Moderate Exercise Increases Platelet Function in Type I Diabetic Patients Without Severe Angiopathy and in Good Control

MARIELLA TROVATI, MD
GIOVANNI ANFOSSI, MD
ROBERTO DE FACIS, MD
ELENA MULARONI, MD
PAOLA MASSUCCO, MD
FRANCO CAVALOT, MD
SERENELLA BURZACCA, MD
LUIGI MATTIELLO, MS
PIETRO GRECO LUCCHINA, MD

OBJECTIVE — The aim of this study was to investigate whether a 45-min moderate exercise, performed postprandially with a timing that partially prevented the risk of hypoglycemia, was able to modify platelet function in patients affected by insulin-dependent (type I) diabetes mellitus without severe late complications and in a good metabolic control.

RESEARCH DESIGN AND METHODS — We submitted 6 male type I diabetic patients (27.2 ± 3.4 yr; body mass index, 21.4 ± 0.6 kg/m^2; HbA_{1c}, 7.6 ± 0.9%) on a daily three-insulin injection regimen, without severe late complications of diabetes, to a 45-min moderate exercise (about 50% of maximal oxygen consumption) with a cycle ergometer, beginning 180 min after breakfast and 195 min after a subcutaneous shot of regular insulin. Serial venous blood samples were conducted to measure plasma glucose, free insulin, counterregulatory hormones (glucagon, growth hormone, cortisol, and catecholamines), platelet sensitivity to ADP, platelet activating factor and collagen, and plasma concentrations of the platelet-specific protein β-thromboglobulin (a marker of the platelet release reaction in vivo).

RESULTS — Exercise was accompanied by a decrease of plasma glucose (from 5.9 ± 1.2 to 4.6 ± 1 mmol/L, $P = 0.067$) and free insulin (from 180 ± 36 to 114 ± 30 pmol/L, $P = 0.003$), and by a significant increase of growth hormone (from 5 ± 1 to 15 ± 4 μg/L, $P = 0.045$), cortisol (from 240 ± 30 to 406 ± 69 nmol/L, $P = 0.018$), epinephrine (from 1005 ± 240 to 5143 ± 1753 pmol/L, $P = 0.077$), and norepinephrine (from 5.04 ± 1.08 to 13.48 ± 2.98 nmol/L, $P = 0.009$). Platelet sensitivity to the agonists and plasma concentrations of β-thromboglobulin increased during the exercise period. In particular, ADP ED_{50} reached during exercise 61 ± 16% of basal values ($P = 0.048$), platelet activating factor ED_{50} reached 73 ± 11% ($P = 0.043$), and collagen ED_{50} reached 68 ± 9% ($P = 0.008$). β-Thromboglobulin rose from 24 ± 2 to 32 ± 3 μg/L ($P = 0.007$).

CONCLUSIONS — Moderate exercise enhances platelet function in type I diabetic patients without severe angiopathy and in a good metabolic control.

FROM THE DIABETIC UNIT, DEPARTMENT OF CLINICAL AND BIOLOGICAL SCIENCES, UNIVERSITY OF TURIN, SAN LUIGI GONZAGA HOSPITAL; AND THE CARDIOLOGICAL UNIT, SAN LUIGI GONZAGA HOSPITAL, ORBASSANO, TURIN, ITALY.

ADDRESS CORRESPONDENCE AND REPRINT REQUESTS TO MARIELLA TROVATI, MD, DEPARTMENT OF CLINICAL AND BIOLOGICAL SCIENCES, UNIVERSITY OF TURIN, SAN LUIGI GONZAGA HOSPITAL, 10043 ORBASSANO, TURIN, ITALY.

Does exercise benefit the diabetic patient? This question, raised by Sherwin and Koivisto (1), has many answers. Actually, if we consider the influence of exercise on the metabolic control in insulin-dependent (type I) diabetic patients, we can admit that it frequently contributes to the deterioration of blood glucose profiles in patients not strictly educated, owing to the risk of exercise-induced hypoglycemic episodes. In well-educated patients, with the sophisticated knowledge of insulin pharmacokinetics and action, we do not need the "horse" of exercise to obtain the "victory" of good control, according to the symbolism of the Joslin medal: insulin and diet are enough.

Another aspect of the complex interrelationships between exercise and diabetes is the role of physical activity on late complications. Episodical reports described the occurrence of acute vascular accidents during exercise (2). Among the possible causes of these phenomena, an exercise-induced platelet activation cannot be ruled out: actually, an increase of platelet aggregability during muscular work has been described in healthy subjects (3–6) and in patients affected by coronary artery disease (7–10), together with an increase of coagulating factors (11,12).

The few studies concerning the exercise-induced changes in platelet function in type I diabetic patients (13–15) differ as far as the metabolic control of patients, the type and duration of exercise, and the methods used to assess platelet aggregability are concerned.

Further studies, therefore, are needed to investigate the relationships between exercise and platelet function in type I diabetic patients in well-defined experimental conditions. A point to be elucidated is the behavior of platelet aggregability not only immediately after exercise, but also during exercise and in the recovery phase.

The aim of this study was to investigate, in a group of type I diabetic

patients in an acceptable control and without severe diabetes late complications, the influence on platelet activation of a 45-min moderate physical exercise performed with a timing that reduces the occurrence of excessive blood glucose decrease (16). Because muscular work is conducted when plasma-free insulin concentrations are falling down—caused by the pharmacokinetics of injected insulin—it imitates the physiological exercise-induced insulin decrease (17). Our aim is, therefore, to observe the dynamics of the platelet responses during, at the end, and after a physical exercise conducted in the conditions usually considered ideal as far as the characteristics of the subjects; the condition of their metabolic control; the type, intensity, and duration of exercise; and, finally, the timing of exercise itself are concerned (2).

RESEARCH DESIGN AND METHODS

— Six male type I diabetic patients, aged 27.2 ± 3.4 (mean ± SEM) yr, with a body mass index of 21.4 ± 0.6 kg/m^2, diabetes duration of 12.6 ± 3.7 yr, and an HbA_{1c} (chromatographic method with microcolumns, BioRad, Richmond, CA) of 7.6 ± 0.9%, were investigated after informed consent. They were on a daily three-insulin injection regimen, receiving subcutaneous shots of regular insulin before breakfast and lunch, and regular + intermediate insulin before supper. They did not present signs or symptoms of macroangiopathy, nephropathy, neuropathy, or proliferative retinopathy. Two of them showed few microaneurysms at fluoroangiography. Subjects were nonsmokers and did not take medications known to affect platelet function within the preceding 4 wk. None of them practiced sports on a regular basis.

After admission to the metabolic ward, subjects received their regular subcutaneous insulin injection (Actrapid Novo, Copenhagen, Denmark), 9.2 ± 0.7 U and, 15 min later, their breakfast (milk and bread). A 45-min exercise on a bicycle ergometer (Kem II, Mijnhardt, Germany) with a 75-W work load (~50% of their maximal oxygen consumption) was begun 180 min after breakfast. After exercise, patients rested for 60 min. During the test, ECG was recorded, and blood pressure was monitored using a cuff manometer.

Throughout the study, venous blood samples were serially drawn from an indwelling butterfly needle kept open with a 0.9% NaCl infusion to measure plasma glucose, lactate, free insulin, and counterregulatory hormones: glucagon, growth hormone, epinephrine, and norepinephrine. Blood for platelet aggregation studies and β-thromboglobulin measurement was obtained by multiple venipunctures without stasis conducted before exercise at 15, 30, and 45 min (end of the exercise) and at 30 and 60 min of the recovery phase. This procedure avoids the artefactual platelet activation occurring, in our experience, when venous blood samples are performed through an indwelling catheter. Previous studies in our laboratory showed that this kind of sampling and infusion procedure does not influence platelet function (18).

Plasma glucose was measured by enzymatic method (Beckman Glucose Analyzer, Fullerton, CA); lactate by means of an enzymatic kit (Boehringer Mannheim, Germany); free insulin by radioimmunoassay (RIA) after extraction with 25% polyethylene glycol, according to Kuzuya et al. (19); glucagon, growth hormone, and cortisol by RIA (using commercial kits); and epinephrine and norepinephrine by high-performance liquid chromatography with electrochemical detectors as previously described (18).

Platelet sensitivity to aggregating agents was measured according to the Born's method (20). Briefly, blood was collected in polypropylene tubes, anticoagulated with 3.8% trisodium citrate (9:1, v/v), and immediately centrifuged at 180 × *g* for 15 min to obtain platelet-rich plasma; platelet-poor plasma was prepared by further centrifugation at 2000 × *g* for 10 min. Platelet count was determined by model S-Plus Coulter Counter (Coulter Electronics, Hialeah, FL), adjusting platelet counts by dilution with platelet-poor plasma when during the tests their increase was >50,000/μl, as previously described (21). Aggregation studies were conducted within 60 min from venous blood samples by means of a double channel aggregometer (model 840, ELVI, Madrid, Spain), at 37°C, with a constant stirring at 900 rpm. Aggregation profiles were measured as increases in light transmission following addition of the aggregating agents supplied by Sigma Chemical Co. (St. Louis, MO): ADP sodium salt, 1-*O*-alkyl-2-*O*-acetyl-*sn*-glycero-3-phosphorylcholine (platelet activating factor [PAF]), and acid-soluble collagen. Aggregation profiles were measured as increases in light transmission following the addition of each aggregating agent. Maximal aggregation was calculated according to the Weiss and Rogers formula (22). To compare the different aggregating profiles, we used the ED_{50} (i.e., the agonist concentration obtained from a dose-response curve able to induce a maximal aggregation of 50%). Because for each time point at least four concentrations of each of the three agonists have been checked, at least 72 ex vivo platelet aggregations have been conducted for each patient during the study. β-Thromboglobulin was measured by RIA (Kit Amersham, Bucks, UK). Venous blood samples for its detection were placed in chilled tubes containing inhibitors of platelet aggregation (Thrombotect tubes, Abbott, Campoverde di Aprilia, Latina, Italy) and immediately centrifuged at +4°C in the J6M Beckman refrigerated centrifuge at 2500 × *g*. Plasma was stored at −70°C until the assay.

Data, in the text and Table 1, are presented as mean ± SEM. For statistical analysis, we used the analysis of variance for repeated measures.

RESULTS

— All patients were able to exercise for the preselected 45 min, but

Table 1—*Influence of a 45-min exercise period at 50% $\dot{V}O_{2\,max}$ on plasma glucose, hormones, and platelet function in insulin-dependent diabetic patients*

	MINUTES				*P*
	0	15	30	45	VALUE
GLUCOSE (MMOL/L)	5.9 ± 1.2	5.1 ± 1.3	4.7 ± 1.0	4.6 ± 1.0	0.067
FREE INSULIN (PMOL/L)	180 ± 36	162 ± 40	126 ± 30	114 ± 30	0.003
GLUCAGON (NG/L)	53 ± 13	56 ± 15	57 ± 13	60 ± 15	NS
GROWTH HORMONE (μG/L)	5 ± 1	7 ± 2	15 ± 4	15 ± 4	0.045
CORTISOL (NMOL/L)	240 ± 30	279 ± 14	350 ± 41	406 ± 69	0.018
EPINEPHRINE (PMOL/L)	1005 ± 240	2026 ± 486	5143 ± 1753	4788 ± 1785	0.077
NOREPINEPHRINE (NMOL/L)	5.04 ± 1.08	9.46 ± 2.9	13.48 ± 2.98	8.84 ± 1.90	0.009
ED_{50} (% OF BASAL VALUES)					
ADP	100	61 ± 16	63 ± 14	138 ± 36	0.048
PAF	100	73 ± 11	80 ± 10	131 ± 21	0.043
COLLAGEN	100	71 ± 8	68 ± 9	79 ± 8	0.008
B-THROMBOGLOBULIN (μG/L)	24 ± 2	29 ± 2	29 ± 3	32 ± 3	0.007

PAF, platelet activating factor; NS, not significant.

some of them were exhausted at the end because they were untrained. No patient experienced hypoglycemic symptoms.

Exercise induced an increase of heart rate from 73 ± 7 to 131 ± 7 beats/min and systolic blood pressure from 115 ± 5 to 135 ± 8 mmHg, whereas diastolic blood pressure did not change (76.6 ± 3.3 and 75.8 ± 3.4 mmHg at the beginning and end of the exercise).

Plasma lactate increased from 1.44 ± 0.11 to 2.53 ± 0.60 mmol/L ($P = 0.052$). Table 1 shows results concerning the exercise-induced changes of plasma glucose, hormones, and platelet function.

Exercise was accompanied by a decrease of plasma glucose concentrations and plasma-free insulin. In the first 15 min of exercise, 1 of the 6 patients reached a hypoglycemic glucose concentration of 2.75 mM, without a further reduction thereafter.

As far as the counterregulatory hormones are concerned, plasma glucagon did not change, whereas growth hormone, cortisol, and catecholamines increased. The patient presenting hypoglycemic glucose levels showed a particularly high catecholamine increase, with values of 6514 pmol/L for epinephrine and 23.69 nmol/L for norepinephrine.

Platelet sensitivity to agonists of five of six patients is reported. In the patient six, an error in the venous blood sampling procedure at rest made comparison impossible with the postexercise detections. Platelet response to agonists increased during exercise in all subjects, as testified by the reduction of ED_{50} values. The greatest decrease was observed in the patient presenting hypoglycemic glucose concentrations, who showed ED_{50} values of the three agonists <50% of the basal level. At the end of the exercise and in the recovery phase, some patients presented a decrease of platelet sensitivity to ADP and PAF (at 30 min of the recovery phase ED_{50} for ADP and PAF, expressed in percent of basal values, were 125 ± 37% and 149 ± 59%, respectively, with a return toward preexercise levels at 60 min). In the six patients investigated, exercise induced a slight but significant increase of β-thromboglobulin plasma concentrations.

CONCLUSIONS— This study demonstrates that a 45-min exercise of moderate intensity—timed to reduce the occurrence of a great blood glucose fall—is able to modify platelet function in type I diabetic patients, who showed an acceptable degree of control and were free of severe diabetic complications determining an increase in platelet responses to agonists ex vivo and a slight release reaction in vivo. This occurred from an increase in the platelet-specific protein β-thromboglobulin. Platelet response is similar for three agonists showing different mechanism of action (i.e., ADP, collagen, and PAF) and demonstrating the presence of a generalized platelet hyperaggregability. Furthermore, our study shows that the increase in platelet function is already present after a short period of moderate exercise (i.e., 15 min).

In this study, we carefully evaluated the hormonal response to exercise by measuring both the concentrations of plasma-free insulin and those of the main counterregulatory hormones.

We previously demonstrated that glucagon, growth hormone, and cortisol at physiological concentrations do not modify platelet function (18), whereas the catecholamine concentrations that are reached in vivo, even if far lower than the aggregating range, can increase platelet function by exerting a synergistic mechanism with other agonists (23,24). This phenomenon occurs both in vitro and in vivo, and it has been described during insulin-induced hypoglycemia

(18,21,25). The particularly great agonist ED_{50} decrease showed by the patient presenting hypoglycemic glucose concentrations could suggest a synergistic effect of exercise and hypoglycemia on platelet aggregability, mediated by a great enhancement of catecholamine concentrations.

The decrease of platelet response to ADP and PAF presented by some patients at the end of the exercise and in the recovery phase is similar to that described after the hypoglycemic nadir (18,21). It can be ascribed to different phenomena: among them, there is the possibility that platelets activated in vivo became less sensitive to agonists ex vivo.

As far as the insulin role on the platelet response to exercise is concerned, we recently demonstrated that this hormone shows antiaggregating properties (26). Because, in our experimental design exercise is conducted when plasma-free insulin concentrations are falling down because of characteristics of injected insulin, it cannot be excluded that this phenomenon could potentially contribute to the observed increase of platelet aggregability.

Enhancement in platelet release reaction occurring in vivo during this kind of exercise is slight, because β-thromboglobulin concentrations remain in the physiological range. It might not be forgotten, on the other hand, that during exercise there is not only an increase of the proaggregatory catecholamines, but also an enhanced release of the antiaggregatory prostanoid prostacyclin. This phenomenon has been demonstrated both in healthy subjects (6) and in type I diabetic patients (27,28). A preserved vascular endothelium is probably very useful to prevent the potential damages induced by exercise on the vessel wall, both by producing prostacyclin and by avoiding the interactions between the activated platelets and the subendothelial structures.

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