Nitric Oxide-cGMP and Prostacyclin-cAMP Pathways in Patients with Type II Diabetes and Different Types of Retinopathy

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Key Words

Nitric oxide · Platelets · Prostacyclin · Endothelial dysfunction · Diabetic retinopathy

Abstract

The aim of this study was to investigate two factors of endothelial dysfunction and their platelet second messengers in patients with type II diabetes and different types of retinopathy. We compared 20 healthy volunteers and 117 patients with type II diabetes (34 with no signs of diabetic retinopathy, 26 with background diabetic retinopathy, 29 with ischemic-proliferative diabetic retinopathy and 28 with edematous diabetic retinopathy). The following parameters were recorded: platelet aggregation, nitrites, 6-keto-prostaglandin-F_{1α} and intraplatelet cAMP and cGMP. Platelet aggregation was greater in patients with diabetic retinopathy. The concentration of ADP that produced 50% maximum intensity of aggregation was 1.81 μ M in patients without diabetic retinopathy, 0.92 μ M in patients with background diabetic retinopathy, 0.85 μ M in patients with ischemic-proliferative diabetic retinopathy and 0.44 μ M in patients with edematous diabetic retinopathy. The platelets in these patients were more resistant to inhibition by SIN-1 (concentrations of SIN-1 that produced 50% inhibition of maximum intensity of collagen-induced aggregation in the four

patient groups: 18.1, 13.6, 16.2 and 33.2 μ M, respectively). Nitrite concentration in patients with ischemic-proliferative diabetic retinopathy was one sixth of the value in healthy controls, but there was no significant difference between the control group and patients with edematous diabetic retinopathy. In the latter group, neutrophils increased nitrite production by $68.7 \pm 3\%$, whereas in patients with ischemic-proliferative diabetic retinopathy, this increase was $18.7 \pm 2.0\%$. We conclude that nitric oxide production is higher in patients with type II diabetes and edematous retinopathy than in those with ischemic-proliferative retinopathy. This finding, together with the possibly greater production of free radicals, may explain the greater impairment of platelet function in the former patients.

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Introduction

After 20–25 years of suffering from diabetes, more than 80% of patients have some type of retinal lesion, and these patients are 20 times more likely to become blind than members of the general population [1–3].

A prolonged increase in levels of blood glucose is recognized as one of the main causes of the appearance and evolution of diabetic microangiopathy, of which retinopa-

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Table 1. Mean characteristics of the healthy volunteers and patients with type II diabetes

	Group 1	Group 2	Group 3	Group 4	Group 5
Diabetes	no	yes	yes	yes	yes
Number	20	34	26	29	28
Retinopathy	no	no	background	ischemic-proliferative	edematous
Age, years	58.2 ± 4.5	62.3 ± 3.6	59.7 ± 3.1	62.4 ± 1.3	63.2 ± 2.4
Gender (F/M)	11/9	14/14	11/15	15/14	13/15
Duration of diabetes, years	0	12.1 ± 2.2	11.8 ± 1.9	10.9 ± 2.1	12.2 ± 2.8
HbA _{1c} , %	3.8 ± 0.1	5.7 ± 0.8	6.4 ± 0.9	7.8 ± 0.9	8.1 ± 1.1
Systolic blood pressure, mm Hg	131 ± 12	139 ± 15	135 ± 15	137 ± 16	133 ± 18
Diastolic blood pressure, mm Hg	65 ± 5.5	68 ± 4.2	70 ± 6.4	68 ± 5.5	75 ± 6.0
Microalbuminuria, mg/dl	0 ± 0	0 ± 0	0 ± 0	2.2 ± 0.9	6.5 ± 1.2

thy is one type [4]. Sustained hyperglycemia leads to a series of interrelated alterations that can cause evident endothelial dysfunction – which in turn can cause vascular lesions in the retina of patients with diabetes and retinopathy [5].

One of the main consequences of endothelial dysfunction is a deficit in nitric oxide (NO) and prostacyclin, substances with vasodilating and platelet-inhibiting functions [6, 7]. In this connection, platelet hyperactivity has been widely documented in patients with diabetes mellitus, and this hyperactivity is greater in patients with microangiopathy [8–12].

In previous studies [13, 14], we showed that platelet alterations in patients with type I diabetes mellitus differed depending on whether their retinopathy was predominantly ischemic or edematous. The aim of the present study was to measure prostacyclin and NO production after activation of these pathways by different substances, i.e. *L*-arginine for NO and a calcium ionophore for prostacyclin, and the activation of their platelet second messengers cAMP and cGMP by prostaglandin E₁ and SIN-1, respectively, in patients with type II diabetes mellitus with different types of retinopathy.

Endothelial cells are the best source to exactly determine prostacyclin and NO, but this is obviously difficult in humans. For that reason, we studied NO production in neutrophils and prostacyclin in mononuclear leukocytes as peripheral blood cells that produce these substances.

Patients and Methods

Patient Selection

The participants in this study were 20 healthy volunteers and 117 patients with diabetes diagnosed as type II (non-insulin-dependent diabetes mellitus; NIDDM). We excluded patients with other types

of vasculopathy (macro- or microangiopathy). Except for oral glucose-lowering drugs, none of the subjects took any medication at the time of the study or during the 15 days before inclusion. All participants were given the same recommendations regarding dietary measures, exercise and lifestyle. All participants gave their informed consent in writing after receiving information about the aims of the study, and the procedure was approved by the Ethics Committee of the Virgen de la Victoria University Hospital in Málaga, Spain.

All patients and healthy volunteers underwent a complete ophthalmologic examination, which included evaluation of the visual field, intraocular pressure, lens and fluorescein angiography (FAG). On the basis of the FAG results, the participants were divided into five groups. Group 1 (*n* = 20) comprised persons without diabetes or signs of retinopathy. Group 2 (*n* = 34) consisted of patients with NIDDM with no signs of retinopathy. Group 3 (*n* = 26) contained patients with NIDDM and background retinopathy, defined as the presence on FAG of small microaneurysms, small, rounded parietal hemorrhages, small, rounded areas of infiltration or any combination of the three, with no evidence of the formation of hard exudates and no more than 6 leaks identified with fluorescein. The patients in group 4 (*n* = 29) had NIDDM and predominantly ischemic-proliferative retinopathy, defined as the presence of new vessel formation (intraretinal, preretinal, papillary or vitreous), with leaks in the new vessels in the early phase of FAG. Group 5 (*n* = 28) contained patients with NIDDM and predominantly edematous retinopathy, defined as the presence of abundant, confluent infiltrates that formed plaques and diffuse retinal edema (more than 6 leaks). Table 1 summarizes the main characteristics of the participants in each group.

At the end of the study, a blood sample was obtained from each participant between 8.00 and 9.00 h, before the subject had anything to eat or drink, and before the patients had injected their daily insulin dose.

Glycosylated hemoglobin (HbA_{1c}), duration of diabetes, daily insulin dose and other findings of interest were recorded from the patient's clinical record. For each patient, HbA_{1c} was measured 2 or 3 times per year, and the value used in this study was the mean of the three most recent measurements.

Laboratory Procedures

All reagents were from Sigma Chemical Co. (St. Louis, Mo., USA) unless otherwise stated. Blood samples were collected with 3.8% sodium citrate as the anticoagulant at a proportion of 1:10. Each

Table 2. EC₅₀ values of the inducers of platelet aggregation

	ND (n = 20)	DWR (n = 34)	DBR (n = 26)	DIPR (n = 29)	DER (n = 28)
ADP in PRP, μM	2.11 $\pm$ 0.29	1.99 $\pm$ 0.21	1.82 $\pm$ 0.20	1.94 $\pm$ 0.25	1.90 $\pm$ 0.26
ADP in WB, μM	2.15 $\pm$ 0.17	1.81 $\pm$ 0.07	0.92 $\pm$ 0.06*	0.85 $\pm$ 0.04*	0.44 $\pm$ 0.06*
Collagen in PRP, $\mu g/ml$	0.71 $\pm$ 0.08	0.77 $\pm$ 0.07	0.81 $\pm$ 0.09	0.77 $\pm$ 0.08	0.41 $\pm$ 0.06*
Collagen in WB, $\mu g/ml$	0.48 $\pm$ 0.05	0.46 $\pm$ 0.05	0.40 $\pm$ 0.03	0.34 $\pm$ 0.03	0.17 $\pm$ 0.02*

* $p < 0.05$ in comparison to all the other groups. ND = Volunteers without diabetes; DWR = patients with diabetes without retinopathy; DBR = patients with diabetes and background retinopathy; DIPR = patients with diabetes and ischemic-proliferative retinopathy; DER = patients with diabetes and edematous retinopathy; WB = whole blood.

sample was divided into aliquots for analyses of whole blood, platelet-rich plasma (PRP), mononuclear leukocytes and neutrophils. PRP was obtained by centrifuging whole blood at 180 g for 10 min at 20 °C. Mononuclear leukocytes and neutrophils were obtained on a Ficoll density gradient (Histopaque 1077 and 1119).

Platelet Aggregation

The maximum intensity of platelet aggregation induced by ADP or collagen (Menarini Diagnóstica SA, Barcelona, Spain) in whole blood or PRP was measured using an electrical impedance method [15] (Chrono-Log 530S aggregometer, Chrono-Log Corporation, Haverton, Pa., USA). The maximum intensity of aggregation was recorded as the maximum change in impedance 10 min after the inducing agent was added. The concentration of the inducers of platelet aggregation that produced 50% maximum intensity of aggregation was defined as EC₅₀. In some experiments with PRP, the samples were incubated with different concentrations of prostaglandin E₁ (adenyl cyclase stimulant) or SIN-1 (guanyl cyclase stimulant), and aggregation was induced by adding 1 $\mu g/ml$ collagen. The concentration of prostaglandin E₁ and SIN-1 that produced 50% inhibition of the maximum intensity of collagen-induced aggregation was defined as IC₅₀.

Nitrites and Nitrates

NO is rapidly converted to NO₂⁻ and NO₃⁻ (NOx) in an oxygenated aqueous solution such as human plasma. Samples were filtered through Ultrafree-MC microcentrifuge filters (Millipore, Gif-Sur-Yvette, France) to remove the hemoglobin from cell lysis. A commercial kit (Cayman Chemical, Ann Arbor, Mich., USA) was used to measure NOx concentration. Concentrations of NOx were measured in plasma and in the supernatant of neutrophils stimulated for 30 min with 100 μM L-arginine.

Prostacyclin Concentration

The concentration of prostacyclin was quantified by determining the concentration of 6-keto-prostaglandin-F_{1 α} in samples of mononuclear leukocytes and cell-free plasma. Calcium ionophore A23187 (1 μM) was added, and the mixture was incubated for 5 min at 37 °C and then centrifuged at 10,000 g for 3 min. The supernatant was frozen at -80 °C until analysis. The concentration of 6-keto-prostaglandin-F_{1 α} was measured by enzyme immunoassay (Amersham International plc, Little Chalfont, Buckinghamshire, UK).

Platelet cAMP

Platelets from PRP were washed in a medium composed of 5.6 g/l NaCl, 0.7 g/l NaH₂PO₄, 0.5 g/l KH₂PO₄, 0.9 g/l glucose, 0.05 g/l apyrase and 50 nM prostaglandin E₁. The sample was centrifuged at 1,000 g for 15 min at 4 °C, and the resulting pellet was resuspended in a solution consisting of 7.7 g/l NaCl, 0.6 g/l NaH₂PO₄, 1 g/l NaHCO₃, 0.3 g/l CaCl₂, 0.2 g/l MgCl₂, 1.2 g/l HEPES, 0.9 g/l glucose, 0.05 g/l apyrase and 0.5 g/l bovine albumin. The number of platelets was adjusted to 2.5×10^{11} cells/l, and the sample was divided into aliquots to which we added 10 μM isobutylmethylxanthine and 1 μM prostaglandin E₁. After incubation for 5 min at 37 °C, the process was stopped by adding 1 N HCl. The sample was centrifuged at 10,000 g for 3 min and the resulting supernatant was neutralized with NaOH. The production of cAMP was measured by enzyme immunoassay (Amersham International).

Intraplatelet cGMP

Samples were prepared as described above for cAMP, except that 100 μM zaprinast was used instead of isobutylmethylxanthine, and 10 μM SIN-1 was used instead of prostaglandin E₁. The production of cGMP was measured by enzyme immunoassay (Amersham International).

Statistical Analysis

The results were expressed as the mean $\pm$ SEM. Differences between groups were detected with one-way analysis of variance followed by testing for the least significant difference. Simple linear correlations were also calculated. Differences were considered significant at $p < 0.05$. The SPSS program (version 10.0 for Windows, SPSS Co., Chicago, Ill., USA) was used for all statistical analyses.

Results

Platelet aggregation increased in a concentration-dependent way after stimulation with collagen or ADP, both in whole blood and PRP (data not shown). Table 2 shows the EC₅₀ values for inducers of platelet aggregation (ADP and collagen). The differences between groups were significant only after induction with collagen in PRP from patients with diabetes and edematous retinopathy. The

Fig. 1. Inhibition of platelet aggregation in PRP induced with 1 $\mu\text{g}/\text{ml}$ collagen, after incubation with increasing concentrations of prostaglandin E_1 (PGE_1) (a) or SIN-1 (b). ● = Volunteers without diabetes ($n = 20$); □ = patients with diabetes but without retinopathy ($n = 34$); △ = patients with diabetes and background retinopathy ($n = 26$); ◇ = patients with diabetes and ischemic-proliferative retinopathy ($n = 29$); * = patients with diabetes and edematous retinopathy ($n = 28$). * $p < 0.05$ in comparison to all other groups.

Fig. 2. Plasma concentrations of $\text{NO}_2^-/\text{NO}_3^-$. ND = Volunteers without diabetes ($n = 20$); DWR = patients with diabetes but without retinopathy ($n = 34$); DBR = patients with diabetes and background retinopathy ($n = 26$); DIPR = patients with diabetes and ischemic-proliferative retinopathy ($n = 29$); DER = patients with diabetes and edematous retinopathy ($n = 28$). * $p < 0.05$ in comparison to all other groups.

Table 3. IC_{50} values of prostaglandin E_1 and SIN-1 in PRP

	ND ($n = 20$)	DWR ($n = 34$)	DBR ($n = 26$)	DIPR ($n = 29$)	DER ($n = 28$)
PGE_1 , μM	$1.63 \pm 1.51^*$	5.01 ± 0.41	7.35 ± 0.55	9.91 ± 0.85	10.30 ± 1.18
SIN-1, μM	$1.08 \pm 0.06^*$	18.10 ± 2.02	13.60 ± 1.59	16.22 ± 1.93	$33.26 \pm 2.28^*$

* $p < 0.05$ in comparison to all the other groups. ND = Volunteers without diabetes; DWR = patients with diabetes without retinopathy; DBR = patients with diabetes and background retinopathy; DIPR = patients with diabetes and ischemic-proliferative retinopathy; DER = patients with diabetes and edematous retinopathy; PGE_1 = prostaglandin E_1 .

EC_{50} for ADP was significantly lower in whole blood from patients with diabetes and retinopathy than in that from the patients without retinopathy and the healthy group. The EC_{50} for collagen was significantly lower in whole blood from patients with diabetes and edematous retinopathy than in that from the other groups.

Prostaglandin E_1 inhibited platelet aggregation induced by collagen in a concentration-dependent way (fig. 1). Inhibition was greater in volunteers without diabetes than in patients with the disease, and among the latter, inhibition was lowest in patients with edematous retinopathy. SIN-1 also inhibited aggregation induced by col-

Fig. 3. a NO_2/NO_3 production in neutrophils under basal conditions (white bars) and after stimulation with $100 \mu\text{M}$ *L*-arginine (hatched bars). b Intraplatelet concentrations of cGMP under basal conditions (white bars) and after stimulation with $10 \mu\text{M}$ SIN-1 (hatched bars). ND = Volunteers without diabetes ($n = 20$); DWR = patients with diabetes but without retinopathy ($n = 34$); DBR = patients with diabetes and background retinopathy ($n = 26$); DIPR = patients with diabetes and ischemic-proliferative retinopathy ($n = 29$); DER = patients with diabetes and edematous retinopathy ($n = 28$). * $p < 0.05$ in comparison to all other groups.

lagen in a concentration-dependent way. Resistance to inhibition by SIN-1 was greatest in patients with edematous retinopathy. Table 3 shows the IC_{50} values for prostaglandin E_1 and SIN-1.

Plasma concentrations of NOx are illustrated in figure 2. In patients with diabetes and background or ischemic-proliferative retinopathy, plasma NOx concentrations were lower than in the other two groups of patients. The concentration of NOx in patients with diabetes but no retinopathy or edematous retinopathy did not differ significantly from that in healthy control participants.

Fig. 4. a 6-Keto-prostaglandin- $\text{F}_{1\alpha}$ (6-keto-PGF $_{1\alpha}$) production in mononuclear leukocytes under basal conditions (white bars) and after stimulation with $1 \mu\text{M}$ calcium ionophore A23187 (hatched bars). b Intraplatelet concentrations of cAMP under basal conditions (white bars) and after stimulation with $10 \mu\text{M}$ prostaglandin E_1 (PGE1) (hatched bars). ND = Volunteers without diabetes ($n = 20$); DWR = patients with diabetes but without retinopathy ($n = 34$); DBR = patients with diabetes and background retinopathy ($n = 26$); DIPR = patients with diabetes and ischemic-proliferative retinopathy ($n = 29$); DER = patients with diabetes and edematous retinopathy ($n = 28$). * $p < 0.05$ in comparison to noninduced samples; ** $p < 0.05$ in comparison to all other groups of diabetic patients.

NO production by neutrophils stimulated with *L*-arginine (fig. 3a) was significantly greater in healthy volunteers than in patients with diabetes and edematous retinopathy. Platelet concentrations of cGMP (fig. 3b) were similar in all five groups, although cGMP production after induction with SIN-1 showed no significant change in platelet samples from patients with ischemic-proliferative or edematous retinopathy.

Plasma levels of 6-keto-prostaglandin- $\text{F}_{1\alpha}$ were significantly higher in healthy people ($18.25 \pm 2.05 \text{ nmol/l}$) than in patients with diabetes; the values in the patient

groups were 15.33 ± 2.35 nmol/l in diabetic patients without retinopathy ($p < 0.05$), 13.49 ± 1.95 nmol/l in patients with diabetes and background retinopathy ($p < 0.05$), 4.51 ± 0.66 nmol/l in patients with diabetes and ischemic-proliferative retinopathy ($p < 0.001$) and 1.23 ± 0.21 nmol/l in patients with diabetes and edematous retinopathy ($p < 0.001$).

Leukocyte production of 6-keto-prostaglandin- $F_{1\alpha}$ increased significantly after stimulation with calcium ionophore (fig. 4a) in all groups, although leukocytes from healthy volunteers showed the largest increase. Intraplatelet concentrations of cAMP (fig. 4b) were significantly lower in patients with diabetes than in healthy controls. In contrast with the results in the control group, stimulation with prostaglandin E_1 led to no significant increase in cAMP levels in any of the patients with diabetes.

Discussion

Our study shows that platelet alterations, as assessed by prostacyclin and NO levels and the activation of their platelet second messengers, in patients with NIDDM diabetes differ depending on the type of retinopathy these patients have.

Our aggregometric findings are similar to those of most authors who have studied this parameter in patients with diabetes, in whom platelet aggregation is more sensitive to stimulation by habitual inducers than in healthy persons [8–12]. This result is compatible with earlier findings by our group in patients with type I diabetes mellitus [13, 14], in whom aggregation was also greater than in healthy controls, and among whom the difference was especially striking for patients with edematous retinopathy. Our present findings show that platelet reactivity is also enhanced in patients with type II diabetes and edematous retinopathy.

Experiments that have investigated endogenous antiaggregant substances have showed that platelets from patients with diabetes are less sensitive to inhibition by these antiaggregants than those from healthy volunteers. These results are similar to earlier findings reported with adenosine or *L*-arginine [16, 17]. Platelet inhibition via the adenylyl cyclase pathway (incubation with prostaglandin E_1) did not differ between the groups of patients with retinopathy, but was lower in patients with no retinopathy. This indicates that this pathway is more severely altered when microangiopathic complications appear. However, when we tested the guanylyl cyclase pathway of platelet inhibition (incubation with SIN-1), we found a

greater degree of resistance to inhibition in patients with edematous retinopathy in comparison to other retinopathies.

Plasma concentrations of NOx, together with the greater platelet activity and lower sensitivity to inhibition by SIN-1, support the existence of a platelet imbalance that might account for the appearance of ischemia [18]. In this connection, it has been shown that nitrite levels in patients with diabetes but without vascular complications are similar to those in the healthy population, whereas in patients with vasculopathies, plasma nitrite levels are lowered [19]. Decreased platelet [20, 21] and vascular constitutive NO synthase activity [22] have been suggested as the causes of reduced NO production in patients with diabetes. Our findings suggest that this reduction is also the result of decreased NO production in neutrophils.

Nonetheless, patients with diabetes and edematous retinopathy stood out because of the absence of any reduction in plasma NOx levels. In theory, we would have assumed that because of the increase in NO, platelet inhibition would be greater, but in fact the data showed that inhibition was less intense in this subgroup of patients. To explain this apparent paradox, we analyzed NO production by neutrophils and the activity of cGMP, its second messenger in platelets. In patients with diabetes, NO synthase activity was lower, as shown by the lower level of NOx in the supernatant after incubation with *L*-arginine. This result is compatible with earlier reports of a lower activity of constitutive NO synthase in patients with diabetes [20, 21]. However, the pattern of NOx production differed in patients with edematous retinopathy; basal NOx production was not significantly lower than in healthy volunteers, and induction with *L*-arginine led to higher NOx levels than in other subgroups of retinopathy.

Intraplatelet cGMP formation was clearly diminished in patients with any type of retinopathy [23]. This may explain the greater platelet activity and blunted sensitivity to inhibition by SIN-1. Hence, the defect in the NO-cGMP axis appears to occur in NO formation. The increased production of NOx in patients with edematous retinopathy might be explainable by the formation of peroxynitrites; in patients with diabetes, the formation of free radicals is greater and the antioxidant defense capacity lower than in healthy persons [24, 25]. In addition, superoxide anions react with NO to form peroxynitrite radicals [26], which are highly oxidizing and markedly increase platelet aggregation [27, 28]. On the basis of this explanation, it might be suspected that in patients with

type II diabetes and edematous retinopathy, more free radicals would form and react with NO to give rise to peroxynitrites, which are detected when NOx production is measured. This would explain the absence of any apparent decrease in plasma NOx in these patients and the increase in NOx production by neutrophils incubated with *L*-arginine. In this connection, the elevated platelet peroxynitrite production in patients with type II diabetes has been shown to result from the 3-fold increase in induced NO synthase activity in comparison to healthy persons [29]. Moreover, Zou and Bachschmid [30] demonstrated that an increase of peroxynitrites leads to prostacyclin inhibition; plasma concentrations of 6-keto-prostaglandin-F_{1α} were decreased in all diabetic groups, but in patients with edematous retinopathy, this decrease was higher than in other groups. In patients with background and ischemic-proliferative retinopathy, the endothelial dysfunction could explain this inhibition; according to the results of Zou and Bachschmid [30], in patients with edematous retinopathy, the lowest plasma levels of 6-keto-prostaglandin-F_{1α} could be a consequence of the formation of peroxynitrites. However, to confirm this hypothesis, further research is needed to show that more free radicals and peroxynitrites are formed in these patients than in patients with diabetes and other types of retinopathy.

The prostacyclin-cAMP axis is also altered in patients with diabetes; the production of the prostanoid is lower, and sensitivity to platelet adenylyl cyclase is diminished.

These alterations are further evidence of the platelet alterations in patients with diabetes; however, we found no differences between patients with different types of retinopathy. Experimental studies have shown a relationship between the vascular production of prostacyclin and retinal vascularization in rats with streptozotocin-induced diabetes [31], and changes in prostacyclin synthesis modify the intensity of retinal ischemia in this model [32–34]. However, in animals made diabetic, only ischemic retinopathy has been reproduced, but not edematous retinopathy.

In conclusion, there is a clear imbalance between platelet activity and the function of endogenous NO-cGMP and prostacyclin-cAMP pathways in patients with type II diabetes mellitus, and this imbalance is especially evident in patients with edematous retinopathy. The differences we found between patients with different types of retinopathy suggest new approaches to research on the mechanisms that favor or impede the appearance of each type of retinopathy and raise the possibility that different treatments may be appropriate for each group. However, further studies will be needed to learn more about these mechanisms in patients with different types of retinopathy.

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References

- Brailsford SC, Davies R, Canning C, Roderick PJ: Evaluating policies for the early detection of retinopathy in patients with non-insulin dependent diabetes. *Health Care Manag Sci* 1998;1:115–124.
- Klein R, Palta M, Allen C, Shen G, Han DP, D'Alessio DJ: Incidence of retinopathy and associated risk factors from time of diagnosis of insulin-dependent diabetes. *Arch Ophthalmol* 1997;115:351–356.
- Reuterving CO, Kratholm J, Wachtmeister L: Ophthalmic health care in diabetes mellitus: A cross-sectional study in northern Sweden. *Ophthalmic Epidemiol* 1999;6:267–278.
- Retinopathy and nephropathy in patients with type 1 diabetes four years after a trial of intensive therapy. The Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications Research Group. *N Engl J Med* 2000;342:381–389.
- De Vriese AS, Verbeuren TJ, Van de Voorde J, Lameire NH, Vanhoutte PM: Endothelial dysfunction in diabetes. *Br J Pharmacol* 2000;130:963–974.
- Moncada S, Vane JR: Unstable metabolites of arachidonic acid and their role in haemostasis and thrombosis. *Br Med Bull* 1978;34:129–135.
- Radomski MW, Moncada S: The biological and pharmacological role of nitric oxide in platelet function. *Adv Exp Med Biol* 1993;344:251–264.
- Michelson AD, Furman MI: Laboratory markers of platelet activation and their clinical significance. *Curr Opin Hematol* 1999;6:342–348.
- Knobler H, Savion N, Shenkman B, Kotev-Emeth S, Varon D: Shear-induced platelet adhesion and aggregation on subendothelium are increased in diabetic patients. *Thromb Res* 1998;90:181–190.
- Iwase E, Tawata M, Aida K, Ozaki Y, Kume S, Satoh K, Qi R, Onaya T: A cross-sectional evaluation of spontaneous platelet aggregation in relation to complications in patients with type II diabetes mellitus. *Metabolism* 1998;47:699–705.
- Roshan B, Tofler GH, Weinrauch LA, Gleason RE, Keough JA, Lipinska I, Lee AT, Delia JA: Improved glycemic control and platelet function abnormalities in diabetic patients with microvascular disease. *Metabolism* 2000;49:88–91.
- Mazzanti L, Mutus B: Diabetes-induced alterations in platelet metabolism. *Clin Biochem* 1997;30:509–515.
- De La Cruz JP, Moreno A, Sintas A, García Campos JM, Sánchez de la Cuesta F: Platelet hyperaggregation in diabetic patients with different types of retinopathy is partially influenced by erythrocytes in whole blood. *Diabetes Res* 1997;32:51–68.

- 14 De La Cruz JP, Moreno A, Guerrero A, Sánchez de la Cuesta F: Antiplatelet effects of prostacyclin and nitric oxide in patients with type I diabetes and ischemic or edematous retinopathy. *Platelets* 2001;12:210–217.
- 15 Cardinal DC, Flower RJ: The electronic aggregometer: A novel device for assessing platelet behavior in blood. *J Pharmacol Methods* 1980;3:135–158.
- 16 Gasser JA, Cooper MB, Tan KC, Saggerson ED, Betteridge DJ: Altered cellular signalling and decreased platelet sensitivity to adenosine in insulin-dependent diabetic patients with proliferative retinopathy. *Cell Signal* 1993;5:145–153.
- 17 Mendez JD, Zarzoza E: Inhibition of platelet aggregation by L-arginine and polyamines in alloxan treated rats. *Biochem Mol Biol Int* 1997;43:311–318.
- 18 Huszka M, Kaplar M, Rejto L, Tornai I, Palatka K, Laszlo P, Udvardy M: The association of reduced endothelium derived relaxing factor-NO production with endothelial damage and increased in vivo platelet activation in patients with diabetes mellitus. *Thromb Res* 1997;86:173–180.
- 19 Ferlito S, Gallina M: Nitrite plasma levels in type 1 and 2 diabetics with and without complications. *Panminerva Med* 1998;40:304–308.
- 20 Martina V, Bruno GA, Trucco F, Zumpano E, Tagliabue M, Di Bisceglie C, Pescarmona G: Platelet cNOS activity is reduced in patients with IDDM and NIDDM. *Thromb Haemost* 1998;79:520–522.
- 21 Rabini RA, Staffolani R, Fumelli P, Mutus B, Curatola G, Mazzanti L: Decreased nitric oxide synthase activity in platelets from IDDM and NIDDM patients. *Diabetologia* 1998;41:101–104.
- 22 Pitei DL, Watkins PJ, Edmonds ME: NO-dependent smooth muscle vasodilation is reduced in NIDDM patients with peripheral sensory neuropathy. *Diabet Med* 1997;14:284–290.
- 23 Michimata T, Murakami M, Iriuchijima T: Nitric oxide-dependent soluble guanylate cyclase activity is decreased in platelets from male NIDDM patients. *Life Sci* 1996;59:1463–1471.
- 24 West IC: Radicals and oxidative stress in diabetes. *Diabet Med* 2000;17:171–180.
- 25 Bonnefont-Rousselot D, Bastard JP, Jaudon MC, Delattre J: Consequences of the diabetic status on the oxidant/antioxidant balance. *Diabetes Metab* 2000;26:163–176.
- 26 Moncada S, Palmer RM, Higgs EA: Nitric oxide: Physiology, pathophysiology, and pharmacology. *Pharmacol Rev* 1991;43:109–142.
- 27 Moro MA, Darley-USmar VM, Goodwin DA, Read NG, Zamora R, Feelisch M, Radomski MW, Moncada S: Paradoxical fate and biological action of peroxynitrite on human platelets. *Proc Natl Acad Sci USA* 1994;91:6702–6706.
- 28 Brown AS, Moro MA, Masse JM, Cramer EM, Radomski MW, Darley-USmar V: Nitric oxide-dependent and independent effects on human platelets treated with peroxynitrite. *Cardiovasc Res* 1998;40:380–388.
- 29 Tannous M, Rabini RA, Vignini A, Moretti N, Fumelli P, Zielinski B, Mazzanti L, Mutus B: Evidence for iNOS-dependent peroxynitrite production in diabetic platelets. *Diabetologia* 1999;42:539–544.
- 30 Zou M-H, Bachschmid M: Hypoxia-reoxygenation triggers coronary vasospasm in isolated bovine coronary arteries via tyrosine nitration of prostacyclin synthase. *J Exp Med* 1999;190:135–139.
- 31 Moreno A, De La Cruz JP, García Campos J, Sánchez de la Cuesta F: Prostacyclin-thromboxane balance and retinal vascular pattern in rats with experimentally induced diabetes. *Can J Ophthalmol* 1995;30:117–123.
- 32 De La Cruz JP, Moreno A, Sánchez de la Cuesta F, García Campos J: Effect of antiplatelet drug therapy on the retinal vascular pattern in experimental diabetes. *Can J Ophthalmol* 1990;25:329–332.
- 33 De La Cruz JP, Moreno A, Muñoz M, García Campos J, Sánchez de la Cuesta F: Effect of aspirin plus dipyridamole on the retinal vascular pattern in experimental diabetes mellitus. *J Pharmacol Exp Ther* 1997;280:454–459.
- 34 Moreno A, De La Cruz JP, Mérida F, García Campos JM, Sánchez de la Cuesta F: Effects of diltiazol on the vascular retinal pattern in experimental diabetes in rats. *Haemostasis* 1995;25:166–171.