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Platelet activity, reactivity and platelet–leukocyte conjugate formation before and after exhaustive or moderate exercise in patients with IDDM

Thomas Hilberg, Evelyn Eichler, Doreen Gläser, Volker Schmidt, Holger H. W. Gabriel

Diabetes mellitus alters blood coagulation and platelet function which supports the suggestion that diabetes mellitus is a hypercoagulable state. Firstly the aim of the study was to investigate if differences in platelet activity, reactivity and platelet–leukocyte conjugate (PLC) formation can be observed in subjects with IDDM; secondly, if differences can be seen between the diabetic and control group concerning exercise-induced changes in platelet activation and conjugate formation; and thirdly, if different types of exercise lead to different patterns in platelet activation. Sixteen subjects with IDDM and 16 controls underwent a maximal step test and an endurance test (90% IAT, 45 min). Blood samples were taken after 30 min rest, and immediately and 1 h after completion of exercise. CD62P expression and differentiated platelet–leukocyte conjugates (CD45, CD14, CD41) were detected flow-cytometrically with and without stimulation with TRAP-6. The rest values of the platelet–granulocyte (PGC) and platelet–lymphocyte conjugates (PLyC) were higher ($P < 0.05$) in the diabetics. After exercise, platelet reactivity (CD62P-TRAP; $P < 0.05$) but not the activity (CD62P-unstimulated), as well as all different conjugates with or without stimulation were increased ($P < 0.05$) independently from the group. Differences according to the type of exercise were barely observable. IDDM without vascular complications leads to higher PCG and PLyC at rest and to identical increases in differentiated platelet–leukocyte formation after exercise in comparison with matched controls.

Introduction

The changes in coagulation parameters measured in patients with diabetes mellitus support the suggestion that diabetes mellitus is a hypercoagulable state.[1] Diabetes appears to influence blood coagulation as well as fibrinolysis and additionally platelet function. The present study concentrates on changes in platelet function. As of yet it remains unclear whether platelet count may or may not be altered by diabetes, as studies have shown both changed as well as unchanged platelet counts.[2–4] However, platelet function is increased which can be determined by aggregation tests[5,6] or by using other markers of platelet activation.[6] It has been hypothesised, that vascular disease present in diabetes may lead to platelet damage, and altered platelet function may in turn contribute to furthered vascular disease.[7] However, on account of the heterogeneity of the different measurements of platelet function it is necessary to use a standard for the description of platelet function *in vivo*. The measurement of P-selectin on the surface of platelets via flow cytometric technique has been the golden standard for investigating platelet activation;[8] an increase in P-selectin on the surface of platelets

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Abbreviations:

BP:	blood pressure
IAT:	individual anaerobic threshold
IDDM:	insulin-dependent diabetes mellitus
mabs:	monoclonal antibodies
%PC:	percent antibody-positive cells
PLC:	platelet–leukocyte conjugates
PGC:	platelet–granulocyte conjugates
PMC:	platelet–monocyte conjugates
PLyC:	platelet–lymphocyte conjugates
TRAP:	thrombin receptor activating peptide.

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and higher plasma concentration of circulation P-selectin could be shown in different studies.[9–11] Thus this method was used in this study. However different studies have also shown that degranulated platelets can lose P-selectin on their surface yet continue to function,[12,13] and in addition platelets can bind to endothelial as well as white blood cells *in vivo*. Therefore, to aid in a detailed depiction of platelet function *in vivo*, determination of platelet–leukocyte conjugate formation is relevant. Michelson *et al*. have even postulated that the investigation of platelet–monocyte conjugate formation is a more sensitive marker compared to P-selectin.[8] Different studies confirm that platelet–leukocyte conjugates are excellent markers for pathological conditions; e.g., the presence of increased platelet–leukocyte conjugate formation has been shown in patients with stable[14] or unstable angina,[15] AMI[16] or coronary interventions.[17] The study of Furman *et al*. demonstrates that platelet–monocyte conjugate (PMC) formation can be used as an early marker of an acute myocardial infarction.[18] Furthermore, an increased PMC formation has also been demonstrated in diabetic patients.[19] Increases in conjugate formation and platelet function are not only observable under pathological conditions, but can also temporarily be observed under physiological conditions, e.g., after physical exercise.[20–22] The determination of platelet–leukocyte conjugate formation was also used in this study.

It is not fully understood, if and how the two effects namely diabetes and exercise, influence each other. However, knowledge of potential interactions between exercise and diabetes is important; moderate exercise is often suggested as a therapeutic intervention, and maximal physical exercise is used in diagnostic procedures, e.g. stress testing (ECG). There also exists no data on how different types of exercising may affect platelet function in diabetic subjects.

The aim of this study was to answer the three following questions: firstly, if differences in platelet activity, reactivity and platelet–leukocyte conjugate formation can be observed in moderately trained subjects with insulin-dependent diabetes mellitus in comparison with controls; secondly, if differences can be shown between the diabetic and control groups concerning exercise-induced changes in platelet activation and conjugate formation; and last, but not least, if different types of exercise lead to different patterns in platelet activation in the diabetic and control group.

Materials and methods

Subjects

The study groups comprised 16 moderately trained male patients with insulin-dependent diabetes mellitus (DM) and 16 control subjects (CO) matched for sex, age, fitness and body mass index (BMI). The main characteristics of the 32 non-smoking participants are shown in Table 1. The groups were not statistically different regarding anthropometric, fitness and other main characteristic data, with the exception of blood pressure, HDL, and diabetes-specific characteristics. Basic physical examination of

Table 1. Main characteristics of the diabetic (DM) and control group (CO)

	DM ($N=16$)	CO ($N=16$)	P
Age (years)	27 ± 2	28 ± 2	NS
Height (m)	1.81 ± 0.02	1.81 ± 0.02	NS
Weight (kg)	78 ± 3	76 ± 2	NS
BMI (kg/m^2)	23.8 ± 0.6	23.2 ± 0.4	NS
Body fat (%)	13.7 ± 0.9	14.1 ± 0.9	NS
HbA_{1c} (%)	7.2 ± 0.2	5.4 ± 0.1	<0.001
Serum glucose (mmol/l)	8.80 ± 0.85	5.00 ± 0.13	<0.01
Serum cholesterol (mmol/l)	4.19 ± 0.22	4.51 ± 0.17	NS
Serum LDL-cholesterol (mmol/l)	2.32 ± 0.16	2.79 ± 0.17	NS
Serum HDL-cholesterol (mmol/l)	1.49 ± 0.10	1.28 ± 0.07	<0.05
Serum VLDL-cholesterol (mmol/l)	0.37 ± 0.05	0.44 ± 0.04	NS
Serum triglycerides (mmol/l)	0.81 ± 0.11	0.95 ± 0.09	NS
Serum creatinine (mmol/l)	74.6 ± 0.5	81.2 ± 2.3	NS
Fibrinogen (mg/dl)	289 ± 13	300 ± 10	NS
VO_2 max (ml/min)	3798 ± 175	3870 ± 118	NS
VO_2 relative (ml/min per kg)	49 ± 3	51 ± 2	NS
Maximum power (W)	264 ± 11	272 ± 11	NS
Maximum power relative (W/kg)	3.4 ± 0.2	3.6 ± 0.2	NS
IAT relative (W/kg)	2.2 ± 0.2	2.3 ± 0.2	NS
Heart rate max (bpm)	191 ± 3	195 ± 2	NS
Lactate max (mmol/l)	10.00 ± 0.48	10.90 ± 0.63	NS
Systolic BP at rest (mmHg)	128 ± 3	120 ± 2	<0.05
Diastolic BP at rest (mmHg)	78 ± 2	73 ± 2	<0.05
Duration of diabetes (years)	14.0 ± 2.7	0	<0.001

Values are the means ± SEM. NS, not significant; BP, blood pressure.

the subjects, ECG and blood pressure measurements at rest, as well as a routine laboratory status and blood coagulation standard tests did not reveal pathological findings, with the exception of fasting blood glucose and HbA_{1c}. Four diabetics were treated by insulin pump therapy, the others by conventional insulin therapy. Diabetes-related complications were screened by history, physical examination, retinal fundoscopy (mild non-proliferative retinopathy in three diabetics), repeated measurements of albumin excretion rate (microalbuminuria in two diabetics) and 128-Hz tuning fork (mild neuropathy present in one diabetic). No patient had any clinical symptoms, manifestations or history of cardio- or peripheral vascular disease. Subjects did not use any medication, apart from insulin, 6 weeks prior to the study until the finish. Procedures used in this study were approved by the Ethics Committee of the Faculty of Medicine of the Friedrich-Schiller-University Jena. Written informed consent was obtained from each subject, prior to the start of the study.

Exercise tests

Maximal exercise test One to 2 weeks before the test program all subjects performed an incremental graded exercise on a cycle ergometer (step test, start 50 W, an increase of 25 W every 3 min until volitional exhaustion) to measure peak oxygen uptake (VO_2 peak) and the individual anaerobic threshold (IAT). The data are shown in Table 1. The VO_2 was measured at 0.5-min intervals using an open spirometric system (Oxycon beta, Jaeger, Hoechberg, Germany). Capillary blood samples were obtained from the previously hyperemized ear lobe at rest, at the end of each level of exercise, and at the end of the first, third, fifth, and tenth minute of the recovery period. Maximal lactate concentration was measured by the EBIO plus (Eppendorf, Hamburg, Germany). The IAT was determined according to Stegmann *et al*.[23]

Exercise program The test program included two standardised exercises. First a maximal step test on a cycle ergometer, starting at 50 W, with an increase of 25 W every 3 min until volitional exhaustion and then an endurance test with 90% of the previous measured IAT over 45 min separated by 7 days were assessed. All the exercise tests were done between 08 : 00 and 12 : 00 in the laboratory.

Analytical methods

Laboratory analysis of the control parameters Blood samples (serum) for the determination of the control parameters were obtained after at least a 10-h overnight fast. The control parameter HbA_{1c} were measured by immunoassay (Tina-quant®), microalbuminuria by semi-quantitative test stripes (both Roche Diagnostics GmbH, Mannheim, Germany); glucose by enzymatic amperometry (EBIO plus Eppendorf; Hamburg, Germany); serum-cholesterol and triglycerides were determined enzymatically, creatinine by single slide method (all Ortho Clinical Diagnostics, Neckargemünd, Germany), LDL and VLDL were calculated by Friedewald's formula, and fibrinogen was measured (derived method) on the ACL 7000 (Instrumentation laboratory, Kirchheim, Germany).

Blood sampling and laboratory methods for platelets analysing Blood samples were taken on the exercise days after at least a 10-h overnight fast and a small standardised breakfast by a clean venipuncture (20-gauge needle) from an antecubital vein under controlled venous stasis (< 30 s) of 40 Torr after 30 min rest, and immediately and 1 h after exercise. All venipunctures were taken from the subjects in reclined position in the following order: 10 ml of blood were taken without any anticoagulants for the assessment of other tests. After this, 2.61 ml of blood were added to 0.29 ml of 0.106 M trisodium citrate for the assessment of the platelet and leukocyte count, hematocrit (Act-Diff, Coulter Electronics, Krefeld, Germany) and measurements by flow cytometry as explained in the following section. Additional blood was taken for other tests. Changes in plasma volume were calculated for platelets and leukocytes as described in the literature according to the method of Dill and Costill.[24]

Flow cytometric analysis

Sample preparation for flow cytometric analysis of platelets Direct immuno-fluorescence technique was used for the determination of changes in platelet activity and response to agonists. The following fluorescein-isothiocyanate (FITC), phycoerythrin (PE)-conjugated, phycoerythrin-texas red (ECD)- and phycoerythrin-cyanin 5.1 (PC5)-conjugated monoclonal antibodies (mabs) were purchased from Coulter-Immunotech Diagnostics (Krefeld, Germany): anti-CD62P-FITC (clone CLB-Thromb/6), anti-CD41-PE (clone P2), anti-CD14-ECD (clone RM052) and anti-CD45-PC5 (clone J33). Immediately after withdrawal of blood, the platelet count in the samples was adjusted to approximately 20 000 platelets/µl with PBS buffer (Life Technologies, Gibco-BRL, Karlsruhe, Germany) including 0.5% BSA (Vitros Ortho, Clinical Diagnostics, Neckargemünd, Germany) and prewarmed to 37°C. The following measurements were exactly done as described in detail in the previous study.[20]

Platelet–leukocyte conjugates One hundred µl of whole blood were diluted with 300 µl PBS buffer (Life Technologies, Gibco-BRL) including 1% BSA (PAA Laboratories GmbH) at 37°C. Fifty µl blood were incubated with 6 µl anti-CD45-PC5, 6 µl anti-CD14-ECD (Coulter-Immunotech Diagnostics, Clone RM052), and 12 µl anti-CD41-PE and in the stimulating test with 6 µl TRAP-6 (37.5 µM) for 10 min at 37°C. After incubation, 10 µl anti-CD62P-FITC were added for 5 min at 37°C for blocking conjugate formation via CD62P–PSGL-1 binding *ex vivo*. After that, 1 ml PBS buffer with 1% BSA was added at 4°C to stop the reaction. The gating was measured via side scatter versus CD45 and, in addition, CD45 versus CD14 to characterize granulocytes, monocytes and lymphocytes. The different platelet–leukocyte conjugates were identified by CD41. For isotype control, 12 µl IgG1-PE (Coulter-Immunotech Diagnostics, Clone 679.1Mc7) instead of CD41-PE was added.

Flow cytometry Immediately after the immunolabeling procedure the samples were measured in a flow cytometer (Coulter® EPICS® XL-MCL™, Beckman Coulter GmbH); laser excitation 488 nm, detection of FITC at 525 nm, PE at 575 nm, ECD at 620 nm, and PC5 at 675 nm as previously described.[20] Data acquisition and analysis were performed with System II™ software (Beckman Coulter GmbH). Testing platelet activity and sensitivity to agonist, the platelet population evaluated was found positive in >98% for the platelet-specific CD41 antigen. Activation status of the platelets measured by antibody binding and different platelet–leukocyte conjugate formation were expressed by antibody-positive cells (isotype control was subtracted).

Statistics

Results are reported as mean ± SEM and the range unless otherwise stated. Using the Kolmogorov–Smirnov test, only a small part of the data showed a normal distribution. Normally distributed data were tested by paired Student's *t*-test, others by Wilcoxon rank test to investigate differences between the matched groups before and after exercise, and between the changes according to the different exercises. The testing of the main rest values between the groups was done with the mean rest value of the step and the endurance test. The level of significance was set at $P < 0.05$. Statistical analysis was done with SPSS-11.0 software.

Results

Exercise performance, heart rates and metabolic data

The performance, heart rate and metabolic data are shown in Table 2. These data were not statistically different in the diabetic compared to the control group, with the exception of the diastolic blood pressure 5 min post and lactate concentration at IAT. However, statistically relevant differences could be observed regarding the two different exercises concerning the maximum power in both groups, as well as the maximal heart rate and lactate at the end of exercise. These data all together confirm the severe cardiac and metabolic stress especially in the step test and the differences between the exercises.

Platelet and leukocyte count

The changes in cell count and hematocrit are shown in Table 3. WBC, granulocytes, and platelet counts at rest were significantly higher in the diabetic group compared to the control group ($P < 0.05$). WBC,

Table 2. Performance parameters in the diabetic (DM) and control group (CO) before and after step and endurance test

	DM ($N = 16$)		CO ($N = 16$)	
	Step test	Endurance test	Step test	Endurance test
Maximum power (W)	287 ± 12	–	289 ± 9	–
IAT (W)	184 ± 13	–	182 ± 9	–
90% IAT (W)	–	166 ± 12§	–	164 ± 8§
IAT relative (W/kg)	2.4 ± 0.2	–	2.4 ± 0.1	–
Lactate at rest (mmol/l)	1.19 ± 0.07	1.14 ± 0.09	1.34 ± 0.12	1.30 ± 0.10
Lactate at IAT (mmol/l)	3.35 ± 0.14*	–	3.96 ± 0.18*	–
Lactate at end of exercise (mmol/l)	10.15 ± 0.60	3.33 ± 0.24§	11.34 ± 0.54	3.65 ± 0.29§
Heart rate at rest (bpm)	68 ± 3	68 ± 3	67 ± 3	66 ± 2
Heart rate max (bpm)	194 ± 3	174 ± 4§	197 ± 2	174 ± 3§
Heart rate 5 min post (bpm)	109 ± 3	103 ± 3§	109 ± 2	97 ± 3§
Systolic BP at rest (mmHg)	130 ± 3	121 ± 3	118 ± 2	117 ± 2
Diastolic BP at rest (mmHg)	74 ± 2	74 ± 2	73 ± 2	70 ± 2
Systolic BP 100 W (mmHg)	158 ± 6	–	150 ± 4	–
Diastolic BP 100 W (mmHg)	85 ± 3	–	85 ± 2	–
Systolic BP 5 min post (mmHg)	128 ± 5	118 ± 2	117 ± 3	118 ± 75
Diastolic BP 5 min post (mmHg)	79 ± 1*	73 ± 2	70 ± 3*	75 ± 2§

Values are the means ± SEM. *$P < 0.05$ differences between the groups; §$P < 0.05$ differences between the type of exercise. BP, blood pressure.

Table 3. Blood cell count and hematocrit before and after the exercises

	$N = 16$	Step test			Endurance test		
		Rest	Post	1 h post	Rest	Post	1 h post
WBC (1/nl)	DM	5.18 ± 0.25*	8.83 ± 0.30#	5.56 ± 0.28	5.30 ± 0.23*	8.10 ± 0.40#§	5.68 ± 0.29
	CO	4.63 ± 0.20	8.29 ± 0.44#	4.75 ± 0.26	4.53 ± 0.18	7.32 ± 0.36#§	5.34 ± 0.28
Granulocytes (1/nl)	DM	3.08 ± 0.23*	4.31 ± 0.27#	3.71 ± 0.28	3.17 ± 0.22*	4.51 ± 0.34#	3.71 ± 0.31
	CO	2.48 ± 0.16	3.58 ± 0.27#	2.95 ± 0.21	2.51 ± 0.16	3.70 ± 0.29#	3.34 ± 0.25
Monocytes (1/nl)	DM	0.31 ± 0.03	0.52 ± 0.04#	0.21 ± 0.03	0.33 ± 0.03	0.44 ± 0.04#§	0.26 ± 0.03
	CO	0.33 ± 0.03	0.55 ± 0.06#	0.24 ± 0.03	0.36 ± 0.03	0.46 ± 0.04#§	0.27 ± 0.02
Lymphocytes (1/nl)	DM	1.79 ± 0.09	4.01 ± 0.22#	1.63 ± 0.07	1.80 ± 0.10	3.15 ± 0.22#§	1.72 ± 0.08
	CO	1.79 ± 0.07	4.20 ± 0.20#	1.56 ± 0.06	1.66 ± 0.06	3.18 ± 0.18#§	1.73 ± 0.08
Platelets (1/nl)	DM	248 ± 9*	306 ± 10#	257 ± 9	247 ± 9*	323 ± 12#§	250 ± 9
	CO	216 ± 8	260 ± 10#	218 ± 8	212 ± 7	278 ± 10#§	221 ± 8
Hematocrit (l/l)	DM	0.42 ± 0.01	0.47 ± 0.01#	0.42 ± 0.01	0.40 ± 0.01	0.44 ± 0.01#	0.41 ± 0.01
	CO	0.41 ± 0.01	0.46 ± 0.01#	0.41 ± 0.01	0.40 ± 0.01	0.43 ± 0.01#§	0.40 ± 0.01

Values are the means ± SEM. *$P < 0.05$ differences between the groups (mean of rest values of step and endurance test); #$P < 0.05$ differences Rest to Post; §$P < 0.05$ differences between the type of exercise. WBC, white blood count.

Table 4. CD62P expression and platelet–leukocyte conjugate formation unstimulated and TRAP-stimulated before and after the exercises

	$N = 16$	Step test			Endurance test		
		Rest	Post	1 h post	Rest	Post	1 h post
%PC unstimulated							
CD62P	DM	0.82 ± 0.10	0.95 ± 0.11	0.81 ± 0.10	0.92 ± 0.10	0.95 ± 0.11	0.94 ± 0.11
	CO	0.91 ± 0.14	1.24 ± 0.13	0.85 ± 0.10	0.82 ± 0.07	0.72 ± 0.08§	0.71 ± 0.07
PGC	DM	5.79 ± 0.37*	8.28 ± 0.36#	5.89 ± 0.26	6.07 ± 0.34*	8.10 ± 0.42#	6.28 ± 0.39
	CO	4.91 ± 0.50	7.57 ± 0.54#	5.43 ± 0.33	3.75 ± 0.48	6.39 ± 0.32#	4.74 ± 0.26
PMC	DM	6.55 ± 1.53	11.64 ± 2.41#	6.00 ± 1.51	6.46 ± 1.21	7.28 ± 1.15	7.25 ± 1.06
	CO	5.10 ± 1.10	8.34 ± 1.61#	5.93 ± 1.48	3.34 ± 0.76	4.76 ± 0.76#	3.51 ± 0.49
PLyC	DM	5.13 ± 0.24*	6.77 ± 0.33#	5.40 ± 0.25	5.42 ± 0.21*	7.10 ± 0.22#	5.44 ± 0.24
	CO	4.38 ± 0.16	6.39 ± 0.28#	4.66 ± 0.18	4.29 ± 0.19	5.96 ± 0.27#	4.66 ± 0.19
%PC TRAP-stimulated							
CD62P	DM	63.6 ± 5.0	70.9 ± 2.9#	67.8 ± 3.7	63.2 ± 5.7	69.7 ± 5.0	65.9 ± 5.1
	CO	58.1 ± 4.6	66.0 ± 4.0#	50.6 ± 6.0	66.0 ± 4.2	64.7 ± 6.3	54.6 ± 6.1
PGC	DM	9.8 ± 0.8*	16.1 ± 1.6#	9.1 ± 0.7	11.6 ± 1.1*	16.4 ± 2.1#	11.2 ± 1.4
	CO	9.4 ± 1.0	14.8 ± 1.5#	8.5 ± 0.6	7.7 ± 1.0	11.9 ± 1.1#	7.4 ± 0.4
PMC	DM	16.0 ± 2.1	28.3 ± 3.9#	13.6 ± 2.0	18.8 ± 2.3	25.0 ± 4.3	16.7 ± 3.1
	CO	13.5 ± 2.1	22.3 ± 2.7#	11.1 ± 1.6	10.4 ± 1.2	15.3 ± 2.2#	8.9 ± 0.6
PLyC	DM	5.9 ± 0.2*	7.7 ± 0.4#	5.7 ± 0.2	6.0 ± 0.2*	8.1 ± 0.3#	5.9 ± 0.2
	CO	4.9 ± 0.2	6.9 ± 0.4#	4.8 ± 0.2	4.9 ± 0.3	6.6 ± 0.3#	5.0 ± 0.2

Values are the means ± SEM. *$P < 0.05$ differences between the groups (mean of rest values of step and endurance test); #$P < 0.05$ differences Rest to Post; §$P < 0.05$ differences between the type of exercise. PGC, platelet–granulocyte conjugates, PMC, platelet–monocyte conjugates; PLyC, platelet–lymphocyte conjugates; %PC, percent positive cells.

granulocyte, monocyte, lymphocyte and platelet counts were significantly ($P < 0.05$) increased after both exercise tests in both groups. No differences could be observed in the increases after exercise in the diabetic versus control group. However, the enhancement in WBC, monocytes, and lymphocytes after exercise was higher in both groups after the step test but lower in the platelets compared with the endurance test ($P < 0.05$).

Platelet activity and platelet–leukocyte conjugates (unstimulated)

The results of the unstimulated experiments are shown in the upper part of Table 4. The rest values in CD62P-positive cells were not different in either group. PGC and PLyC were higher in the diabetic group in comparison with the controls. These results could also be observed in the absolute cell count of CD41-positive granulocytes ($P < 0.01$) and lymphocytes ($P < 0.05$), as demonstrated in Figure 1. After exercise, PGC, PMC, PlyC were all increased ($P < 0.05$) in both groups after both exercises, with the exception of PMC in the diabetic group after the endurance exercise. In addition, the increase ($P < 0.01$) in the PGC absolute cell count after the different exercises is shown in Figures 2 and 3. No differences of the changes after exercise could be demonstrated in the diabetic versus control group. The comparison of changes after the two exercises

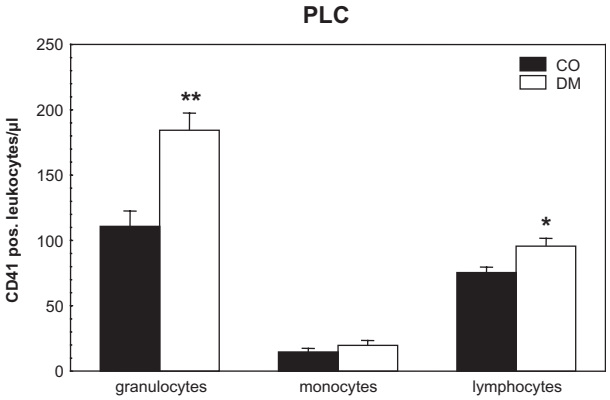

Figure 1. Absolute count of platelet–leukocyte conjugate subpopulations (PLC)/µl; DM, diabetic group ($N = 16$); CO, control group ($N = 16$). Differences between the groups: $^{*}P < 0.05$; $^{**}P < 0.01$; mean ± SEM (mean of rest values of step and endurance test).

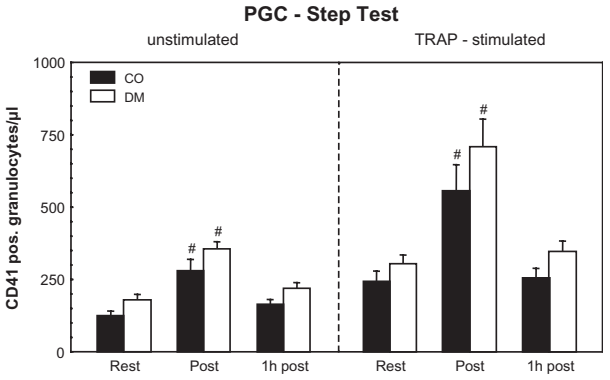

Figure 2. Absolute count of platelet–granulocyte conjugates (PGC)/µl in the step test; DM, diabetic group ($N = 16$); CO, control group ($N = 16$); differences between Rest and Post: $\#P < 0.001$; mean ± SEM.

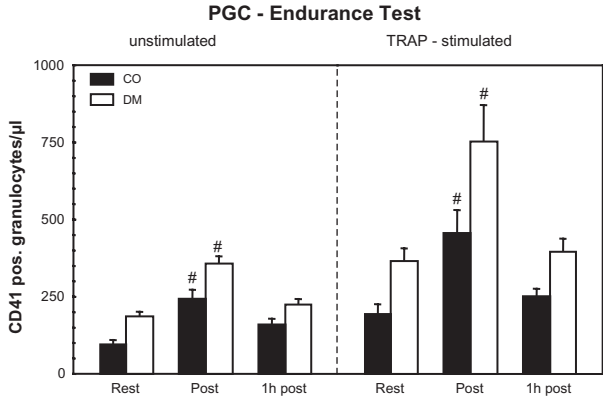

Figure 3. Absolute count of platelet–granulocyte conjugates (PGC)/µl in the endurance test; DM, diabetic group ($N = 16$); CO, control group ($N = 16$); differences between Rest and Post: $\#P < 0.001$; mean ± SEM.

only showed a difference in CD62P in the control group ($P < 0.05$).

Platelet reactivity and platelet–leukocyte conjugates (TRAP-stimulated)

In the stimulation experiment, the rest values in PGC and PLyC were also significantly higher in the diabetic group ($P < 0.05$) (Table 4, lower part). All parameters showed an increase ($P < 0.05$) after the step test, which was similar to the endurance test, with the exception of CD62P-positive cells in both groups and PMC in the diabetic group. These increases after exercise were independent of IDDM. In addition, the rise ($P < 0.01$) in TRAP-stimulated PGC absolute cell count after the exercises is shown in Figures 2 and 3. Differences induced by the type of exercises were not observed.

Discussion

Diabetes mellitus increases the mortality risk. Eighty percent of diabetics (type II) die a thrombotic death and 75% of these deaths are a result of cardiovascular complications.[1] These complications are due to endothelial abnormalities and changes in blood coagulation and platelet function.[5,25–27] Increased platelet adhesion and aggregation have been reported in subjects with diabetes.[28] In addition, using flow cytometry, Tschoepe *et al.*[10,29] have shown that P-selectin, as a marker for platelet activation, is increased in diabetic patients independent of metabolic control. Higher P-selectin levels have also been observed in islet cell antibody-positive healthy relatives (first degree) of IDDM patients.[30] In the present study, platelet and granulocyte count and also WBC were higher in the diabetic group in comparison with the control group. Higher granulocyte count in diabetics has been shown in different studies,[31,32] but again no differences were observed.[33] Elevated platelet counts are frequently observed in diabetics with a long duration of disease or complicated by nephropathy.[3] In the study of Brown *et al.*, platelet count was increased in diabetics both with or without vascular disease, but only significantly in the group with vascular complications.[4] Platelet activity measured by CD62P in the unstimulated experiment was not different in our diabetic group compared to controls. This is somewhat in contrast to the literature.[9,10,29,30] The discrepancy of the results may be due to the different experimental groups; in the present study, moderately trained type I diabetics without vascular complications were included. Vascular complications alter platelet function but it is not clearly proved if the training status influences P-selectin expression at rest, while Kestin *et al.* demonstrated that fitness level can influence exercise-induced receptor regulation in platelets.[34] Of course, fitness training can alter other hemostatic markers in type I diabetic patients.[35] After TRAP-stimulation, CD62P expression was slightly higher in the diabetic group but this was statistically not relevant.

In contrast, PGC and PLyC unstimulated and TRAP-stimulated were higher in the diabetic group. These parameters are possibly more sensitive, showing small differences as seen in the present experiment. Mainly platelets and leukocytes, granulocytes

and monocytes form the conjugates via P-selectin on the surface of activated platelets and its counterpart P-selectin glycoprotein ligand-1 (PSGL-1) and CD15, and in addition by fibrinogen bridging between glycoprotein (GP) IIb/IIIa and CD11b/CD18.[36,37] This conjugate formation may facilitate platelet–leukocyte interaction with modifications in leukocyte rolling, adhesion and migration *in vivo*[38] and *in vitro*.[39] Figure 1 shows the differences in absolute platelet–leukocyte conjugate count per µl of blood, which depicts the situation *in vivo*.

Not only vascular diseases but also exercise itself can influence platelet function. A small rise in CD62P unstimulated after an exhaustive step test on a treadmill ergometer but not after long-term exercise on a treadmill and short-term exercise on a cycle ergometer has been investigated in healthy non-smokers.[20–22] Of course, the different exercises in these studies led to an enhanced platelet reactivity measured by CD62P expression after stimulation and increased platelet–leukocyte conjugate formation, which could be shown in two of these studies, when conjugate formation was also investigated. Although exercise is appropriate for diagnostic and rehabilitation interventions, little is known about the combination of exercise and diabetes mellitus regarding platelet function. Hendra *et al.* failed to find any differences in the exercise induced increase of β-thromboglobulin concentration and agonist-induced aggregation in diabetics versus controls.[40] In the present study, maximal exercise initiated by an incremental step test led to an increase in all blood cell counts and hematocrit in the diabetic group, as well as in the control group. However, only the control group showed a small increase in platelet activity after maximal exercise which was not statistically relevant ($P = 0.056$). In contrast, platelet reactivity was increased together with all the platelet–leukocyte conjugate subpopulations after maximal exercise in the unstimulated and TRAP-6-stimulated experimental tests, but these changes were not different within the groups. The level of the conjugates was higher in the diabetics, which has been demonstrated in the rest values, but the effect of exercise was similar between the diabetics and controls. Figure 2 shows the changes in PGC absolute cell counts regarding the step test and clearly demonstrates the situation *in vivo*. The comparison of step test and endurance test was done to investigate differences between maximal and submaximal exercise. A step test is normally used in the standardising stress test ECG; the endurance test was initiated with 90% of the individual anaerobic threshold (IAT) and a duration of 45 min, which can be recommended for rehabilitative training. The IAT is a commonly used method to control exercise intensity; at IAT lactate production and elimination are in a steady state, but above this point lactate concentration will increase until exhaustion of the subjects due to the pH decrease. Above this IAT, the catecholamines will increase over-proportionally, as demonstrated by Urhausen *et al.*[41] The endurance test also led to a rise in blood cell counts and hematocrit, but this was lower in the majority of cases in comparison with the maximal step test. Endurance exercise induced neither platelet activity nor platelet reactivity in a statistically relevant dimension. However, conjugate formation seemed to be the more sensitive parameter again. After the endurance exercise, PGC and PLyC in both, and PMC only in the control group were enhanced in the unstimulated samples identical to the TRAP-stimulated samples. Figure 3 shows the changes in the absolute PGC count *in vivo* in this experiment. The peak level of the conjugates after the endurance exercise appeared somewhat lower in some cases, but that was statistically not relevant. Concerning the type of exercise, only the change of CD62P expression after the step test in comparison with the endurance test was significantly different.

It can be summarised that in the present study the PCG and PLyC at rest were higher in the diabetics compared to controls, but differences between the groups concerning CD62P expression on the surface of the platelets have not been verified. Yet, if measurement of platelet–leukocyte conjugates offers the possibility to detect early changes in diabetes, this should be further investigated in additional studies. A maximal step test leads to an increase in platelet reactivity (CD62P in TRAP-stimulated samples) and PCG, PMC, and PLyC formation but this was independent of IDDM. An endurance test resulted in lower increases in absolute blood cell count but the increase in differentiated platelet–leukocyte conjugate formation was similar to the changes seen after an exhaustive step test.

The subjects with IDDM but without vascular complications in this study only showed a slightly altered platelet function at rest which was barely able to be investigated by measuring platelet–leukocyte conjugates. The exercise-induced changes in platelet function in these diabetics were comparable to control subjects and did not result in a hypercoagulable state.

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References

1. Carr M E. Diabetes mellitus: a hypercoagulable state. *J Diabetes Complications* 2001; **15**: 44–54.
2. Borsey D Q, Prowse C V, Gray R S, Dawes J, James K, Elton R A, Clarke B F. Platelet and coagulation factors in proliferative diabetic retinopathy. *J Clin Pathol* 1984; **37**: 659–64.
3. Sterner G, Carlson J, Ekberg G. Raised platelet levels in diabetes mellitus complicated with nephropathy. *J Intern Med* 1998; **244**: 437–41.

4. Brown A S, Hong Y, de Belder A, Beacon H, Beeso J, Sherwood R, Edmonds M, Martin J F, Erusalimsky J D. Megakaryocyte ploidy and platelet changes in human diabetes and atherosclerosis. *Arterioscler Thromb Vasc Biol* 1997; **17**: 802–7.
5. Garcia Frade L J, de la Calle H, Alava I, Navarro J L, Creighton L J, Gaffney P J. Diabetes mellitus as a hypercoagulable state: its relationship with fibrin fragments and vascular damage. *Thromb Res* 1987; **47**: 533–40.
6. Hughes A, McVerry B A, Wilkinson L, Goldstone A H, Lewis D, Bloom A. Diabetes, a hypercoagulable state? Hemostatic variables in newly diagnosed type 2 diabetic patients. *Acta Haematol* 1983; **69**: 254–9.
7. Vinik A I, Erbas T, Park T S, Nolan R, Pittenger G L. Platelet dysfunction in type 2 diabetes. *Diabetes Care* 2001; **24**: 1476–85.
8. Michelson A D, Barnard M R, Krueger L A, Valeri C R, Furman M I. Circulating monocyte-platelet aggregates are a more sensitive marker of in vivo platelet activation than platelet surface P-selectin: studies in baboons, human coronary intervention, and human acute myocardial infarction. *Circulation* 2001; **104**: 1533–7.
9. Omoto S, Nomura S, Shouzu A, Nishikawa M, Fukuhara S, Iwasaka T. Detection of monocyte-derived microparticles in patients with Type II diabetes mellitus. *Diabetologia* 2002; **45**: 550–5.
10. Tschoepe D, Roesen P, Esser J, Schwippert B, Nieuwenhuis H K, Kehrel B, Gries F A. Large platelets circulate in an activated state in diabetes mellitus. *Semin Thromb Hemost* 1991; **17**: 433–8.
11. Jilma B, Fasching P, Ruthner C Rumplmayr A, Ruzicka S, Kapiotis S, Wagner O F, Eichler H G. Elevated circulating P-selectin in insulin dependent diabetes mellitus. *Thromb Haemost* 1996; **76**: 328–32.
12. Michelson A D, Barnard M R, Hechtman H B, MacGregor H, Connolly R J, Loscalzo J, Valeri C R. In vivo tracking of platelets: circulating degranulated platelets rapidly lose surface P-selectin but continue to circulate and function. *Proc Natl Acad Sci USA* 1996; **93**: 11877–82.
13. Berger G, Hartwell D W, Wagner D D. P-Selectin and platelet clearance. *Blood* 1998; **92**: 4446–52.
14. Furman M I, Benoit S E, Barnard MR Valeri C R, Borbone M L, Becker R C, Hechtman H B, Michelson A D. Increased platelet reactivity and circulating monocyte-platelet aggregates in patients with stable coronary artery disease. *J Am Coll Cardiol* 1998; **31**: 352–8.
15. Ott I, Neumann F J, Gawaz M, Schmitt M, Schomig A. Increased neutrophil-platelet adhesion in patients with unstable angina. *Circulation* 1996; **94**: 1239–46.
16. Neumann F J, Marx N, Gawaz M, Brand K, Ott I, Rokitta C, Sticherling C, Meinl C, May A, Schomig A. Induction of cytokine expression in leukocytes by binding of thrombin-stimulated platelets. *Circulation* 1997; **95**: 2387–94.
17. Mickelson J K, Lakkis N M, Villarreal-Levy G, Hughes B J, Smith C W. Leukocyte activation with platelet adhesion after coronary angioplasty: a mechanism for recurrent disease? *J Am Coll Cardiol* 1996; **28**: 345–53.
18. Furman M I, Barnard M R, Krueger L A, Fox M L, Shilale E A, Lessard D M, Marchese P, Frelinger AL 3rd, Goldberg R J, Michelson A D. Circulating monocyte-platelet aggregates are an early marker of acute myocardial infarction. *J Am Coll Cardiol* 2001; **38**: 1002–6.
19. Kaplar M, Kappelmayer J, Veszpremi A, Szabo K, Udvardy M. The possible association of in vivo leukocyte-platelet heterophilic aggregate formation and the development of diabetic angiopathy. *Platelets* 2001; **12**: 419–22.
20. Hilberg T, Schmidt V, Glaser D, Schammne D, Losche W, Gabriel H H. Platelet activity, sensitivity to agonist, and platelet-leukocyte conjugate formation after long-term exercise. *Platelets* 2002; **13**: 273–7.
21. Hilberg T, Schmidt V, Losche W, Gabriel H H. Platelet activity and sensitivity to agonists after exhaustive treadmill exercise. *J Sports Sci Med* 2003; **2**: 15–22.
22. Hilberg T, Glaser D, Schmidt V, Losche W, Franke G, Schneider K, Gabriel H H. Short-term exercise and platelet activity, sensitivity to agonist, and platelet-leukocyte conjugate formation. *Platelets* 2003; **14**: 67–74.
23. Stegmann H, Kindermann W, Schnabel A. Lactate kinetics and individual anaerobic threshold. *Int J Sports Med* 1981; **2**: 160–5.
24. Dill D B, Costill D L. Calculation of percentage changes in volumes of blood, plasma, and red cells in dehydration. *J Appl Physiol* 1974; **37**: 247–8.
25. Williams E, Timperley W R, Ward J D, Duckworth T. Electron microscopical studies of vessels in diabetic peripheral neuropathy. *J Clin Pathol* 1980; **33**: 462–70.
26. Reverter J L, Reverter J C, Tassies D, Rius F, Monteagudo J, Rubies-Prat J, Escolar G, Ordinas A, Sanmarti A. Thrombomodulin and induced tissue factor expression on monocytes as markers of diabetic microangiopathy: a prospective study on hemostasis and lipoproteins in insulin-dependent diabetes mellitus. *Am J Hematol* 1997; **56**: 93–9.
27. Lopez Y, Paloma M J, Rifon J, Cuesta B, Paramo J A. Measurement of prethrombotic markers in the assessment of acquired hypercoagulable states. *Thromb Res* 1999; **93**: 71–8.
28. Colwell J A, Halushka P V. Platelet function in diabetes mellitus. *Br J Haematol* 1980; **44**: 521–6.
29. Tschoepe D, Driesch E, Schwippert B, Nieuwenhuis H K, Gries F A. Exposure of adhesion molecules on activated platelets in patients with newly diagnosed IDDM is not normalized by near-normoglycemia. *Diabetes* 1995; **44**: 890–4.
30. Tschoepe D, Driesch E, Schwippert B, Lampeter E F. Activated platelets in subjects at increased risk of IDDM. DENIS Study Group. Deutsche Nikotinamid Interventionsstudie. *Diabetologia* 1997; **40**: 573–7.
31. Jackson M H, Collier A, Nicoll J J, Muir A L, Dawes J, Clarke B F, Bell D. Neutrophil count and activation in vascular disease. *Scott Med J* 1992; **37**: 41–3.
32. Collier A, Jackson M, Bell D, Patrick A W, Matthews D M, Young R J, Clarke B F, Dawes J. Neutrophil activation detected by increased neutrophil elastase activity in type 1 (insulin-dependent) diabetes mellitus. *Diabetes Res* 1989; **10**: 135–8.
33. Thomson G A, Fisher B M, Gemmell C G, MacCuish A C, Gallacher S J. Attenuated neutrophil respiratory burst following acute hypoglycaemia in diabetic patients and normal subjects. *Acta Diabetol* 1997; **34**: 253–6.
34. Kestin A S, Ellis P A, Barnard M R, Errichetti A, Rosner B A, Michelson A D. Effect of strenuous exercise on platelet activation state and reactivity. *Circulation* 1993; **88**: 1502–11.
35. Rigla M, Fontcuberta J, Mateo J, Caixas A, Pou J M, de Leiva A, Perez A. Physical training decreases plasma thrombomodulin in type I and type II diabetic patients. *Diabetologia* 2001; **44**: 693–9.
36. Hagberg I A, Lyberg T. Evaluation of circulating platelet-leukocyte conjugates: a sensitive flow cytometric assay well suited for clinical studies. *Platelets* 2000; **11**: 151–60.
37. Li N, Hu H, Lindqvist M, Wikstrom-Jonsson E, Goodall A H, Hjemdahl P. Platelet-leukocyte cross talk in whole blood. *Arterioscler Thromb Vasc Biol* 2000; **20**: 2702–8.
38. Diacovo T G, Puri K D, Warnock R A, Springer T A, von Andrian U H. Platelet-mediated lymphocyte delivery to high endothelial venules. *Science* 1996; **273**: 252–5.
39. Fernvik E, Lundahl J, Magnusson C G, Hallden G. The effect of in vitro activation and platelet interaction on the CD9 distribution and adhesion properties of human eosinophils. *Inflamm Res* 1999; **48**: 28–35.
40. Hendra T J, Oughton J, Smith C C, Betteridge D J, Yudkin J S. Platelet function in uncomplicated insulin-dependent diabetic patients at rest and following exercise. *Diabet Med* 1988; **5**: 469–73.
41. Urhausen A, Weiler B, Coen B, Kindermann W. Plasma catecholamines during endurance exercise of different intensities as related to the individual anaerobic threshold. *Eur J Appl Physiol Occup Physiol* 1994; **69**: 16–20.