Serum Elastase Activity, Serum Elastase Inhibitors, and Occurrence of Carotid Atherosclerotic Plaques

The Etude sur le Vieillissement Artériel (EVA) Study

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Background—In the last decades, interest has increased in the potential deleterious atherogenic effects of some cellular elastase activities. The results of experimental and clinical investigations were inconsistent. In this report, we assessed the associations of serum elastase activity and serum elastase inhibitors with carotid plaque occurrence during the 4-year follow-up in a population of 859 subjects free of coronary heart disease and stroke (age, 59 to 71 years).

Methods and Results—Serum elastase activity and serum elastase inhibitors were measured at baseline examination. Carotid B-mode ultrasound examination was performed at baseline and 2 years and 4 years later. The occurrence of carotid plaques in subjects with the lowest serum elastase activity values (quartile 1), in those with the intermediate values (quartiles 2 to 3), and in those with the highest values (quartile 4) was, respectively, 24.6%, 18.9%, and 12.2% (P<0.001 for trend). The multivariate odds ratios of carotid plaque occurrence associated with the three groups (adjusted for major known cardiovascular risk factors) were, respectively, 1.00, 0.67 (CI, 0.44 to 1.02; P=0.06), and 0.40 (CI, 0.23 to 0.70; P<0.001). For serum elastase inhibitors, the occurrence of carotid plaques in quartile 1 (lowest values), quartiles 2 to 3, and quartile 4 (highest values) was, respectively, 11.7%, 18.8%, and 25.2% (P for trend<0.001). The corresponding multivariate adjusted odds ratios were 1.00, 1.98 (CI, 1.19 to 3.31; P<0.01), and 3.18 (CI, 1.80 to 5.60, P<0.001).

Conclusions—Low values of serum elastase activity and high values of serum elastase inhibitors were strongly and independently associated with increased 4-year carotid plaque occurrence. Further studies are necessary to elucidate the nature of the associations between elastase parameters and atherosclerosis. (Circulation. 2002;105:2638-2645.)

Key Words: atherosclerosis • plaque • metalloproteinase • inflammation
Methods
Details of the EVA study have been reported previously. The initial study population was composed of 1389 volunteers between 59 and 71 years of age who were recruited from the electoral rolls of the city of Nantes (western France). Subjects were contacted both through the mail and, to a lesser extent, through information campaigns. When a subject was recruited, his or her spouse was systematically asked to participate in the study if he or she was in the required age range. After the baseline visit, which took place between June 1991 and July 1993, subjects were invited to participate in 2-year (mean±SD, 23.8±1.0 months; range, 22 to 27) and 4-year (mean±SD, 47.7±1.4 months; range, 45 to 53) follow-up examinations. The study protocol was approved by the Comité d’Ethique du Centre Hospitalier Universitaire du Kremlin-Bicêtre, and written informed consent was obtained from all participants.

Medical History and Standard Biological Procedures
Medical information, obtained at baseline examination by a standardized questionnaire, included demographic background, medical history, and personal habits such as cigarette consumption and alcohol consumption.

Subjects were classified as a never smoker, former smoker, or current smoker. Two independent measurements of systolic and diastolic blood pressure were made with a digital electronic tensiometer (SP9 Spengler) after a 10-minute rest, and the mean value was used in the analysis. Subjects with systolic blood pressure ≥140 mm Hg, diastolic blood pressure ≥90 mm Hg, and/or subjects who were using antihypertensive drugs were considered to be hypertensives. Measurements methods of standard biochemical parameters are described elsewhere. Hypercholesterolemia was defined as total cholesterol level ≥7.2 mmol/L or use of lipid-lowering drugs. Subjects who had a medical history of diabetes, used antidiabetic drugs, or had a fasting plasma glucose level ≥7.0 mmol/L were considered as diabetics. The body mass index was computed as weight (in kilograms) divided by squared height (in meters).

Serum Elastase Activity and Serum Elastase Inhibitor Measurements
Serum elastase-type activity and serum elastase inhibitors were measured only at baseline. Determination of elastase-type activity was performed in serum samples diluted 1 to 20 in 100 mmol/L Tris buffer containing 0.02% NaN3, 0.01% Brij at pH 8.0; 10 UL of the substrate (succinylala3-p-nitroanilide, 125 mmol/L in N-methylpyrrolidone-2) was added, and 200 UL of this mixture was incubated at 37°C for 10 hours. The optical density was read at 410 nm in a Dynatech ELISA plate reader. The calibration curve was obtained with crystall pure pancreatic elastase (type IV 100 U/mg, Sigma) between 0.1 and 1.0 U. One unit was defined as the concentration of enzyme hydrolyzing 1 mg elastin in 20 minutes in the above conditions. Results are expressed as units per milliliter of serum. Determinations were carried out in duplicate; every plate carries a standard curve, also in duplicate. The coefficient of variation of serum elastase-type activity measurements was 7%. Other details of the method are described elsewhere. The determination of elastase inhibitor titers has been previously described. Briefly, 10 µL of serum samples was pipetted into 5-µL polypropylene centrifuge tubes, and 100 µL of pancreatic elastase was added (type IV Sigma, 20 U/µL) with 10 µL of an elastin fiber suspension (100 mg/5 mL) kept at constant agitation to ensure a uniform distribution of the fibers during pipetting. The tubes were incubated for 2 hours at 37°C with constant shaking, then centrifuged at 5000 rpm for 10 minutes at 4°C and the radioactivity of the suspensate measured in a 1209 Rack β-counter (Pharmacia). Inhibitory titers were calculated from the ratio of radioactivity released in the absence of serum to activities determined in the presence of serum. All determinations were carried out in duplicate and expressed as percentage inhibition. The coefficient of variation of serum elastase inhibitors measurements was 4%.

Ultrasonography
Ultrasound examinations at baseline, 2-year follow-up, and 4-year follow-up were performed with the use of the Aloka SSD-650, with a transducer frequency of 7.5 MHz. Acquisition, processing, and storage of B-mode images were computer-assisted with software specially designed for longitudinal studies (EUREQUA).

Details of the protocol have been described elsewhere. At each examination, it involved scanning of the common carotid arteries (CCAs), of the carotid bifurcations, and of the origin (first 2-cm) of the internal carotid arteries. At the time of examination, the intima-media thickness (IMT) was measured on the far wall of the mid and distal common carotid artery as the distance between the lumen-intima interface and the media-adenitia interface, by using an automated edge detection algorithm. Two longitudinal measurements of CCA-IMT (at a site free of any discrete plaque) were completed on both the right and left common carotid arteries. Right CCA-IMT, left CCA-IMT, and mean CCA-IMT (the mean of the four right and left CCA-IMT measurements) were used in the analysis.

Both near and far walls of all arterial segments (ie, common carotid artery or bifurcation origin of the internal carotid artery) were scanned longitudinally and transversally 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-adenitia interface and the internal side of the lesion was ≥1 mm. When a plaque was present, optimal frozen images (one longitudinal and one transversal view), showing the plaque in its greatest thickness, were selected by the sonographer and stored on an optical disk. When several plaques were present on the same arterial segment, the number of plaques was recorded, and examination was centered on that showing the greatest encroachment into the lumen. In the absence of plaques, no measurement was made in the carotid bifurcations–internal carotid arteries, but optimal frames were selected and stored on the optical disk. For each image stored at baseline or at 2-year follow-up, a mask was constructed. It consisted of recording the anatomic situation of the investigated territory, the type of echographic cut, and the orientation of the anterior lateral or posterior cuts of the ultrasound beam in relation to the neck.

At 2-year examination, the sonographer recalled information from the corresponding optical disk, which had been defined at baseline examination on the right and left segments. The real-time echographic image and the fixed contours recorded during the baseline examination were superimposed on the screen. The sonographer then produced a beam that coincided with the echographic and contour image. At 4-year examination, masks constructed during the 2-year follow-up were recalled. All these procedures were used both for CCA-IMT measurements and plaque detection.

The same four sonographers performed ultrasound examinations at baseline, 2-year follow-up, and 4-year follow-up. For each subject, we attempted to have the 2-year and 4-year follow-up examinations performed by the sonographer who had performed the baseline examination. This was the case for 76% of subjects. Reproducibility of the scanning and reading procedures for both carotid plaques and CCA-IMT have been reported elsewhere.

Data Analysis
The occurrence of carotid plaques during the follow-up (at 2-year examination or/and at 4-year examination) was defined as the occurrence of one plaque (or more) in previously normal segments and/or the occurrence of new plaques in segments that previously had plaques. The distributions of serum elastase activity and serum elastase inhibitors values in the EVA study were described previously. The current analysis, serum elastase activity and serum elastase inhibitors values were used as continuous as well as categoric variables. They were divided into 3 categories according approximately to the 25th and 75th sex-specific values (quartile 1, quartiles 2 to 3, and quartile 4). For serum elastase activity values, the cutoff points were 0.20 U/mL and 0.48 U/mL for men and 0.19 U/mL and 0.47 U/mL for women. For serum elastase inhibitors, the
cutoff points were 42.7% and 58.4% for men and 45.9% and 60.2% for women.

Standard procedures from the Statistical Analysis System (SAS) were used for univariate and multivariate analyses. Associations of the three categories of serum elastase activity and serum elastase inhibitors values were assessed by χ² tests and ANOVA. Multivariate-adjusted odds ratios (ORs) and 95% CIs were estimated by means of multiple logistic regression models with plaque occurrence (yes, no) as the dependent variable and categories of serum elastase activity and serum elastase inhibitors values as independent variables.

Subjects who were examined during the echocardiographic training period (between June and December 1991) were considered to have unreliable initial ultrasound examinations on the basis of interreader reproducibility studies and were systematically excluded from statistical analysis (n=235). A further 57 subjects who reported at baseline examination a history of angina, myocardial infarction, or stroke were excluded from the analysis. Of the 1092 remaining subjects, serum elastase activity values and serum elastase inhibitors values were not available for 119 subjects. Of the 973 remaining subjects, 859 (88.3%) had complete data on baseline cardiovascular risk factors and underwent at least one follow-up B-mode ultrasound examination (790 had two follow-up examinations and 69 had only one). At baseline, there were no statistically significant differences between subjects who participated and those who did not participate in the 4-year follow-up survey for serum elastase parameters, cardiovascular risk factors, and ultrasound examination findings.

**Results**

The mean (±SD) values of serum elastase activity and serum elastase inhibitors were, respectively, 0.43±0.47 U/mL and 51.9±12.2%. No association was observed between these two parameters (correlation coefficient=0.04, P=0.25). The associations of baseline population characteristics with sex-specific categories of serum elastase activity and serum elastase inhibitors are shown in Tables 1 and 2. Subjects with

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**TABLE 1. Baseline Population Characteristics and Baseline Ultrasound Examination Findings According to Sex-Specific Categories of Serum Elastase Activity Values**

<table>
<thead>
<tr>
<th>Serum Elastase Activity</th>
<th>Quartile 1 (low) (n=211)</th>
<th>Quartiles 2–3 (n=434)</th>
<th>Quartile 4 (high) (n=214)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Women, %</strong></td>
<td>59.7</td>
<td>60.6</td>
<td>60.3</td>
<td>0.98</td>
</tr>
<tr>
<td><strong>Age, y</strong></td>
<td>65.0±3.1*</td>
<td>65.3±3.0</td>
<td>64.7±2.8</td>
<td>0.05</td>
</tr>
<tr>
<td><strong>BMI, kg/m²</strong></td>
<td>24.6±3.8</td>
<td>25.5±3.8</td>
<td>26.1±3.9</td>
<td>0.002</td>
</tr>
<tr>
<td><strong>Smoking habits, %</strong></td>
<td></td>
<td></td>
<td></td>
<td>0.09</td>
</tr>
<tr>
<td>Never</td>
<td>64.0</td>
<td>62.7</td>
<td>55.6</td>
<td></td>
</tr>
<tr>
<td>Exsmokers</td>
<td>29.4</td>
<td>27.4</td>
<td>36.9</td>
<td></td>
</tr>
<tr>
<td>Smokers</td>
<td>6.6</td>
<td>9.9</td>
<td>7.5</td>
<td></td>
</tr>
<tr>
<td><strong>Alcohol consumption, %</strong></td>
<td></td>
<td></td>
<td></td>
<td>0.25</td>
</tr>
<tr>
<td>0 mL/d</td>
<td>33.2</td>
<td>31.6</td>
<td>23.8</td>
<td></td>
</tr>
<tr>
<td>1–20 mL/d</td>
<td>41.2</td>
<td>42.4</td>
<td>46.7</td>
<td></td>
</tr>
<tr>
<td>&gt;20 mL/d</td>
<td>25.6</td>
<td>26.0</td>
<td>29.4</td>
<td></td>
</tr>
<tr>
<td><strong>Systolic BP, mm Hg</strong></td>
<td>129.2±16.0</td>
<td>131.4±17.3</td>
<td>131.5±16.8</td>
<td>0.26</td>
</tr>
<tr>
<td><strong>Diastolic BP, mm Hg</strong></td>
<td>77.4±9.4</td>
<td>79.0±11.1</td>
<td>79.4±10.0</td>
<td>0.10</td>
</tr>
<tr>
<td><strong>Hypertension, %</strong></td>
<td>43.6</td>
<td>41.7</td>
<td>43.9</td>
<td>0.59</td>
</tr>
<tr>
<td><strong>Glucose, mmol/L</strong></td>
<td>5.33±1.09</td>
<td>5.41±0.67</td>
<td>5.69±1.16</td>
<td>0.001</td>
</tr>
<tr>
<td><strong>Diabetes, %</strong></td>
<td>4.3</td>
<td>4.4</td>
<td>12.6</td>
<td>0.001</td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>6.33±0.95</td>
<td>6.41±0.98</td>
<td>6.46±1.04</td>
<td>0.37</td>
</tr>
<tr>
<td>Hypercholesterolemia, %</td>
<td>41.2</td>
<td>37.8</td>
<td>38.3</td>
<td>0.69</td>
</tr>
<tr>
<td>HDL cholesterol, mmol/L</td>
<td>1.71±0.45</td>
<td>1.64±0.44</td>
<td>1.60±0.43</td>
<td>0.05</td>
</tr>
<tr>
<td>LDL cholesterol, mmol/L</td>
<td>1.60±0.34</td>
<td>1.63±0.36</td>
<td>1.64±0.37</td>
<td>0.54</td>
</tr>
<tr>
<td>Triglycerides, mmol/L</td>
<td>1.00±0.99</td>
<td>1.06±0.48</td>
<td>1.22±0.66</td>
<td>0.001†</td>
</tr>
<tr>
<td>Apolipoprotein B, mg/dL</td>
<td>122.9±32.1</td>
<td>124.8±28.5</td>
<td>131.2±29.5</td>
<td>0.009</td>
</tr>
<tr>
<td>Fibrinogen, mg/dL</td>
<td>332.6±107.8</td>
<td>349.0±108.1</td>
<td>362.6±114.1</td>
<td>0.009†</td>
</tr>
<tr>
<td>C-reactive protein, mg/L</td>
<td>1.48±2.52</td>
<td>1.93±2.81</td>
<td>2.93±5.07</td>
<td>0.001†</td>
</tr>
<tr>
<td>Right CCA-IMT, mm</td>
<td>0.65±0.12</td>
<td>0.65±0.12</td>
<td>0.64±0.11</td>
<td>0.58</td>
</tr>
<tr>
<td>Left CCA-IMT, mm</td>
<td>0.68±0.13</td>
<td>0.67±0.14</td>
<td>0.67±0.13</td>
<td>0.46</td>
</tr>
<tr>
<td>Mean CCA-IMT, mm</td>
<td>0.67±0.11</td>
<td>0.66±0.12</td>
<td>0.65±0.10</td>
<td>0.56</td>
</tr>
<tr>
<td>Baseline carotid plaques, %</td>
<td>19.0</td>
<td>16.4</td>
<td>21.0</td>
<td>0.33</td>
</tr>
</tbody>
</table>

BMI indicates body mass index; CCA-IMT, common carotid intima-media thickness.

*Mean±SD.
†Based on log-transformed values.
the highest serum elastase activity values (quartile 4) had higher means of body mass index, glucose, triglycerides, apolipoprotein B, fibrinogen and C-reactive protein, and higher prevalence of diabetes (Table 1). In contrast, only plasma fibrinogen and C-reactive protein were significantly and positively related to categories of serum elastase inhibitors (Table 2).

The proportion of subjects who had occurrence of carotid atherosclerotic plaques during follow-up was 18.6%. In a recent article, we reported that age, sex, smoking habits, hypertension, hypercholesterolemia, mean CCA-IMT, and presence of carotid plaques at baseline were related to carotid plaque occurrence in this population.8

The distribution and ORs of carotid plaque occurrence associated with quartiles of serum elastase activity are shown in Table 3. Age- and sex-adjusted ORs of carotid plaque occurrence were 0.70 (P<0.07) in subjects having the intermediate serum elastase activity values (quartiles 2 to 3) and 0.43 (P<0.001) in those having the highest serum elastase activity values (quartile 4), compared with those having the lowest values (quartile 1) (P for trend<0.002). Multivariate adjustment for major known cardiovascular risk factors did not markedly change these findings (Table 3). These associations were also consistently observed both in men and in women (Table 3).

Table 4 shows the distribution and ORs of carotid plaque occurrence associated with quartiles of serum elastase inhibitors values. The age- and sex-adjusted ORs for quartile 1 (lowest values), quartiles 2 to 3, and quartile 4 (highest values) of serum elastase inhibitors were, respectively, 1.00, 1.73 (P<0.05), and 2.55 (P<0.001) (P for trend<0.001). The corresponding multivariate ORs were 1.00, 1.98, and 3.18

The distribution and ORs of carotid plaque occurrence according to sex-specific categories of serum elastase inhibitor values are shown in Table 2. Baseline Population Characteristics and Baseline Ultrasound Examination Findings According to Sex-Specific Categories of Serum Elastase Inhibitor Values

<table>
<thead>
<tr>
<th>Serum Elastase Inhibitors</th>
<th>Quartile 1 (low) (n=214)</th>
<th>Quartiles 2–3 (n=431)</th>
<th>Quartile 4 (high) (n=214)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Women, %</td>
<td>60.3</td>
<td>60.3</td>
<td>60.3</td>
<td>1.00</td>
</tr>
<tr>
<td>Age, y</td>
<td>64.9±3.0</td>
<td>65.2±3.0</td>
<td>65.2±3.1</td>
<td>0.48</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>25.6±3.7</td>
<td>25.5±4.1</td>
<td>25.0±3.8</td>
<td>0.62</td>
</tr>
<tr>
<td>Smoking habits, %</td>
<td></td>
<td></td>
<td></td>
<td>0.06</td>
</tr>
<tr>
<td>Never</td>
<td>59.4</td>
<td>62.4</td>
<td>60.8</td>
<td></td>
</tr>
<tr>
<td>Exsmokers</td>
<td>31.8</td>
<td>29.5</td>
<td>30.4</td>
<td></td>
</tr>
<tr>
<td>Smokers</td>
<td>8.9</td>
<td>8.1</td>
<td>8.9</td>
<td></td>
</tr>
<tr>
<td>Alcohol consumption, %</td>
<td></td>
<td></td>
<td></td>
<td>0.15</td>
</tr>
<tr>
<td>0 mL/d</td>
<td>27.1</td>
<td>29.7</td>
<td>33.6</td>
<td></td>
</tr>
<tr>
<td>1–20 mL/d</td>
<td>49.1</td>
<td>40.6</td>
<td>42.5</td>
<td></td>
</tr>
<tr>
<td>&gt;20 mL/d</td>
<td>23.8</td>
<td>29.7</td>
<td>23.8</td>
<td></td>
</tr>
<tr>
<td>Systolic BP, mm Hg</td>
<td>130.4±16.1</td>
<td>130.5±17.1</td>
<td>132.1±17.3</td>
<td>0.47</td>
</tr>
<tr>
<td>Diastolic BP, mm Hg</td>
<td>78.2±9.5</td>
<td>78.5±10.5</td>
<td>79.5±11.1</td>
<td>0.37</td>
</tr>
<tr>
<td>Hypertension, %</td>
<td>42.8</td>
<td>41.1</td>
<td>45.8</td>
<td>0.52</td>
</tr>
<tr>
<td>Glucose, mmol/L</td>
<td>5.47±0.81</td>
<td>5.48±0.99</td>
<td>5.42±0.91</td>
<td>0.70</td>
</tr>
<tr>
<td>Diabetes, %</td>
<td>5.6</td>
<td>7.0</td>
<td>6.1</td>
<td>0.78</td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>6.54±1.03</td>
<td>6.34±0.94</td>
<td>6.39±1.04</td>
<td>0.06</td>
</tr>
<tr>
<td>Hypercholesterolemia, %</td>
<td>36.9</td>
<td>39.4</td>
<td>39.3</td>
<td>0.81</td>
</tr>
<tr>
<td>HDL cholesterol, mmol/L</td>
<td>1.66±0.43</td>
<td>1.64±0.45</td>
<td>1.66±0.44</td>
<td>0.76</td>
</tr>
<tr>
<td>LDL cholesterol, mmol/L</td>
<td>1.67±0.37</td>
<td>1.61±0.34</td>
<td>1.61±0.38</td>
<td>0.16</td>
</tr>
<tr>
<td>Triglycerides, mmol/L</td>
<td>1.09±0.53</td>
<td>1.06±0.56</td>
<td>1.12±0.97</td>
<td>0.63†</td>
</tr>
<tr>
<td>Apolipoprotein B, mg/dL</td>
<td>128.9±30.1</td>
<td>124.7±28.4</td>
<td>125.5±32.2</td>
<td>0.26</td>
</tr>
<tr>
<td>Fibrinogen, mg/dL</td>
<td>338.1±115.1</td>
<td>339.0±100.0</td>
<td>377.6±119.0</td>
<td>0.001†</td>
</tr>
<tr>
<td>C-reactive protein, mg/L</td>
<td>1.53±1.88</td>
<td>1.83±3.03</td>
<td>3.08±5.06</td>
<td>0.001†</td>
</tr>
<tr>
<td>Right CCA-IMT, mm</td>
<td>0.64±0.12</td>
<td>0.64±0.12</td>
<td>0.65±0.13</td>
<td>0.83</td>
</tr>
<tr>
<td>Left CCA-IMT, mm</td>
<td>0.67±0.13</td>
<td>0.67±0.13</td>
<td>0.67±0.14</td>
<td>0.94</td>
</tr>
<tr>
<td>Mean CCA-IMT, mm</td>
<td>0.66±0.11</td>
<td>0.66±0.11</td>
<td>0.66±0.12</td>
<td>0.97</td>
</tr>
<tr>
<td>Baseline carotid plaques, %</td>
<td>16.8</td>
<td>19.3</td>
<td>17.3</td>
<td>0.70</td>
</tr>
</tbody>
</table>

BMI indicates body mass index; CCA-IMT, common carotid intima-media thickness.

*Mean±standard deviation.
†Based on log-transformed values.
Similar patterns of results were observed in men and in women (Table 4). When analysis was performed in combined categories of serum elastase activity and serum elastase inhibitors, subjects who had both the lowest values of serum elastase activity (quartile 1) and the highest values of serum elastase inhibitors (quartile 4) had a 6-fold (OR = 5.99; 95% CI, 1.81 to 19.83) higher risk of carotid plaque occurrence compared to those with the highest values of serum elastase activity and the lowest values of serum elastase inhibitors.

### TABLE 3. Odds Ratios (ORs) [95% CI] for Carotid Plaque Occurrence During Follow-Up Associated With Sex-Specific Quartiles of Serum Elastase Activity

<table>
<thead>
<tr>
<th>Serum Elastase Activity</th>
<th>Quartile 1 (Low)</th>
<th>Quartile 2–3</th>
<th>Quartile 4 (High)</th>
<th>P for Trend</th>
</tr>
</thead>
<tbody>
<tr>
<td>All</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>211</td>
<td>434</td>
<td>214</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Plaque occurrence, %</td>
<td>24.6</td>
<td>18.9</td>
<td>12.2</td>
<td></td>
</tr>
<tr>
<td>Age- and sex-adjusted OR</td>
<td>1</td>
<td>0.70 [0.47–1.05]</td>
<td>0.43 [0.26–0.72]</td>
<td>&lt;0.002</td>
</tr>
<tr>
<td>Multivariate-adjusted OR*</td>
<td>1</td>
<td>0.67 [0.44–1.02]</td>
<td>0.40 [0.23–0.70]</td>
<td>&lt;0.002</td>
</tr>
<tr>
<td>Men</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>85</td>
<td>171</td>
<td>85</td>
<td>0.02</td>
</tr>
<tr>
<td>Plaque occurrence, %</td>
<td>32.9</td>
<td>24.0</td>
<td>17.7</td>
<td></td>
</tr>
<tr>
<td>Age-adjusted OR</td>
<td>1</td>
<td>0.64 [0.36–1.14]</td>
<td>0.43 [0.21–0.90]</td>
<td>0.02</td>
</tr>
<tr>
<td>Multivariate-adjusted OR*</td>
<td>1</td>
<td>0.59 [0.32–1.09]</td>
<td>0.34 [0.15–0.78]</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Women</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>126</td>
<td>263</td>
<td>129</td>
<td>0.02</td>
</tr>
<tr>
<td>Plaque occurrence, %</td>
<td>19.1</td>
<td>15.6</td>
<td>8.5</td>
<td></td>
</tr>
<tr>
<td>Age-adjusted OR</td>
<td>1</td>
<td>0.74 [0.42–1.29]</td>
<td>0.38 [0.18–0.83]</td>
<td>0.01</td>
</tr>
<tr>
<td>Multivariate-adjusted OR*</td>
<td>1</td>
<td>0.72 [0.40–1.30]</td>
<td>0.40 [0.18–0.88]</td>
<td>0.02</td>
</tr>
</tbody>
</table>

*Adjusted for sex (where analyses were performed in combined men and women), age, body mass index, smoking habits, alcohol consumption, systolic blood pressure, serum glucose, serum total cholesterol, serum triglycerides (log-transformed values), plasma fibrinogen (log-transformed values), C-reactive protein (log-transformed values), and baseline CCA-IMT.

### TABLE 4. Odds Ratios (ORs) [95% CI] for Carotid Plaque Occurrence During Follow-Up Associated With Sex-Specific Quartiles of Serum Elastase Inhibitors

<table>
<thead>
<tr>
<th>Serum Elastase Inhibitors</th>
<th>Quartile 1 (Low)</th>
<th>Quartile 2–3</th>
<th>Quartile 4 (High)</th>
<th>P for Trend</th>
</tr>
</thead>
<tbody>
<tr>
<td>All</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>214</td>
<td>431</td>
<td>214</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Plaque occurrence, %</td>
<td>11.7</td>
<td>18.8</td>
<td>25.2</td>
<td></td>
</tr>
<tr>
<td>Age- and sex-adjusted OR</td>
<td>1</td>
<td>1.73 [1.07–2.82]</td>
<td>2.55 [1.51–4.30]</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Multivariate-adjusted OR*</td>
<td>1</td>
<td>1.98 [1.19–3.31]</td>
<td>3.18 [1.80–5.60]</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Men</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>85</td>
<td>171</td>
<td>85</td>
<td>0.02</td>
</tr>
<tr>
<td>Plaque occurrence, %</td>
<td>14.1</td>
<td>27.5</td>
<td>29.4</td>
<td></td>
</tr>
<tr>
<td>Age-adjusted OR</td>
<td>1</td>
<td>2.31 [1.15–4.66]</td>
<td>2.54 [1.18–5.50]</td>
<td>0.02</td>
</tr>
<tr>
<td>Multivariate-adjusted OR*</td>
<td>1</td>
<td>2.65 [1.26–5.61]</td>
<td>2.94 [1.25–6.94]</td>
<td>0.02</td>
</tr>
<tr>
<td>Women</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>129</td>
<td>260</td>
<td>129</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Plaque occurrence, %</td>
<td>10.1</td>
<td>13.1</td>
<td>22.5</td>
<td></td>
</tr>
<tr>
<td>Age-adjusted OR</td>
<td>1</td>
<td>1.35 [0.68–2.67]</td>
<td>2.64 [1.30–5.39]</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Multivariate-adjusted OR*</td>
<td>1</td>
<td>1.65 [0.79–3.42]</td>
<td>3.68 [1.68–8.07]</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

*Adjusted for sex (where analyses were performed in combined men and women), age, body mass index, smoking habits, alcohol consumption, systolic blood pressure, serum glucose, serum total cholesterol, serum triglycerides (log-transformed values), plasma fibrinogen (log-transformed values), C-reactive protein (log-transformed values), and baseline CCA-IMT.
with subjects who had both the highest values of serum elastase activity (quartile 4) and the lowest values of serum elastase inhibitors (quartile 1). These results indicate multiplicative effects, and none of the interaction terms was statistically significant.

Four-year longitudinal changes in mean CCA-IMT were computed as the difference between 4-year follow-up and baseline values. Neither serum elastase activity nor serum elastase inhibitors was associated with 4-year change in mean CCA-IMT. The multivariate-adjusted mean of 4-year change in mean CCA-IMT (±SEM) was 0.047 mm (±0.007) in subjects having the lowest serum elastase activity values (quartile 1), 0.043 mm (±0.005) in those having intermediate serum elastase activity values (quartiles 2 to 3), and 0.048 mm (±0.007) in those having the highest serum elastase activity values (quartile 4) (P=0.49). For serum elastase inhibitors, the multivariate-adjusted mean of 4-year change in mean CCA-IMT in quartile 1, quartiles 2 to 3, and quartile 4 were, respectively, 0.041 mm (±0.007), 0.045 mm (±0.005), and 0.051 mm (±0.007) (P=0.59). The use of 4-year change in left CCA-IMT or right CCA-IMT instead of 4-year change in mean CCA-IMT yielded similar results (data not shown).

**Discussion**

Low values of serum elastase activity and high values of serum elastase inhibitors were strongly and independently associated with increased carotid plaque occurrence in this 4-year longitudinal study performed in a large sample of relatively aged subjects. The magnitude of the associations and the consistency of the results are noteworthy, and this is the first investigation that reports the relations between serum elastase parameters and subsequent development of atherosclerosis.

In our study, no association was observed between serum elastase activity and serum elastase inhibitors. The elastase-type activity measured in this study was derived, up to 90%, from metallo-endopeptidases such as MMP-2 and MMP-9. Their specific inhibitors, TIMPs (tissue inhibitors of metalloproteinases), were not measured. The major source of the elastase inhibitors determined in this investigation was α1-proteinase inhibitor and, to a lesser extent, α2-macroglobulin. Proteinase inhibitor has been shown to be an acute phase reactant and might be considered as a marker of inflammation. The relations of serum elastase inhibitors with fibrinogen and C-reactive protein (Table 2) tend to confirm the acute phase reaction nature of serum elastase inhibitors.

Serum elastase activity was also associated with fibrinogen and C-reactive protein and, in addition, with some components of the metabolic syndrome (Table 1). All these factors, contrary to the major conventional cardiovascular risk factors, were not related to the occurrence of carotid plaques in our study, in accordance with the longitudinal results reported from the Bruneck Study. Thus, they could not confound the observed associations between serum elastase parameters and plaque occurrence. Nevertheless, the opposite direction of the associations of carotid plaques occurrence with serum elastase activity and serum elastase inhibitors was unexpected and remains difficult to explain.

In the literature, contradictory results have been reported about the associations of serum elastase parameters with atherosclerosis. In a cross-sectional study of 140 male subjects with ischemic vascular disease and 60 control subjects, serum elastase-type activity was found to be significantly lower and inhibitor capacity higher in patients than in control subjects. These results are in accordance with ours. In a case-control study of 40 subjects with occlusive arterial disease of the legs and 40 healthy subjects, serum elastase-type protease activity was higher in patients than in control subjects, but the difference was limited to older subjects. In contrast, elastase levels in the sera of 52 individuals presenting clinical symptoms of atherosclerosis were not different from serum elastase levels in 41 control subjects.

Neither the presence nor the severity of coronary atherosclerosis was associated with serum elastase inhibitors (α1-antitrypsin and α2-macroglobulin) in 203 patients admitted for arteriography. The presence of elastase-type activity in the vessel wall has been reported by several investigations. Both aortic smooth muscle cells and fibroblasts from several origins were shown to synthesize elastase activity. Previously, mainly in vitro and experimental animals studies have investigated the association of elastase activity, directly related to the cell content of vascular wall, with the degree of atherosclerosis. The results of these studies suggest that release of elastase activity, in a porcine aortic organ culture model, was associated with intimal smooth muscle cell proliferation and intimal hyperplasia. In this model, the inhibition of elastase activity with α1-proteinase inhibitor markedly repressed the proliferation of neointimal smooth muscle cells. Elastase inhibitors might also prevent the development of coronary artery disease experimentally induced after heart transplantation and intimal hyperplasia after balloon angioplasty in rabbits.

Several Japanese studies have been investigated the effects on atherosclerosis of an oral drug containing porcine pancreatic elastase. Their results suggest that elastase intake may prevent development of atherosclerosis and arterial stiffness. In experimental animal studies, long-term administration of elastase prevents the fragmentation of elastic fiber in immunized rabbits with elastin peptides, the stiffening of the aortic wall in rabbits who have been fed a high cholesterol diet, the development of atherosclerotic lesions in rats fed an atherogenic diet, and the increase in intima thickness of the aorta of rabbits after balloon catheter injury. In human studies, the aortic pulse-wave velocity in atherosclerotic patients was decreased by the oral administration of elastase for 3 months. Elastase was also effective in maintaining graft patency in femoral-popliteal arterial bypass for arteriosclerosis obliterans. However, it is not known whether or not oral intake of elastase can modify serum elastase activity. Several limits of our study should be mentioned. The EVA cohort study comprised volunteers who differ from the general population of the same age in several characteristics. Fourteen percent of the EVA participants had achieved 12 years of schooling or more, as compared with 7% of the general population. The proportion of women is similar, but the prevalence of coronary heart diseases was slightly lower.
Survival bias also could have occurred in this study performed in an elderly population. Subjects with low values of serum elastase activity and/or high values of serum elastase inhibitors might die at an earlier age or might have clinically manifest cardiovascular diseases. This could partly explain the lack of cross-sectional associations between elastase parameters and carotid plaques. Another explanation of the lack of associations at baseline is that the effects of major potential confounding factors (including age) are likely to be stronger in a cross-sectional than in a longitudinal design. Taking into account the high rate of participation (88%) in the follow-up survey, the potential effects of selection and/or survival biases on the observed longitudinal associations are probably small, even though they could not entirely be ruled out.

A third potential explanation of the lack of cross-sectional associations might be that elastase parameters are markers of biological phenomena acting in a short-term period. Follow-up studies on plaque occurrence might thus be more appropriate to detect these effects than cross-sectional designs in which the presence of plaque resulted from both lifelong phenomena. A last potential explanation might be that elastase parameters play a role only at an older age, whereas cross-sectional levels of atherosclerosis are a result of lifelong exposure to risk factors.

Our ultrasound protocol at baseline and follow-up was highly standardized (see Methods). In addition, acquisition, processing, and storage of B-mode images were computer-assisted, with software specially designed for longitudinal studies. Follow-up examinations were performed by the sonographer who had performed the baseline examination for three quarters of the population, and similar patterns of results between elastase parameters and carotid plaques were observed in the remaining quarter (data available from authors). One can argue that in subjects with carotid plaque at baseline, two adjacent plaques at one location may be difficult to be discriminated from one large plaque, and occurrence of a new plaque is difficult to be discriminated from enlargement of an existing plaque. However, the associations between baseline elastase parameters and carotid plaque occurrence were observed both in subjects with carotid plaque at baseline and in those without (data available from authors). In the present study, CCA-IMT change over time was not significantly related to elastase parameters. We used a methodological approach for carotid imaging that clearly differentiates between diffuse intima-media thickening and plaque. We have previously reported that the two types of lesions were interrelated, but some factors could be specifically associated with increased IMT alone or with plaques alone. We do not think that the differential associations of carotid plaques and change in CCA-IMT with elastase parameters could be explained by large measurement errors for IMT. The protocol for IMT at baseline and follow-up was also highly standardized, the results of the reproducibility study was far satisfactory, and change in CCA-IMT in the EVA study was associated with some classic risk factors such as age, body mass index, HDL cholesterol, and systolic blood pressure.

In conclusion, our 4-year longitudinal results suggest that low values of serum elastase activity and high values of serum elastase inhibitors predict increased carotid plaque occurrence in the elderly. Further investigations are needed to elucidate the underlying mechanisms of these associations and to determine the predictive values of elastase activity and inhibition on the risk for clinical cardiovascular events. We are presently following up the EVA cohort for cardiovascular morbidity and mortality rates, and we hope to address these issues in the near future.

Acknowledgments

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References


Serum Elastase Activity, Serum Elastase Inhibitors, and Occurrence of Carotid Atherosclerotic Plaques: The Etude sur le Vieillissement Artériel (EVA) Study

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