Tissue Factor Pathway Inhibitor and Other Endothelium-Dependent Hemostatic Factors in Elderly Individuals With Normal or Impaired Glucose Tolerance and Type 2 Diabetes

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OBJECTIVE — Impaired glucose tolerance (IGT) is believed to be a prediabetic phase that precedes the development of type 2 diabetes. In elderly subjects, IGT and diabetes are both independently associated with the occurrence of cardiovascular disease. Endothelial damage precedes atherosclerotic changes of the vascular wall. Therefore, several markers of endothelial dysfunction were examined in elderly subjects with IGT and elderly patients with type 2 diabetes.

RESEARCH DESIGN AND METHODS — Von Willebrand factor (vWF), tissue plasminogen activator (t-PA), plasminogen activator inhibitor type-1 (PAI-1), and thrombomodulin were studied as markers of endothelial dysfunction in a population-based study of elderly subjects with normal glucose tolerance (NGT) or IGT and type 2 diabetes. In addition to these endothelium-dependent factors, we also investigated tissue factor pathway inhibitor (TFPI) activity in relation to metabolic parameters and cardiovascular risk factors.

RESULTS — All data were adjusted for age. Increased levels of vWF antigen, t-PA antigen, and PAI-1 activity were seen in the IGT and diabetic group compared with the NGT group. TFPI activity and thrombomodulin levels were increased in all elderly subjects, and no differences were seen between the groups. There was a positive association between HbA_{1c} and TFPI activity and vWF antigen. Fasting blood glucose levels correlated with vWF antigen, t-PA antigen, and PAI-1 activity, whereas urine albumin excretion correlated with TFPI activity, vWF antigen, and PAI-1 activity. Serum insulin levels correlated strongly not only with vWF antigen and t-PA antigen but also with PAI-1 activity. This correlation did not change after further adjustment for serum glucose and HbA_{1c}, which may suggest that in the elderly subjects, impaired fibrinolysis is probably associated with insulin resistance. There were no associations between the endothelium-dependent hemostatic factors and lipids, except for a negative correlation between HDL cholesterol and thrombomodulin.

CONCLUSIONS — In elderly subjects with IGT, several endothelium-dependent hemostatic factors are already consistently increased, indicating endothelial damage in this stage.

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Abbreviations: CV, coefficient of variation; ELISA, enzyme linked immunosorbent assay; F_{1+2}, prothrombin fragments 1 + 2; IGT, impaired glucose tolerance; NGT, normal glucose tolerance; PAI-1, plasminogen activator inhibitor type-1; TFPI, tissue factor pathway inhibitor; t-PA, tissue-plasminogen activator; vWF, von Willebrand factor.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

The state of impaired glucose tolerance (IGT) is considered a transitional phase to the development of type 2 diabetes. The prevalence of IGT and type 2 diabetes is increasing with age (1–3). Aging itself is not the only risk factor for atherosclerosis; IGT and diabetes are also independently associated with the occurrence of cardiovascular disease (4,5). Several factors contribute to the development of cardiovascular disease in diabetic and nondiabetic subjects, such as dyslipidemia, (micro)albuminuria, and hypertension (6–12). Hyperinsulinemia as an independent risk factor is controversial (13). It has been shown that endothelial damage precedes atherosclerotic changes of the vascular wall. Therefore, several markers of endothelial (dys)function, such as von Willebrand factor (vWF), tissue plasminogen activator (t-PA), plasminogen activator inhibitor type-1 (PAI-1), and thrombomodulin have been studied in the past to assess whether they can predict the risk of developing cardiovascular disease (14–17). Recently, we and others have demonstrated increased tissue factor pathway inhibitor (TFPI) activity in patients with diabetes (18,19). This seems to be particularly true in patients with diabetes and microalbuminuria (19,20). Because TFPI is mainly produced by and bound to the vascular endothelium, possibly by glycosaminoglycans (21), these data suggest that TFPI may also reflect endothelial (dys)function. In addition, TFPI is partly associated with lipoproteins (22,23). In a population-based study, we studied TFPI activity and other endothelium-dependent factors in elderly subjects with NGT, IGT, and type 2 diabetes.

RESEARCH DESIGN AND METHODS

Subjects

The study was conducted in participants of the Rotterdam Study, a population-

Table 1—*Characteristics of subjects with normal and IGT and type 2 diabetes*

	NGT	IGT	Type 2 diabetes
Sex (M:F)	18:33	23:25	29:18
Age (years)	65 ± 6	69 ± 8*	69 ± 8*
BMI (kg/m^2)	25.5 ± 3	28.0 ± 3.5*	27.3 ± 3.3*
Systolic blood pressure (mmHg)	133 ± 23	150 ± 20*	146 ± 21*
Diastolic blood pressure (mmHg)	75 ± 11	82 ± 9‡	79 ± 12
HbA_{1c} (%)	5.7 ± 0.5	6.0 ± 0.5	7.2 ± 1.3†
Fasting glucose (mmol/l)	5.4 ± 0.3	6.3 ± 0.7*	7.0 ± 3.0†
Glucose 120 min (mmol/l)	5.1 ± 1.2	9.0 ± 0.9*	14.4 ± 3.9†
Fasting serum insulin (mU/l)	9.9 (5.3–22.1)	17.4 (5.8–41.5)*	20 (5.8–55.5)*
Serum insulin 120 min (mU/l)	55 (10.5–189.6)	191.4 (28.3–1,874)*	194.2 (17.0–532.7)*
Total cholesterol (mmol/l)	6.6 ± 1.2	6.6 ± 1.1	6.4 ± 1.1
HDL cholesterol (mmol/l)	1.3 ± 0.4	1.3 ± 0.3	1.3 ± 0.3
Triglycerides (mmol/l)	1.4 (0.8–11.3)	1.4 (0.7–3.6)	1.4 (0.6–3.0)
Albuminuria (mg/day)	5.4 (1.3–261)	6.5 (1.4–105)	7.3 (2.1–685)
Smoking	12	9	7
Retinopathy (men:women)	None	1:2	2:2
Peripheral artery disease	1:4	2:4	5:3
Angina pectoris	None	1:3	2:1
Myocardial infarction	None	1	4:1

Data are means ± SD or median (range). *$P < 0.001$ versus normal; †$P < 0.001$ versus normal and IGT; ‡$P < 0.01$ versus normal.

based prospective cohort study of determinants of chronic disabling diseases in the elderly (24). The baseline examinations were conducted between 1990 and 1993 and included 7,983 subjects (response rate 78%). Informed consent was obtained from all subjects, and the study was approved by the medical ethics committee of the Erasmus University Medical School. The participants were evaluated with respect to microvascular and macrovascular complications (history, physical examination, urinary albumin excretion, and electrocardiography), diabetes control, and lipid profile. Grade of retinopathy was assessed by an ophthalmologist by means of funduscopic examination. During the first follow-up examination of the Rotterdam Study in 1993–1994, a sample of participants aged 55–75 years at baseline was invited to participate in an additional diabetes examination, including an oral glucose tolerance test. The overall response rate for the follow-up examination was 90%; 1,107 subjects participated in the diabetes study. The present study population consists of a random sample of 144 men and women who were divided according to their glucose tolerance at follow-up, using the World Health Organization (WHO) criteria, into a group with NGT, a group with IGT, and a group with diabetes. With the oral glucose tolerance test, 39 cases of newly diagnosed diabetes were identified. The subjects did not use medication, which could influence hemostasis. The characteristics of the subjects studied are shown in Table 1.

Methods

Fasting blood samples for this cross-sectional survey were collected in tubes containing sodium citrate 3.25% (dilution 1:10) for determining TFPI activity, vWF antigen, thrombomodulin, t-PA antigen and PAI-1 activity, HbA_{1c}, and lipid profile at baseline.

TFPI activity was measured using the chromogenic substrate assay according to Sandset after pretreatment with polybrene (expressed in percent with regard to standardized TFPI activity, measured in a plasma pool obtained from 45 healthy donors with a mean age 36 ± 5 years; coefficient of variation [CV] 7.4% at 100% level in our laboratory). In vivo thrombin formation was assessed by determination of prothrombin fragments 1 + 2 (F_{1+2}) using enzyme-linked immunosorbent assay (ELISA; Enzygnost Behring, Marburg, Germany; intra-assay CV 5–7 ± 5%, interassay CV 6–13%). Plasma levels of vWF antigen were determined by an ELISA method using rabbit anti-human vWF (Dako, Denmark; CV 7%, normal range 60–180%). Thrombomodulin (Diagnostica Stago, France; CV 8%, mean 24 ng/ml) and t-PA antigen (Innogenetics, Belgium; CV 7%, normal range 1.3–10.4 ng/ml, mean 4.1 ± 2.4 ng/ml) were measured by enzyme immunoassay. PAI-1 activity was photometrically measured (Kabi Diagnostica, Sweden; intra-assay CV 6%, normal range 1–20 AU/ml, median 8 AU/ml). HbA_{1c} was measured by high-performance liquid chromatography (Diamat; Bio-Rad, Richmond, CA). Serum total cholesterol, HDL cholesterol, and triglyceride levels were determined with enzymatic methods (Unimate 5 and 7; Roche, Basel, Switzerland), and apolipoprotein A1 and apolipoprotein B were measured with immunoturbidimetry (Uni-kit; Roche). An enzymatic hexokinase method (Unimate 5; Roche) was used for measuring serum glucose concentrations. Serum free insulin was measured with a double antibody radioimmunoassay after pretreatment with polyethylene glycol (Pharmacia Diagnostics, Uppsala, Sweden; within-assay CV 3.4–6.1% in the range of 3–50 mU/l). For determination of urinary albumin, an immunoturbidimetric technique was applied (Uni-kit; Roche; interassay CV 3%).

All data are expressed as means ± SD or as medians and ranges when not normally distributed. For comparing more than two groups, ANOVA with Student-Newman-Keuls correction for multiple comparisons was used. If data were not

Table 2—*Hemostatic endothelium-dependent factors and albuminuria by categories of glucose intolerance*

	NGT	IGT	Type 2 diabetes
TFPI-act (%)	111 ± 16	113 ± 15	116 ± 16
vWF (%)	127 ± 30	179 ± 44*	168 ± 83*
t-PA (ng/l)	10.7 ± 3.5	14.0 ± 4.2*	15.4 ± 6.8*
PAI-1 (AU/l)	13 (2–26)	23 (3–53)†	18 (2–80)*
Thrombomodulin	39.7 ± 10.3	44.9 ± 12.8	41.8 ± 15.7
F_{1+2}	1.85 (0.54–9.31)	1.98 (0.49–13.57)	1.47 (0.18–13.57)

Data are means ± SD or median (range). Test for trend, adjusted for age. *$P < 0.001$ vs normal; †$P < 0.01$ versus normal.

normally distributed, the Kruskall-Wallis ANOVA was applied. Categorical variables were analyzed by the χ^2 test. Multivariate logistic regression analyses (based on the maximum-likelihood method) were used to investigate the association of TFPI activity with other endothelium-dependent parameters and cardiovascular risk factors. *P* values ≤0.05 were considered statistically significant.

RESULTS — There were no statistically significant differences in sex among the NGT, IGT, and diabetes groups (Table 1). The mean age, BMI, and systolic blood pressure of the NGT subjects were significantly lower than in the subjects with IGT and diabetes. The diastolic blood pressure was significantly higher in the IGT subjects than in the NGT subjects. Serum insulin levels were higher in the IGT and diabetes groups compared with the NGT group. No differences in serum insulin levels were found between the IGT and diabetic subjects. The lipid profile was similar in the three groups. Because TFPI activity, t-PA antigen, PAI-1 activity, and vWF antigen increase with age, the results were adjusted for age (21,25,26). Smoking did not influence the results. Compared with the NGT subjects, vWF antigen, t-PA antigen, and PAI-1 activity were all significantly higher in the IGT and diabetic subjects (Table 2). TFPI activity was increased in all groups as compared with the younger healthy volunteers, from which the standardized plasma pool was obtained. The TFPI activity was highest in the patients with diabetes, although it was higher in the IGT group compared with the NGT group. However, the differences were small and not statistically significant. Thrombomodulin levels showed the same pattern. Albumin excretion rate was not different among the three groups. In total, 17 subjects had microalbuminuria, of which four had NGT, four had IGT, and nine had diabetes. One patient with diabetes had macroalbuminuria. Also, with regard to the procoagulant state, as measured by the prothrombin F_{1+2} fragments, the three groups did not differ.

When correlating the different hemostatic endothelium-dependent factors with the metabolic parameters, it seemed that TFPI activity, vWF antigen, and PAI-1 activity were significantly positively correlated with HbA_{1c} (Table 3). However, these correlations were moderate. There was a correlation between fasting blood glucose and vWF antigen, t-PA antigen, and PAI-1 activity, whereas serum insulin was strongly correlated with vWF antigen ($r = 0.34$), t-PA antigen ($r = 0.60$), and PAI-1 activity ($r = 0.47$). The strong relation between insulin levels and PAI-1 activity and t-PA antigen remained after further adjustment for serum glucose and HbA_{1c}. Total cholesterol was only correlated with PAI-1 activity. In addition, there was a negative correlation between HDL cholesterol and t-PA antigen, PAI-1 activity, and thrombomodulin. PAI-1 activity and t-PA antigen were positively associated with triglycerides. The partial correlation coefficients between the various endothelial-dependent factors and cardiovascular risk factors are shown in Table 4. In addition to TFPI activity, vWF antigen and PAI-1 activity were positively correlated with the urine albumin excretion. Table 5 shows the interrelationships between the endothelium-dependent factors. PAI-1 activity and thrombomodulin were associated with vWF antigen and t-PA antigen, whereas the latter was also correlated with vWF antigen. There seemed to be a borderline correlation between TFPI activity and vWF antigen and PAI-1 activity.

CONCLUSIONS — In the present study, elderly subjects with IGT were shown to have already increased levels of several endothelium-dependent hemostatic factors, indicating endothelial damage in this stage. Atherosclerotic change of the arterial vascular bed is one of the

Table 3—*Correlations between endothelium-dependent factors and metabolic parameters.*

	TFPI	vWF	t-PA	PAI-1	Thrombomodulin	F_{1+2}
HbA_{1c}	0.18†	0.20†	0.17*	0.16†	−0.11	−0.11
Glucose	0.15*	0.23‡	0.25‡	0.32§	−0.09	−0.09
Insulin	0.11	0.34§	0.60§	0.47§	0.05	−0.06
Glucose 120 min	−0.00	0.21†	0.31§	0.31§	0.08	−0.12
Insulin 120 min	0.05	0.19†	0.39§	0.22†	−0.01	−0.10
Total cholesterol	0.04	−0.10	0.01	0.16†	−0.12	0.03
HDL cholesterol	−0.001	−0.12	−0.33§	−0.22‡	−0.16†	−0.07
Triglycerides	0.07	0.02	0.21†	0.30§	−0.03	−0.04

Data are partial coefficients, adjusted for age. *$P < 0.10$; † $P < 0.05$; ‡ $P < 0.01$; §$P < 0.001$.

Table 4—*Correlations between endothelium-dependent factors and cardiovascular risk factors*

	TFPI	VWF	t-PA	PAI-1	Thrombomodulin	F_{1+2}
Systolic blood pressure	0.05	0.02	0.08	0.17†	0.11	0.10
Diastolic blood pressure	0.03	0.01	0.04	0.17†	0.01	0.19†
Albuminuria	0.16†	0.21†	0.16*	0.22‡	0.13	−0.02
BMI	0.04	0.20†	0.42§	0.45§	0.09	0.13

Data are partial correlation coefficients, adjusted for age. *$P < 0.10$; †$P < 0.05$; ‡$P < 0.01$; §$P < 0.001$.

main events of aging. Disturbances in glucose metabolism accelerate this process of atherosclerosis. Because endothelial damage plays a central role in the pathogenesis of cardiovascular disease, one may thereby assume that in subjects with a disturbed glucose metabolism, more vascular endothelial dysfunction is present in comparison with age-matched healthy control subjects. More vascular dysfunction may thereby be reflected by higher levels of circulating endothelium-derived factors.

We observed significantly increased levels of vWF antigen, t-PA antigen, and PAI-1 activity in the IGT group as well as in the diabetes group as compared with the NGT group. This confirms earlier reports in which it was also suggested that these alterations resulted from damage of endothelial cells (27). Because there were no differences between the IGT and diabetes groups, the increases in levels of vWF antigen, t-PA antigen, and PAI-1 activity could suggest that part of the endothelial damage may already occur in the prediabetic phase. This may explain the reported increased incidence of cardiovascular disease in these two groups. It is known that the levels of vWF antigen, t-PA antigen, and PAI-1 activity are influenced by glucose metabolism (28–30). Compared with the NGT group, fasting glucose was highest in the IGT and diabetes groups, whereas HbA_{1c} was significantly higher in the subjects with type 2 diabetes. Correlation analysis showed a positive association not only between HbA_{1c} and vWF antigen but also between fasting blood glucose and vWF antigen, t-PA antigen, and PAI-1 activity. In addition, serum insulin correlated also with these three endothelial hemostatic factors. In particular, there was a strong relation between insulin levels and PAI-1 activity and t-PA antigen, which remained after further adjustment for serum glucose and HbA_{1c}. These findings suggest that higher insulin levels, and therefore probably insulin resistance, are associated with impaired fibrinolysis in elderly subjects with or without diabetes.

Thrombomodulin levels were increased in the three groups, but no differences between the groups could be demonstrated. However, thrombomodulin did not correlate with the risk factors or any of the metabolic parameters, with the exception of HDL cholesterol. This is in contrast to an earlier report in which no correlation between lipids and thrombomodulin was found (31), whereas others had the same findings (32).

Although we found a slightly higher level of TFPI activity in the IGT and diabetes groups, this difference was not statistically significant. However, we were able to demonstrate a significant association between TFPI activity and HbA_{1c}. This is in agreement with the findings of Kario et al. (19), who also found increased TFPI antigen levels in patients with type 2 diabetes, especially those with overt albuminuria, compared with healthy control subjects. In the present study, a significant correlation between TFPI activity and the albumin excretion rate could also be demonstrated. Because microalbuminuria is a sign of generalized angiopathy, TFPI may be a marker of this condition. The same correlation could also be demonstrated between albuminuria and vWF antigen and PAI-1 activity. However, in comparison with type 1 diabetes, type 2 diabetes is considered a different disease regarding vascular complications. Although type 2 diabetes is characterized by the occurrence of macrovascular complications, in patients with type 1 diabetes, mainly microvascular complications are observed before macrovascular abnormalities occur. TFPI expression is restricted to the endothelium of the microvasculature and is not believed to be synthesized by the endothelium of larger vessels (33). This may explain, in part, why no significant differences between the diabetes and NGT groups were found in the present study. In addition, the elderly subjects in the present study population seemed to be relatively free of cardiovascular disease. In addition, only a total of 17 subjects in all three groups had microalbuminuria, whereas one subject with diabetes had macroalbuminuria. The presence of extensively generalized angiopathy would therefore be less likely. Others found increased levels of TFPI only in patients with type 2 diabetes and overt albuminuria (19).

It is widely accepted that in patients with diabetes, a procoagulant state can be found (34,35). Because TFPI is a coagulation inhibitor, one may suggest that TFPI activity is influenced by this procoagulant state. In an earlier study of patients with type 1 diabetes, no correlation between a procoagulant status and TFPI activity could be demonstrated (20). In the present study, we were unable to show a difference in procoagulation, as measured by the prothrombin fragments 1 + 2 (F_{1+2}), between the three groups. There

Table 5—*Correlations between endothelium-dependent factors*

	TFPI	VWF	t-PA	PAI-1	Thrombomodulin
vWF	0.14*				
t-PA	0.02	0.42§			
PAI-1	0.14*	0.25‡	0.48§		
Thrombomodulin	−0.05	0.22‡	0.18†	−0.04	
F_{1+2}	−0.002	−0.03	0.03	−0.09	0.10

Data are partial correlation coefficients, adjusted for age. *$P < 0.10$; †$P < 0.05$; ‡$P < 0.01$; §$P < 0.001$.

was also no significant correlation between TFPI activity and the F_{1+2} fragments in this patient population. Others were also unable to demonstrate a correlation between TFPI and factor VIIa levels (19). In addition, we could not find a relation between F_{1+2} and the other endothelial hemostatic factors.

Dyslipidemia is a well-recognized risk factor for cardiovascular disease in subjects with IGT and type 2 diabetes (4,6). Because TFPI in plasma is mainly associated with lipoproteins, especially with LDL and HDL (22,23), TFPI activity could be influenced by the levels of lipoproteins. The levels of lipoproteins were similar in the three groups, and no significant relationship between TFPI activity and lipids was found. This is in agreement with our earlier report of patients with type 1 diabetes, whereas others have found a positive relation in nondiabetic hypercholesterolemic subjects (18,36). Only PAI-1 activity was associated with total cholesterol, whereas there was a negative correlation between HDL cholesterol and t-PA antigen, PAI-1 activity, and thrombomodulin.

The endothelium-dependent factors are also known to be affected by blood pressure (37–39). The systolic blood pressure was higher in the subjects with IGT and diabetes, whereas the diastolic blood pressure was higher in the IGT group. However, the systolic and diastolic blood pressures were only positively correlated with PAI-1 activity. Obesity is another well-known risk factor for cardiovascular complications (40). The BMI was higher in the IGT and diabetes groups. In addition, a significant positive correlation between BMI and t-PA antigen, PAI-1 activity, and vWF antigen could be demonstrated. This is in concordance with the findings of earlier studies (41–43). In contrast to Vambergue et al. (44), we could not find a correlation between BMI and TFPI activity.

We concluded that several hemostatic endothelium-dependent factors are already increased in elderly subjects, relatively free of cardiovascular disease, with IGT, which is believed to precede the development of type 2 diabetes. This seems to be especially true for vWF antigen, t-PA antigen, and PAI-1 activity. TFPI activity and thrombomodulin levels were increased in all elderly subjects with NGT or IGT and type 2 diabetes, but there were no differences among the groups. However, the positive correlation between HbA_{1c} and TFPI activity may indicate an influence of overall glycemic control on TFPI activity. Because of the strong correlation between PAI-1 activity and t-PA antigen and insulin levels after adjustment for glucose and HbA_{1c}, one may hypothesize that endothelial dysfunction may precede insulin resistance in elderly individuals.

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