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Acute, Short-Term Hyperglycemia Enhances Shear Stress-Induced Platelet Activation in Patients With Type II Diabetes Mellitus

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OBJECTIVES The aim of our study was to assess whether acute, short-term hyperglycemia affects platelet reactivity in patients with Type II diabetes mellitus (T2DM).

BACKGROUND Hyperglycemic spikes are thought to precipitate ischemic events in T2DM. Previous studies have shown in vivo platelet activation in diabetes; however, no studies have assessed whether acute in vivo hyperglycemia induces further activation of platelets.

METHODS In a cross-over, randomized, double-blind study, 12 patients with T2DM underwent 4 h of either acute hyperglycemia (13.9 mmol/l, 250 mg/dl) or euglycemia (5.5 mmol/l, 100 mg/dl). Shear stress-induced platelet activation, P-selectin and lysosomal integral membrane protein (LIMP) expression on platelets in the bleeding-time blood, urinary 11-dehydro-thromboxane B_2 (TxB_2) excretion, von Willebrand factor:antigen (vWF:Ag), and von Willebrand factor: activity (vWF:activity) were measured before and after hyperglycemia or euglycemia.

RESULTS Shear stress-induced platelet activation, P-selectin and LIMP expression on platelets in the bleeding-time blood, and urinary 11-dehydro-TxB_2 excretion increased significantly after hyperglycemic clamping, whereas no changes were observed after euglycemic clamping. Plasma vWF:Ag and vWF:activity increased strikingly in parallel fashion after hyperglycemic clamping, whereas no changes were observed after euglycemic clamping.

CONCLUSIONS Our data demonstrate that acute, short-term hyperglycemia induces an increased activation of platelets exposed to high shear stress conditions in vitro (filtration method) or in vivo (bleeding time). In vivo platelet activation is reflected by an increased urinary excretion of 11-dehydro-TxB_2. The increased levels of vWF in the circulation correlate with the increase in platelet activation markers and may indicate some degree of causation. Acute, short-term hyperglycemia in T2DM may precipitate vascular occlusions by facilitating platelet activation. (J Am Coll Cardiol 2003;41:1013–20) © 2003 by the American College of Cardiology Foundation

Compared with non-diabetics, patients with Type II diabetes mellitus (T2DM) have a two- to four-fold increased risk of ischemic cardiovascular disease, a risk largely independent of concomitant hypertension, hypercholesterolemia, and smoking (1–3). Increased platelet reactivity has been suggested as a potential mechanism of the accelerated atherosclerosis and enhanced incidence of arterial thrombosis seen in diabetics (4,5).

Several in vitro studies have shown hyper-reactivity of platelets from T2DM patients, as indicated by enhanced aggregation, increased fibrinogen binding, and thromboxane production (6–8). However, the pathophysiologic relevance of in vitro aggregometric tests to the involvement of platelets in the in vivo atherothrombotic complications of diabetes is questionable (5).

More interesting, enhanced biosynthesis of thromboxane A_2 by platelets has been shown in vivo in T2DM, by the measurement of urinary excretion of 11-dehydro-thromboxane B_2 (TxB_2) (8).

Enhanced in vivo aggregation in diabetics is a consequence of a metabolic alteration and not of large-vessel arterial disease (9). Indeed, tight glycemic control is associated with a clear reduction of in vivo platelet activation (8,10).

The correlation between fasting plasma glucose and the incidence of cardiovascular events is well established (2,3), but several clues point to an important role of hyperglycemic spikes in determining atherosclerotic complications (11) and in precipitating ischemic events (12,13).

However, at present, no conclusive data on the effect of a hyperglycemic spike on platelet activation in vivo in diabetics are available. High shear stress-induced platelet activation is crucial in determining the thrombotic complications occurring at an arterial site of atherosclerotic narrowing (14–16), and plasma von Willebrand factor (vWF) plays an important role in these phenomena. Shear-induced platelet adhesion and aggregation on subendothelium are increased in diabetic patients under good metabolic control (17), and abnormalities of vWF have been described in diabetes (18).

The aim of our study was to establish whether acute, short-term hyperglycemia, in the range of that commonly

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Abbreviations and Acronyms

ADP = adenosine diphosphate
Ag = antigen
AUC = area under the curve
HbA_{1c} = glycosylated hemoglobin
LIMP = lysosomal integral membrane protein
T2DM = Type II diabetes mellitus
TxB_2 = thromboxane B_2
vWF = von Willebrand factor

seen in diabetics in the post-prandial condition, induces platelet hyper-reactivity in patients with T2DM, with particular reference to parameters of platelet function relevant to the pathophysiology of arterial thrombotic complications.

METHODS

Subjects. Twelve patients with T2DM (7 males and 5 females; age 60 ± 2 years [range 48 to 68]; duration of diabetes 7 ± 1 years; body mass index 29 ± 0.5 kg/m^2), as defined in accordance with the American Diabetes Association criteria (19), were studied on two separate occasions. They had not ingested any drugs interfering with platelet function within the previous 10 days and were studied in the morning, under fasting conditions. None had a history of ischemic cardiovascular disease, but the physical examination or instrumental diagnosis was positive for macrovascular complications. At the time of the study, they were being treated with diet (n = 5), oral hypoglycemic agents (metformin and/or sulfonylureas) (n = 5), or insulin (n = 2). None had a history of hemorrhagic diathesis or abnormalities of platelets, renal failure, hepatic failure, alcohol abuse, or heart valve stenosis.

Study design. The patients were enrolled in a cross-over, randomized, double-blind study to assess the effects of 4 h of controlled hyperglycemia or euglycemia on platelet function, using the glucose clamp technique, as previously described (20). The patients underwent the two clamp procedures, according to a randomized sequence, 7 to 10 days apart. The study was approved by our institutional Board Review Committee, and written, informed consent was given by all patients. In the hyperglycemic study, glucose was infused at a variable rate, based on plasma glucose measured every 5 min, to maintain steady state glucose at 250 mg/dl (139 mmol/l). In the euglycemic study, insulin was infused at the rate of 0.4 mU/kg per min, and glucose was infused at a variable rate to maintain plasma glucose at 100 mg/dl (5.5 mmol/l) (20).

Plasma glucose was measured by means of a Beckman glucose analyzer (Glucose Analyzer II, Beckman Instruments, Fullerton, California). Plasma insulin was measured by a previously described assay (21). Glycosylated hemoglobin A_{1c} (HbA_{1c}) was determined by high-performance liquid chromatography, using the HI-Auto A_{1c} TM HA 8121 apparatus (DIC, Kyoto Daiichi, Kogaku Co., Ltd., Kyoto, Japan) (range in non-diabetic subjects: 3.8% to 5.5%).

Blood was collected before and at the end of each clamp. A bleeding-time test was carried out before pre-clamp blood sampling, and another bleeding-time test was done after post-clamp blood sampling. All laboratory tests were carried out by investigators who were unaware of the type of clamping procedure.

Bleeding time. A standardized bleeding-time test was performed using an automatic template device (Simplate II, Organon Teknika Corp., Jessup, Maryland; batch no. 102985), as previously described (22,23). The mean bleeding time was calculated from the two cuts. The blood emerging from the skin wound inflicted for the measurement of the bleeding time was collected for platelet activation measurements (23).

Platelet activation studies. Shear-induced platelet activation was assessed with the O'Brien filtration test (24,25). Two aliquots (5 ml each) of citrated venous whole blood were pushed through polycarbonate glass fiber filters (Pall U100, batch no. 53503; Pall Corp., Port Washington, New York), at a constant pressure of 40 mm Hg. The filter closure time and percentage of platelets retained were measured.

In particular, platelets retained between 20 and 40 s were calculated by the difference of the counts in the blood that had flowed through the filter between 20 and 40 s from the start of the test and the counts in the blood before it passed through the filter.

Platelet aggregation induced by adenosine diphosphate (ADP) 2 μmol/l was performed as previously described (22,26), and the maximal amplitude of aggregation was recorded at 3 min. Spontaneous platelet aggregation was assessed as described (27).

Flow cytometric analysis of platelets was carried out in samples from peripheral venous blood and in the whole blood emerging from the bleeding-time wounds, as previously described (23), by assessing the expression on the platelet surface of the activation antigens P-selectin and LIMP. Expression of activation antigens is given as the percentage of platelets positively stained with the monoclonal antibodies of interest (23).

Von Willebrand factor measurements. Plasma obtained by centrifugation of citrated venous blood at 3,000*g* for 20 min was immediately frozen and stored at −80°C for later assay.

Levels of vWf:antigen (Ag) were measured with an enzyme-linked immunosorbent assay kit (Asserachrom vWF, Boehringer Mannheim, Mannheim, Germany), whereas vWF: activity was tested with a system (vWF Activity Kit, Shield Diagnostic Ltd., Dundee, U.K.; kindly provided by Dr. Poggesi, Bouty S.p.A., Milan, Italy) that utilizes a monoclonal antibody recognizing an epitope of vWF involved in the interaction with glycoprotein Ib-alpha. The results are expressed as the percentage of control.

Urinary excretion of 11-dehydro-TxB_2. The urinary excretion of 11-dehydro-TxB_2 was evaluated by a radioimmu-

Table 1. Metabolic and Blood Count Characteristics of Patients Before and After the Glycemic Clamps

Parameter	Before/After		p Value
	Euglycemia	Hyperglycemia	
HbA_{1c} (%)	7.2 ± 0.18	7.2 ± 0.16	NS
Fasting plasma glucose (mmol/l)	7.8 ± 0.4/5.4 ± 0.1*	7.8 ± 0.3/13.8 ± 0.2*	NS/$p < 0.05$
Plasma insulin (μU/ml)	7.4 ± 0.9/53 ± 1.5*	7.4 ± 0.9/51.3 ± 3.5*	NS/NS
Platelet count ($\times 10^9$/l)	181 ± 5.2/177 ± 6.2*	177 ± 6.4/169 ± 6.9*	NS/$p < 0.05$
Hematocrit (%)	38.9 ± 0.9/37.8 ± 0.8	38.1 ± 0.8/37.3 ± 0.6	NS/NS

*Significantly different from "before" value. Data are presented as the mean value ± SEM.
HbA_{1c} = glycosylated hemoglobin.

noassay, as previously described (9). Briefly, urine subsamples (40 ml) were taken from the 24-h urine collection ending at the start of the clamp (pre-samples), from the 4-h urine collection of the clamp (acute samples), and from the 24-h urine collection starting from the moment of the beginning of the clamp (post-samples) and then snap-frozen in liquid nitrogen and stored at −80°C until assay.

Immunoreactive 11-dehydro-TxB_2 was assessed by previously gas chromatography–mass spectrometry-validated radioimmunoassay techniques (8,28). Plasma glucose, insulin, and HbA_{1c} were measured by standard techniques (20).

Statistical analysis. Statistical analysis was carried out according to the method of Jones and Kenword (29) for crossover designs with two baselines, as previously described (30). Only after carryover and direct-by-period effects were shown as not significant, thus confirming the adequacy of the washout period, data were analyzed with repeated measures analysis of variance followed by Tukey's multiple comparisons test. For the correlation between different parameters, data from each time point of the single patients were analyzed as separate observations by the Pearson first degree index. Cumulative in situ platelet activation during the bleeding-time procedure was calculated as the area under the curve (AUC) by the trapezoidal rule, using data of P-selectin and LIMP expression on platelets up to the third minute, and interpolating them by the GraphPad-Prism 2 software. Statistical analysis and correlation studies were performed using the GraphPad-Prism 2 package. Data are expressed as the mean value ± SEM. Differences were considered as significant at $p < 0.05$.

RESULTS

Patients. The metabolic and blood count characteristics of the patients are reported in Table 1. The HbA_{1c} levels before euglycemic and hyperglycemic clamping were similar (7.2 ± 0.18% and 7.2 ± 0.16%, respectively; p = NS).

Fasting plasma glucose levels were also similar before the two clamps, with values of 140 ± 7 mg/dl (7.8 ± 0.4 mmol/l) and 141 ± 6 mg/dl (7.8 ± 0.3 mmol/l), with comparable metabolic conditions at baseline on the two study days. Baseline plasma insulin was also similar on the two study days (7.4 ± 0.9 and 7.4 ± 0.9 μU/ml). In the euglycemic study, plasma glucose between 30 and 240 min was 98 ± 2 mg/dl (5.4 ± 0.1 mmol/l; coefficient of variation 4.5 ± 0.1%), whereas it was 249 ± 3 mg/dl (13.8 ± 0.2 mmol/l) with the hyperglycemic clamp (coefficient of variation 5.1 ± 0.2%). The glucose infusion rate was 4.35 ± 0.4 mg/kg per min during hyperglycemic clamping and 3.8 ± 0.6 mg/kg per min during euglycemic clamping ($p < 0.05$). Plasma insulin between 30 and 240 min was 53 ± 1.5 μU/ml during euglycemic clamping and 51.3 ± 3.5 μU/ml during hyperglycemic clamping (p = NS).

Platelet counts were also similar before the euglycemic and hyperglycemic clamps (181 ± 5.2 and 177 ± 6.4 × 10^9/l, respectively) and were slightly but significantly reduced after clamping (177 ± 6.2 and 169 ± 6.9 × 10^9/l, respectively; p = 0.01 vs. pre-clamp).

The hematocrit values were 38.9 ± 0.9% and 38.1 ± 0.8% before the euglycemic and hyperglycemic clamps, respectively, and were slightly but not significantly reduced after clamping (37.8 ± 0.8% and 37.3 ± 0.6%, respectively).

Bleeding time. The bleeding time at baseline was similar to that before the euglycemic and hyperglycemic clamps (368 ± 33 and 360 ± 34 s, respectively). It tended to be shortened after the euglycemic clamp, but not significantly (340 ± 25 s, p = NS), whereas it was significantly shortened after the hyperglycemic clamp (275 ± 20 s, p = 0.0007). All values, before and after the clamps, were within normal ranges (138 to 570 s).

Shear-induced platelet activation. Shear stress-induced platelet activation was similar at baseline on the two study days (filter closure time: 49.2 ± 2.2 and 49.2 ± 1.9 s; retained platelets: 81.6 ± 1.5% and 81.9 ± 3.5%, before the euglycemic and hyperglycemic clamps, respectively).

Shear-induced platelet activation was unaffected by the euglycemic clamp (filter closure time: 48.3 ± 1.8 s, p = NS vs. pre-clamp; retained platelets: 81.2 ± 1.4%, p = NS vs. pre-clamp). In contrast, after the hyperglycemic clamp, it was enhanced (filter closure time: 42.5 ± 1.9 s, p = 0.0012; retained platelets: 87.1 ± 3.2%, p = 0.0002) (Fig. 1).

Platelet aggregometry. Platelet aggregation induced by ADP was similar at baseline before the two clamps and did not change significantly after either. Transmittance was 78.4 ± 6.6% and 77.3 ± 7.9% before euglycemic and hyperglycemic clamping and 81 ± 6.3% and 79.8 ± 6.6% after these clamps, respectively. Spontaneous platelet aggregation was not observed in any of the patients either before or after the euglycemic and hyperglycemic clamps.

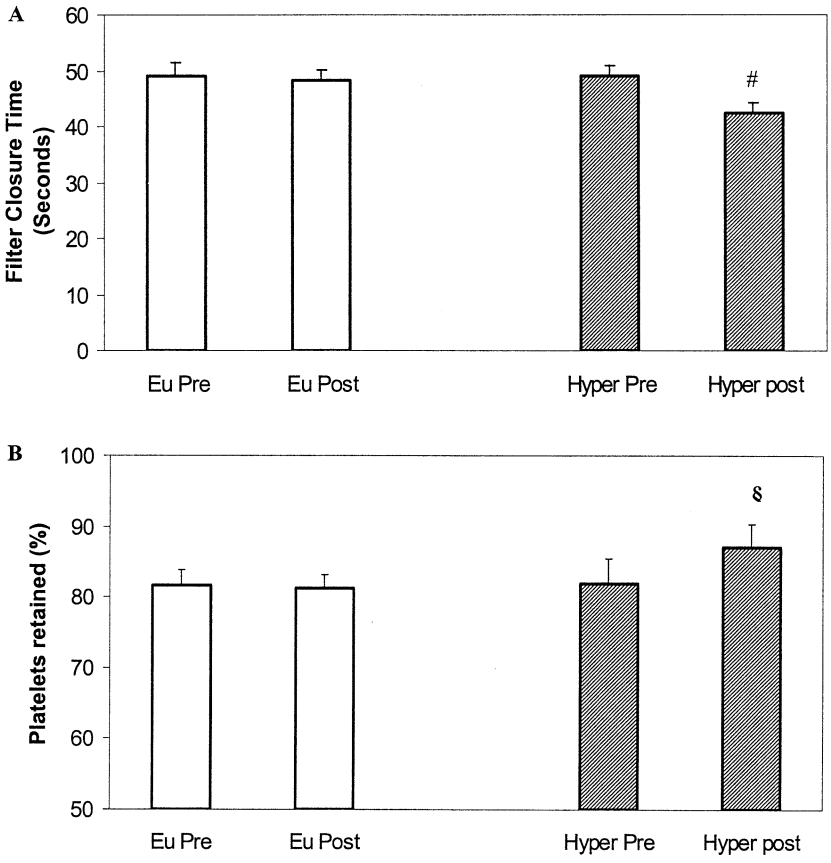

Figure 1. Shear stress-induced platelet activation before and after the euglycemic and hyperglycemic clamps. **(A)** Filter closure time (s). **(B)** Platelets retained between 20 and 40 s (% of total). #p = 0.0012 vs. hyper-pre (i.e., before hyperglycemic clamp). §p = 0.002 vs. hyper-pre. Eu = euglycemic clamp.

Flow cytometry. PLATELET ACTIVATION MARKERS IN VENOUS BLOOD. P-selectin expression on platelets circulating in venous blood was similar at baseline on the two study days (7.9 ± 1.6% and 7.5 ± 1.9% positive cells, respectively). These values are somewhat higher than the average values found in healthy controls in our laboratory (4.0 ± 0.5% positive cells).

P-selectin expression on platelets did not change significantly after either the euglycemic or hyperglycemic clamp (6.6 ± 1.2% and 8.5 ± 1.8%, respectively, p = NS).

Expression of LIMP on platelets circulating in venous blood was similar at baseline on the two study days (9.1 ± 1.9% and 7.5 ± 1.9% positive cells, respectively). Also, these values were somewhat higher than those found in healthy controls in our laboratory (7.0 ± 0.3%). Expression of LIMP on platelets did not change significantly after either the euglycemic or hyperglycemic clamp (9.7 ± 2.1% and 8.2 ± 2%, respectively; p = NS).

PLATELET ACTIVATION MARKERS IN BLEEDING-TIME BLOOD. At baseline, before the clamps, the expression of P-selectin on platelets in the blood emerging from the bleeding-time wound increased progressively up to the fourth minute, in a comparable way on both study days, compatible with ongoing platelet activation, as previously reported (23,31). A maximum of 80.6 ± 10.3% and 78.3 ± 2.15% of P-selectin–positive platelets was reached before euglycemic or hyperglycemic clamping, respectively (data not shown).

After the euglycemic clamp, the time-dependent rise in P-selectin expression on platelets in shed blood was similar to that observed before the clamp, whereas after the hyperglycemic clamp, it was considerably greater, with a significantly larger increase starting from the second minute (Fig. 2A).

Similarly, at baseline, the expression of LIMP on platelets in the blood emerging from the bleeding-time wound increased progressively up to the fourth minute, compatible with ongoing platelet activation, as previously reported (23,31), reaching a maximum of 64.4 ± 4.4% and 63.7 ± 5.9% of positive platelets before the euglycemic and hyperglycemic clamps, respectively (data not shown).

After the euglycemic clamp, the rise in LIMP expression on platelets in the bleeding-time blood was similar to that observed before the clamp, whereas after the hyperglycemic clamp, it was greater, with a significantly larger increase starting from the second minute (Fig. 2B).

Determinations of vWF. Von Willebrand factor antigen in plasma at baseline was similar before the euglycemic and hyperglycemic clamps (93.8 ± 12.8% and 100.4 ± 10.5%,

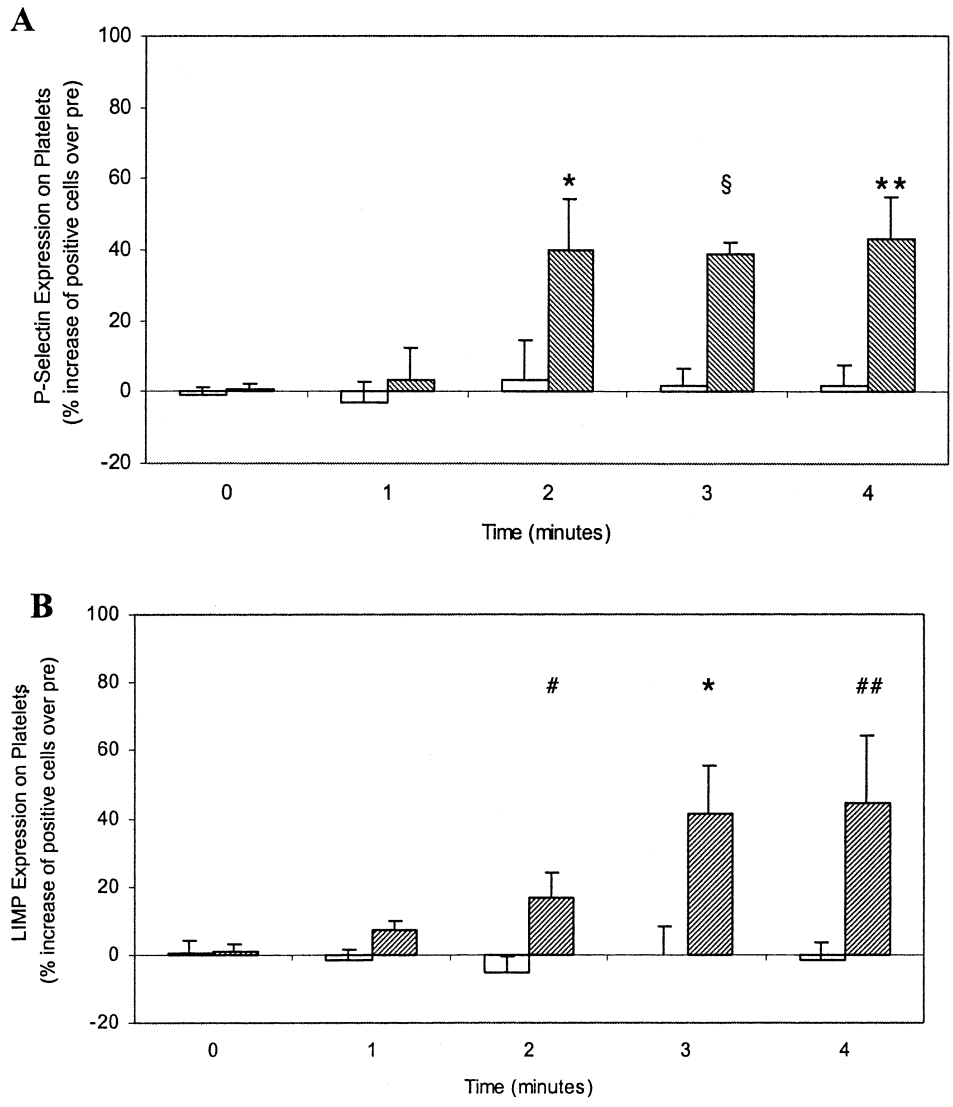

Figure 2. Platelet activation in bleeding-time blood before and after the euglycemic and hyperglycemic clamps. **(A)** P-selectin expression on platelets. **(B)** Expression of LIMP on platelets. **Open bars** = euglycemic; **striped bars** = hyperglycemic. Data are reported as the percent increase in positive platelets between samples after versus before clamping. $^{*}p < 0.04$, §$p < 0.02$ vs. before clamping. $^{**}p < 0.004$, $^{\#}p < 0.03$ vs. before clamping, $^{\#\#}p < 0.005$ vs. before clamping.

respectively; p = NS) and not substantially different from the average normal values found in our laboratory (98.4 ± 2.3%). After the hyperglycemic clamp, vWF:Ag increased to 144.3 ± 14% (p = 0.002), whereas after the euglycemic clamp, it did not change (98.2 ± 11.4%, p = NS).

The vWF:activity at baseline was also comparable before the two clamp studies (71.7 ± 7.2% and 81.9 ± 6.7%, respectively; p = NS) and increased markedly after the hyperglycemic clamp, reaching 124.5 ± 17.3% (p = 0.04), whereas it was unchanged after the euglycemic clamp (82.3 ± 14.3%).

Urinary 11-dehydro-TxB_2. Basal urinary 11-dehydro-TxB_2 excretion before the clamps was comparable on the two study days (1,274 ± 223 and 1,357 ± 301 pg/mg creatinine before the euglycemic and hyperglycemic clamps, respectively), and it was higher than the values of healthy controls in our laboratory (150 to 450 pg/mg creatinine). Urinary 11-dehydro-TxB_2 excretion was unchanged during euglycemic clamping (1,140 ± 248 pg/mg creatinine, p = NS), whereas it was significantly increased during hyperglycemic clamping (1,878 ± 376 pg/mg creatinine, p = 0.02). Finally, 11-dehydro-TxB_2 during the 24 h after the clamp procedure did not differ significantly on the two study days (1,080 ± 201 pg/mg creatinine after the euglycemia clamp and 1,296 ± 269 pg/mg creatinine after the hyperglycemic clamp, p = NS).

Correlations. Plasma vWF:Ag and vWF:activity showed a highly significant correlation. A statistically significant correlation was found between plasma vWF:activity and shear-induced platelet activation (filter closure time), between vWF:activity and urinary 11-dehydro-TxB_2 excretion during the clamps, and between vWF:activity and the AUC of P-selectin and LIMP expression in bleeding-time blood (Fig. 3).

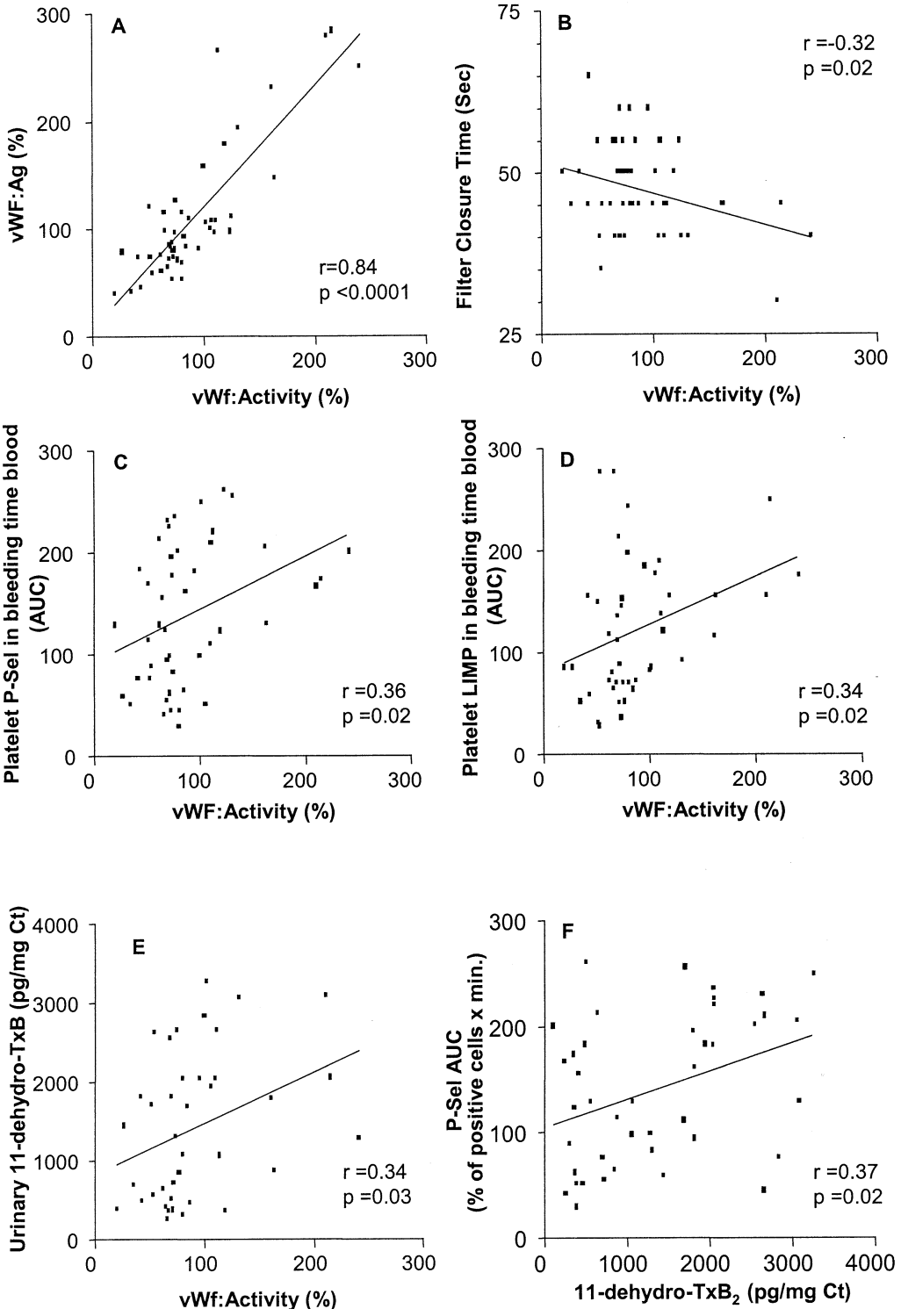

Figure 3. Correlations between different parameters. **(A)** Correlation between vWF:activity and vWF:Ag ($r = 0.84$, $p \leq 0.0001$). **(B)** Correlation between vWF:activity and shear stress-induced platelet activation (filter closure time) ($r = -0.32$, $p = 0.02$). **(C)** Correlation between vWF:activity and P-selectin (P-Sel) expression on platelets in bleeding-time blood (AUC) ($r = 0.36$, $p = 0.03$). **(D)** Correlation between vWF:activity and LIMP expression on platelets in bleeding-time blood (AUC) ($r = 0.34$, $p = 0.02$). **(E)** Correlation between vWF:activity and urinary 11-dehydro-TxB_2 excretion during clamping ($r = 0.34$, $p = 0.03$). **(F)** Correlation between urinary 11-dehydro-TxB_2 excretion during clamping and P-selectin expression on platelets in bleeding-time blood (AUC) ($r = 0.37$, $p = 0.02$). Ct = creatinine.

A statistically significant correlation was also found between 11-dehydro-TxB_2 excretion and P-selectin expression on platelets in the bleeding-time blood (AUC) (Fig. 3). No correlations were found between vWF:activity and ADP-induced platelet aggregation or P-selectin and LIMP expression on platelets in venous blood (data not shown).

DISCUSSION

Platelet activation and cardiovascular complications in T2DM. Previous studies, including data from our group, have demonstrated in vivo platelet activation and platelet hyper-reactivity in T2DM patients (6–10), and chronic hyperglycemia has been clearly identified as a causal factor (8–10). Although the reduction of platelet hyper-reactivity following maintenance of tight metabolic control in diabetics has been demonstrated (8–10), no data are available on whether acute, short-term hyperglycemia further enhances platelet activation. Indeed, several clues point to an important role of hyperglycemic spikes in triggering ischemic cardiovascular complications in T2DM (11–13), but no definite information is available on the effector mechanisms through which acute hyperglycemia may elicit arterial thrombosis.

The present study was designed to evaluate, in experimentally controlled conditions, whether acute, short-term, hyperglycemia enhances platelet activation and especially the activation induced by high shear stress, which is of particular pathophysiologic relevance to platelet thrombus formation at arterial sites of atherosclerotic stenosis (14–17).

Role of high shear stress in the acute hyperglycemia-induced platelet activation. Our results show that acute, short-term hyperglycemia induces an increased activation of platelets exposed to high shear stress in vitro or in vivo, with a mechanism dependent on a raised plasma level of vWF. Indeed, shear stress activation of platelets ex vivo—a parameter highly sensitive to the interaction between vWF and platelets (24,25)—was clearly enhanced after 4 h of hyperglycemia and was not changed after 4 h of euglycemia. Moreover, platelet activation at a localized site of vessel wall damage (i.e., that occurring during the platelet plug formation of the bleeding-time procedure in a condition of average to high shear forces [19 to 60.8 dynes/cm^2]) (32) was increased after acute hyperglycemia but not after short-term euglycemia.

High shear stress-induced platelet activation is strictly dependent on plasma vWF (15,24,25), and we have shown that short-term hyperglycemia induces a significant rise in plasma vWF, whereas after 4 h of euglycemia, no changes in vWF were observed. Urinary 11-dehydro-TxB_2, an in vivo index of platelet activation, also shows that short-term hyperglycemia induces a spike in platelet activation in diabetics, with subsequent rapid restoration to pre-hyperglycemic conditions.

Because urinary excretion of 11-dehydro-TxB_2 gives an integrated view of platelet thromboxane A_2 produced in a given period, wherever in the circulation (33), it is conceivable that upon hyperglycemia, platelets get activated by high shear at localized sites of arterial stenosis, as a consequence of raised circulating vWF, but to an extent not sufficient to acutely modify the expression of P-selectin on platelets recovered in venous blood.

On the other hand, platelet aggregation studied by photometric aggregometry, typically a platelet function test performed under conditions of low shear stress (34), or the expression of P-selectin or LIMP on platelets circulating in venous blood was unaffected by short-term hyperglycemia.

Interestingly, variations in plasma vWF correlated significantly with ex vivo shear stress–induced platelet activation, with P-selectin expression on platelets undergoing in vivo activation during hemostatic plug formation (bleeding time), and with urinary excretion of 11-dehydro-TxB_2—all tests involving the exposure of platelets to high shear conditions—whereas no correlations were evident between vWF and P-selectin on platelets in venous blood or with ADP-induced platelet aggregation in vitro—tests not involving exposure of platelets to high shear stress.

Study limitations. The patients we studied were under fairly good glycemic control, as indicated by their HbA_{1c} values, and it is conceivable that in patients with poorer metabolic control, a stronger pre-existing in vivo platelet activation could have been present. Whether or not acute hyperglycemia would further enhance platelet activation in these patients remains to be determined. In addition, the intravenous glucose clamp technique does not fully mimic the condition of post-prandial hyperglycemia, which involves a concomitant rise in plasma triglycerides and oxidants, and more. However, most of the studies linking acute hyperglycemia to cardiovascular complications utilized the oral glucose tolerance test (11,13), a rather artificial hyperglycemia as well.

The method we used for inducing acute hyperglycemia implies the generation of hyperinsulinemia, which, on the other hand, is common in the post-prandial condition of T2DM (35). Hyperinsulinemia, per se, has been reported to represent a cardiovascular risk factor (36,37), and this might theoretically have confounded our results. However, the present studies were well matched for hyperinsulinemia; thus, the differences observed in terms of platelet function may be entirely attributed to the different plasma glucose rather than insulin concentrations.

Conclusions. Acute, short-term hyperglycemia in patients with T2DM induces a transient hyper-reactivity of platelets to high shear stress, and thus it may potentially contribute to precipitating arterial thrombotic occlusion at stenotic sites. This observation may be of special relevance for patients with T2DM undergoing surgery or for those who are in the early hours of an acute coronary event or in stress situations typically associated with acute hyperglycemia (38), all of which are conditions accompanied by an enhanced incidence of cardiovascular events. Finally, platelet activation induced by high shear stress is poorly sensitive to inhibition by aspirin (39–41), and this might imply the use of alternative therapeutic strategies in patients with T2DM, with a special emphasis on those interventions able to prevent shear stress–induced platelet activation.

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