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Erectile and endothelial dysfunction in Type II diabetes: a possible link

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Abstract

Aims/hypothesis. The aim of this study was to evaluate the relation between erectile dysfunction and endothelial functions, coagulation activation, peripheral and autonomic neuropathy in men with Type II (non-insulin-dependent) diabetes mellitus.
Methods. We studied 30 Type II diabetic patients with symptomatic erectile dysfunction and 30 potent diabetic patients matched for age and disease. Endothelial functions were assessed with the L-arginine test, plasma thrombomodulin and cell adhesion molecules circulating concentrations. Haemostasis was evaluated with markers of thrombin activation and fibrinolysis. Quantitative sensory testing (vibratory, warming, and heat-pain thresholds), cardiovascular reflex tests and 24-h blood pressure monitoring were used to assess peripheral or autonomic neuropathy.
Results. Mean erectile score and HbA_{1c} were 10.5 ± 5.8 and 8.3 ± 1.6 % in patients with erectile dysfunction, and 24.0 ± 0.7 and 6.8 ± 1.4 % in those without erectile dysfunction, respectively ($p < 0.001$); there was a significant relation between HbA_{1c} and erectile function score in patients with erectile dysfunction ($r = -0.45$, $p = 0.02$). The decrease in blood pressure and platelet aggregation in response to L-arginine was lower ($p < 0.05$–0.02) in patients with erectile dysfunction, whereas soluble thrombomodulin, P-selectin and intercellular cell ahhesion molecule-1 concentrations were higher ($p < 0.05$–0.02). Indices of coagulation activation (F1 + 2 and D-dimers) and reduced fibrinolysis (PAI-1) were also found to be higher in erectile dysfunction patients. Heat-pain and warm perception thresholds, as well as cardiovascular reflex tests, were most commonly abnormal in patients with erectile dysfunction ($p < 0.05$). In multivariate analysis, HbA_{1c}, MBP response to L-arginine, P-selectin, indices of coagulation, and quantitative sensory testing were independent predictors of erectile function score.
Conclusion/interpretation. Erectile dysfunction in diabetic men correlates with endothelial dysfunction. A reduced nitric oxide activity might provide a unifying explanation. [Diabetologia (2001) 44: 1155–1160]

Keywords Erectile dysfunction, endothelial functions, L-arginine test, thrombomodulin, coagulation, fibrinolysis, adhesion molecules, peripheral neuropathy.

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Abbreviations: ED, erectile dysfunction; IIEF, international index of erectile function; CAMs, cellular adhesion molecules; QSTs, quantitative sensory testing; ADP, adenosine diphosphate; CASE IV, computer assisted sensory examination; MBP, mean blood pressure

Erectile dysfunction (ED) is a common complication and an important cause of decreased quality of life in men with diabetes. These patients present a risk of ED three-fold higher than the general population; the prevalence of ED increases with age, but in diabetic men it can occur 10 to 15 years earlier regardless of their insulin dependency status [1].

The causes of ED in diabetic patients can be multifactorial, involving mainly vascular, neurological and

pharmacological factors [2]. There is a greater incidence of peripheral neuropathy [3], microangiopathy [4] and arterial insufficiency [5] in diabetic patients with ED than in potent diabetic men. Furthermore, diabetic patients could have other co-morbid conditions that are associated with ED without diabetes, such as renal failure, hypertension and chronic liver disease, thus the precise association of diabetes with ED could be circumstantial.

Nitric oxide is widely recognized as the most important factor involved in corpus cavernosal smooth muscle relaxation, and hence in erection [6]. Nitric oxide is synthesized and released directly from parasympathetic nonadrenergic, noncholinergic nerves [7], and from vascular endothelial cells after stimulation by acetylcholine [8]. Neurogenic and endothelium-mediated corpus cavernosal smooth muscle relaxation in tissue from diabetic men is impaired [9]. This situation is reminiscent of that occurring in other vascular tissues in which endothelial-mediated smooth muscle relaxation is inhibited by high glucose concentrations [10]. Hyperglycaemia could be the link between ED and endothelial dysfunction in diabetic men. Although it has been suggested that a defect in nitric oxide activity might play a part in the pathogenesis of both ED and ischaemic heart disease [11], so far no study has assessed the association between ED and endothelial functions in diabetic patients without vascular complications. Moreover, the role of coagulation abnormalities has not been evaluated in patients with ED.

Our aim was to evaluate the association between ED and endothelial functions, haemostasis, peripheral and autonomic neuropathy in men with Type II diabetes mellitus. For this purpose, we did a case-control study comparing two groups of Type II diabetic patients, with or without ED.

Subjects and methods

We studied 30 Type II diabetic patients with symptomatic erectile disfunction (ED) from at least six months, manifested as a persistent inability to attain and maintain an erection sufficient to permit sexual activity [12]. All patients presented for routine follow-up to Diabetes Clinics and then referred to the Center for Metabolic Diseases at the teaching Hospital of the Second University of Naples. None had clinical evidence of any peripheral vascular disease or psychological disorder. Impaired renal function, including macrolbuminuria, pelvic trauma, prostatic disease, non-diabetic neuropathy, proliferative retinopathy, and other ED-risk factors, such as use of drugs or alcohol, were also considered exclusion criteria. Participants who satisfied the inclusion criteria completed questions 1 to 5 of the IIEF (International Index of Erectile Function), which is a multidimensional questionnaire for assessing ED [13]. The erectile function score represents the sum of questions 1 to 5 of the IIEF questionnaire, with a maximum score of 25 (5 for each question). Another group of 30 Type II diabetic patients, matched for age, body mass index and duration of diabetes, but without ED, served as the control group (Table 1). All patients gave informed consent to participate in the study and the protocol was approved by the review board of the Second University of Naples.

Table 1. Characteristics of Type II diabetic patients

	Patients with ED	Patients without ED
n	30	30
Age (years)	55 ± 5.5	52.9 ± 5.0
BMI (kg/m2)	27.2 ± 2.7	26.1 ± 2.5
IIEF score	10.5 ± 5.8	24.7 ± 7.9[a]
Duration of diabetes (years)	9.5 ± 6.6	10.2 ± 5.0
HbA_{1c} (n. v. = 4.6–5.6 %)	8.3 ± 1.6	6.8 ± 1.4[a]
Plasma glucose (mmol/l)	10.2 ± 3.6	9.7 ± 3.2
Serum cholesterol (mmol/l)	4.8 ± 1.4	4.7 ± 1.5
Serum triglycerides (mmol/l)	1.35 ± 0.75	1.25 ± 0.7
Smokers (%)	70	80
Mean blood pressure (mmHg)	90.4 ± 10.1	88.9 ± 6.9
Dipper (%)	40	33.3
Heart rate (beat/min)	71.6 ± 7.5	69.0 ± 6.6
Platelet aggregation (%)	30.7 ± 18.2	25.8 ± 15.5
Microalbuminuria (%)[b]	30	20
Diabetes treatment (%)		
Diet	0	10
Oral drugs	70	60
Insulin	30	30

Data are means ± SD; n. v. = normal values.
[a] $p < 0.001$
[b] (> 30 mg/24 h)

All patients were studied after a 12-h overnight fast, avoiding the morning dose of insulin, if scheduled in their treatment. Endothelial function was assessed with the L-arginine test [14]. The diabetic patients were placed in a supine position with a room temperature kept between 20 and 24 °C. All patients were instructed to refrain from smoking and from drinking alcoholic beverages or coffee from the night before the test. Intravenous lines were inserted in a large antecubital vein of one arm for infusion and in a dorsal vein of the contralateral arm for blood sampling. Patency was preserved by a slow saline infusion (0.9 % NaCl). Automatic measurements of blood pressure and heart rate (Finapres, Omheda 2300, Englewood, Calif., USA) were carried out. The study began after the patient had rested for 30 min. An intravenous bolus of 3 g L-arginine (10 ml of a 30 % solution of L-arginine monochloride), the natural precursor of nitric oxide, was injected within 60 s. Blood pressure and platelet aggregation responses to 1.25 micromolar adenosine diphosphate (ADP) were measured before L-arginine injection and after 10 min. The overall reproducibility of L-arginine test in the same patient was 0.75 %. L-arginine mimics some of the effects of nitric oxide, including vasodilation and antiplatelet activity [15]; since the vascular effects of L-arginine are thought to derive from metabolic conversion to nitric oxide, the L-arginine test has been used for evaluating endothelial function [14].

The assessment of peripheral neuropathy was based on the quantitative sensory examination using the vibratory, thermal and pain sensory thresholds. Quantitative Sensory Testing (QSTs) was done by a computerized system (Computer Assisted Sensory Examination, CASE IV, Stillwater, MN) designed to evaluate vibratory and warming detection thresholds, as well as heat-pain via a numerical visual analogue scale (1 to 10) of pain. This battery of tests allows noninvasive assessment of large-fibre (vibratory), and small-fibre sensory function (warming, pain). Sensory abnormalities measured by CASE

IV provide a reliable, reproducible, and sensitive index of diabetic neuropathy [16].

All patients were evaluated for cardiac autonomic neuropathy by cardiovascular reflex tests. Autonomic nerve function tests included the heart rate variation during deep breathing and the heart rate responses to the squatting test [17]. The squatting test gives information an both sympathetic and parasympathetic activity, and is better than other single tests in identifying early sympathetic involvement [18]. R-R intervals were recorded with a standard 12-lead ECG. The patients also monitored their blood pressure during 24 h (DynaPulse 5000, Pulse Metric Inc, San Diego, Calif., USA). Based on the blood pressure night-time decrease, the patients were categorized in non-dipper and dipper, respectively, for a night-time reduction less than 10%, and between 10 and 20%.

Blood for routine chemical analyses were taken after an overnight fast. Blood for assessing haemostatic parameters, soluble thrombomodulin, P-selectin and intercellular adhesion molecule-1 (ICAM-1) was collected through a silicone-treated needle and was allowed to flow freely into silicone-treated glass tubes, where it was mixed with 1/10 of its volume of 0.1 mol/l sodium citrate. The blood was immediately centrifuged at 1700 $g \cdot 20$ min at 4 °C and frozen at –80 °C until assayed. Plasma glucose was measured by the glucose-oxidase method; serum cholesterol and triglyceride concentrations were measured by standard enzymatic methods; HbA_{1c} concentration was measured by high pressure liquid chromatography. Coagulative parameters were measured with immunosorbent commercially available kits (F1 + 2: Dade Behring, Marburg, Germany; D-dimer: Diagnostica Stago, Asmières, France; PAI-1 and tPA: Byk-Sangtek Diagnostica, Germany). Thrombomodulin (Asserachrom, Diagnostica); P-selectin and ICAM-1 (R&D Systems, Minneapolis, Minn., USA) were also measured by immunosorbent kits. For all, the intra-assay and interassay coefficients of variation were below 5% and 7%, respectively.

Statistical analysis. Data are presented as means ± SD. Mean blood pressure (MBP) was calculated as diastolic blood pressure plus one-third of pulse pressure. Individual changes of parameters recorded during the L-arginine test were calculated as the difference between the values found at 10 min and baseline values. Data were analysed statistically by ANOVA; individual means were compared using paired or unpaired student's *t* tests. The chi-square test was used for determining the frequency of pathological findings between groups. Multivariate analysis was used to measure the association of ED with glycaemic control, indices of endothelial function, and haemostasis after adjusting covariates. Statistical analyses were done using computer software. A p value of less than 0.05 was chosen as the level of statistical significance.

Results

All 60 participants completed the 5 questions of the questionnaire. Mean erectile function score was 10.5 ± 5.8 (range 1 to 20) in diabetic patients with ED and 24.0 ± 0.7 (range 23 to 25) in diabetic patients without ED ($p < 0.001$); mean HbA_{1c} was 8.3 ± 1.6% (range 5.8 to 13) and 6.8 ± 1.4% (4.6 to 8.4), respectively ($p < 0.001$). There was an inverse association between HbA_{1c} and erectile function score in the group of patients with ED ($r = –0.45$, $p = 0.02$). The other clinical characteristics of the patients did not differ between the two groups; in particular, the percentage of dipper and non-dipper blood pressure profiles was not different, nor was there any difference in the prevalence of microalbuminuria (Table 1). However, markers of coagulation activation (F1 + 2 and D-dimer) and reduced fibrinolysis (PAI-1) were higher in the ED group (Table 2).

Table 2. Coagulation and fibrinolysis parameters in diabetic patients with or without erectile dysfunction

	Patients with ED ($n = 30$)	Patients without ED ($n = 30$)
F1 + 2 (nmol/l) (n. v. = 0.2–1.2)	1.7 ± 0.5	1.1 ± 0.4[a]
D-dimer (g/l) (n. v. = 1.5–2.8)	3.8 ± 0.9	2.5 ± 0.7[a]
PAI-1 (μg/l) (n. v. = 4–43)	55 ± 14	41 ± 10[a]
tPA (μg/l) (n. v. = 1.3–10.4)	10 ± 3	9.2 ± 2.8
PAI-1/tPA	5.6 ± 1.4	4.5 ± 0.9[a]

[a] Indicates significant differences ($p < 0.05$ or less) between groups

The changes in blood pressure recorded during the L-arginine test were expressed as MBP variations from baseline. In patients without ED, the L-arginine bolus produced a decrease of MBP (–4.5 ± 2.1 mmHg) which was different compared with prestimulatory values ($p < 0.01$), and also greater ($p < 0.02$) than that obtained in patients with ED, in whom no change from baseline was observed after L-arginine (+ 0.3 ± 1.4 mmHg) (Fig. 1). Similarly, the decrease from baseline of platelet aggregation response to ADP (–9.7 ± 4.3%) was greater ($p < 0.05$) in patients without ED than in patients with ED (–1.7 ± 1.3%). There was a positive association ($r = 0.38$, $p < 0.05$) between the fall in MBP after L-arginine and the erectile function score in the diabetic patients with ED. Soluble thrombomodulin was higher in the ED group (37 ± 9 vs 26 ± 8 μg/l, $p < 0.02$) and correlated with the erectile function score ($r = –0.34$, $p < 0.05$). P-selectin and ICAM-1 concentrations were increased in patients with ED (61 ± 17 and 268 ± 42μg/l, respectively), compared with patients without ED (43 ± 13 and 220 ± 38 μg/l, $p < 0.05$), and showed a correlation with the erectile function score ($r = –0.41$, $p < 0.02$ for P-selectin; $r = –0.30$, $p < 0.05$ for ICAM-1).

The results of QSTs examination showed pathological changes in about 40% of patients with ED; QSTs were abnormal in about 15% of patients in the group without ED. Pain and warmth perception thresholds were most commonly abnormal in diabetic patients with ED ($p < 0.01$ and $p < 0.05$, respectively); the vibratory threshold abnormalities were only marginally more common in the ED group (Table 3). The diabetic patients with abnormal perception thresholds (pain and warmth) had a lower erectile function score ($p < 0.05$). The percentage of patients

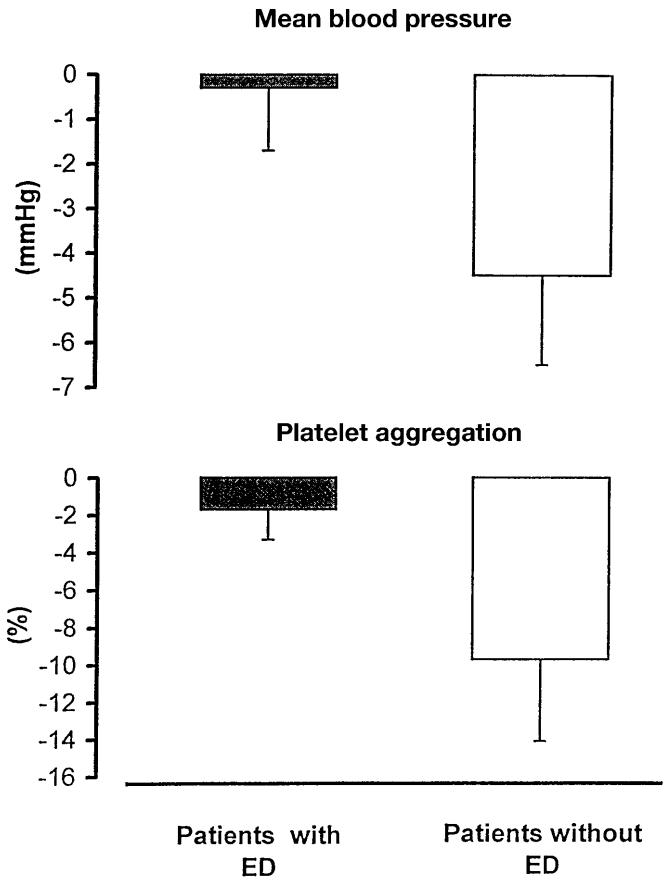

Fig. 1. Variations of mean blood pressure and platelet aggregation to 1.25 μmol/l ADP after L-arginine bolus (3 g within 30 s) in diabetic patients. The decrease of both parameters was ($p < 0.05$) higher in patients with erectile dysfunction

with abnormalities of autonomic cardiovascular tests was higher in the group with ED compared with the group without ED (Table 3).

Multivariate analysis showed that HbA_{1c} was an independent predictor of erectile function score ($p < 0.01$), even after adjusting for MBP response to L-arginine, indices of coagulation, P-selectin, and QSTs, which were also independent predictors ($p < 0.05$).

Discussion

The association between diabetes mellitus and ED is well established [19]. In our study, Type II diabetic patients with medically documented ED presented a poorer glycaemic control than that of a matched control group of diabetic patients without ED. Glycaemic control of diabetes, as assessed by HbA_{1c} concentrations, was inversely and independently associated with the level of ED, even after adjusting for other variables, such as endothelial dysfunction, coagulation activation, and neuropathy, which were also independent predictors of ED. It is possible that the

Table 3. Abnormal (%) autonomic and sensory tests in diabetic patients with or without erectile dysfunction

	Patients with ED ($n = 30$)	Patients without ED ($n = 30$)	p
Deep breathing	30	13.3	0.05
Squatting vagal test	70	30	0.02
Squatting sympathetic test	60	30	0.02
Heat-pain threshold	40	10	0.01
Warm threshold	50	30	0.05
Vibratory threshold	20	13.3	NS

various aspects of long-lasting hyperglycaemia, either acute, chronic or post-prandial [20], all contributing to raising HbA_{1c}, could be involved in the development of ED in men with diabetes mellitus.

The association between global glycaemic control and ED has been reported. In the largest study of prevalence and risk association [21, 22], an Italian multicentre cross-sectional study of 9868 diabetic men 20 to 69 years old, ED was reported by 3534 (35.8%) of patients. After accounting for the effect of age, Type II diabetic men (37/100) tend to report ED less frequently than Type I men (51/100); in both, a positive correlation was observed between ED and poor glycaemic control and smoking; BMI increased only the risk of ED in Type I diabetic men. Since the groups we studied were matched for BMI and smoking, glycaemic control of diabetes remains the only significant predictor of ED in our diabetic population.

A link between the pathogenesis of ED and decreased local nitric oxide activity has been suggested because in isolated corpus cavernosum strips from diabetic patients with ED both neurogenic and endothelium-dependent relaxation were impaired [5]. In our study, diabetic patients with ED presented evidence of abnormal endothelial functions. Blood pressure and platelet aggregation responses to L-arginine, the natural precursor of nitric oxide, were significantly reduced ($p < 0.02$) compared with diabetics without ED. Moreover, the ED group showed plasma thrombomodulin and CAMs concentrations significantly higher ($p < 0.02$ and $p < 0.05$ respectively) than those recorded in potent diabetic patients. Thrombomodulin is mostly located on endothelial cells; after proteolytic cleavage from the endothelial surface, soluble thrombomodulin can be detected in circulating plasma and has been suggested as a laboratory marker of endothelial cell damage and of endothelial cell function [23]. Type II diabetic patients could have plasma concentrations of soluble thrombomodulin higher than age-matched control subjects [24] and an increase in soluble thrombomodulin reflects an increased risk of symptomless carotid atherosclerosis [25]. Soluble forms of CAMs are also considered to be an index of endothelial activation

or even a molecular marker of early atherosclerosis [26]. In one study, plasma CAMs were associated with the risk of deterioration of peripheral neuropathy in diabetic patients [27]. Our findings suggest that diabetic patients with ED present an impairment of endothelial functions and indices of endothelial activation more marked than diabetic patients without ED.

Hyperglycaemia impairs nitric oxide availability to target cells, mimicking the endothelial dysfunction seen in the diabetic patient. Hyperglycaemia could increase free radical formation which could quench and deactivate nitric oxide, reducing its availability for target cells [28–30]. Among free radicals, superoxide anion seems the more likely candidate in mediating the haemodynamic changes of hyperglycaemia because it is rapidly generated in hyperglycaemic conditions, it rapidly reacts with nitric oxide and its serum concentrations correlate with indices of glycaemic control, such as plasma glucose and glycated proteins [31]. Data [32] also support this interpretation; in their hands, glycated haemoglobin directly impairs acetylcholine-mediated relaxation of rat corpus cavernosum in a dose-dependent manner, an effect mimicked by pyrogallol, a donor of superoxide anions, and completely reversed by superoxide dismutase, the enzyme involved in the degradation of superoxide anions. The results also fit with previous data showing a significant increase of the specific AGE pentosidine in the diabetic human penis, but not in the serum, suggesting a tissue-specific effect of the AGEs [33]. Thus, an impairment in nitric oxide bioavailability, due to nitric oxide quenching mediated by superoxide anions, could play a part in the pathogenesis of ED associated with diabetes mellitus.

Diabetes mellitus is characterized by the existence of a thrombosis-prone condition [34]. When coagulation is activated, cleavage parameters, including F1 + 2 and D-D, are released into circulation [35]. Our data show for the first time that patients with ED present markers of coagulation activation and reduced fibrinolysis higher than patients without ED, which points for a role of unbalanced haemostasis in the pathogenesis of diabetic ED. Hyperglycaemia seems to be linked to coagulation activation through an increased generation of free radicals [36].

Erectile dysfunction also depends on a complex interaction of sensory and autonomic fibres [37]. A study found abnormal warm thresholds in all their patients with neurogenic ED; however, they did not evaluate a matched control group of non-ED diabetics [38]. Another study found the prevalence of neuropathy to be 38% in diabetic patients with ED [39]. More recently, a report showed a higher prevalence of sensory threshold abnormalities in a group of 68 diabetic men with ED, with a preferential involvement of axon-reflex vasodilation (89%), followed by warmth perception (81%) [40]. In our study, the best discriminator of neurological involvement in diabetic men with ED was heat-pain threshold for peripheral neuropathy and the squatting test for autonomic neuropathy. As a whole, tests of small-fibre sensory function and cardiovascular reflex tests are most commonly abnormal in Type II diabetic patients with ED.

Our data show that erectile dysfunction in diabetic men correlates with the level of glycaemic control, with endothelial functions, and with neuropathy. A defective nitric oxide activity, linked to reduced nitric oxide availability, could provide a unifying explanation for the association between ED and endothelial dysfunction in Type II diabetic patients.

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