Endothelial Cell Markers and the Risk of Coronary Heart Disease

The Prospective Epidemiological Study of Myocardial Infarction (PRIME) Study

P.E. Morange, MD, PhD; C. Simon, MD, PhD; M.C. Alessi, MD, PhD; G. Luc, MD; D. Arveiler, MD; J. Ferrieres, MD, MSc; P. Amouyel, MD, PhD; A. Evans, MD, FRCP; P. Ducimetiere, PhD; I. Juhan-Vague, MD, PhD; on behalf of the PRIME Study Group

Background—Tissue factor pathway inhibitor (TFPI), von Willebrand factor (vWF), and thrombomodulin (TM) are 3 major hemostatic regulatory molecules synthesized by endothelium. Data from epidemiological studies aiming to evaluate the relation between plasma levels of these molecules and the development of coronary heart disease (CHD) are sparse or contradictory.

Methods and Results—We examined the association between these endothelial-cell markers and the incidence of fatal or nonfatal myocardial infarction (hard CHD) and stable or unstable angina (angina pectoris) in a prospective cohort (the PRIME Study) of nearly 10,000 healthy men recruited in France and Northern Ireland. We measured baseline plasma concentration of the free form of TFPI (f-TFPI), vWF, and the soluble form of TM (sTM) among 296 participants who subsequently developed CHD over the 5-year follow-up (158 with hard CHD and 142 with angina pectoris) and in 563 control subjects by use of a nested case-control design. Individuals with plasma vWF levels in the highest quartile showed a 3.04-fold increase in the risk of hard CHD compared with those in the lowest quartile (95% CI, 1.59 to 5.80). Individuals with f-TFPI levels below the 10th percentile had a 2.13-fold increased risk of hard CHD compared with those with levels above it (95% CI, 1.08 to 4.18). The risk for both molecules persisted after control for inflammatory parameters. Individuals with vWF levels in the highest quartile and f-TFPI levels below the 10th percentile presented a 6.9-fold increased risk of hard CHD compared with those with vWF levels in the lowest quartile and f-TFPI levels above the 10th percentile (95% CI, 1.3 to 37.8).

Conclusions—vWF and f-TFPI plasma levels were independent risk factors for hard CHD events. (Circulation. 2004;109:1343-1348.)

Key Words: endothelium-derived factors ■ epidemiology ■ coronary disease ■ von Willebrand factor

The endothelium is pivotal in the control of hemostasis and thrombosis because it is the primary source of many of the major hemostatic regulatory molecules, such as von Willebrand factor (vWF), tissue factor pathway inhibitor (TFPI), and thrombomodulin (TM).1 vWF mediates platelet adhesion and plays a role in thrombus formation.1 TFPI is the main physiological inhibitor of tissue factor (TF)–induced coagulation.2 Intravascular TFPI exists in several pools.3 The free form of TFPI in plasma (f-TFPI) generally reflects the level of endothelial cell–associated TFPI and displays a potent anticoagulant activity, whereas lipid-bound TFPI seems to have only a small or no anticoagulant function.4–6 TM is an endothelial cell–surface receptor for thrombin that functions as an anticoagulant by greatly accelerating thrombin-induced activation of protein C.7 A soluble truncated form of TM (sTM) circulates in plasma; until now, its physiological role has been unknown.8

The plasma levels of these molecules have been shown to increase with endothelial damage, suggesting that they could be reliable markers of endothelial dysfunction.8–10 Furthermore, an alteration in the production of these molecules by the endothelium could directly contribute to atherothrombotic disease. However, epidemiological data aiming to evaluate the association between plasma levels and the risk of coro-
nary heart disease (CHD) are sparse or contradictory. Several prospective studies of vWF conducted in healthy individuals are available but have produced mixed results. Only a few data are available for TFPI in humans: f-TFPI levels are elevated in patients with unstable angina and increase the risk of a poor outcome. Very recently, a case-control study indicated that low levels of f-TFPI were a risk factor for deep vein thrombosis. Interestingly, mean TFPI levels were not lower in patients with deep vein thrombosis, but f-TFPI levels below the 10th percentile proved to be a risk factor. This finding supported the hypothesis of a threshold effect in which low levels of TFPI increased the risk of thrombosis. To the best of our knowledge, no prospective study data on which low levels of TFPI increased the risk of thrombosis. To find supported the hypothesis of a threshold effect in below the 10th percentile proved to be a risk factor.

Given the lack of homogeneity of the results of these epidemiological studies, we aimed to simultaneously analyze these 3 markers in the same cohort to determine the most predictive ones and their relation with inflammatory markers on risk. We used a nested case-control study design within the Prospective Epidemiological Study of Myocardial Infarction (PRIME) prospective cohort. The PRIME Study is a large multicenter cohort study of men age 50 to 59 years at baseline with a 5-year follow-up, which aimed to investigate the association of different markers and the development of CHD in France and Northern Ireland. Its design afforded us the opportunity to study the predictive ability of parameters for both hard CHD (MI and coronary death) and angina pectoris.

Methods

The PRIME Study has been described in detail. It was set up to investigate risk factors in ischemic heart disease and to identify those explaining the difference in CHD incidence between France and Northern Ireland. From 1991 to 1994, 9758 men age 50 to 59 years with no previous CHD events, living in the area of Lille, Strasbourg, Toulouse, and Belfast, were recruited and followed up over a period of 5 years. At entry, venous blood (9 volumes) was collected between 9 and 10 AM after a 12-hour fast into siliconized vacuum tubes (Vacutainer, Becton Dickinson) containing 0.11 mol/L trisodium citrate (1 volume). Platelet-poor plasma was obtained by centrifugation at 4500g and 20°C for 15 minutes. Without delay, aliquots of plasma were transferred into plastic tubes and frozen on-site to −80°C and were then sent weekly to the central laboratory at the Pasteur Institute of Lille, where they were stored in liquid nitrogen until analysis.

For subjects reporting a possible clinical event, clinical information was sought directly from the hospital or general practitioners’ files. All details of ECG, hospital admissions, enzymes, surgical operations, angioplasty, treatment, etc., were collected and classified according to MONICA criteria. Death certificates were also used to complete information on the cause of death. A medical committee provided independent validation and classification of coronary events. CHD categories retained for analysis were nonfatal MI or coronary death (grouped as hard coronary event), unstable angina pectoris, and effort angina (grouped as angina pectoris). A total of 335 subjects had a confirmed coronary event during follow-up. Each was matched with 2 control subjects (controls). Matched controls were study participants recruited in the same center at approximately the same day (±3 days) and with the same age as the corresponding case subject (case) and free of CHD on the date of the ischemic event of the case.

Table 1 shows the baseline clinical characteristics of the study participants. As expected, initially healthy men who subsequently developed CHD (cases) were more likely at

| TABLE 1. Baseline Patient Characteristics (Mean±SD or Percentage) of Study Participants: The PRIME Study |
|-----------------|-----------------|-----------------|
|                 | Controls (n=563) | Cases (n=296)   | P    |
| Age, y          | 55.2±2.7        | 55.4±2.9        | 0.63 |
| Diabetes, %     | 5.5             | 8.8             | <0.05|
| Hypertension, % | 10.7            | 20.9            | <0.0001|
| Current smokers, % | 29.3           | 39.5            | <0.01|
| Obesity, %      | 13.5            | 20.6            | <0.01|
| High alcohol intake, % | 36.4     | 30.4            | 0.07 |
| Waist/hip ratio | 0.96±0.06       | 0.97±0.06       | <0.01|
| Total cholesterol, mg/dL | 224±42       | 233±40          | <0.01|
| HDL cholesterol, mg/dL | 47±13         | 44±13           | <0.001|
| Triglycerides, mg/dL | 161±127       | 174±128         | 0.16 |
| CRP, mg/L       | 2.39±3.98       | 4.35±10.44      | <0.001|
| IL-6, pg/mL     | 1.82±2.32       | 2.42±2.66       | <0.001|
| Fibrinogen, mg/dL | 3.38±0.99     | 3.62±1.06       | <0.001|
| TNF-α, pg/mL    | 4.81±5.77       | 5.33±9.58       | 0.33 |

P values are derived from conditional logistic regression.

Stored plasma obtained at baseline from 296 cases (158 with hard CHD and 142 with angina pectoris, 4 presenting both types of event during follow-up) and 563 control subjects were sent from the central plasma bank on dry ice to the Laboratory of Hemostasis of La Timone Hospital in Marseille, France. vWF, f-TFPI, and sTM antigens were determined with commercially available ELISAs from Diagnostica Stago. Blood specimens were analyzed in blinded pairs, with the position of the case specimen varied at random within pairs to reduce the possibility of systematic bias and minimize interassay variability. Interassay variation coefficients were 8%, 10%, and 10% for vWF, f-TFPI, and sTM, respectively. The methods used to evaluate baseline lipid parameters, fibrinogen, C-reactive protein (CRP), and interleukin (IL)-6 have been described elsewhere. Tumor necrosis factor (TNF)-α was measured by ELISA (R&D Systems) according to the instructions available from the supplier.

Statistical Analysis

Continuous variables are presented as mean±SD and qualitative variables as percentages. Because of skewed distributions, vWF, f-TFPI, and sTM were categorized before further analyses. Quartiles were used for vWF, f-TFPI, and sTM, whereas f-TFPI was also categorized according to the 10th percentile of the distribution in controls, as previously suggested. A conditional regression analysis suitable for a nested case-control design was performed to identify discriminating predictive parameters. The same analysis was used to determine the relative risk of future CHD events after control for different sets of variables: (1) body mass index (BMI), total cholesterol, HDL cholesterol, systolic blood pressure, smoking, alcohol, and diabetes; (2) further adjustment for CRP, TNF-α, IL-6, and fibrinogen. The final model included all the above variables and vWF, f-TFPI, and TM. Estimated relative risks are presented with 95% CIs.

Associations of endothelial-cell markers with anthropometric characteristics, metabolic parameters, and inflammatory markers were tested by means of Pearson’s correlation coefficient in the control group. The statistical analyses were performed with SAS software (Version 8, SAS Institute). All tests were considered as significant at the 0.05 level.

Results

Table 1 shows the baseline clinical characteristics of the study participants. As expected, initially healthy men who subsequently developed CHD (cases) were more likely at
baseline to have a history of hypertension, smoking, hyperlipidemia, or diabetes compared with men who remained free of reported disease (controls). The effect of these conventional cardiovascular risk factors was similar in France and Northern Ireland. Levels of inflammatory markers such as fibrinogen, CRP, and IL-6 were higher in cases than in controls.

Associations between the different parameters studied were tested in the control subjects. Among these, f-TFPI values were associated positively with BMI (r = 0.14; P < 0.05), waist/hip ratio (r = 0.11; P < 0.05), triglycerides (r = 0.15; P < 0.05), and total cholesterol (r = 0.11; P < 0.05) and negatively with HDL cholesterol (r = 0.10; P < 0.05) but not with CRP, IL-6, or fibrinogen. sTM was associated negatively only with HDL cholesterol (r = 0.16; P < 0.05) and vWF with CRP (r = 0.14; P < 0.05). Endothelial-cell markers correlated strongly with one another. f-TFPI correlated strongly with TM (r = 0.53; P < 0.01) and to a lesser extent with vWF (r = 0.12; P < 0.01). vWF correlated with TM (r = 0.12; P < 0.01).

The mean levels of endothelial-cell markers across the categories of events are shown in Table 2. Baseline levels of vWF were higher in individuals who subsequently developed hard CHD but not angina pectoris. Mean f-TFPI and sTM did not differ significantly between cases and controls irrespective of the type of event. Because it was recently shown that f-TFPI levels below the 10th percentile were a risk factor for venous thrombosis, supporting a threshold effect, we performed a similar analysis in the present study. Of the individuals with hard CHD, 13.3% presented values below the cutoff, compared with 8.3% of the controls (P = 0.07).

The relative risks of CHD during follow-up according to plasma levels of endothelial-cell markers were assessed (Table 3). After adjustment for conventional cardiovascular risk factors, hard CHD incidence was higher in individuals with plasma concentrations of vWF in the highest quartile than in those in the lowest quartile (RR = 3.04; 95% CI 1.59 to 5.80; Table 3, model A). The association remained after further adjustment for inflammatory parameters (Table 3, model B). Individuals with plasma concentrations of f-TFPI below the 10th percentile presented an increased risk of hard CHD compared with those above the 10th percentile (RR = 2.13; 95% CI 0.75 to 6.10; Table 3, model B). As with vWF, the association remained significant after further adjustment for inflammation parameters (Table 3, model C). A decreasing trend, albeit not significant, of the risk of hard CHD in the 2 highest quartiles of f-TFPI was observed. No association was observed between incident hard CHD and sTM, whichever model was used. After inclusion of the 3 endothelial-cell markers in the same model (Table 4), both plasma levels of vWF in the highest quartile and f-TFPI above the 10th percentile remained independently associated with the risk of hard CHD.

### Table 3. Adjusted Relative Risks of Hard CHD During Follow-Up According to Successive Plasma Levels of Each Endothelial Cell Marker: The PRIME Study

<table>
<thead>
<tr>
<th>Model</th>
<th>vWF</th>
<th>f-TFPI</th>
<th>sTM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Model A</td>
<td>2nd quartile</td>
<td>1.47 (0.80–2.69)</td>
<td>1.26 (0.70–2.25)</td>
</tr>
<tr>
<td></td>
<td>3rd quartile</td>
<td>0.95 (0.51–1.77)</td>
<td>0.60 (0.32–1.11)</td>
</tr>
<tr>
<td></td>
<td>4th quartile</td>
<td>3.04 (1.59–5.60)</td>
<td>1.26 (0.70–2.25)</td>
</tr>
</tbody>
</table>

**Note:** Values in quartiles for vWF, f-TFPI, thrombomodulin (sTM), and in two classes for f-TFPI according to the 10th percentile of the distribution in controls. Relative risks (95% CIs) are derived from conditional logistic regression.

**Model A:** Adjusted for BMI, total cholesterol, HDL cholesterol, systolic blood pressure, smoking, alcohol, and diabetes.

**Model B:** Further adjustment for inflammation parameters: fibrinogen, CRP, TNF-α, and IL-6.

*P < 0.05; †P < 0.01.*
TABLE 4. Relative Risks of Hard CHD During Follow-Up According to Plasma Levels of Endothelial Cell Markers: The PRIME Study

<table>
<thead>
<tr>
<th>OR (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>VWF</td>
<td></td>
</tr>
<tr>
<td>1st quartile</td>
<td>1</td>
</tr>
<tr>
<td>2nd quartile</td>
<td>1.16 (0.59–2.28)</td>
</tr>
<tr>
<td>3rd quartile</td>
<td>0.99 (0.48–2.00)</td>
</tr>
<tr>
<td>4th quartile</td>
<td>3.34 (1.59–7.00)</td>
</tr>
<tr>
<td>f-TFPI</td>
<td></td>
</tr>
<tr>
<td>&gt;10th P</td>
<td>1</td>
</tr>
<tr>
<td>&lt;10th P</td>
<td>2.384 (1.59–5.13)</td>
</tr>
<tr>
<td>STM</td>
<td></td>
</tr>
<tr>
<td>1st quartile</td>
<td>1</td>
</tr>
<tr>
<td>2nd quartile</td>
<td>1.43 (0.74–2.76)</td>
</tr>
<tr>
<td>3rd quartile</td>
<td>1.53 (0.78–3.03)</td>
</tr>
<tr>
<td>4th quartile</td>
<td>0.92 (0.46–1.86)</td>
</tr>
</tbody>
</table>

Variables included in the models: BMI, total cholesterol, HDL cholesterol, systolic blood pressure, smoking, alcohol, diabetes, fibrinogen, CRP, TNF-α, IL-6, vWF, f-TFPI, and sTM. Relative risks (95% CIs) are derived from conditional logistic regression.

Given the prothrombotic and antithrombotic properties of endothelial cells, a combined analysis of vWF and f-TFPI on risk was performed (Figure). Individuals with vWF levels in the highest quartile and f-TFPI levels below the 10th percentile exhibited a 6.9-fold increased risk of hard CHD compared with those with vWF levels in the lowest tertile and f-TFPI levels above the 10th percentile (95% CI, 1.3 to 37.8).

Discussion
The main findings of this study are that f-TFPI and vWF plasma levels are independent predictors of hard CHD but not angina pectoris. Individuals with both f-TFPI below the 10th percentile and vWF in the highest quartile presented a 6.9-fold increase in the risk of hard CHD. sTM plasma levels were not associated with CHD whatever the cardiovascular end point studied.

Thompson et al. reported that plasma vWF antigen was an independent predictor of subsequent MI or sudden death from CHD in the ECAT study. In individuals healthy at baseline, a recent meta-analysis yielded a combined odds ratio of 1.5 (95% CI, 1.1 to 2.0). However, among the studies included in this analysis, the predictive ability of vWF depends strongly on the variables controlled for. The precise mechanism by which vWF is associated with cardiovascular risk is unclear. vWF, an acute-phase reactant, could be only a marker of the inflammatory process, which is known to play a major role in CHD. Accordingly, the ECAT study showed in subjects with angina pectoris that the independent relative risk of cardiovascular mortality associated with vWF disappeared after adjustment for variables related to inflammation, i.e., CRP and/or fibrinogen. In contrast, the Hoorn study found that the dual adjustment of vWF and CRP did not alter the risk estimate of coronary death. In the PRIME Study, incident hard CHD was significantly associated with CRP, IL-6, and fibrinogen, but only IL-6 remained significantly associated with hard CHD when the 3 inflammatory markers were included in the same model. In our analysis, we have included these 3 inflammatory markers and TNF-α to confirm that the contribution of vWF to CHD risk is independent of any inflammatory effect. After adjustment for these inflammatory variables, high vWF plasma levels remained a risk factor for hard CHD, suggesting different pathways through which vWF and inflammation determine hard CHD risk.

Several reports have demonstrated the colocalization of TFPI and TF in atherosclerotic plaques, suggesting a significant role for TFPI in the regulation of TF activity. Several studies in mice have shown that TFPI, through the inhibition of tissue factor activity, may influence plaque thrombogenicity. In humans, epidemiological data concerning the association between CHD and TFPI are sparse and based on retrospective studies. Plasma f-TFPI is elevated in patients with unstable angina and increases the risk of an unfavorable outcome. To the best of our knowledge, this is the first prospective study on the association of CHD and f-TFPI. Individuals with f-TFPI levels below the 10th percentile have a 2.13-fold increased risk of hard CHD. Mean f-TFPI level, however, was not lower in patients with hard CHD, supporting the hypothesis of a threshold effect in which moderately reduced f-TFPI level may slightly increase the risk of hard CHD, as previously suggested for venous thrombosis. In the ARIC study, high sTM levels were found to be protective against CHD. The present study did not find the same association between sTM levels and any type of CHD events. The explanation for these different results is not clear but may be because of ethnic or age differences between the 2 populations. The proportion of black people tended to decline with increasing quintile of sTM, and sTM was positively related to age in the ARIC Study. Moreover, for CHD incidence, a significant interaction was found between plasma sTM concentration and age in this latter [The age range at inclusion was larger in the ARIC study (45 to 65 years) than in the PRIME Study (50 to 59 years)].

Endothelium synthesizes both procoagulant (vWF) and anticoagulant (f-TFPI and sTM) factors, suggesting the need for a combined analysis of prothrombotic and antithrombotic factors to provide a more precise estimation of the risk. This
is underlined by the strong correlations observed between the different endothelial-cell markers in the present study. After inclusion of f-TFPI, vWF, and sTM in the same model, f-TFPI below the 10th percentile and vWF in the highest quartile remained independently associated with hard CHD. When these risk factors where considered together, individuals with both f-TFPI below the 10th percentile and vWF in the highest quartile presented a 6.9-fold increased risk of hard CHD. It should be noted that the small number of cases with hard disease constituted a limitation to the analyses of the interaction between these 2 endothelial markers and hard CHD. One of the aims of the PRIME Study is to explain the differences in risk of CHD between France and Northern Ireland. When analyses were performed separately for France and Northern Ireland, the relationships of endothelial-cell markers with hard CHD were similar, although statistical significance was lost because of the smaller number of subjects in each group.

In addition to the clinical manifestations of thrombosis, the contribution of platelets and the coagulation system to the progression of atheroma itself is recognized. However, in the present study, no association was observed between f-TFPI or vWF plasma levels and the risk of angina pectoris. This lack of association could be explained by the fact that the PRIME Study, after 5 years of follow-up, does not yet have sufficient power to detect a modest effect.

We conclude that plasma f-TFPI below the 10th percentile and vWF in the highest quartile are independently associated with the risk of hard CHD. Combined analysis of the 2 factors could provide a more precise estimation of the risk.

Appendix: The PRIME Study Group

The PRIME study is organized under an agreement between IN- SERM and the Merck, Sharpe and Dohme-Chibret Laboratory, with the following participating laboratories:

The Strasbourg MONICA Project, Department of Epidemiology and Public Health, Faculty of Medicine, Strasbourg, France (D. Arveiller, B. Haas).

The Toulouse MONICA Project, INSERM U558, Department of Epidemiology, Paul Sabatier-Toulouse Purpan University, Toulouse, France (J. Ferrières, J.B. Ruidavets).

The Lille MONICA Project, INSERM U508, Institut Pasteur de Lille, France (P. Amouyel, M. Montaye).

The Department of Epidemiology and Public Health, Queen’s University Belfast, Northern Ireland (A. Evans, J. Yarnell).

The Department of Atherosclerosis, INSERM UR545, Lille, France (G. Luc, J.M. Bard, L. Elkhalil, J.C. Fruchart).

The Department of Hematology, Hôpital de la Timone, Marseilles, France (I. Juhan-Vague).

The Laboratory of Endocrinology, INSERM U326, Toulouse, France (B. Perret).

The Vitamin Research Unit, The University of Bern, Bern, Switzerland (F. Gey).

The Trace Element Laboratory, Department of Medicine, Queen’s University Belfast, Northern Ireland (D. McMaster, Jayne Woodside, Ian Young).

The DNA Bank, INSERM U525, Paris, France (F. Cambrien).

The Coordinating Center, INSERM U258, Paris-Villejuif, France (P. Ducimetière, A. Bingham).

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on behalf of the PRIME Study Group

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