Tissue Plasminogen Activator, von Willebrand Factor, and Risk of Type 2 Diabetes in Older Men

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OBJECTIVE — The objective of this study was to assess the relationship between putative markers of endothelial dysfunction (tissue plasminogen activator [t-PA] antigen and von Willebrand factor [vWF] antigen) and development of type 2 diabetes, as well as the role of inflammation, adipokines, hepatic function, and insulin resistance in modifying these relationships.

RESEARCH DESIGN AND METHODS — This was a prospective study of 3,562 nondiabetic men aged 60–79 years followed up for an average of 7 years during which there were 162 incident cases of type 2 diabetes.

RESULTS — Elevated t-PA (top third) was associated with a near threefold increase in risk of diabetes compared with the risk in those in the bottom third after adjustment for lifestyle factors and waist circumference (relative risk [RR] 2.98 [95%CI 1.79–5.00]; $P_{trend} < 0.0001$); weaker but significant (marginal) associations were seen with vWF (1.24 [0.83–1.85]; $P = 0.05$ for trend). Both biomarkers of endothelial dysfunction correlated significantly with markers of inflammation (interleukin-6 [IL-6] and C-reactive protein [CRP]), hepatic function (γ-glutamyl transferase [GGT]), and insulin resistance, with t-PA showing stronger associations with adiposity, hepatic function, and insulin resistance than vWF. t-PA was also significantly and inversely associated with adiponectin. Adjustment for IL-6, adiponectin, and GGT attenuated the association of incident diabetes with vWF (1.06 [0.71–1.60]), but the relationship seen with t-PA remained significant (adjusted RR 2.19 [1.29–3.70]). Subsequent adjustment for insulin attenuated the association further, but t-PA was still associated with a significant increase in risk (1.66 [0.96–2.85]; $P_{trend} = 0.02$).

CONCLUSION — t-PA antigen, but not vWF antigen, is independently associated with risk of type 2 diabetes.

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Endothelial dysfunction plays a pivotal role in the development of atherosclerosis (1) and may be one of the underlying causes (or "common soil") of both coronary heart disease (CHD) and type 2 diabetes (2). There is substantial evidence suggesting a potential role for endothelial dysfunction in insulin resistance, although the relationship is almost certainly bidirectional (3). A number of prospective studies have shown that circulating biomarkers of endothelial dysfunction such as E-selectin, intercellular adhesion molecule 1, von Willebrand factor (vWF), and, in particular, plasminogen activator inhibitor type 1 (PAI-1) predict incident diabetes (4–9), although the findings have not always been consistent. Some studies have shown the above associations to be independent of inflammation and insulin resistance (6,7). Adipokines, in particular low adiponectin, and hepatic function as measured by the hepatic enzymes alanine aminotransferase (ALT) and γ-glutamyl transferase (GGT) have been associated with risk of subsequent type 2 diabetes (10–13). Adipokines and liver dysfunction have also been linked to PAI-1 and markers of endothelial dysfunction (14–16). Few population studies have addressed the associations between liver dysfunction and markers of endothelial dysfunction. Whether the association between markers of endothelial dysfunction and diabetes is independent of adipokines and hepatic function has not been studied, but it is important to do so to assess possible residual confounding.

Tissue plasminogen activator (t-PA) is released from vascular endothelium; hence, circulating levels of t-PA antigen may be a marker of endothelial dysfunction (17). However, a higher plasma t-PA antigen level represents largely inactive circulating t-PA–PAI-1 complexes, which, in turn, may reflect both endothelial disturbance (t-PA and PAI-1 release) and hepatic PAI-1 release (17). An elevated t-PA antigen level is considered to be an integral feature of the insulin resistance syndrome and is also related to the inflammatory response (18). The results of one population study suggested that t-PA is predictive of future diabetes independent of the metabolic syndrome (19), although this study was limited in the small number of incident cases, and the findings were not statistically significant. This question is worthy of study because t-PA is more stable than PAI-1 and also appears to be more strongly linked to incident CHD events in population cohorts (17). We have therefore examined the relationships between t-PA and vWF (two circulating markers of endothelial dysfunction) and the risk of incident type 2 diabetes and have assessed whether these associations are independent of adiponectin, hepatic function as measured

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Abbreviations: ALT, alanine transaminase; CHD, coronary heart disease; CRP, C-reactive protein; ELISA, enzyme-linked immunosorbent assay; GGT, γ-glutamyl transferase; HOMA-IR, homeostasis model assessment of insulin resistance; IL, interleukin; PAI-1; plasminogen activator inhibitor type 1; t-PA, tissue plasminogen activator antigen; vWF, von Willebrand factor.

by ALT and GGT levels, inflammation as measured by levels of C-reactive protein (CRP) and interleukin-6 (IL-6), and insulin resistance.

RESEARCH DESIGN AND METHODS

— The British Regional Heart Study is a prospective study of cardiovascular disease involving 7,735 men aged 40–59 years selected from the age-sex registers of one general practice in each of 24 British towns, who were screened between 1978 and 1980 (20). In 1998–2000, all surviving men, now aged 60–79 years, were invited for a 20th year follow-up examination. All relevant local research ethics committees provided approval. All men provided informed written consent for the investigation, which was performed in accordance with the Declaration of Helsinki. They completed a questionnaire (Q20) that included questions on their medical history, lifestyle behavior, and family history of diabetes. The men were requested to fast for a minimum of 6 h, during which time they were instructed to drink only water and then to present for measurement at a specified time between 0800 and 1800 h. All men were asked to provide a blood sample, collected using the Sarstedt Monovette system. A total of 4,252 men (77% of survivors) presented for examination.

Cardiovascular risk factors

Details of measurement and classification methods for smoking status, physical activity, BMI, social class, alcohol intake, blood pressure, and blood lipids in this cohort have been described (20–22). Anthropometric measurements including body weight, height, and waist circumference were obtained. Subjects were measured in light clothing without shoes in the standing position. BMI (weight in kilograms divided by height in meters squared) was calculated for each man. From the combined information at the initial screening and follow-up questionnaires in 1996 (Q96) and at rescreening (Q20), the men were classified into five cigarette-smoking groups: *1*) those who had never smoked; *2*) ex-smokers since screening; *3*) smokers at baseline who gave up between screening and Q96; *4*) smokers at baseline and at Q96 who gave up after 1996; and *5*) current cigarette smokers at Q20. The longest held occupation of each man was recorded at screening, and the men were grouped into one of six social classes: I, II, or III nonmanual (nonmanual groups); III manual; and IV and V (manual groups). Those whose longest occupation was in the armed forces formed a separate group. Heavy drinking was defined as >5 drinks/day. On the basis of a physical activity score (21), the men were classified into four groups: inactive, light, moderate, and moderately vigorous/vigorous. Plasma glucose was measured by a glucose oxidase method using a Falcor 600 automated analyzer. Serum insulin was measured using an enzyme-linked immunosorbent assay (ELISA) that does not cross-react with proinsulin. Triglycerides, blood glucose, and insulin concentrations were adjusted for the effects of fasting duration and time of day (22). Insulin resistance was estimated according to homeostasis model assessment (HOMA-IR) (the product of fasting glucose [millimoles per liter] and insulin [units per milliliter] divided by the constant 22.5) (23). Plasma levels of t-PA antigen were measured with an ELISA (Biopool AB, Umea, Sweden), as was vWF antigen (DAKO, High Wycombe, U.K.) (21). CRP was assayed by ultrasensitive nephelometry (Dade Behring, Milton Keynes, U.K.). Plasma adiponectin concentrations were determined using an ELISA (R&D Systems, Oxford, U.K.) (10). IL-6 was assayed using a high-sensitivity ELISA (R&D Systems) (10). Hepatic enzymes including GGT and ALT were measured using a Hitachi 747 automated analyzer.

Study subjects

Endothelial marker measurements (t-PA or vWF) were available for 4,083 men at Q20. Men with a doctor's diagnosis of diabetes, men with a diagnosis of diabetes in the year of reexamination, and those with a fasting glucose of >7 mmol/l (World Health Organization criteria) were considered to have prevalent diabetes and were excluded ($n = 484$). We further excluded men with missing data for IL-6 ($n = 37$). Thus, analysis is based on 3,562 men.

Follow-up

All men were followed up for all-cause mortality, cardiovascular morbidity, and development of type 2 diabetes from the initial examination to July 2006 (24), and follow-up was achieved for 99% of the cohort. This analysis is based on follow-up from rescreening in 1998–2000, a mean follow-up period of 7 years (6–8 years). Information on deaths was collected through the established tagging procedures provided by the National Health Service registers. Evidence regarding diabetes was obtained by reports from general practitioners with biennial reviews of the patients' notes (including hospital and clinic correspondence) through to the end of the study period. Cases of diabetes are based on self-reported diagnoses confirmed by primary care records, an approach that has been validated in the present study (25).

Statistical methods

The men were divided by tertiles of the inflammatory markers. The Cox proportional hazards model was used to assess the multivariate-adjusted relative risk for each third compared with the reference group (lowest third). In the adjustment, smoking (never, long-term ex-smokers [>15 years], recent ex-smokers, and current smokers), social class (seven groups), physical activity (four groups), alcohol intake (five groups), parental history of diabetes (yes or no), preexisting CHD (yes or no) and stroke (yes or no), and use of statins (yes or no) were fitted as categorical variables. Waist circumference, HOMA-IR, GGT, adiponectin, IL-6, and CRP were fitted as continuous variables.

RESULTS

— During the mean follow-up period of 7 years (range 5–7 years) there were 162 incident cases of diabetes in the 3,562 nondiabetic men. Table 1 shows the baseline characteristics in the men who developed diabetes and in men who remained free of diabetes. Men who developed diabetes had higher BMI and waist circumference than those who did not. They were also more likely to be physically inactive, to have a parental history of diabetes, to have a higher prevalence of CHD, to have higher levels of metabolic risk factors, and to have significantly higher levels of markers of inflammation (CRP and IL-6), endothelial dysfunction markers (vWF and t-PA), and hepatic enzymes (GGT and ALT) and lower adiponectin levels than men who did not develop diabetes.

Table 2 shows the correlations between endothelial dysfunction markers and their correlations with metabolic risk factors, inflammation, adiponectin, and hepatic enzymes. t-PA antigen was strongly correlated with central adiposity (waist circumference) and was significantly associated with all components of the metabolic syndrome, insulin resistance, adiponectin, and hepatic enzymes. These associations persisted even after adjustment for waist circumference (Table

Table 1—*Distribution of risk factors and inflammatory/hemostatic markers in 3,562 nondiabetic subjects aged 60–79 years at reexamination according to diabetes status at follow-up*

	Developed diabetes		*P*
	No	Yes	for difference
n	3,400	162	
Age (years)	68.7 (5.6)	68.4 (5.4)	0.56
BMI (kg/m^2)	26.6 (3.5)	29.7 (4.1)	<0.0001
Waist circumference (cm)	96.2 (9.9)	104.1 (10.4)	<0.0001
Current smokers (%)	13.3	9.3	0.14
Inactive (%)	32.4	45.2	0.0008
Manual (%)	53.1	64.6	0.004
Heavy drinkers (>5 drinks/day) (%)	3.7	2.5	0.42
Parental history of diabetes (%)	5.4	9.9	<0.0001
CHD (%)	17.6	32.1	<0.0001
Use of statins (%)	6.5	13.6	0.0005
Stroke (%)	5.0	6.2	0.57
SBP (mmHg)	148.2 ± 23.9	153.0 ± 22.7	0.01
Triglyceride (mmol/l)	1.57 (1.12–2.13)	2.10 (1.54–2.81)	<0.0001
HDL (mmol/l)	1.34 ± 0.34	1.17 ± 0.29	<0.0001
Glucose (mmol/l)	5.52 (5.21–5.89)	5.99 (5.59–6.52)	<0.0001
Log HOMA-IR	0.65 ± 0.58	1.27 ± 0.57	<0.0001
CRP (mg/l)	1.67 (0.80–3.30)	2.59 (1.27–4.42)	<0.0001
IL-6 (pg/ml)	2.41 (1.55–3.42)	2.92 (1.93–4.23)	<0.0001
Adiponectin (μg/ml)	7.11 (4.52–11.46)	4.96 (3.11–7.39)	<0.0001
GGT (IU/l)	27.4 (18–37)	37.0 (24–52.5)	<0.0001
ALT (IU/l)	15.2 (12–20)	19.5 (13.5–28.0)	<0.0001
vWF (IU/dl)	137.8 ± 44.4	146.4 ± 48.1	0.02
t-PA (ng/ml)	10.74 ± 4.21	13.37 ± 4.79	<0.0001

Data are means ± SD or geometric means (interquartile range) for skewed variables. SBP, systolic blood pressure.

2). vWF showed weaker but significant associations with waist circumference, hepatic enzymes, and insulin resistance. Less consistent associations were seen for the metabolic risk factors. All showed similar significant associations with inflammatory markers (CRP and IL-6).

Table 3 shows the incidence rates for diabetes and the relative risk of diabetes by tertiles of the biomarkers of endothelial dysfunction with adjustment for demographic factors (age, social class, smoking, physical activity, alcohol intake, parental history of diabetes, preexisting CHD, and use of statins) and the effects of adjustment for waist circumference, inflammation (IL-6), adiponectin, hepatic enzymes (GGT), and insulin resistance (HOMA-IR). IL-6 was used in the adjustment, as IL-6 and CRP are strongly correlated ($r = 0.57$), and CRP showed no independent association with diabetes after adjustment for IL-6. Similarly GGT and ALT are strongly correlated ($r = 0.43$), and GGT was a stronger predictor of diabetes than ALT (17) and showed stronger associations with the endothelial dysfunction markers.

Elevated t-PA was associated with a >2-fold increase in risk of diabetes even after adjustment for demographic factors and waist circumference (model 2). Waist circumference and BMI are highly correlated ($r = 0.86$). The findings were virtually unchanged if BMI was included in the adjustment instead of waist circumference. A weaker association was seen between vWF and diabetes. To further assess whether the relationship between diabetes and markers of endothelial dysfunction was independent of inflammation, adipocytes, and hepatic enzymes, we adjusted in turn for adiponectin, IL-6, and GGT. Simultaneous adjustment for these factors attenuated the associations, but the increased risk of diabetes associated with elevated t-PA remained significant. No association was seen with vWF after adjustment.

Insulin resistance could mediate the association between t-PA and risk of diabetes. Adjustment for HOMA-IR in addition to IL-6 and adiponectin (model 3) considerably attenuated the relationships for t-PA, but it remained significantly associated with increased risk of diabetes. When GGT and HOMA-IR were both included in the adjustment (model 3 + GGT + HOMA-IR), elevated t-PA was still associated with a 60% increase in risk.

The increased risk of diabetes associated with elevated t-PA was seen in both obese and centrally obese (waist circumference >102 cm or BMI >30 kg/m^2) and nonobese (waist ≤102 cm) men and in those with normal glucose levels (<6.1 mmol/l) and impaired glucose levels (>6.1 mmol/l) (data not shown). No significant interaction was seen between obesity or glucose levels and t-PA and risk of diabetes ($P = 0.41$ and $P = 0.50$ for obesity and high glucose, respectively).

CONCLUSIONS — In this large random sample of nondiabetic British men aged 60–79 years, we have confirmed the findings of several previous studies (4–9) that circulating markers of endothelial dysfunction are associated with incident diabetes. We have shown that t-PA antigen, but not vWF antigen, is independently associated with the development of diabetes in men. Our findings extend those of other studies on circulating endothelial markers and risk of type 2 diabetes by assessing a wider range of risk factors and adjusting for correlates reflective of perturbances in other relevant pathways including markers of inflammation and insulin resistance as well as adiponectin and hepatic enzymes, which have not previously been assessed or comprehensively adjusted for. The association between vWF and diabetes was largely dependent on inflammation. Adjustment for IL-6 attenuated the association between vWF and risk of diabetes. In contrast, the association between t-PA antigen and diabetes was independent of markers of adiposity or inflammation, adiponectin, and hepatic enzymes, despite being correlated with markers in each pathway. The increased risk associated with elevated t-PA partially reflected insulin resistance, but there still remained a 60% increase in risk even after adjustment for HOMA-IR. We considered vWF and t-PA to reflect endothelial dysfunction; however, the modest correlation between these two biomarkers ($r = 0.21$) and the differing associations seen between t-PA and vWF with insulin resistance and features of the metabolic syndrome support the view that these endothelial biomarkers are regulated differently. Therefore, one should be cautious in labeling such markers equally or purely under the endothelial function umbrella.

Table 2—*Spearman correlation coefficients of endothelial markers with metabolic and anthropometric variables, inflammatory markers, liver enzymes, and adiponectin*

	t-PA				vWF			
	Unadjusted		Adjusted for WC		Unadjusted		Adjusted for WC	
	r	*P*	*r*	*P*	*r*	*P*	*r*	*P*
t-PA	1.00				0.21	<0.0001	0.20	<0.0001
vWF	0.21	<0.0001	0.20	<0.0001	1.00			
Metabolic factors								
WC	0.31	<0.0001			0.06	<0.001		
HDL cholesterol	−0.20	<0.0001	−0.12	<0.0001	−0.07	<0.001	−0.04	<0.01
Triglycerides	0.34	<0.0001	0.26	<0.0001	0.03	NS	0.02	NS
SBP	0.09	<0.0001	0.07	<0.0001	−0.01	NS	−0.02	NS
Glucose	0.13	<0.0001	0.11	<0.0001	0.04	NS	0.03	NS
HOMA-IR	0.35	<0.0001	0.25	<0.0001	0.11	<0.0001	0.10	<0.01
Inflammatory markers and adiponectin								
CRP	0.25	<0.0001	0.20	<0.0001	0.26	<0.0001	0.25	<0.0001
IL-6	0.20	<0.0001	0.16	<0.0001	0.25	<0.0001	0.24	<0.0001
Adiponectin	−0.15	<0.0001	−0.12	<0.0001	0.06	<0.001	0.07	<0.001
Liver enzymes								
ALT	0.17	<0.0001	0.11	<0.0001	0.005	NS	−0.005	NS
GGT	0.31	<0.0001	0.27	<0.0001	0.13	<0.0001	0.12	<0.0001

SBP, systolic blood pressure; WC, waist circumference.

vWF is produced mainly by vascular endothelial cells activated by proinflammatory cytokines (26). Although vWF was shown to be predictive of diabetes independent of a marker of inflammation (CRP) and insulin in the Framingham Offspring Study (7), others have reported no association (8) or no independent association between vWF and diabetes (27). Our results, generated in a more homogeneous population of predominantly white men, suggest that the association between vWF and diabetes is explained by levels of the proinflammatory cytokine IL-6, which is known to be associated with incident diabetes (10) and which stimulates release of vWF from vascular endothelium (28). Thus, any link between vWF and incident diabetes may be indirect rather than direct.

Circulating t-PA antigen reflects not only endothelial synthesis and release of t-PA but also circulating complexes of t-PA with its major inhibitor, PAI-1 (17), which is synthesized by adipocytes, hepatocytes, and endothelial cells and is also an acute-phase reactant. t-PA (and PAI-1) levels increase as part of the inflammatory response (29), and in the present study t-PA correlated with several inflammatory markers including CRP and IL-6. Numerous studies have shown PAI-1 to be predictive of type 2 diabetes (4,6–8) and, in

Table 3—*Incidence rates and adjusted relative risk of type 2 diabetes according to thirds of inflammatory and endothelial markers*

	Thirds			
Endothelial markers	Lowest	Middle	Top	P_{trend}
t-PA (ng/ml)	<8.7	8.7	12.1	
Rate/1,000 person-years	2.6	6.3	11.6	
Model 1	1.00	2.36 (1.40–3.99)	4.54 (2.76–7.47)	<0.0001
Model 2	1.00	1.89 (1.12–3.20)	2.98 (1.79–5.00)	<0.0001
Model 3	1.00	1.81 (1.07–3.07)	2.65 (1.58–4.45)	<0.0001
Model 3 + GGT	1.00	1.65 (0.97–2.81)	2.19 (1.29–3.70)	0.0003
Model 3 + HOMA-IR	1.00	1.35 (0.78–2.32)	1.92 (1.12–3.27)	0.001
Model 3 + GGT + HOMA-IR	1.00	1.25 (0.72–2.16)	1.66 (0.96–2.85)	0.02
vWF (IU/dl)	<115	115	155	
Rate/1,000 person-years	5.8	6.1	8.4	
Model 1	1.00	1.04 (0.70–1.56)	1.39 (0.94–2.03)	0.007
Model 2	1.00	0.94 (0.62–1.41)	1.24 (0.83–1.85)	0.05
Model 3	1.00	0.91 (0.61–1.38)	1.18 (0.79–1.78)	0.11
Model 3 + GGT	1.00	0.87 (0.57–1.31)	1.06 (0.71–1.60)	0.31
Model 3 + HOMA-IR	1.00	0.87 (0.57–1.32)	1.10 (0.73–1.66)	0.27
Model 3 + GGT + HOMA-IR	1.00	0.84 (0.55–1.28)	1.01 (0.67–1.53)	0.49

Data are relative risk (95% CI). Model 1, adjusted for age, social class, smoking, alcohol intake, physical activity, parental history of diabetes, preexisting CHD, stroke, and use of statins; model 2, model 1 plus waist circumference; and model 3, model 2 plus adiponectin and IL-6.

some, to be independent of adiposity and markers of inflammation (6,7). Similar relationships were seen for t-PA in this study, as one might expect, given its strong correlation with PA1-I (26). Inflammation and adiponectin appeared to have only modest attenuating effects on the relationship between t-PA and diabetes. In our previous report we have shown that IL-6 (positively) and adiponectin (inversely) are predictive of diabetes (11). Because t-PA levels correlated with IL-6 and adiponectin, t-PA may explain the positive relationship between IL-6 and diabetes seen in this and other studies and the significant inverse association seen between adiponectin and diabetes in obese men (11). However, these relationships remained significant after adjusting for t-PA. Thus, the associations between t-PA, adipokines (IL-6 and adiponectin), and diabetes appear to be independent of each other.

Insulin has been shown to stimulate expression of PAI-1 and t-PA (30). t-PA was associated with insulin resistance and all components of the metabolic syndrome, and the relationship between t-PA and diabetes was to some extent explained by insulin resistance, but there remained a significant independent association after adjustment for HOMA-IR. Clearly, more direct measures of insulin resistance, e.g., clamping, may have attenuated the relationship between t-PA and incident diabetes further. Indeed, we have previously reported strong associations between clamp-measured insulin resistance and the t-PA antigen concentration in women with polycystic ovary syndrome ($r = -0.59$; $P < 0.05$) and healthy control subjects ($r = -0.62$; $P < 0.05$) (31). Thus, t-PA antigen concentrations, partly by virtue of strong correlations with insulin resistance, may be useful as part of a multiple biomarker portfolio to predict future diabetes risk.

The association of t-PA and diabetes may also partially occur via common associations with liver disturbance. Indeed, metformin, which is thought to work principally at the liver, has been consistently shown to lower t-PA concentrations in different groups and to do so in correlation with improvements in insulin resistance measures (32). The association between t-PA and diabetes was attenuated further but not eliminated after inclusion of both insulin resistance and hepatic function in the adjustment. Prospective studies relating t-PA to diabetes are extremely limited. Our finding that t-PA predicted diabetes is consistent with a much smaller previous report of t-PA in the Northern Sweden Monitoring of Trends and Determinants in Cardiovascular Disease (MONICA) Study (19), which also considered only a limited number of potential confounders. Thus, our work extends the available data on the link between t-PA and incident diabetes.

Our study is not without some limitations. It was performed in an older, predominantly white Caucasian male population, and we cannot generalize our findings to women, younger men, or other ethnic groups, although, as noted above, t-PA has been shown to be strongly correlated with measured insulin resistance in women with polycystic ovary syndrome and control subjects (31), suggesting that the t-PA–diabetes relationship is likely to be seen in women as well. Diabetes incidence in this study was determined by documented doctor-diagnosed cases of diabetes, which would inevitably result in underascertainment of cases. It may be argued that those with elevated markers of endothelial dysfunction are more likely to be obese and to have had contact with their general practitioner and thus are more likely to have had diagnostic tests for diabetes. However, among the prevalent diabetic subjects at rescreening who had been excluded, t-PA and vWF were positively associated with both diagnosed diabetes (physician diagnosis) and undiagnosed diabetes (using fasting glucose concentration criteria of >7.0 mmol/l). Thus, the increased risk associated with elevated markers of endothelial dysfunction is unlikely to be due to bias in ascertainment. Finally we did not measure PAI-1 directly, but note that t-PA is the more stable marker (33).

Circulating levels of t-PA (17) and vWF (26) are associated with risk of CHD, and their elevations in individuals with type 2 diabetes (34) might therefore promote their atherothrombotic complications. The present study adds to the literature on the potential importance of t-PA in the development of diabetes. We suggest that further detailed studies of endothelial markers (in particular t-PA) and risk of diabetes be performed to clarify their value in prediction of diabetes, ideally in combination with other markers, and their possible roles in pathogenesis.

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