Platelet Sodium-Proton Exchanger and Phospholipid-Dependent Procoagulant Activity in Patients With Type 2 Diabetes

Beata Telejko, Marian Tomasiak, Halina Stelmach, and Ida Kinalska

Platelet Na^+/H^+ exchanger (NHE) activity, phospholipid-dependent thrombin generation, and platelet factor 3 (PF3) availability were measured in 83 type 2 diabetics and in 40 age- and sex-matched healthy subjects. Na^+/H^+ exchanger activity was significantly increased in diabetic patients in comparison to the controls ($\kappa = 4.29 \pm 0.71 \times 10^{-3} \times s^{-1}$ *v* $3.21 \pm 0.64 \times 10^{-3} \times s^{-1}$, $P < .00001$). However, there was no significant difference between subjects with ($\kappa = 4.28 \pm 0.75 \times 10^{-3} \times s^{-1}$) and without ($\kappa = 4.26 \pm 0.32 \times 10^{-3} \times s^{-1}$) arterial hypertension, as well as between patients with normo- and microalbuminuria or overt proteinuria ($\kappa = 4.26 \pm 0.58 \times 10^{-3} \times s^{-1}$, $\kappa = 4.47 \pm 0.93 \times 10^{-3} \times s^{-1}$ and $\kappa = 4.07 \pm 0.38 \times 10^{-3} \times s^{-1}$, respectively). Comparatively high NHE activity was observed in the group of patients with hemoglobin A_{1c} (HbA_{1c}) less than 7.5%. Multiple regression analysis revealed that the factors independently related to platelet Na^+/H^+ exchanger activity were: total PF3 activity ($\beta = 0.77$, $P = .011$) and triglyceride (TG) concentration ($\beta = 0.44$, $P = .039$). Phospholipid-dependent thrombin generation and PF3 availability were also enhanced in all plasma fractions of diabetic patients, especially in platelet-poor plasma (PPP) and platelet-free plasma (PFP) ($P < .0001$ and $P < .00001$, respectively). There was a positive correlation between NHE activity and thrombin generation, as well as with PF3 availability in all plasma fractions. Our results suggest that enhanced platelet Na^+/H^+ exchanger activity associated with raised phospholipid-dependent procoagulant activity may increase the risk of vascular damage in type 2 diabetic patients.

SODIUM-PROTON exchangers (NHE) constitute a family of membrane proteins that regulate intracellular pH, cellular volume, and cell proliferation.[1] NHE overactivity has been shown to be associated with arterial hypertension[2,3] and type 1 diabetic nephropathy.[4,5] In 1993 Ruiz-Palomo and Toledo[6] hypothesized that primary dysfunction of Na^+/H^+ membrane transport may be also a common feature in subjects with metabolic syndrome. However, there are very few reports on platelet NHE activity in type 2 diabetes.[7,8] A small study using a cell-swelling method revealed an increase of platelet Na^+/H^+ exchange in nephropathic versus normoalbuminuric patients.[7] Another study, in which the fluorimetric method was applied, did not find significant differences in platelet Na^+/H^+ exchange kinetics between type 2 diabetics and healthy subjects.[8]

The clinical importance of an altered NHE function is under discussion. In the previous study[9] we demonstrated that intracellular Na^+ accumulation via activated Na^+/H^+ exchange may result in the development of phospholipid-dependent procoagulant activity on the platelet surface. Therefore the aim of the present study was the measurement of platelet Na^+/H^+ exchanger activity and phospholipid-dependent procoagulant activity—expressed as thrombin generation and platelet factor 3 (PF3) availability—in type 2 diabetic patients, as well as their relationship to a range of metabolic factors and glycemic control.

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MATERIAL AND METHODS

Patients

The group studied consisted of 83 type 2 diabetic patients (39 males and 44 females, aged 35 to 63 years) recruited consecutively from the outpatient clinic and 40 healthy subjects (17 males and 23 females, aged 37 to 62 years). Subjects with an impaired renal function (creatinine $\geq$ 2.0 mg/dL) were excluded. Thirty-six diabetics had been previously diagnosed as having arterial hypertension and treated with angiotensin-converting enzyme (ACE) inhibitors, selective β-blockers, and/or indapamide. Antihypertensive therapy was discontinued 48 hours before the study. Twenty-eight patients were treated with sulfonylureas, 20 with insulin (Eli-Lilly, Fegersheim, France) and 35 with insulin plus sulfonylurea. None of the women participating in the study had been receiving hormonal replacement therapy during the previous 6 months. None of the participants had taken any medications affecting platelet function for at least 14 days. The study protocol was approved by the local ethical committee (Medical Academy Bialystok) and informed consent was obtained from all subjects.

Blood Collection

Blood samples were collected after an overnight fasting period, into tubes containing 3.2% sodium citrate (10:1 vol/vol); platelet counts were measured using a Coulter STKS. Platelet-rich plasma (PRP) was prepared by centrifugation of the whole blood at 200 $\times$ *g* for 15 minutes at room temperature; platelet-poor plasma (PPP) was obtained by centrifuging PRP at 2,000 $\times$ *g* for 6 minutes, and platelet-filtrated plasma (PFP) was obtained by centrifuging PPP at 11,000 $\times$ *g* for 10 minutes and then passing it through 0.1-μm cellulose filter (Whatman International Ltd, Maidstone, UK).

Platelet Sodium-Proton Exchanger Activity

The activity of the Na^+/H^+ exchanger was measured as changes in platelet volume in acid-loaded cells, using an optical swelling assay, according to Rosskopf et al.[2] Sodium propionate in a concentration of 140 mmol/L was used for induction of cell swelling. The free propionate anion is in equilibrium with the protonated acid, which is lipid-soluble and can readily permeate the plasma membrane.[10] Intracellular dissociation of propionic acid causes cytoplasmic acidification, which in turn activates the exchange of extracellular Na^+ for intracellular H^+ in order to re-establish the intracellular pH.[2,10] However, since the entry of propionic acid continues, intracellular pH remains acidic and the

Table 1. Clinical Characteristics of the Population Studied

Parameter	Control Group (n = 40)	Type 2 Diabetes (n = 83)
Age (yr)	54.4 ± 6.2	56.7 ± 7.8
Duration of diabetes (yr)	—	9.1 ± 6.7
BMI (kg/m^2)	27.9 ± 4.7	30.3 ± 4.5
Platelet count ($\times 10^3/\mu L$)	212.7 ± 48.5	208.7 ± 39.0
Creatinine (nmol/L)	76.1 ± 7.9	84.1 ± 21.2
HbA_{Ic} (%)	4.4 ± 0.8	9.9 ± 2.3
		$P < .01$
Total cholesterol (mmol/L)	5.1 ± 0.8	5.1 ± 1.3
HDL-C (mmol/L)	1.3 ± 0.3	0.96 ± 0.3
		$P < .01$
LDL-C (mmol/L)	3.3 ± 0.8	3.0 ± 1.0
Triglycerides (mmol/L)	1.0 ± 0.6	2.1 ± 1.0
		$P < .0001$

continuous maximal activation of NHE leads to an accumulation of Na^+.[2,10] The uptake of osmotically obliged water is then manifested as cell swelling, which is a measure of Na^+ influx.[2,10] The propionate medium (140 mmol/L sodium propionate, 20 mmol/L HEPES, 10 mmol/L glucose, 5 mmol/L KCL, 1 mmol/L $MgCl_2$ and 1 mmol/L $CaCl_2$, pH = 6.7) was prewarmed to 37°C and then samples of PRP were directly added, with continuous stirring at 300 rpm. The changes in optical density (OD) were continuously monitored for 2 minutes, at a wavelength of 680 nm, using spectrophotometer UNICAM-Helios γ (Unicam, Cambridge, UK) connected with a personal computer. Aurora Software for Helios (Unicam) was used to analyze the changes in OD. The activity of platelet Na^+/H^+ exchanger was expressed as the rate constant (κ) of a swelling process ($\times 10^{-3} \times s^{-1}$).

Phospholipid-Dependent Thrombin Generation

Assay of thrombin generation was adapted from Rota et al. [11] Defibrinated plasma was prepared by adding one part of reptilase (20 BU of Batroxobin, DADE Behring, Marburg, Germany) to 200 parts of fresh pooled plasma obtained from 12 healthy donors. The clot was left to form for 10 minutes at 37°C and discarded after centrifugation at 2,000 × *g* for 10 minutes. The defibrinated plasma was filtered through a 0.1-μm cellulose filter (Whatman International) and stored in 1-mL aliquots at −70°C. Thrombin generation was measured in PRP, PPP, and PFP by adding 20 μL of the sample studied and 10 μL of Russell's viper venom (RVV, Sigma Chemicals, St Louis, MO; 80 μg/mL) to 120 μL of the defibrinated plasma, preincubated in 37°C. After 60 seconds, 25 μL of 0.1 mol/L $CaCl_2$ in buffer I (0.05 mol/L TrisHCl, 0.1 mol/L NaCl, 0.1 mol/L $CaCl_2$, 0.5% bovine serum albumin [BSA], pH = 7.5) was rapidly added; after the next 4 minutes, 40-μL aliquots of the mixture were transferred into microtiter wells containing 197.5 μL of buffer II (0.05 mol/L Tris HCl, 0.1 mol/L NaCl, 20 mmol/L EDTA, 0.5% BSA, pH = 7.5) to stop the reaction. Then 12.5 μL of 4 mmol/L S-2238 (a chromogenic substrate specifically cleaved by thrombin; Chromogenix, Milano, Italy) was added into each well. The absorbance of the released color product was read during 6 minutes at a wavelength of 405 nm, using a Sunrise (Tecan, Salzburg, Austria) plate reader. The amidolytic activity was expressed in milli-international units as nanomoles of the substrate cleaved in 1 minute per 1.0 mL of the plasma studied.[12]

Availability of PF3

The availability of phospholipids with the activity of PF3 was measured in PRP, PPP, and PFP fraction, using modified RVV time.[13,14] Samples (100 μL) of plasma studied were transferred to the coagulometer cuvette (Boehring Fibrintimer, Liederbach, Germany) and incubated at 37°C for 2 minutes with 100 μL of RVV at the final concentration of 5 μg/mL, with continuous stirring. Then 0.1 mL of 0.025 mol/L $CaCl_2$ was rapidly added and the clotting time was recorded.

Other Laboratory Studies

Patients weight and height were measured and body mass index (BMI) was calculated. Glycated hemoglobin (HbA_{1c}) was measured using a high-performance liquid chromatography (HPLC) technique (HPLC variant, BIORAD Laboratories, München, Germany). Total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and triglyceride (TG) concentrations were determined by enzymatic methods, using commercial kits produced by ANALCO-GBG (Warsaw, Poland). Urinary albumin excretion (AER) was measured in 12-hour urine samples, collected during 3 consecutive days, using an immunoturbidimetric method (Orion Diagnostica, Espoo, Finland).

Statistics

Statistical analysis was done using analysis of variance (ANOVA) and the Mann-Whitney *U* test. Simple linear or multiple regression was also performed to establish factors independently related to the sodium-proton exchanger activity. Data are expressed as the mean ± SD, and *P* values less than .05 are regarded significant.

RESULTS

Clinical Characteristics

Patients with type 2 diabetes had greater BMI and higher TG levels ($P < .0001$), as well as lower HDL-C concentration ($P < .01$), than healthy individuals (Table 1). Among diabetic subjects, 64 were normoalbuminuric (albumin excretion < 20 μg/min; mean, 9.4 ± 6.2 μg/min), eight were microalbuminuric (mean, 49.3 ± 34.9 μg/min), and 11 had overt proteinuria (mean, 1.27 ± 0.93 g/24 h).

Platelet NHE Activity

To determine the intra-assay reproducibility of the swelling method, at least 5 measurements were made in each subject. The determination of swelling rate constants was highly reproducible and intra-assay variation coefficients were 9.2% ± 2.1% in nondiabetics and 10.8% ± 2.6% in type 2 diabetics.

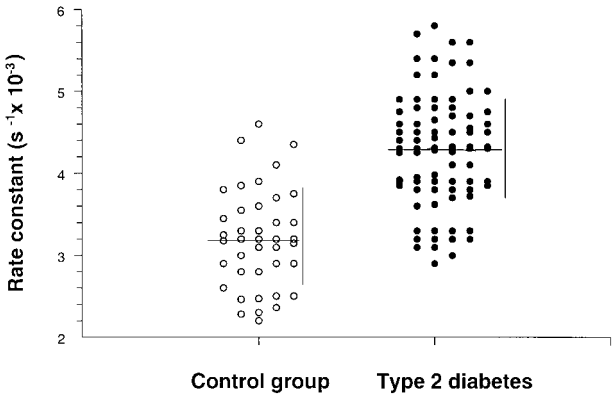

Fig 1. Swelling rate constants in the nondiabetic (○) and diabetic group (●). Each point represents the mean of 5 measurements for each individual. The mean ± SD is indicated for both groups.

Table 2. Platelet NHE Activity, Thrombin Generation, and PF3 Activity in the Groups Studied

Parameter	Control Group (n = 40)	Type 2 Diabetes (n = 83)	*P* Value
NHE activity ($\times 10^{-3} \times s^{-1}$)	3.21 ± 0.64	4.29 ± 0.71	$P < .00001$
Thrombin generation in PRP (mIU/min)	88.13 ± 18.5	107.0 ± 32.7	$P < .001$
Thrombin generation in PPP (mIU/min)	42.01 ± 6.4	49.67 ± 9.3	$P < .0001$
Thrombin generation in PFP (mIU/min)	14.85 ± 5.1	39.19 ± 8.1	$P < .00001$
RVV time in PRP (s)	27.03 ± 4.43	24.85 ± 4.24	$P < .05$
RVV time in PPP (s)	33.99 ± 5.17	28.21 ± 5.30	$P < .0001$
RVV time in PFP (s)	54.39 ± 18.5	36.11 ± 7.49	$P < .000001$

Figure 1 shows the rate constant for each individual from the nondiabetic and diabetic groups. The mean NHE rate was significantly higher in diabetic patients in comparison to controls ($P < .00001$, Table 2). There was no difference in NHE activity between subjects with ($\kappa = 4.28 \pm 0.75 \times 10^{-3} \times s^{-1}$) and without ($\kappa = 4.26 \pm 0.32 \times 10^{-3} \times s^{-1}$) arterial hypertension, or between patients with normo- and microalbuminuria or overt proteinuria ($\kappa = 4.26 \pm 0.58 \times 10^{-3} \times s^{-1}$, $\kappa = 4.47 \pm 0.93 \times 10^{-3} \times s^{-1}$, and $\kappa = 4.07 \pm 0.38 \times 10^{-3} \times s^{-1}$, respectively). Comparatively high NHE values were observed in the group of patients with HbA_{1c} less than 7.5% (Table 3).

We did not find any differences in NHE activity between men and women, both in the diabetic (males: $\kappa = 4.34 \pm 0.79 \times 10^{-3} \times s^{-1}$; females: $\kappa = 4.26 \pm 0.68 \times 10^{-3} \times s^{-1}$) and the nondiabetic group (males: $\kappa = 3.31 \pm 0.45 \times 10^{-3} \times s^{-1}$; females: $\kappa = 3.35 \pm 0.9 \times 10^{-3} \times s^{-1}$).

In the total cohort studied (diabetics + nondiabetics) NHE activity correlated significantly with phospholipid-dependent thrombin generation in PPP ($r = 0.50$, $P = .01$) and PFP ($r = 0.39$, $P = .023$), and with PF3 availability in all plasma fractions (PRP: $r = 0.36$, $P = .016$; PPP: $r = 0.53$, $P = .0001$; PFP: $r = 0.43$, $P = .005$). There was no significant association between NHE activity and other clinical or metabolic parameters studied (age, gender, duration of diabetes, BMI, lipoprotein concentration, and HbA_{1c}). Multiple regression analysis revealed that the factors independently related to platelet NHE activity were total PF3 activity ($\beta = 0.77$, $P = .011$) and TG concentration ($\beta = 0.44$, $P = .039$).

Phospholipid-Dependent Thrombin Generation

Phospholipid-dependent thrombin generation was significantly enhanced in diabetic patients in comparison to healthy subjects (Table 2). There was no difference in the total thrombin generation (in PRP) between diabetics with arterial hypertension (mean, 106.9 ± 37.7 mIU/min), microalbuminuria or proteinuria (mean, 100.6 ± 22.6 mIU/min), and patients without complications (mean, 105.4 ± 26.6 mIU/min). Comparatively high thrombin potential was observed in the group with HbA_{1c} values less than7.5% (Table 3). Phospholipid-dependent thrombin generation did not correlate significantly with any of the parameter studied, both in diabetics and control subjects. However, in the total population, there was a significant correlation between thrombin generation in PPP ($r = 0.50$, $P = .01$) and PFP ($r = 0.39$, $P = .023$) and platelet NHE activity.

PF3 Availability in Plasma Fractions

PF3 activity was significantly enhanced in all plasma fractions of diabetic patients and the most apparent difference was observed in PPP ($P < .0001$) and PFP ($P < .000001$) fraction (Table 2). PF3 activity was also increased in subjects with arterial hypertension (PRP: 24.5 ±3.9 seconds *v* 27.2 ± 6.4 seconds, $P < .05$; PPP: 27.5 ± 4.9 seconds *v* 32.1 ± 7.2 seconds, $P < .001$; PFP: 35.5 ± 6.9 seconds *v* 37.9 ± 10.2 seconds, $P =$ not significant [NS]). We did not find significant differences in PF3 availability in patients with normo- and microalbuminuria or overt proteinuria (data not shown) nor in subjects with poor and satisfactory metabolic control; however,

Table 3. Platelet NHE Activity, Thrombin Generation, and PF3 Activity in Type 2 Diabetic Patients in Relation to HbA_{1c} Values

Parameter	HbA_{1c} (%) 6.1–7.5 (n = 19)	7.6–9.0 (n = 13)	> 9.0 (n = 51)
NHE activity ($\times 10^{-3} \times s^{-1}$)	4.52 ± 0.67	3.71 ± 0.77	3.94 ± 0.52
		$P < .005$	$P < .001$
Thrombin generation in PRP (mIU/min)	127.23 ± 22.9	100.62 ± 43.3	102.71 ± 32.8
		$P < .05$	$P < .005$
Thrombin generation in PPP (mIU/min)	51.13 ± 9.4	49.03 ± 9.6	49.31 ± 9.2
Thrombin generation in PFP (mIU/min)	39.46 ± 7.1	37.28 ± 8.4	39.46 ± 8.5
RVV time in PRP (s)	23.70 ± 3.66	26.70 ± 4.91	24.80 ± 4.19
		$P < .05$	
RVV time in PPP (s)	27.10 ± 4.99	28.68 ± 5.83	28.53 ± 5.35
RVV time in PFP (s)	35.90 ± 6.46	37.53 ± 8.50	35.91 ± 7.77

the shortest RVV times were observed in the group with HbA_{1c} less than 7.5% (Table 3). In healthy individuals there was a significant association between total PF3 activity and BMI ($r = 0.69$, $P = .041$), whereas in diabetics PF3 activity correlated with platelet count (PRP: $r = 0.23$, $P = .02$; PPP: $r = 0.21$, $P = .043$) and TG concentration (PRP: $r = 0.23$, $P = .042$; PFP: $r = 0.32$, $P = .006$).

We did not find any significant differences in the parameters studied between patients treated with oral drugs, insulin alone, or combined therapy.

DISCUSSION

Our study demonstrated that the activity of platelet NHEs significantly enhanced in type 2 diabetic patients, irrespective of the presence of microalbuminuria or proteinuria and arterial hypertension. Moreover, the high NHE activity found in patients with well-controlled diabetes strongly suggests that this is a primary defect, not caused by chronic hyperglycemia.

There are few data concerning platelet NHE activity in type 2 diabetes available in the literature.[7,8] Herman et al[7] in a cell-swelling study, reported an increase in the maximal amiloride-sensitive change in platelet volume (which reflects enhanced NHE activity) in type 2 diabetics with proteinuria (> 0.5 g/24 h) in comparison to patients with urinary protein excretion less than 0.15 g/24 h. Although noteworthy, these results were limited by 3 main shortcomings: the small number of patients enrolled in the study, lack of comparison with healthy individuals, and no study of the kinetics of NHE. In contrast, analysis of the maximal rate of NHE (V_{max}) and K_m for extracellular sodium, performed by Foyle et al[8] by loading platelets with the pH_i-sensitive fluorescent dye (BCECF), did not reveal any significant differences between diabetic and control subjects. The sodium-proton exchange kinetics were not related to age, BMI, blood pressure, lipids, or albumin excretion rate. It could be due to the extremely wide range of the values observed both in the diabetic and, especially, in the control subjects, making any comparison and a final conclusion difficult.

Each of the procedures tha has been used to assess NHE in platelets has certain advantages and shortcomings. Evaluation of NHE by fluorimetric determination of pH_i, allows on the one hand a detailed analysis of separate kinetic components of the exchange mechanism, but on the other hand also has some disadvantages.[10] First, it requires a multistep preparation of platelets, with one or two steps of gel filtration, loading with the fluorescent intracellular pH indicator, and treatment with NH_4Cl to obtain cytoplasmic alkalization and then acidification.[3, 8, 10] Second, the intracellular buffering power is a crucial factor for this method and must be taken into account for calculation of the proton efflux rate.[3,8,10] Moreover, artifacts may be possible because of the "leakage" of the fluorescent dye out of the platelets, since even after a second gel filtration procedure, approximately 20% of the total fluorescence may be present in the medium.[10]

The indirect swelling assay was introduced by Livne et al in 1987[10] as a rapid and simple test for assessment of NHE in platelets. However, when using electronic cell sizing, the activity of NHE could not be monitored continuously as only single measurements every 30 seconds could be performed.[7,10]

The optical swelling assay, applied for the first time by Rosskopf et al[2] is also an indirect method, that does not directly measure the properties of the NHE itself, but only the NHE-dependent cell swelling. However, the continuous monitoring of volume gain possible by this method makes it sensitive and reproducible enough to yield reliable estimates of NHE activity in platelets from different donors.[2] The intra-assay reproducibility may be regarded as sensitive enough for discrimination of increased NHE rates in various groups of subjects.[2] The rate constants of the changes in optical density are independent of the platelet concentration in the range of 0.4 to 1.2×10^8 cells/mL.[2] An additional advantage of the swelling procedure is that a small amount of platelets with only minimal manipulation is required, because PRP can be used directly.[2]

In contrast to the fluorimetric method, the optical swelling procedure under given experimental conditions (sodium propionate concentration of 140 mmol/L and $pH_o = 6.7$), seems to be quite insensitive to the effect of platelet buffering power. Livne et a[10] found that buffering capacity affected cell swelling only at low (23 to 70 mmol/L) propionate concentration. The use of 140 mmol/L propionate–induced acidification keeps the pH_i permanently low throughout measurement, due to the constant presence of weak acid outside the cell, which induces a persistent activation of the Na^+/H^+ exchanger. Even when operating a the maximal rate, the NHE is unable to increase the pH_i.[2,10] Therefore, at a high propionate concentration (140 mmol/L) the cytoplasmic acidification system is very efficient and remains active throughout the experiment, the rate of swelling is high, and the effect of platelet buffering capacity seems to be negligible.

For these reasons, we chose the optical swelling assay as a rapid and reproducible test, requiring only minimal platelet manipulation and therefore suitable for clinical studies. It is also noteworthy that in our study the intra-assay reproducibility (9.2% ± 2.1% in nondiabetics and 10.8% ± 2.6% in type 2 diabetics) was even better than that found by Rosskopf et a[2] (12.1% ± 6.4% in normotensive and 12.2% ± 5.2% in hypertensive subjects).

According to Rosskopf et al,[3] the activity of platelet NHE does not seem to be modified by antihypertensive treatment. However, erythrocyte NHE activity may decrease in patients with type 2 diabetes[15] and essential hypertension[16] receiving ACE inhibitors (lisinopril and captopril). This effect was not observed in subjects treated with doxazosin and nifedipine.[15]

The clinical importance of enhanced NHE activity is controversial. We found that the increase of platelet NHE activity was associated with higher phospholipid-dependent procoagulant activity, assessed by the availability of PF3 and the direct measurement of thrombin generation. We showed that phospholipid-dependent thrombin generation was significantly increased in type 2 diabetics, irrespective of their vascular complications and glycaemic control. Aoki et al[17] also found enhanced platelet-dependent thrombin generation in type 2 diabetic patients, the highest values were observed in subjects with HbA_{1c} levels greater than 9%. In our study, the enhancement of thrombin potential might result from an increased availability of anionic phospholipids with the activity of PF3, exposed on platelet surface, which was also demonstrated in our patients. The highest increase of PF3 activity was found in

PPP and PFP. As clearly demonstrated by Jy et al,[14] we can assume that the source of that activity was cell-derived microparticles (microvesicles, platelet microparticles)—small fragments of the cellular membrane shed by platelets and in to a minor degree by erythrocytes and leukocytes into the extracellular space upon activation.[18,19] These vesicles are enriched in anionic phospholipids, facilitating the ordered assembly of coagulation factors and the explosion of thrombin generation.[19,20] Circulating microparticles provide additional procoagulant activity at a distance from the site of platelet activation[20,21] and deliver bioactive lipids to adjacent platelets, endothelial cells, and monocytes, promoting adhesive interactions and occlusion of the vessel lumen.[21] Elevated levels of microparticles have been reported in type 2 diabetic patients,[22,23] especially in those with lipid disturbances, as a potential atherogenic factor.[23]

In conclusion, our results suggest that increased platelet NHE activity associated with raised phospholipid-dependent procoagulant activity may enhance the risk of vascular damage in patients with type 2 diabetes.

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