Prothrombin Fragment 1+2 Is Associated With Carotid Intima-Media Thickness in Subjects Free of Clinical Cardiovascular Disease

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Background and Purpose—Thrombin, a central enzyme in the clotting cascade, plays a role not only in thrombosis but also in the progression of atherosclerosis. We studied the relationship between prothrombin fragment 1+2 (F1+2), a specific marker of thrombin generation in vivo, and carotid intima-media thickness (IMT), an index of subclinical atherosclerosis.

Methods—We examined 181 asymptomatic middle-aged subjects (mean age 55.6 years, 76.7% men) free of overt clinical atherosclerotic disease. F1+2 was measured by enzyme-linked immunosorbent assay and IMT by duplex ultrasonography of carotid artery. Multiple linear regression analysis was used to assess the relationship between the 2 parameters.

Results—Compared with individuals in the lowest tertile of F1+2, those in the upper tertile (>0.55 mmol/L) showed significantly higher IMT (P<0.01). In correlation analysis, a positive relationship was found between plasma F1+2 and carotid IMT. F1+2 also correlated positively with cholesterol (P<0.008) and low-density lipoprotein cholesterol (P<0.005), but not with blood pressure or body mass index. In the multivariate analysis, the association of F1+2 with carotid IMT remained significant (P<0.001) after adjustment for age, sex, body mass index, systolic blood pressure, cholesterol, diabetes, and smoking.

Conclusions—In a population sample of adults without clinically overt atherosclerotic disease, the plasma levels of F1+2 were significantly associated with carotid IMT, suggesting a relationship between thrombin generation and the development atherosclerosis. (Stroke. 2004;35:1085-1089.)

Key Words: prothrombin ■ atherosclerosis ■ carotid arteries ■ intima-media thickness ■ thrombin

Increasing evidence indicates that the hemostatic system plays an important role in the pathogenesis of atherosclerotic vascular disease.1 Thrombin, a central enzyme of this system, is considered a key mechanism in the pathophysiology of cardiovascular diseases (CVD).2 The incidence of CVD and the recurrence of coronary events have been found to be positively associated with several coagulation factors, such as fibrinogen, factor VII, and factor VIII.3–6

The conversion of prothrombin to thrombin is a central event in the coagulation cascade. Prothrombin fragment 1+2 (F1+2) is a polypeptide released from the prothrombin during its activation to thrombin by the prothrombinase complex. Measurement of circulating levels of F1+2 has been considered a specific marker of thrombin generation in vivo.7,8 Elevated F1+2 has been found in patients with peripheral arterial disease, coronary atherosclerosis, and in relation to the presence of conventional CAD risk factors, such as age, smoking, and dyslipidemia.9–12 In a previous study, we also reported a possible predictive value of F1+2 in relation to graft occlusion in patients undergoing aorto-coronary bypass surgery.13 However, other reports did not find F1+2 to be predictive of future ischemic events.14

Intima-media thickness (IMT) of the carotid artery is a marker of preclinical atherosclerosis, which has been shown to predict incident CVD events,15–17 and has been associated with cardiovascular risk factors.18,19 Whether markers of thrombin generation are associated only with the thrombotic component of CVD or also with the atherosclerotic process is still debated.20 The existence of a relationship between F1+2 and thickness of the arterial wall would be an important issue, because this might make it possible to identify asymptomatic subjects who might benefit from antithrombotic strategies. The aim of this study was, therefore, to examine the relationship between F1+2 and carotid IMT in middle-aged adults free of clinically overt cardiovascular disease.

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Patients and Methods

Subjects
A total of 181 consecutive apparently healthy subjects (76.7% men, mean age 55.6 years) attending the outpatient clinic for vascular risk assessment by the Internal Medicine Department at the University Hospital of Navarra were studied. Subjects were free from clinically apparent atherosclerotic disease based on: (1) absence of history of coronary disease, stroke, or peripheral arterial disease; and (2) normal ECG and chest-x-ray results. Coronary heart disease was defined by: (1) self-reported myocardial infarction, angina, or use of nitroglycerin; and (2) self-reported history of coronary angioplasty or coronary artery bypass surgery. Cerebrovascular disease was defined as self-reported stroke, transient ischemic attack, or carotid endarterectomy. Symptoms of intermittent claudication were queried in a questionnaire, together with the physician’s interview. Patients were also excluded if they had advanced carotid atherosclerosis according to carotid IMT measurements (>1.7 mm). Additional exclusion criteria were the presence of severely impaired renal function, arteritis, collagenosis, a history of alcohol abuse, and administration of antiinflammatory agents or any antithrombotic medication in the previous 2 weeks. Patients with significant acute infection, according to clinical criteria by the attending physician, were also excluded.

Assessment of Cardiovascular Risk Factors
In addition to questions about symptoms of ischemic heart disease, peripheral vascular disease, and stroke, the cardiovascular risk factors, diabetes mellitus, arterial hypertension, and smoking habits were obtained. Blood pressure was measured twice on the right upper arm with a random-zero mercury sphygmomanometer in patients in the sitting position (average of 2 measurements). Patients were considered to be hypertensives if they had systolic blood pressure >140 mm Hg and/or diastolic pressure >90 mm Hg or were using antihypertensive drugs. Subjects with a positive history of diabetes mellitus or with fasting glucose levels >7.0 mmol/L were considered as having diabetes. Smoking was defined as “current smokers” or “nonsmokers.” Obesity was defined as >130% ideal body mass estimated by the body mass index (kg/m²). Dyslipidemia was diagnosed in the presence of at least 1 of the following measurements: total cholesterol >200 mg/dL, low-density lipoprotein (LDL) cholesterol >130 mg/dL, and high-density lipoprotein (HDL) cholesterol <35 mg/dL.

Written informed consent was obtained from the subjects, the study was performed in accordance with the Declaration of Helsinki, and the local committee on human research approved the study protocol.

Determination of Plasma Levels of F1+2
Blood was collected by venipuncture between 9:00 and 11:00 AM after 12 hours of fasting. Venous blood were mixed (9:1) with sodium citrate solution 0.11 mol/L and immediately centrifuged at 2000 g for 20 minutes at 4°C to avoid in vitro thrombin activation. The platelet-poor plasma was then frozen at −80°C until assayed. F1+2 was measured in citrated plasma by ELISA using a commercial kit (Enzygnost F1+2 micro; Dade Behring), following manufacturer instructions. The reference range (5th to 95th percentile) was 0.4 to 1.1 nmol/L. Inter-assay and intra-assay correlation coefficients of variation were <8%.

Lipid Measurements
Serum cholesterol, HDL, and LDL cholesterol, triglycerides, and glucose were measured on fasting blood samples by standard enzymatic techniques.

Measurement of Carotid IMT
All subjects underwent ultrasonography of the common carotid arteries. Ultrasonography was performed with a 5- to 12-MHz linear-array transducer (ATL 500 HDI). The measurement of IMT was made 1 cm proximal to the carotid bulb of each common carotid artery at plaque-free sites. For each individual, the IMT was determined as the average of near-wall and far-wall measurements of each common carotid artery. Carotid artery IMT has been shown to be reproducible. Subjects were examined by the same 2 certified sonographers blinded to all clinical information. The reproducibility of IMT measurements between and within sonographers had previously been checked in 20 individuals who returned 2 weeks later for a second examination. The intraobserver and interobserver coefficients of variance were 5% and 10%, respectively.

Statistical Analysis
Distribution of continuous variables in groups was expressed as mean±SD. Triglyceride and HDL concentrations were log-transformed before analysis to normalize their distributions. Differences in the baseline characteristics according to tertiles of F1+2 were evaluated by ANOVA followed by Tukey B post-hoc test. Outliers defined as ±3SD from the mean were included in analysis. The χ² statistic was used to test differences for categorical variables. Pearson correlation test was used for assessing univariate correlations between F1+2 and all continuous variables. Multivariate linear regression analysis was performed to evaluate factors related to carotid IMT and to the possibility of interactions. The odds ratio for percentiles of carotid IMT was estimated by logistic regression model adjusted for cardiovascular risk factors. Statistical analysis was performed with SPSS for Windows 11.0. Statistical significance was established as P<0.05.

Results
After exclusion criteria, 181 subjects free from clinical CVD (76.7% men, mean age 55.6 years) were included. Frequency distribution of plasma F1+2 is shown in Figure 1. The mean plasma levels of F1+2 in the studied population were 0.5 nmol/L (range 0.04 to 1.5 nmol/L). Outliers (n=5) were included in all of the analysis.

Table 1 shows the demographic and clinical characteristics of subjects stratified by tertiles of plasma F1+2. Compared with individuals in the lowest tertile, those in the upper tertile (>0.55 nmol/L) had significantly higher carotid IMT (P<0.02) and HDL cholesterol (P<0.01). The percentage of smokers was also significantly elevated in this group (P<0.05). No differences among tertiles were found for age, body mass index, cholesterol, LDL cholesterol, and blood pressure.

In the overall population, there was a significant positive bivariate correlation between plasma F1+2 and carotid IMT (r=0.19, P<0.017). As shown in Table 2, the relation...
Table 1. Baseline Characteristics of the Studied Population Stratified by Tertiles of Plasma F1+2 (Mean± SD)

<table>
<thead>
<tr>
<th>Variable</th>
<th>F1+2 (n=61)</th>
<th>0.30–0.55 nmol/L (n=59)</th>
<th>&gt;0.55 nmol/L (n=61)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>54.8±9.3</td>
<td>57.9±12.5</td>
<td>54.7±9.4</td>
</tr>
<tr>
<td>Sex (M/F)</td>
<td>54/7</td>
<td>40/19†</td>
<td>45/16*</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>28.1±3.6</td>
<td>28.3±4.7</td>
<td>27.4±3.6</td>
</tr>
<tr>
<td>SBP (mm Hg)</td>
<td>128.3±18.5</td>
<td>136.3±21.6</td>
<td>133.8±22.2</td>
</tr>
<tr>
<td>DBP (mm Hg)</td>
<td>82.6±9.9</td>
<td>83.9±9.8</td>
<td>83.1±10.7</td>
</tr>
<tr>
<td>Smokers (%)</td>
<td>27.1</td>
<td>30.5</td>
<td>47.5*</td>
</tr>
<tr>
<td>Arterial hypertension (%)</td>
<td>40.7</td>
<td>61.0*</td>
<td>57.4</td>
</tr>
<tr>
<td>Diabetes mellitus (%)</td>
<td>22.0</td>
<td>18.6</td>
<td>8.2*</td>
</tr>
<tr>
<td>Total cholesterol (mg/dL)</td>
<td>222.4±43.0</td>
<td>228.9±43.8</td>
<td>237.9±41.4</td>
</tr>
<tr>
<td>HDL-cholesterol (mg/dL)§</td>
<td>48.8±14.3</td>
<td>51.7±13.6*</td>
<td>53.1±13.1†</td>
</tr>
<tr>
<td>LDL-cholesterol (mg/dL)</td>
<td>147.2±40.6</td>
<td>154.8±35.2</td>
<td>159.4±39.9</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)§</td>
<td>137.3±98.6</td>
<td>110.6±66.1</td>
<td>118.3±70.8</td>
</tr>
<tr>
<td>Carotid IMT (mm)</td>
<td>0.74±0.2</td>
<td>0.75±0.2</td>
<td>0.82±0.1**‡</td>
</tr>
</tbody>
</table>

BMI indicates body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure.

*P<0.05 †P<0.01 as compared to the lowest tertile.
‡P<0.01 as compared to the medium tertile.
§Based on log-transformed values.

Discussion

In a population sample of middle-aged adults free of clinically overt atherosclerotic disease, we demonstrate that plasma levels of F1+2, a specific marker of thrombin generation, showed a significant association with carotid IMT, an index of subclinical atherosclerosis, independent of a wide range of important confounding variables, including conventional cardiovascular risk factors.

Table 2. Correlation Between F1+2 Levels and All Other Parameters Evaluated

<table>
<thead>
<tr>
<th>Variable</th>
<th>R</th>
<th>P</th>
<th>Pc</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>—</td>
<td>0.29</td>
<td>—</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>—</td>
<td>—</td>
<td>0.97</td>
</tr>
<tr>
<td>SBP (mm Hg)</td>
<td>0.11</td>
<td>0.46</td>
<td>0.18</td>
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<td>DBP (mm Hg)</td>
<td>0.011</td>
<td>0.18</td>
<td>0.89</td>
</tr>
<tr>
<td>Total cholesterol (mg/dL)</td>
<td>0.20</td>
<td>0.008</td>
<td>0.01</td>
</tr>
<tr>
<td>HDL cholesterol (mg/dL)</td>
<td>—0.02</td>
<td>0.38</td>
<td>0.81</td>
</tr>
<tr>
<td>LDL cholesterol (mg/dL)</td>
<td>0.22</td>
<td>0.005</td>
<td>0.009</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td>0.03</td>
<td>0.34</td>
<td>0.67</td>
</tr>
<tr>
<td>Carotid IMT (mm)</td>
<td>0.34</td>
<td>0.017</td>
<td>0.001</td>
</tr>
</tbody>
</table>

P values for bivariate correlations. R and Pc values of partial correlations after controlling for age and sex.

Table 3. Correlation of the Carotid IMT With All Other Variables Investigated

<table>
<thead>
<tr>
<th>Variable</th>
<th>R</th>
<th>P</th>
<th>Pc</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>—</td>
<td>0.001</td>
<td>—</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>0.01</td>
<td>0.2</td>
<td>0.85</td>
</tr>
<tr>
<td>SBP (mm Hg)</td>
<td>0.19</td>
<td>0.001</td>
<td>0.02</td>
</tr>
<tr>
<td>DBP (mm Hg)</td>
<td>0.06</td>
<td>0.6</td>
<td>0.43</td>
</tr>
<tr>
<td>Total cholesterol (mg/dL)</td>
<td>0.12</td>
<td>0.7</td>
<td>0.14</td>
</tr>
<tr>
<td>HDL cholesterol (mg/dL)</td>
<td>—0.11</td>
<td>0.2</td>
<td>0.18</td>
</tr>
<tr>
<td>LDL cholesterol (mg/dL)</td>
<td>0.14</td>
<td>0.33</td>
<td>0.10</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td>—0.13</td>
<td>0.60</td>
<td>0.12</td>
</tr>
<tr>
<td>F1+2 (nmol/L)</td>
<td>0.34</td>
<td>0.017</td>
<td>0.001</td>
</tr>
</tbody>
</table>

P values for bivariate correlations. R and Pc values of partial correlations after controlling for age and sex.

between the 2 parameters remained highly statistically significant (r=0.34, P<0.001) after controlling for age and gender. F1+2 was also significantly associated with total cholesterol (r=0.20, P<0.008) and LDL cholesterol (r=0.21, P<0.005).

Carotid IMT significantly correlated with age (r=0.26, P<0.001) and systolic blood pressure (r=0.27, P<0.001), with the latter also remaining statistically significant after controlling for age and sex (Table 3). Analysis by percentiles of IMT showed that subjects in the 75th percentile (>0.87 mm) had significantly higher plasma levels of F1+2 than those in the lowest percentile (0.64±0.3 nmol/L versus 0.43±0.2 nmol/L, P<0.01) (Figure 2).

A significant association between F1+2 and IMT (P<0.001) was also found by multiple linear regression analysis after adjusting for all the potential confounders (Table 4), with the F1+2 explaining 6.5% of IMT variance after adjustment for the effects of traditional risk factors. The interaction between F1+2 and carotid IMT was not affected by exclusion of outliers (β=0.13 P<0.007, model R=30.3%).

When considering wall thickness (values higher or lower than 75th percentile of carotid IMT), major IMT (>0.87 mm) was significantly associated with increased F1+2 (fully adjusted OR: 4.8; 95% CI: 1.2 to 18.6; P<0.02).
F1+2 mmol/L

Figure 2. Plasma levels of F1+2 according to the 75th percentile of carotid IMT.

Univariate analysis showed that F1+2 levels were significantly associated with IMT, but also with some but not all traditional cardiovascular risk factors as previously reported.10,11 The association between F1+2 and carotid IMT remained significant after adjusting for cardiovascular risk factors in a multiple linear regression model. Moreover, the adjusted odds ratio for F1+2 and 75th percentile of carotid IMT was 4.8 (95% CI: 1.2 to 18.6; P<0.02). Taken together, our data indicate that thrombin generation may have an unfavorable effect on the thickening of the arterial wall.

F1+2, which comes from in vivo cleavage of prothrombin by factor Xa, provides an important diagnostic tool in the evaluation of hypercoagulable states and in the diagnosis of different clinical conditions related to thrombotic phenomena.22-24 The half-life, approximately 90 minutes, makes its measurement in plasma more reliable than other markers for ongoing coagulation, such as thrombin–antithrombin complexes. However, whether F1+2 provides a useful marker of atherosclerosis is still debated. Several studies have found elevated plasma levels of F1+2 associated with the presence and severity of atherosclerotic disease25,26 and in old high-risk patients with clinical signs of CVD,27 whereas others did not find a relationship with severity of angiographically assessed atherosclerosis9 or with other noninvasive measurements of atherosclerosis, such as the ankle–brachial index.10 Finally, a prospective study reported no association between F1+2 and risk of future cardiovascular events.14

Ultrasonographically determined carotid IMT is a useful method to study early atherosclerosis and has been extensively validated by direct and indirect methods. IMT has been found to correlate positively with the presence of atherosclerotic plaques in the carotid and femoral regions, and with CVD as well as with conventional cardiovascular risk factors, such as age, hypertension, hypercholesterolemia, and smoking.15-17,28,29 consistent with the findings observed in the present study.

Data on the relation between carotid IMT and hemostatic variables are still limited. In some studies, including recent data from our group, fibrinogen was the only hemostatic variable associated with carotid atherosclerosis.22,30,31 Other studies have found a significant correlation between plasma factors VII and VIII and von Willebrand factor with carotid IMT in peripheral arterial disease32 and in patients with clinical signs of CVD,27 but the association between thrombin generation markers and atherosclerotic disease is far from being established.33,34 Results presented herein demonstrate that F1+2 is significantly associated with carotid IMT in middle-aged subjects free of clinical overt atherosclerotic disease, which may be relevant to a better understanding of the pathophysiological relation between thrombin generation and cardiovascular events. Because thrombin, a central enzyme in the coagulation cascade, is also known to play a role in atherosclerosis progression, measurement of plasma levels of F1+2 levels might be of value in predicting subclinical atherosclerosis and in the identification of patients at high vascular risk who may benefit from prophylactic antithrombotic strategies.35,36

Increased F1+2 may be related to several factors. Subclinical atherosclerosis could be induced by thrombin generation through damaged endothelial surface, reduced fibrinolysis, or increased platelet activation, but the mechanism at this time is unclear. Other mechanisms, such as inflammation, may explain part of the association of F1+2 with carotid IMT.37 In addition, unknown genetic or environmental factors may cause increased thrombin action by an unknown mechanism.

One limitation of the data set is that although smoking can have long-term effects on hemostasis, there is no information on smoke cessation. Also, our interpretation of the results is restricted by the cross-sectional analysis and the small sample size, which represent a potential bias for complex multifactorial analysis. A prospective study would undoubtedly increase our understanding of the relations between F1+2 and CVD. Because few women and no elderly individuals were included, results cannot be extrapolated to other populations at risk of CVD. In the multivariate analysis, we tried to adjust for some, but not all, of the possible confounders that could also influence the F1+2.10,11

In the present study of a group of subjects without clinically overt atherosclerotic disease, we found that plasma F1+2 proved to be significantly associated with carotid IMT. Because F1+2 represents a marker of thrombin generation, our results suggest interrelationships between thrombin generation and progression of atherosclerosis. We tentatively suggest, therefore, that interfering with thrombin generation, as assessed by reduction of F1+2, might be a means of reducing the progression of atherosclerotic disease.

<table>
<thead>
<tr>
<th>Independent Variables</th>
<th>β</th>
<th>SE (β)</th>
<th>P*</th>
<th>Partial R² (%)</th>
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</thead>
<tbody>
<tr>
<td>F1+2 (nmol/L)</td>
<td>0.127</td>
<td>0.039</td>
<td>0.001</td>
<td>6.5</td>
</tr>
<tr>
<td>Sex (F/M)</td>
<td>0.096</td>
<td>0.029</td>
<td>0.001</td>
<td>6.9</td>
</tr>
<tr>
<td>Age (y)</td>
<td>0.003</td>
<td>0.001</td>
<td>0.016</td>
<td>3.1</td>
</tr>
<tr>
<td>SBP (mm Hg)</td>
<td>0.001</td>
<td>0.001</td>
<td>0.023</td>
<td>3.3</td>
</tr>
<tr>
<td>Diabetes (N/Y)</td>
<td>0.143</td>
<td>0.033</td>
<td>0.001</td>
<td>9.5</td>
</tr>
<tr>
<td>Smoking (N/Y)</td>
<td>0.052</td>
<td>0.026</td>
<td>0.047</td>
<td>2.5</td>
</tr>
</tbody>
</table>

*Adjusted for age, sex, BMI, SBP, cholesterol, diabetes and smoking. The model R² for the total population was 30.4%.
Acknowledgments
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References
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