks AP, Boutouyrie PH, Safar ME. Arterial alterations with aging and high blood pressure: a noninvasive study of carotid and femoral arteries. *Arterioscler Thromb.* 1993;13:90–97.
37. Schmidt-Trucksass A, Grathwohl D, Schmid A, Boragk R, Upmeier C, Keul J, Huonker M. Structural, functional, and hemodynamic changes of the common carotid artery with age in male subjects. *Arterioscler Thromb Vasc Biol.* 1999;19:1091–1097.
38. Joakimsen O, Bonna KH, Stensland-Bugge E, Jacobsen BK. Age, and sex differences in the distribution and ultrasound morphology of carotid atherosclerosis: the Tromsø Study. *Arterioscler Thromb Vasc Biol.* 1999;19:3007–3013.
39. Gronholdt ML, Nordestgaard BG, Nielsen TG, Sillesen H. Echolucent carotid artery plaques are associated with elevated levels of fasting and postprandial triglyceride-rich lipoproteins. *Stroke.* 1996;27:2166–2172.
40. Gronholdt ML, Nordestgaard BG, Wiebe BM, Wilhelm JE, Sillesen H. Echo-lucency of computerized ultrasound images of carotid atherosclerotic plaques are associated with increased levels of triglyceride-rich lipoproteins as well as increased plaque lipid content. *Circulation.* 1998;97:34–40.
41. Mathiesen EB, Bonna KH, Joakimsen O. Low levels of high-density lipoprotein cholesterol are associated with echolucent carotid artery plaques: the Tromsø study. *Stroke.* 2001;32:1960–1965.
42. Willeit J, Kiechl S, Oberhollenzer F, Rungger G, Egger G, Bonora E, Mitterer M, Muggeo M. Distinct risk profiles of early and advanced atherosclerosis: prospective results from the Bruneck Study. *Arterioscler Thromb Vasc Biol.* 2000;20:529–537.
43. Asmar RG, Topouchian JA, Benetos A, Sayegh FA, Mourad JJ, Safar ME. Non-invasive evaluation of arterial abnormalities in hypertensive patients. *J Hypertens.* 1997;15(suppl 2):S99–S107.
44. De Bray JM, Baud JM, Dauzat M. Consensus concerning the morphology and the risk of carotid plaques. *Cerebrovasc Dis.* 1997;7:289–296.
45. Sabetai MM, Tegos TJ, Nicolaides AN, Dhanjil S, Pare GJ, Stevens JM. Reproducibility of computer-quantified carotid plaque echogenicity: can we overcome the subjectivity? *Stroke.* 2000;31:2189–2196.
46. Joakimsen O, Bonna KH, Stensland-Bugge E. Reproducibility of ultrasound assessment of carotid plaque occurrence, thickness, and morphology: the Tromsø Study. *Stroke.* 1997;28:2201–2207.
47. Geroulakos G, Ramaswami G, Nicolaides A, James K, Labropoulos N, Belcaro G, Holloway M. Characterization of symptomatic and asymptomatic carotid plaques using high-resolution real-time ultrasonography. *Br J Surg.* 1993;80:1274–1277.
48. El-Barghouty N, Geroulakos G, Nicolaides A, Androulakis A, Bahal V. Computer-assisted carotid plaque characterization. *Eur J Vasc Endovasc Surg.* 1995;9:389–393.