Plasma 6-keto-PGF_{1 α} , thromboxane B₂ and PGE₂ in Type 1 (insulin-dependent) diabetic patients during exercise

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Summary. The capacity of prostacyclin production determined as plasma 6-keto-PGF_{1 α} was investigated in 12 Type 1 (insulin-dependent) diabetic patients with a median duration of diabetes of 14 years during ordinary metabolic control. Using high pressure liquid chromatography preceding radioimmunoassay, the plasma concentration of 6-keto-PGF_{1 α} the stable metabolite of prostacyclin, was determined at rest and after a standardised bicycle exercise test. The plasma 6-keto-PGF_{1 α} in diabetic patients at rest did not differ from that of 25 healthy volunteers; 2.9 pg/ml (range <0.2–15.3) versus 1.7 pg/ml (range <0.2–16.6). During the exercise test plasma 6-keto-PGF_{1 α} increased significantly in the diabetic patients

as well as in the control group ($p < 0.05$). The increment of 6-keto-PGF_{1 α} in the diabetic patients was neither related to the metabolic regulation, duration of diabetes nor to changes in platelet volume, platelet number or the production of thromboxane B₂ and prostaglandin E₂. Our results do not support the hypothesis that Type 1 diabetic patients have a decreased capacity of prostanoid production, as suggested from in vitro studies.

Key words: Type 1 diabetic patients, exercise, plasma prostanoids: 6-keto-PGF_{1 α} , TXB₂ and PGE₂.

Recent in vitro studies have suggested an altered metabolism of the prostanoids in the vascular endothelium and in platelets from patients with diabetes mellitus [1–5]. It has been suggested that decreased production of the antiaggregatory and vasodilatory prostacyclin (PGI₂) in the vascular endothelium and increased platelet production of thromboxane (TXA₂) – a potent vasoconstrictor and proaggregator – are changes which have contributed to the development of microvascular complications in patients with diabetes mellitus.

The impact of these changes on endothelium and platelet function in vivo is, however, still unknown. Previous measurements of prostacyclin production assessed from the stable plasma metabolite 6-keto-PGF_{1 α} and of TXA₂ determined as the plasma metabolite TXB₂ have often resulted in conflicting and what seems unrealistically high and varying results, obviously due to problems in methodology, e.g. cross-reactions with other substances present in plasma and interference from other plasma eicosanoids [6–16].

In order to minimize these problems in methodology, we isolated the different prostanoids, 6-keto-PGF_{1 α} , TXB₂ and PGE₂, by reverse phase high pressure liquid chromatography before their determination by radioimmunoassay.

When we used this more specific method in a previous study, we did not find decreased values of plasma 6-keto-PGF_{1 α} at rest in Type 1 diabetic patients [17]. The aim of the present study was to find out if prostacyclin production, determined as plasma 6-keto-PGF_{1 α} , was impaired during standardised exercise in Type 1 diabetic patients, and to relate the results to changes in platelet number, platelet volume and TXB₂ and PGE₂ production, since these factors might influence the prostacyclin production.

Subjects and methods

Twelve Type 1 diabetic patients (10 males, 2 females) without obvious macrovascular complications were selected for the study. Patients with other diseases, including cardiovascular and pulmonary conditions, were excluded. Patients with preretinal bleeding or ongoing laser treatment for proliferative retinopathy were also excluded from the study. None was receiving medication other than insulin, nor had any taken non-steroid anti-inflammatory drugs (NSAID) within the previous week. None were alcohol-dependent, nor had they ingested alcohol during the 48 h prior to the examination. None were allowed to smoke during the 2 h before testing.

Retinopathy was present in 9 of the patients; 2 had proliferative retinopathy and 7 simple retinopathy. None of the patients had clinical signs of diabetic nephropathy. Table 1 provides further details of the clinical data.

Table 1. The clinical data from 12 Type 1 diabetic patients and 25 control subjects selected for the study

	Age (Years)	Non-fasting blood glucose (mmol/l)	HbA _{1c} (%)	SE-creatinine (µmol/l)	Smoking (%)	Duration of diabetes (years)	Insulin intake (IE/24 h)
Diabetic patients							
Median	30	13.0	8.2	89.0	50	14	36
Range	15–46	5.0–22.2	5.3–13.0	73.0–104.0		0.25–19	22–62
Control subjects							
Median	31	5.0	4.9	81.5			
Range	18–44	4.3–6.5	4.0–6.1	62.0–106.0	56		

Table 2. The plasma prostanoid concentration in 12 Type 1 diabetic patients and 25 control subjects before and after standardised exercise

	Plasma 6-keto-PGF _{1α} (pg/ml)		Plasma TXB ₂ (pg/ml)		Plasma PGE ₂ (pg/ml)	
	Diabetic patients	Control subjects	Diabetic patients	Control subjects	Diabetic patients	Control subjects
Pre-exercise						
Median	2.9	1.7	28.7	20.9	1.0	0.4
Range	<0.2–15.3	<0.2–16.6	10.3–91.7	1.4–170.5	<0.02–4.2	<0.02–16.0
Post-exercise						
Median	9.0 ^{b, a}	5.3 ^b	22.5	24.7	0.7	0.7
Range	5.0–16.4	0.3–33.3	10.2–161.8	1.8–95.4	<0.02–12.9	<0.02–13.4

Significance of difference values is as follows: ^a diabetic versus control. ^b post-exercise versus pre-exercise. ^{a, b} $p < 0.05$

Twenty-five age-matched healthy volunteers (13 males, 12 females) served as a control group.

Informed consent was obtained from all subjects, and the study was accepted by the local ethical committee.

A bicycle ergometer test was performed between 12.00–14.00 hours, after at least 15 min of rest in the supine position.

In order to avoid hypoglycaemia during the exercise, blood glucose was determined just before the test. If the blood glucose was ≤ 3 mmol/l, the test was cancelled. The test was completed by all the participants without symptoms of hypoglycaemia. All the subjects were monitored by ECG during the exercise test. The blood pressure was measured before and after the test, which was started at a work-load of 300 kpm/min. A graduated work-load of 150–300 kpm/min was added every 4 min until a heart rate of 85% of the age-predicted maximum was achieved. The test lasted 16 min.

The groups were not matched for physical fitness, but there was no significant difference in the basal heart rate, and the absolute exercise load between the two groups.

Blood samples for prostanoid analysis were taken from a cubital vein just before and immediately after the test, with a maximum of 50 mmHg stasis [17].

Precooled polypropylene tubes were used containing 200 µl of a mixture of dipotassium ethylenediamine tetra-acetic acid (K₂EDTA) 95 mg/ml and indomethacin 1.8 µg/ml. The blood was kept on ice until centrifugation for 10 min at 2000 g and 4 °C to obtain platelet free plasma which was enriched with 1–2 pg/ml of a mixture of tritium labelled 6-keto-PGF_{1α}, TXB₂ and PGE₂ as internal standards. Immediately after, the plasma was adjusted to pH 3.5 with 1 mmol hydrochloric acid (HCL) to bring the prostanoids to a non-ionic state. The prostanoids were extracted from the plasma using a SEP-PAK C₁₈ Cartridge, and stored overnight at –21 °C until further processing. The individual prostanoids 6-keto-PGF_{1α}, TXB₂ and PGE₂ were isolated using a reverse phase high pressure liquid chromatography system (Waters Associates, Milford, Mass, USA), and determined with a ¹²⁵I-Radio-immunoassay kit (NEN International, Boston, Mass, USA). The RIA-results were corrected for recovery after the plasma blank value and the internal standards were subtracted. The intra/inter series variation (CV%) of the assay procedure was 8.5/12.2; 6.4/7.8; 6.2/14.6 for 6-keto-PGF_{1α} and TXB₂ and PGE₂

respectively [17]. The sensitivity of the radioimmunoassay, defined as two times the standard deviation at zero binding, was 0.2, 0.7 and 0.02 pg/ml for 6-keto-PGF_{1α}, TXB₂ and PGE₂ respectively.

HbA_{1c} was determined using microcolumn (Biorad, Richmond, Calif, USA). Blood glucose and serum creatinine were measured using routine laboratory methods.

Platelet number and volume were determined on a Coulter S+ (Coulter Electronic Limited, Luton, Beds, UK), using test tubes containing 0.875 ml ACD/Na₂EDTA medium to avoid changes in the platelet volume during the procedure [18].

Statistical analysis

Wilcoxon's test for paired samples, the Mann-Whitney test for non-paired samples, and Spearman's rank correlation test were used for statistical analysis. Statistical significance was established at $p < 0.05$.

Results

The pre-exercise plasma concentrations of the prostanoids 6-keto-PGF_{1α}, TXB₂ and PGE₂ (Table 2) in the diabetic patients were not significantly different from those of the control subjects. The platelet number and the platelet volume at rest were also not significantly different (Table 3).

During the exercise test the plasma 6-keto-PGF_{1α} increased significantly in both the diabetic and control groups, median increment 4.7 pg/ml and 2.8 pg/ml, respectively ($p < 0.05$).

The post-exercise plasma level of 6-keto-PGF_{1α} in the diabetic patients was significantly higher than that of the control group ($p < 0.05$) (Table 2). However, the difference between the increments in the two groups was not significant.

Table 3. The platelet number and the platelet volume in 12 Type 1 diabetic patients and 25 control subjects before and after standardised exercise

	Platelet number $\times 10^9/l$		Platelet volume fl.	
	Diabetic patients	Control subjects	Diabetic patients	Control subjects
Pre-exercise				
Median	230	210	8.5	7.9
Range	152–334	163–318	6.8–9.4	6.6–9.9
Post-exercise				
Median	286 ^{2b}	245 ^{3b}	8.4	8.1 ^{1b}
Range	197–426	202–355	6.8–9.5	6.6–10.2

Significance of difference values is as follows: ^b post-exercise versus pre-exercise. ^{1b} $p < 0.05$; ^{2b} $p < 0.01$; ^{3b} $p < 0.001$

Plasma TXB₂ and PGE₂ did not change significantly during exercise, neither in the diabetic patients nor in the control group (Table 2). The platelet number increased significantly in the diabetic group ($p < 0.01$) as well as in the control group ($p < 0.001$), whereas the platelet volume only increased significantly in the control group ($p < 0.05$) (Table 3). The increase in plasma 6-keto-PGF_{1 α} in the diabetic patients was not correlated to age, duration of diabetes, diabetes regulation based on HbA_{1c} and actual blood glucose, or intake of insulin, nor to the changes in plasma TXB₂, PGE₂, platelet number, or platelet volume.

Discussion

Our results indicated that the production of prostacyclin determined as plasma 6-keto-PGF_{1 α} was not reduced in Type 1 diabetic patients.

We did not find any sign of increased TXB₂ production, although the diabetic patients were in suboptimal metabolic regulation and 75% of the patients had microvascular complications.

These results are in accordance with an earlier study [17].

The significant exercise-induced increase of plasma 6-keto-PGF_{1 α} indicated that the in vivo capacity of prostacyclin production determined as 6-keto-PGF_{1 α} in diabetic patients was not reduced, compared to non-diabetic control subjects.

The increment of 6-keto-PGF_{1 α} during exercise in the diabetic patients was not related to the TXB₂ nor PGE₂ production from the platelets or other sources because plasma TXB₂ and plasma PGE₂ did not change significantly during the exercise. The correlation between the changes of 6-keto-PGF_{1 α} and PGE₂ was not significant. Neither the platelet number, which increased significantly during the exercise, nor the platelet volume, which tended to be larger in diabetic patients, correlated with the increase of plasma 6-keto-PGF_{1 α} . The blood glucose and the HbA_{1c} did not correlate with changes in plasma 6-keto-PGF_{1 α} , which

indicates that the glucose regulation per se and the degree of glycosylation are not directly involved in exercise stimulated 6-keto-PGF_{1 α} production.

The increase of plasma 6-keto-PGF_{1 α} in our non-diabetic control group is in accordance with earlier reports [19–23].

In some of these studies exercise also induced increase in plasma TXB₂, but only in patients with cardiovascular disease [20, 22].

In one study [24] the plasma TXB₂ increased significantly in healthy volunteers, but only during anaerobic exercise; and the plasma level of 6-keto-PGF_{1 α} was unchanged. However, the assay sensitivity for 6-keto-PGF_{1 α} in this assay system was low.

In another study [25] the plasma TXB₂ increased significantly during a maximal exercise test, and the increment continued in the recovery period. The resting level of plasma TXB₂ was rather high, even higher than the serum TXB₂ level. Plasma 6-keto-PGF_{1 α} also increased during this test, but the level of plasma 6-keto-PGF_{1 α} was not presented in the study. These results might be due to problems in methodology. In particular, the use of an intravenous catheter and a free flow technique might stimulate an artifactual biosynthesis of prostanoids during the collection of the blood samples [26] and so invalidate the results.

High levels of plasma 6-keto-PGF_{1 α} (60–2000 pg/ml) have been reported in most of the exercise studies [19–23] and in studies in diabetic patients [14–16]. Recent reports [6, 9] indicate a normal basal level of 6-keto-PGF_{1 α} about 3 pg/ml, when determined by gas chromatography/negative ion chemical ionisation mass spectrometry (NICI-MS), it therefore seems clear that the plasma 6-keto-PGF_{1 α} might only represent a very small fraction of the high levels reported. This very small level of plasma 6-keto-PGF_{1 α} cannot explain the reported big differences of 6-keto-PGF_{1 α} between groups of diabetic patients with different degree of complications [14–16].

In one study using NICI-MS methods the basal level of 6-keto-PGF_{1 α} was only a few pg/ml, and the exercise-induced increment of plasma 6-keto-PGF_{1 α} was about 10 pg/ml in healthy subjects [9]. Our results are in accordance with this study.

The exercise-induced increase in plasma 6-keto-PGF_{1 α} in Type 1 diabetic patients has not been reported previously; our results contradict the finding that diabetic patients have a decreased vascular capacity of prostacyclin production, as suggested from in vitro studies [4, 5]. However, as our diabetic patients only had moderate microvascular changes, it is still possible that diabetic patients with extensive macrovascular disease might exhibit a decreased capacity in prostacyclin production during exercise stress.

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