Risk Factors for Early Carotid Atherosclerosis in Middle-Aged French Women

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The prevalence of carotid atherosclerosis and of its risk factors was examined in 517 apparently healthy French women, aged 45-54 years. Early phases of carotid atherosclerosis were assessed by B-mode ultrasonography. An intimal-medial thickening was found in 30.4% of the women and atheromatous plaques in 8.7%. The prevalence rate of carotid atherosclerosis increased with age, smoking, and postmenopausal status. However, after adjustment for the effect of age, postmenopausal women did not have more atherosclerotic lesions than did premenopausal women. No significant associations were found between carotid atherosclerosis and triglyceride, apolipoprotein A-I, body mass index, blood glucose, fibrinogen, plasma viscosity, or hematocrit. The mean age-adjusted levels of total cholesterol, low density lipoprotein cholesterol, apolipoprotein B, and systolic and diastolic blood pressures significantly increased with the severity of carotid atherosclerosis, whereas high density lipoprotein cholesterol significantly decreased. Multiple regression analysis showed that age, smoking, high density lipoprotein cholesterol, low density lipoprotein cholesterol (or apolipoprotein B), and systolic (or diastolic) blood pressure were significantly and independently related to the severity of carotid atherosclerosis. In conclusion, the association of early carotid lesions with major cardiovascular risk factors suggests that carotid atherosclerosis may be used as a marker of the general atherosclerotic process. (Arteriosclerosis and Thrombosis 1991;11:966-972)

Although risk factors for stroke have been extensively investigated, relatively few studies have related risk factors to atherosclerosis of the extracranial and/or intracranial arterial system. Some of these studies are autopsy studies whose results, obtained for populations that were not randomly selected, may be difficult to generalize. Most other studies are angiographic or ultrasound studies of patients or other highly selected populations, providing information about quite advanced atherosclerotic lesions of the intracranial and/or extracranial arteries. In addition, few studies included women, and generally the small sample size of these studies does not allow examination of the specific risk factors for atherosclerosis in women. The recent availability of noninvasive procedures such as high-resolution B-mode ultrasonography allows the detection of minimal lesions of the arterial wall without hemodynamic disturbances in subjects from the general population. The investigation of the risk factors for early atherosclerosis may lead to a better understanding of the pathogenesis of atherosclerosis and may provide new insight on strategies for early prevention of atherosclerosis in the population. Thus, the present study examines the risk factors for early carotid atherosclerosis assessed by high-resolution B-mode ultrasonography in a population of apparently healthy middle-aged French women.

Methods

Population

The study population consisted of apparently healthy Caucasian women who volunteered for a standard health checkup. Under the French Social Security System, all members are entitled to a free checkup every 5 years. Women who requested a checkup at the Centre d’Investigations Pré-Cliniques de Paris were invited to participate in the study if they were aged 45-54 years, born in France, and gave an informed consent to further clinical and biologic investigations. The proportion of women who refused to participate was very low (=5%). During their visit, history of coronary heart disease and diabetes, smok-
ing habits, sex hormone use, and menopausal status were obtained by standardized self-administered questionnaire. Women were classified as smokers if they smoked at least one cigarette per day and as nonsmokers if they had never smoked or had stopped smoking at the time of the examination. They were considered premenopausal if they had menstruated within the last 3 months. They were considered postmenopausal if their menopause was surgically induced (bilateral oophorectomy and/or hysterectomy) or if the menses had ceased naturally for at least 6 months before the examination. Blood pressure was measured with a sphygmomanometer in the supine position after a 10-minute rest. The body mass index was computed as weight (kg) divided by height squared (m²).

**Ultrasonographic Evaluation of the Carotid Arteries**

The ultrasonographic evaluation of the carotid arteries was made in 517 women free of coronary heart disease and diabetes who were consecutively recruited between January 1988 and November 1989. B-mode ultrasound imaging with 7.5-MHz transducers having an axial resolution of 0.5 mm was performed by a single trained physician. Subjects were examined in the supine position. The study protocol entailed scanning the carotid bifurcations and the near and far walls of the right and left common carotid arteries (CCAs) in their mid and distal portions. The carotid bifurcation was defined on a longitudinal B-mode scan as the intermediate portion between the distal part of the CCA and the origins of the internal carotid artery (ICA) and the external carotid artery (ECA). The increasing diameter of the distal part of the CCA served as a landmark for the proximal limit of the carotid bifurcation. Lines drawn through the tip of the flow divider perpendicular to the axes of both the ICA and the ECA served as distal limits of the carotid bifurcation. All measurements were made at the time of scanning with the instrument's electronic calipers to the nearest 0.25 mm. Two types of lesions were noted by the physician: intimal-medial thickening and atheromatous plaque.

**Measurement of intimal–medial thickening.** The intimal–medial thickness was measured on a longitudinal B-mode scan of the mid CCA. Only the far wall was considered. The midportion of the CCA was chosen because of its parallelism with the axis of the neck. Its B-mode scan pattern is characterized by two echogenic lines separated by a hypoechoic or anechoic space. The outer line corresponds to the medial–adventitial interface, and the inner line corresponds to the luminal–intimal interface. Thus, the distance between the two parallel lines represents the intimal–medial thickness. Previous studies showed that the mean intimal–medial thickness of the CCA assessed by B-mode ultrasonography in normal subjects was about 0.50 mm. The distribution of intimal–medial thickness in these studies led us to consider the arterial wall thickness as abnormal if it was greater than or equal to 0.75 mm. Because curved interfaces do not reflect the ultrasound perpendicularly, the ultrasonographable interfaces are less sharp in the carotid bifurcation. Thus, no attempt was made to measure the intimal–medial thickening at this site.

**Measurement of plaque thickness.** The mid CCA, distal CCA, and carotid bifurcation of both sides were scanned. Measurement of plaque thickness was generally made on longitudinal B-mode scans. Transverse B-mode scans were used only if they provided a better visualization of plaque than did the longitudinal B-mode scans (plaques of the anterior wall). A localized echo structure encroaching into the vessel lumen was considered to be a plaque if the distance between the medial–adventitial interface and the internal side of the lesion was greater than or equal to 1.75 mm. In view of the characteristics of our B-mode system, we chose a cut point of 1.75 mm to avoid any confusion with an intimal–medial thickening, especially in the carotid bifurcation.

**Categories of carotid atherosclerosis.** As shown in Table 1, the severity of carotid atherosclerosis was graded into three categories: normal, intimal–medial thickening (when no other lesion was noted in the CCA and the carotid bifurcation), and plaque. The interobserver reproducibility of the severity of carotid atherosclerosis was assessed in 24 subjects who were hospitalized either for vascular or orthopedic rehabilitation. None had any history or symptoms of cerebrovascular disease. The patients were scanned by two independent observers 1 day apart (one of these observers also performed the B-mode scans in the population study). A single B-mode scan reading was made by each observer at the time of scanning. κ Statistics were used to assess the interobserver reproducibility. The κ coefficient was 0.62, indicating that agreement between the two observers was substantial (95% confidence interval, 0.35–0.89; p<0.001).

**Biologic Measurements**

Blood samples were drawn from each subject between 9 AM and 12 PM. Total cholesterol, triglyceride,
and glucose were assayed enzymatically on a Hitachi 737 Analyzer (Boehringer Mannheim GmbH, Mannheim, FRG). High density lipoprotein (HDL) cholesterol was measured enzymatically after precipitation with phosphotungstic acid and Mg\textsuperscript{2+} ions, a method selected by the lipids–lipoproteins commission of the French Society of Clinical Biology.\textsuperscript{20} Low density lipoprotein (LDL) cholesterol was computed with the Friedewald formula.\textsuperscript{21} Apolipoproteins (apos) A-I and B were assayed with an immunonephelometric fixed-time method on a Behring nephelometric analyzer (Behringwerke AG, Marburg, FRG) with Behring antisera and standards. Citrated platelet-poor plasma was used to measure plasma fibrinogen according to the method of von Clauss.\textsuperscript{22} Plasma viscosity was measured in duplicate by the Myrenne capillary viscosimeter. Most determinations were performed daily. Plasma samples for determinations of apolipoproteins were stored at 4°C until assay within a week, and those for determinations of plasma viscosity were stored at -180°C until assay within 2 weeks. The majority of women had fasted, but 15% of them had taken a light continental breakfast (without milk, butter, and sugar) before blood collection. No significant differences in blood glucose or blood lipids were seen between fasting and nonfasting blood samples except for HDL cholesterol, which was slightly increased in nonfasting women. However, as the inclusion of these women did not alter the relations between the biologic variables and carotid atherosclerosis, statistical analysis was performed on data from the whole population.

**Statistical Analysis**

Standard procedures from Statistical Analysis Systems (SAS, Cary, N.C.) were used for univariate and multivariate analyses. Mean age-adjusted levels of cardiovascular risk factors according to the three classes of carotid atherosclerosis were obtained by analysis of covariance and significance tests from logistic regression. The independent associations between the severity of atherosclerosis and the cardiovascular risk factors were tested with multiple logistic regressions in which a three-class severity of carotid atherosclerosis was introduced as a dependent variable and the cardiovascular risk factors were introduced as independent variables (either quantitative or dichotomous variables). Probability values lower than 0.05 were considered significant.

**Results**

The prevalence rates for intimal–medial thickening and plaques in women aged 45–54 were 30.4% and 8.7%, respectively. Only 60.9% of the women were free of any carotid lesions. Ninety-five percent of the atheromatous plaques were located at the carotid bifurcations and only 4.5% at the CCAs. Fifteen percent of the women with plaques had bilateral atheromatous lesions. As shown in Figure 1, the prevalence rate of all types of carotid lesions significantly increased with age; two thirds of the women had no carotid lesions in the 45–49-year age group, whereas it was the case for only half the women in the 50–54-year age group. The significant positive association between smoking and the severity of atherosclerosis is shown in Figure 2. After controlling for the effect of age (as a continuous variable) in multiple logistic regression, this association became highly significant ($p<0.004$). The prevalence rate for carotid lesions was also significantly higher in postmenopausal women than in premenopausal women (see Figure 3). However, the association between menopausal status and the severity of atherosclerosis was no longer significant after adjustment for age. Similar results were found after exclusion of women who had received sex hormones.
significant associations were observed with logarithmically transformed triglyceride, apo A-I, blood glucose, body mass index, fibrinogen, plasma viscosity, and hematocrit values. Table 2 shows the mean age-adjusted levels of the major cardiovascular risk factors according to the severity of carotid atherosclerosis. Total cholesterol, LDL cholesterol, apo B, and systolic and diastolic blood pressures significantly increased after adjustment for age, whereas HDL cholesterol significantly decreased with the severity of carotid atherosclerosis. The decrease in apo A-I in women with atherosclerotic lesions was only of borderline significance after adjustment for age, whereas the rise in fibrinogen in these women did not reach the significance level. The independent associations between carotid atherosclerosis and age, systolic blood pressure, smoking, HDL cholesterol, or LDL cholesterol were examined in a five-variable logistic model presented in Table 3. All variables independently contributed to the prediction of the severity of carotid atherosclerosis. The strongest predictors were age and systolic blood pressure. Similar results were obtained when apo A-I and apo B were introduced in the regression model instead of HDL cholesterol and LDL cholesterol (\( p < 0.077 \) for apo A-I, \( p < 0.022 \) for apo B). The substitution of diastolic blood pressure (\( p < 0.0011 \)) for systolic blood pressure did not change the results.

**Discussion**

The prevalence of carotid atherosclerosis is not well documented in the general population, particularly in women. Until noninvasive procedures were developed, ethical considerations prevented any investigation of the carotid arteries in healthy subjects. Most of the previous studies were performed either in subjects with symptoms of cerebral ischemia or other vascular disease or in subjects at high risk for ischemic arterial disease. Moreover, they used different methods to assess carotid atherosclerotic lesions, which makes comparisons between them difficult.
cult. The prevalence rates and the clinical and biologic determinants of carotid atherosclerosis assessed by B-mode ultrasonography have not yet been investigated in healthy women.

The prevalence rates of early carotid lesions were relatively high in these apparently healthy women aged 45–54. Because the study subjects were recruited among volunteers, the generalization of our results to other female populations should be applied with caution. Although the participation rate was very high, we cannot exclude a self-selection bias leading to an overrepresentation of women at high risk of cardiovascular disease. On the other hand, women who voluntarily have a health checkup are known to have a high socioeconomic status and to pay great attention to their health; therefore, an underrepresentation of high-risk women is also possible. However, the percentage of women with atheromatous plaques of the carotid arteries (8.7%) was comparable to that found in another study. Among 66 women of the same age range referred to a neurological clinic in Paris for a general checkup or symptoms usually unrelated to cerebrovascular disease, 13.6% presented minimal plaques with a less than 15% diameter reduction at the ultrasound examination that included continuous-wave Doppler and duplex scanning. Women with intimal–medial thickening were considered to have normal carotid arteries. None had stenosis of 15–50% diameter reduction, which is not surprising given the small sample size.

The present study shows that the major coronary risk factors, that is, age, blood pressure, cigarette smoking, and blood lipid and lipoprotein levels, are independently associated with early phases of carotid atherosclerosis. Women with intimal–medial thickening also have a higher risk profile than do women without any lesions. Although risk factors for symptomatic cerebrovascular disease have been extensively investigated, relatively few studies have related these risk factors to carotid atherosclerosis in populations including women. These studies largely differ in the type of population and methods used to assess carotid atherosclerosis, which makes comparisons difficult. Angiography can be used only in highly selected populations, whereas ultrasonography allows the study of healthy subjects as well as symptomatic patients. Whereas angiography and pulse-wave Doppler sonography measure arterial stenosis, B-mode ultrasonography measures the arterial wall thickness and thus is capable of detecting early atheromatous lesions before they induce disturbed flow patterns.

Age and hypertension are the two factors that have been most consistently associated with stroke and carotid atherosclerosis. High blood pressure (or hypertension) was found to be a risk factor for advanced carotid atherosclerosis in patients with symptomatic cerebrovascular disease examined either with angiography or with B-mode ultrasonography. It was also found in neurologically asymptomatic patients hospitalized for coronary angiography examined with B-mode ultrasonography. It has also been reported that high systolic and diastolic blood pressures were significantly associated with the progression of angiographic atherosclerotic lesions in the carotid arteries. However, two ultrasonographic studies performed with hypertensive subjects failed to find any association between blood pressure and carotid atherosclerosis. These negative findings may be partly explained by the small variation of blood pressure in hypertensive populations. However, the severity of carotid atherosclerosis assessed with B-mode ultrasonography was not related to blood pressure levels or duration of hypertension in a randomly selected population sample of Finnish men with a wide range of blood pressure. In contrast with this latter study, the present work suggests that, at least in women, increased blood pressure is a strong predictor of early carotid atherosclerosis. It is interesting to note that the increase in systolic or diastolic blood pressure was still apparent in women with intimal–medial thickening. The significance of this finding is questionable. Whether increased blood pressure is a cause or a consequence of the thickening of the arterial wall cannot be determined from this cross-sectional study.

The relation of cigarette smoking with the risk of stroke is not well established. However, a recent meta-analysis suggested that there was a small increased risk in smokers and ex-smokers compared with that for nonsmokers. There is also some evidence that cigarette smoking is positively associated with carotid atherosclerosis in patients with symptomatic ischemic arterial disease, as based on both angiographic and ultrasonographic studies. In accordance with our results, smoking is suggested to be an independent risk factor for carotid atherosclerosis in healthy populations examined either with B-mode or duplex ultrasonography. Smoking seems to be associated not only with the presence or the severity of atheromatous plaques but also with an increase in the intimal–medial thickness of the carotid arteries and with the progression of the intimal–medial thickening.

Although there is no clear evidence that blood lipid levels are risk factors for clinical manifestations of cerebral ischemia, studies linking total cholesterol and/or lipoproteins to carotid atherosclerosis have yielded more consistent results, as recently reviewed. Most studies performed in men and women with symptomatic cerebrovascular disease or in asymptomatic patients at high risk for vascular disease showed an association between an adverse lipid profile and the prevalence or the severity of carotid atherosclerosis assessed by angiography or ultrasonography. One study, however, failed to find any differences in plasma lipids and lipoproteins in patients with angiographically documented carotid atherosclerosis compared with those without atherosclerotic lesions. Only two ultrasonographic studies have examined the relation between plasma lipid
concentration and carotid atherosclerosis in apparently healthy male populations. One of them found that men with carotid stenosis measured by multigate pulsed Doppler had lower HDL to total cholesterol ratios than did men without carotid stenosis.13 The other study showed that LDL cholesterol but not HDL cholesterol was significantly associated with the degree of carotid atherosclerosis assessed by B-mode ultrasonography.27 Our results indicate that, at least in women, both HDL and LDL cholesterol are independent risk factors for early carotid atherosclerosis but are less powerful predictors than other risk factors such as age, smoking, or blood pressure. Although apolipoproteins have been reported to be more strongly associated with coronary atherosclerosis than are lipoproteins,32 few data are available with respect to carotid atherosclerosis. One study of 30 men and women with familial hypercholesterolemia showed that the severity of carotid stenosis assessed by duplex ultrasonography was positively associated with LDL cholesterol and apo B and negatively with HDL cholesterol and apo A.8 Our study suggests that apolipoproteins and lipoproteins show a similar pattern of associations with the carotid lesions. Apolipoproteins do not seem to improve the prediction of carotid atherosclerosis.

The present study failed to find any significant associations between the severity of carotid atherosclerosis and body mass index, triglyceride, blood glucose, hemorheological parameters, and menopausal status. Fibrinogen is a strong predictor of myocardial infarction and stroke in men.33-36 and it has been reported to be a risk factor for myocardial infarction but not for stroke in women.36 Its relations with carotid atherosclerosis are somewhat conflicting. In one angiographic study of subjects with symptomatic cardiovascular disease, fibrinogen was associated with the presence of atheroma at the carotid bifurcation but not with its progression within 2 years.13 On the other hand, fibrinogen was no longer significantly related to B-mode carotid lesions in healthy Finnish men after adjustment for the other cardiovascular risk factors.27

The role of the menopause on the incidence of cardiovascular disease is debated.37,38 A better knowledge of the menopausal effects on the atherosclerotic process may help clarify the situation. Our study suggests that menopause has little influence on the development of carotid atherosclerotic lesions. However, menopause may act as a promoting factor for atherosclerotic lesions in other arterial sites. It has been reported that aortic atherosclerosis diagnosed by radiographic detection of calcified deposits in the abdominal aorta is more common in postmenopausal women, even after adjustment for other cardiovascular risk factors.39 It is clear that further ultrasonographic studies are needed to ascertain the menopausal effects on early atherosclerosis in various arterial sites.

Our failure to find any significant association between carotid atherosclerosis and menopausal status or some biologic risk factors may also be due to a lack of statistical power. There is, especially, some variability in assessing atherosclerotic lesions with B-mode ultrasonography. In the present study the interobserver reproducibility of the severity of carotid atherosclerosis (in three classes) assessed in a small group of patients was 0.62. Sources of variability also exist in the interpretation of the B-scans.40 A previous study reported that the between-reader agreement ranged from 0.34 for the ECA to 0.65 for the ICA, and the within-reader agreement ranged from 0.64 for the ECA to 0.73 for the ICA. In a few cases, our B-mode scans might have been of poor quality either because of anatomic characteristics or because of obesity. Thus, it is likely that misclassifications have occurred in some instances, resulting in an underestimation of the actual relation of the severity of atherosclerosis to risk factor levels.

Despite these limitations, B-mode ultrasonography represents a unique tool to detect minimal changes of the arterial wall in population studies. Until now, little has been known about the anatomic basis and the clinical relevance of an intimal-medial thickening. An increase in the arterial wall thickness was found in hypercholesterolemic subjects compared with controls.19 A population-based study showed that age, LDL cholesterol, smoking, blood leukocyte count, and platelet aggregability were the strongest predictors of the progression of the intimal-medial thickening within 2 years in men.29 In the present study, the high-risk profile found in women with intimal-medial thickening suggests that arterial wall thickening may be the early phase of the atherosclerotic process. Its later development in atheromatous plaques needs to be confirmed in longitudinal studies.

In conclusion, B-mode ultrasonography may be a useful method to detect early atherosclerotic lesions and to monitor their progression in a general population. Whether early carotid atherosclerosis may be relevant to clinical manifestations of ischemic arterial disease deserves further investigation.

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