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Postprandial thrombin activatable fibrinolysis inhibitor and markers of endothelial dysfunction in type 2 diabetic patients

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Abstract

The aim of this study was to assess postprandial changes in thrombin activatable fibrinolysis inhibitor (TAFI) antigen, a thrombin-dependent fibrinolysis inhibitor with anti-inflammatory properties, and soluble markers of endothelial dysfunction in normotriglyceridemic type 2 diabetic patients. Fasting and postprandial TAFI antigen, thrombomodulin, tissue factor pathway inhibitor (TFPI), and plasminogen activator inhibitor 1 were assessed in 12 normotriglyceridemic type 2 diabetic patients treated with diet (hemoglobin A_{1c}, 6.80% ± 0.67%) and 14 controls. Fasting low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, free fatty acids and apolipoprotein B, and fasting and postprandial triglyceride, glucose, and insulin were also measured. Fasting TAFI was higher in the control group (102% ± 16.9% vs 72.9% ± 15.9%; $P < .0005$) and was inversely correlated with glycemic control. It decreased 4 hours after the meal (31.8% reduction [$P < .005$] for controls and 12.6% [$P < .05$] for diabetic patients) and returned to fasting levels after 8 hours. This decrement was correlated with fasting TAFI, glucose and hemoglobin A_{1c}, and the area under the curve of glucose. Thrombomodulin, TFPI, and plasminogen activator inhibitor 1 were similar in both groups, with thrombomodulin and TFPI showing a transient postprandial increase. A fat-rich meal produces a transient increase in markers of endothelial dysfunction and a temporary reduction in TAFI, an anti-inflammatory molecule whose concentration is low in type 2 diabetes mellitus.

1. Introduction

Endothelial cells produce several substances involved in the regulation of thrombosis and fibrinolysis. The soluble forms of some of these substances, such as thrombomodulin (TM), tissue factor pathway inhibitor (TFPI), and the plasminogen activator inhibitor 1 (PAI-1), have been proposed as markers of endothelial damage [1-3]. Thrombin activatable fibrinolysis inhibitor (TAFI) is a glycoprotein that, once activated by the thrombin-TM complex, inhibits fibrinolysis by decreasing plasminogen binding on the surface of partially degraded fibrin and that has recently been described to have anti-inflammatory properties [4,5]. The ingestion of either fat or glucose is followed by a reversible postprandial impairment in endothelium-dependent vasodilation measured by ultrasound [6]. The mechanisms underlying vessel dysfunction are not fully understood, but seem to depend on the increase in oxidative stress in endothelial cells, which thereby initiates a pro-inflammatory cascade and reduces nitric oxide availability [7].

Diabetes is a systemic inflammatory disease and a well-known predisposing factor for endothelial dysfunction, an early step in both micro- and macrovascular complications, and patients with type 2 diabetes show increased circulating levels of markers of endothelial dysfunction [8,9]. On the other hand, postprandial hyperglycemia [10] and hypertriglyceridemia [11] have been found to be associated with increased cardiovascular risk. The aim of this study was to assess fasting and postprandial TAFI antigen and soluble markers of endothelial damage in well-controlled, type 2 diabetic patients. To control for the fact that hypertriglyceridemia is associated with a reduced endothelium-mediated

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Table 1
Main features of the subjects included in the study [13]

	Type 2 diabetic patients	Nondiabetic controls
n (male/female)	12 (8/4)	14 (8/6)
Age (y)	52.4 ± 10.4	54.0 ± 6.1
BMI (kg/m^2)	25.8 ± 2.6	24.3 ± 2.4
Waist circumference (cm)	91.6 ± 3.0	85.8 ± 3.2
Diabetes duration (y)	3.5 (2-13)	–
Fasting glucose (mmol/L)	7.77 (6.19-10.25)**	5.72 (4.79-6.20)
HbA_{1c} (%)	6.80 ± 0.67**	5.41 ± 0.34
Fasting triglyceride (mmol/L)	0.92 ± 0.31	0.81 ± 0.20
Total cholesterol (mmol/L)	4.71 ± 0.85	4.99 ± 0.64
HDL-C (mmol/L)	1.12 ± 0.26*	1.40 ± 0.28
LDL-C (mmol/L)	3.25 ± 0.72	3.28 ± 0.60
VLDL-C (mmol/L)	0.32 ± 0.17	0.30 ± 0.11
Apolipoprotein B (g/L)	0.95 ± 0.22	0.95 ± 0.13
Free fatty acids (mmol/L)	0.50 ± 0.16	0.42 ± 0.13
HOMA index	4.90 (3.91-14.87)**	3.61 (3.02-10.86)

Qualitative variables (sex) are expressed as number of subjects affected, and continuous variables are expressed as mean ± SD (gaussian distribution) or as median (range) (non–gaussian distribution, ie, glucose, diabetes duration and HOMA index). BMI indicates body mass index; HDL-C, high-density lipoprotein cholesterol; VLDL-C, very low-density lipoprotein cholesterol.
* $P < .05$ vs controls.
** $P < .0005$ vs controls.

arterial vasorelaxation [7,12], we selected a specific, normotriglyceridemic group of patients.

2. Methods

2.1. Patients

A total of 12 nonobese (body mass index, <30 kg/m^2), normotriglyceridemic (triglyceride, <2.25 mmol/L) type 2 diabetic patients with hemoglobin A_{1c} (HbA_{1c}) of less than 8%, treated with diet only, were included in the study. Their main features are shown in Table 1 and have previously been described in detail [13]. Patients taking drugs or being in situations known to interfere with lipoprotein metabolism were excluded (established nephropathy, lipid-lowering drugs, steroids, nonselective β-blockers, or high-dose diuretics). A group of 14 nondiabetic normolipidemic, nonobese, control subjects of similar age, body mass index, and fasting triglycerides was also included (see Table 1). A test meal was given, and laboratory determinations were made. The protocol was approved by the local ethics committee, and all of the subjects signed written informed consent.

2.2. Test meal

After a 10- to 12-hour overnight fast, a peripheral intravenous catheter was inserted, and a first blood sample was obtained in Vacutainer tubes (Becton Dickinson, Plymouth, UK) containing EDTA, fluoride, or sodium citrate.

A test meal was given, consisting of 600 mL of a vanilla- or nut-flavored beverage (Nepro, Abbott Laboratories, Columbus, OH) and which contained 5021 kJ (1200 kcal) as fat (58 g), protein (42 g), and carbohydrate (134 g). Patients were asked not to perform unusual exercise or drink alcohol the day before the test. Water was allowed, but no other beverages, food, strenuous, exercise, or smoking were permitted during the test.

2.3. Laboratory tests

Total triglyceride and cholesterol (enzymatic methods); low-density lipoprotein cholesterol (LDL-C) (β-quantification), high-density lipoprotein cholesterol, (direct method) and very low-density lipoprotein cholesterol (β-quantification); apolipoprotein B (immunoturbidimetry); free fatty acids (enzymatic method); HbA_{1c} (automatic DCA 2000 reader [Bayer, Elkhart, IN; ref no. 5035B]; normal values, 4.3%-5.7%); insulin (immunochemoluminescence, Immulite 2000, Diagnostic Products, Los Angeles, CA); and glucose (enzymatic method) were determined in the fasting

Table 2
Fasting and postprandial hemostatic variables

	Baseline	P (0-4 h)	4 h	P (4-8 h)	8 h	P (0-8 h)
TAFI (%)						
Controls	102 ± 16.9	<.0005	70.3 ± 15.2	<.0005	100 ± 25.7	NS
Patients	72.9 ± 15.9***	<.05	65.5 ± 20.6	.091	73.1 ± 15.2**	NS
TM (μg/L)						
Controls	27.1 ± 6.8	.002	35.3 (27.2-60.3)	.002	25.5 ± 6.1	.004
Patients	27.9 ± 7.5	<.05	32.2 (20.0-45.7)	.005	26.9 ± 8.1	NS
TFPI (%)						
Controls	13.2 (0.94-19.9)	.002	18.6 ± 4.8	<.0005	14.1 ± 4.1	NS
Patients	12.6 (5.1-18.0)	.005	17.0 ± 5.4	<.0005	12.0 ± 4.3	NS
PAI-1 (AU/mL)						
Controls	8.93 ± 5.7	.002	0.96 (0.26-7.29)	NS	1.62 (0.26-8.27)	.004
Patients	9.43 (0.38-20.4)	<.05	2.16 (0.29-13.1)	<.05	2.78 (1.00-40.4)	NS
Prothrombin F1 + F2 (nmol/L)						
Controls	1.09 ± 0.15	.003	1.27 ± 0.17	<.0005	0.89 ± 0.13	<.0005
Patients	0.93 (0.72-1.77)*	NS	1.24 (0.88-1.87)	<.05	0.91 (0.53-1.43)	NS

NS indicates nonsignificant.
* $P < .05$ vs controls.
** $P < .005$ vs controls.
*** $P < .0005$ vs controls.

state. All these enzymatic methods were from Roche Diagnostics, Basel, Switzerland. Insulin and glucose were also measured at 1, 2, 3, 4, 5, and 6 hours after the meal, and triglyceride concentrations at 2, 3, 4, 5, 6, and 8 hours. The methods used have previously been described in detail [13]. The homeostasis assessment model (HOMA) was used to estimate insulin resistance, and areas under the curve (AUCs) were calculated by the trapezoid rule.

For hemostatic variables, whole-blood samples were collected in 1/10 volume of 0.129 mol/L sodium citrate at baseline and 4 and 8 hours after the test meal. These time points were chosen to include the postprandial peak in endothelial dysfunction, previously reported to happen 3 to 4 hours after the meal [12]. The samples were stored at −80°C, within 2 hours after they were drawn, until used. Plasma TAFI antigen (TAFI0, TAFI4, and TAFI8) was performed with a commercially available kit from HYPHEN BioMed (Zymutest TAFI, Andrésy, France). This assay is based on a 2-site enzyme-linked immunosorbent assay, and TAFI levels are expressed as percentage of pooled plasma from normal individuals. Thrombomodulin (TM0, TM4, and TM8) was determined by an immunoenzymatic assay as previously described [9]. Free TFPI (TFPI0, TFPI4, and TFPI8; Asserachrom free TFPI, Diagnostica Stago, Asnières, France), functional PAI-1, and prothrombin fragment 1 + 2 (F1 + F2) were also measured [9]. All of the components measured are stable in samples kept at temperatures below −40°C. Coefficients of variation range between 5% and 13%.

2.4. Statistical analysis

Analysis was performed using SPSS 10.0 statistical package for Windows (SPSS, Chicago, IL). Quantitative data are expressed as mean and standard deviation (gaussian distribution) or as median and range (non–gaussian distribution), and qualitative data as percentages. Comparison between groups was performed using Student t test (gaussian distribution) and Mann-Whitney U test (non–gaussian distribution) for quantitative data, and χ^2 test for qualitative variables. Comparisons within a group were made using Student t test for paired data or Wilcoxon test. Bivariate correlations were performed between continuous data (Spearman ρ). Multiple regression analysis was performed including hemostatic variables and their changes as the dependent variable and other continuous variables correlated with them in bivariate analysis as independent variables.

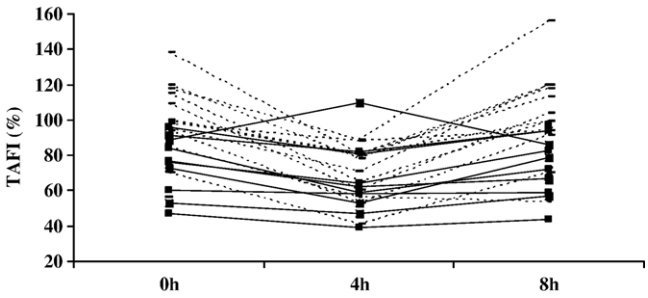

Fig. 1. TAFI antigen concentrations in type 2 diabetic (continuous line) and controls (dotted line) at baseline (0h), 4 hours (4h), and 8 hours (8h) after the test meal. For more details, including statistical analysis, see Table 2.

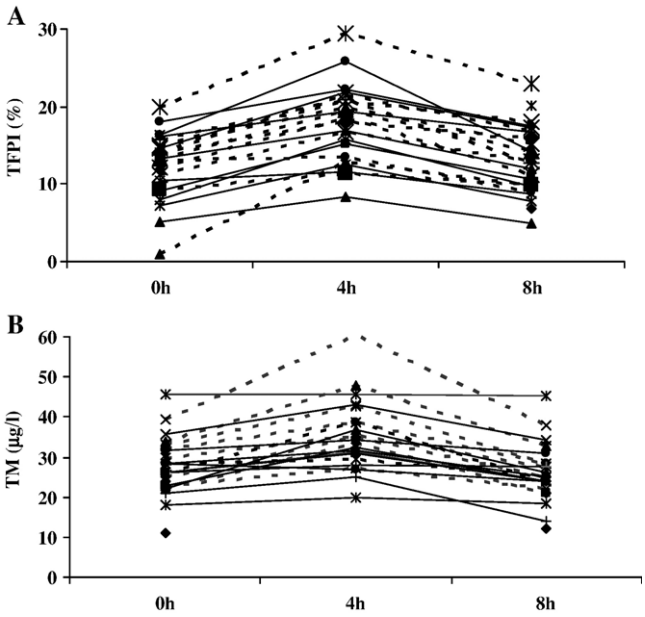

Fig. 2. TFPI (A) and TM (B) concentrations in type 2 diabetic (continuous line) and controls (dotted line) at baseline (0h), 4 hours (4h), and 8 hours (8h) after the test meal. For more details, including statistical analysis, see Table 2.

3. Results

Main fasting laboratory results in patients and control subjects are shown in Table 1 and have been reported in more detail elsewhere [13]. As expected, postprandial glucose was higher in patients than in controls, but no significant differences were found between both groups in postprandial triglycerides [13]. Fasting TAFI antigen was lower in type 2 diabetic patients ($P < .0005$) and decreased significantly 4 hours after the meal in both groups, but in a lower proportion in the patient group (12.6% vs 31.8%, $P < .005$) (Table 2 and Fig. 1). Markers of endothelial dysfunction and F1 + F2 are shown in Table 2. Briefly, both TM and TFPI increased significantly 4 hours after the meal and returned to previous values at 8 hours, following the same pattern in type 2 diabetic patients and controls (Table 2 and Fig. 2). Plasminogen activator inhibitor 1 levels, on the other hand, decreased significantly in both groups and remained stable after 8 hours in the control group, but increased again in the patient group (Table 2).

Table 3 shows the correlations between TAFI and other variables. For fasting TAFI (TAFI0), only the correlations with HbA_{1c} ($r = -0.469$, $P < .01$) and total cholesterol ($r = 0.518$, $P < .005$) remained significant in multiple regression analysis, whereas for the change in TAFI 4 hours after the

Table 3
Significant correlations between TAFI and other variables

	Fasting TAFI	Increase in TAFI 4 h after the meal	TAFI 8 h after the meal
Fasting glucose	−0.571; <.05	0.537; <.05	
Glucose 1 h after the meal		0.538; <.05	−0.418; <.05
Glucose 4 h after the meal	−0.450; <.05		−0.391; <.05
Glucose 6 h after the meal	−0.445; <.05		
AUC of glucose	−0.571; <.005	0.440; <.05	
HbA_{1c}	−0.542; <.05	0.580; <.005	−0.413; <.05
HOMA	−0.522; <.01		
Total cholesterol	0.458; <.05		
HDL-C	0.405; <.05		
LDL-C			
Fasting TAFI		−0.628; <.005	0.825; <.0005
Increment in TAFI 4-8 h after the meal		−0.742; <.0001	
TAFI 4 h after the meal			0.636; <.005

R and *P* values are shown.

meal, HbA_{1c} ($r = 0.385$, $P < .05$) and TAFI0 ($r = -0.489$, $P < .05$) showed correlation in multivariate analysis. TM0, TM4, and TM8 were all correlated with triglycerides at 4 hours ($r = 0.516$, 0.424, and 0.555; $P < .01$, .05, and .005, respectively), and TM4, also with glucose at 4 hours ($r = 0.449$, $P < .05$). The increase in TM from baseline to hour 4 was correlated with the AUC of glucose ($r = 0.477$, $P < .05$). TFPI0 and TFPI4 were correlated with LDL-C ($r = 0.465$ and 0.520, respectively, $P < .05$ for both) and glucose at 6 hours ($r = -0.445$ and −0.469, respectively, $P < .05$ for both).

4. Discussion

In the present study, the administration of a high-fat meal leads to 2 main findings. On the one hand, TAFI antigen, which is lower in the type 2 diabetic patients studied, suffers a transient postprandial reduction, which is negatively correlated with HbA_{1c} and with glycemia, expressed as AUC. On the other hand, TM and TFPI, 2 markers of endothelial damage, increase immediately and return to previous values thereafter. Although not described before, these results are in agreement with the well-known endothelial dysfunction, which takes place after a high-fat meal [2]. With the ingestion of a meal, the entry of chylomicrons into the bloodstream overruns the capacity to clear triglyceride-rich lipoproteins, leading to an accumulation of these atherogenic particles. Consequently, the endothelium is exposed to the latter, which induce transient endothelial dysfunction, as shown by a reduced flow-dependent dilatation in the brachial artery. This impairment in endothelial function reaches its peak 3 to 4 hours after the ingestion of the fat-rich meal [12] and is greater in diabetic patients than in healthy subjects [14].

Although TAFI has been hypothesized to play a role in atherothrombosis [4,15], data from observational studies are controversial. Differences could be at least partially be explained by the method used to quantify TAFI, the clinical situation of the study subjects, and the site where the samples were obtained [15-17]. In some studies, both functional TAFI and TAFI antigen are high in patients with cardiovascular disease [15-17], but a large, prospective European study showed that TAFI antigen was slightly lower in men who had a myocardial infarction in the previous 6 months than in age-matched controls (74.5% vs 77.5%, respectively; $P = .008$) and that TAFI antigen above the 90th percentile was associated with a reduced risk of myocardial infarction (odds ratio, 0.55; 95% confidence interval, 0.34-0.91) [18]. Similar results were obtained in the Prospective Epidemiological Study of Myocardial Infarction (PRIME) study, a case-control study in which nearly 10,000 healthy men were recruited [19].

There are few studies assessing TAFI levels in patients with diabetes [18,20-22] and their results are controversial. Studies performed in Japanese population show increased TAFI levels in patients with type 2 diabetes mellitus and microalbuminuria [21] that seem to be related with insulin resistance, whereas European studies find no independent association between TAFI and the main clinical components of the metabolic syndrome [18,22]. In the present study, performed in lean, well-controlled type 2 diabetic patients and normolipidemic controls, an inverse correlation was found between TAFI antigen and HbA_{1c} and the HOMA index. In a group of type 1 diabetic patients, with and without microvascular complications, there was a trend toward lower TAFI concentrations than in healthy controls [20]. The authors hypothesized that this reduction could be due to TAFI consumption in relation to generalized vasculopathy.

Thrombin activatable fibrinolysis inhibitor is activated by the thrombin-TM complex on the endothelial cell surface [4] and has recently been described to have anti-inflammatory properties, that is, inactivation of bradykinin and the extremely potent anaphylatoxin C5a [5,23]. Furthermore, intracoronary plasma TAFI levels are correlated with acute-phase reactants in a group of patients with coronary artery disease [16]. Because inflammation plays a crucial role in the development of atherosclerosis, decreased TAFI levels may contribute to the increased vascular risk associated with diabetes. In the present study, fasting TAFI levels were lower in diabetic patients and were inversely correlated with the HOMA index and parameters of glycemic control (HbA_{1c} and fasting and postprandial glucose). Furthermore, TAFI is a glycoprotein with a 20% carbohydrate content, whose activation peptide contains 4 potential glycosylation sites [24]. These data suggest a possible role of insulin resistance and glycemic control as determinants of TAFI levels in patients with type 2 diabetes mellitus.

To our knowledge, there are no previous studies on TAFI in postprandial conditions. Indeed, this is the first time a transient postprandial reduction of TAFI antigen is

described, both in diabetic patients and in healthy controls. The difference between both groups in the postprandial TAFI response might be explained by the difference in fasting levels. Although low concentrations of TM stimulate the activation of TAFI by thrombin, higher TM concentrations have the opposite effect, dampening TAFI activation through the inhibition of activated protein C–mediated thrombin generation [25]. Because a nonfunctional method was used for its measurement, changes in TAFI antigen levels in this study cannot be explained by the changes in TM. In addition, no correlation was found between TAFI and TM at any time point, and the decrement in TAFI antigen was not correlated with the increment in TM levels. Alternatively, the postprandial decrease in TAFI concentrations might be caused by its consumption because of the activation of thrombosis, reflected by the immediate increase in F1 + F2. The main predictors of the postprandial reduction in TAFI in this study were fasting TAFI and HbA_{1c}. These data suggest a possible role of glucose also in the postprandial results encountered in the present study.

Thrombomodulin is an integral membrane glycoprotein of endothelial cells, which has anticoagulant properties. Because it is not constitutively secreted from the cells, but is released as a result of endothelial damage, circulating TM levels are considered to reflect endothelial impairment. Moreover, TM is increased in a variety of diseases associated with endothelial injury [26], including type 1 and type 2 diabetes mellitus. For the first time, the present study describes the changes experienced by TM concentrations in the postprandial state. An increase in TM levels was found 4 hours after the fat-rich test meal both in the diabetic patients and in the healthy controls and was correlated with postprandial triglycerides, as well as with glucose and with the AUC of glucose. This fast increase in the soluble fraction of TM is probably a reflection of the postprandial endothelial damage induced by the increase in glycemia and triglyceridemia. The similar postprandial changes in both groups might be explained by the fact that the patients had good glycemic control and normal postprandial triglycerides.

Fasting TFPI levels were similar in the well-controlled diabetic patients and in the controls, in agreement with our previous data [9]. The postprandial increase in TFPI is also in agreement with a previous study [27], but, to our knowledge, this is the first to show such an increase in diabetic patients. The endothelium is known to be the main pool of this tissue factor–dependent coagulation inhibitor, but the mechanisms by which TFPI is bound to and released from the endothelial cells have not been entirely elucidated [28]. In a manner similar to TM, circulating TFPI concentrations mirror the transient endothelial dysfunction associated with the fat-rich test meal. Nevertheless, most circulating TFPI is bound to lipoproteins [29], and we found a direct correlation between TFPI and fasting LDL-C. The latter is not expected to increase postprandially, but lipoprotein particles suffer compositional changes and become more prone to oxidation after a meal [30]. The fact that these modifications might lead to a reduction in the affinity of the particles for TFPI cannot be ruled out.

Finally, the postprandial decrease in PAI-1 activity found in the present study is in agreement with previous studies performed in nondiabetic populations [31-33], with circadian variability being the common explanation for the decrease in PAI-1 antigen and activity, although an increased consumption (or other explanations) cannot be excluded.

In conclusion, the ingestion of a fat-rich meal leads to an increase in TM and TFPI plasma concentrations, and a temporary reduction in TAFI antigen, an anti-inflammatory molecule whose circulating levels are reduced in this group of well-controlled, normotriglyceridemic, type 2 diabetic patients. Although endothelial dysfunction was not directly measured, these results support the known deleterious effect a fatty meal has on it. Of course, other markers, not assessed in this study, may also be involved.

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