

Original Article: Complications

Chronic administration of Sildenafil improves markers of endothelial function in men with Type 2 diabetes

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Abstract

Objective Diabetic patients have a reduced endothelial response to phosphodiesterase-5 inhibitors. The aim of this study was to determine the effects of chronic therapy with sildenafil on endothelial function in patients with Type 2 diabetes mellitus (DM2).

Methods In a double-blind, placebo-controlled parallel design, 20 patients without erectile dysfunction randomly received a loading dose of sildenafil (100 mg) for 3 days, followed by either sildenafil 25 mg three times a day (t.d.s.) for 4 weeks or sildenafil 25 mg t.d.s. for 4 days followed by placebo t.d.s. for 3 weeks.

Results After 1 week, flow-mediated dilatation (FMD) improved significantly (> 50% compared with baseline) in patients allocated to both sildenafil arms (62 and 64%, respectively). In patients allocated to chronic sildenafil, a progressive increase in percentage of patients with FMD improvement was noted (78, 86 and 94% at 2, 3 and 4 weeks, respectively) while a progressive decrease in the placebo group occurred (45, 18 and 6% at 2, 3 and 4 weeks, respectively). At the end of the study, a significant improvement in FMD compared with baseline was noted after chronic sildenafil (FMD from 6.8 ± 0.5 to $12.5 \pm 0.7\%$, $P = 0.01$ vs. baseline). A decrease in endothelin-1 levels and an increase in nitrite/nitrate levels were found after chronic sildenafil; significant changes from baseline in C-reactive protein, interleukin 6, intercellular adhesion molecule and vascular adhesion molecule levels were also found.

Conclusions In DM2 patients, daily sildenafil administration improves endothelial function and reduces markers of vascular inflammation, suggesting that the diabetes-induced impairment of endothelial function may be improved by prolonged phosphodiesterase-5 inhibition.

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Keywords diabetes, endothelial dysfunction, erectile dysfunction, phosphodiesterase type 5, rehabilitation

Abbreviations cGMP, cyclic guanosine 3'-5'-monophosphate; CRP, C-reactive protein; DM2, Type 2 diabetes mellitus; ED, erectile dysfunction; eNOS, endothelial nitric oxide synthase; FMD, flow-mediated dilatation; ICAM, intercellular adhesion molecule; IL-6, interleukin 6; NO, nitric oxide; PDE5i, phosphodiesterase type-5 inhibitor; RH-PAT, reactive hyperaemia by peripheral arterial tonometry; t.d.s., three times daily; VCAM, vascular adhesion molecule

Introduction

Patients with diabetes mellitus have an increased risk of future cardiovascular events comparable with that of patients with coronary artery disease [1–3]. Thus, the risk of future cardiovascular events is equivalent in diabetes and established coronary artery disease [3]. Endothelial dysfunction, defined as an

abnormal response leading to a reduction in the bioavailability of nitric oxide (NO) and impaired vasodilatation, is thought to play a major role in the development of atherosclerosis and acute coronary syndromes, which are in turn frequent complications of diabetes. Recently, it has been suggested that endothelial dysfunction is often present in diabetic patients who do not have clinically evident atherosclerotic diseases [4]. Diabetic patients often have blunted endothelial function along with a reduced response to vasoactive agents [5].

Sildenafil is the first specific phosphodiesterase type-5 inhibitor (PDE5i) marketed for the treatment of erectile dysfunction (ED). It facilitates erection by increasing NO availability

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through the inhibition of cyclic-GMP (cGMP) breakdown in endothelial cells [6–8]. However, as the PDE5 isozyme is widely expressed in the vasculature [9], sildenafil enhances NO-mediated responses in vascular beds other than the penis. The efficacy of PDE5i is lower in diabetic patients with ED compared with those without diabetes [10]. Similarly, the endothelial effectiveness of drugs known to improve endothelial function is lower in patients with diabetes compared with patients without diabetes [11]. Chronic sildenafil administration may regulate the transduction pathway leading to the activation of endothelial nitric oxide synthase (eNOS), with no effect on NO bioavailability or on the cGMP pathway and without concerns for tachyphylaxis [12]. Furthermore, chronic administration of PDE5i may improve endothelial function in the long term [13]. Thus, chronic administration of PDE5i should improve the responsiveness of the dysfunctional endothelium in patients with diabetes. The aim of the present study was to determine the effects of chronic therapy with sildenafil on vascular reactivity and on markers of endothelial function in patients with Type 2 diabetes mellitus (DM2).

Methods

Baseline screening protocol

The study population included DM2 male patients aged 40–75 years and without overt ED, as determined by the administration of the International Index of Erectile Function-5 items, a validated questionnaire for the assessment of erectile function [14]. All subjects gave a medical history and underwent a complete physical examination. Exclusion criteria were: use of androgens or anti-androgens within 3 months from study initiation; clinically significant findings on the physical examination; uncontrolled diabetes [glycated haemoglobin (HbA_{1c}) > 9.0%] or uncontrolled arterial hypertension; the presence of a known, clinically significant disease that would interfere with the study procedures [i.e. unstable angina and/or arrhythmia, uncorrected hypokalaemia, recent acute myocardial infarction (MI; < 3 months), renal or liver dysfunction, primary valvular or congenital heart disease, myocardial, pericardial or endocardial disease, congestive heart failure; known HIV or hepatitis C-positive serologies]; or were taking medications known to

interfere with forearm blood flow (in the case of concurrent use of cardiac medication, a washout period longer than five times the half lives of the active molecule was observed before inclusion). Patients with ED were excluded in order not to unblind the study. All patients gave informed written consent to the study, which had been approved by the local Ethic Committees. A patient could be withdrawn from the study at any time for any of the following reasons: refusal to comply with the parameters of the study; desire to withdraw; medical condition which the investigator considered to contraindicate the continuation of the study, such as unstable angina; requirement to take a concomitant drug that could interfere with endothelial function; or the occurrence of an adverse event.

Study design

After receiving a loading dose of sildenafil 100 mg for 3 days (at bedtime), patients received sildenafil 25 mg t.d.s. for a further 4 days and were then randomized in a double-blind fashion to receive either sildenafil 25 mg t.d.s. or matched placebo t.d.s. for 3 weeks in order to achieve the same degree of inhibition of phosphodiesterase type-5 enzyme in all areas of the vascular bed, according to previous data on its tolerability in patients with pulmonary hypertension. Our institution's pharmacy provided the identical placebo and active drug tablets used, and then controlled the double-blind key for this study. Patients were instructed to take sildenafil and sildenafil-placebo tablets fasting or at least 4 h after lunch. Compliance was assessed by counting the number of returned tablets at the end of the study. Forearm blood flow was measured at baseline, 1 h after the first loading dose of sildenafil and at the end of each of the 4 weeks, and 2 and 4 weeks after the discontinuation of the study drugs (Fig. 1). Endothelial function studies were performed 2 h after the self administration of the study drug by the same trained investigator. Serum NO and endothelin-1 levels were measured at baseline and after each treatment period, as were markers of endothelial inflammation such as C-reactive protein (CRP), interleukin-6 (IL-6), vascular adhesion molecule (VCAM) and intercellular adhesion molecule (ICAM) according to a previously published procedure [15].

Study of endothelial function

Endothelial function was assessed by measuring changes from baseline in the caliber of the brachial artery during reactive

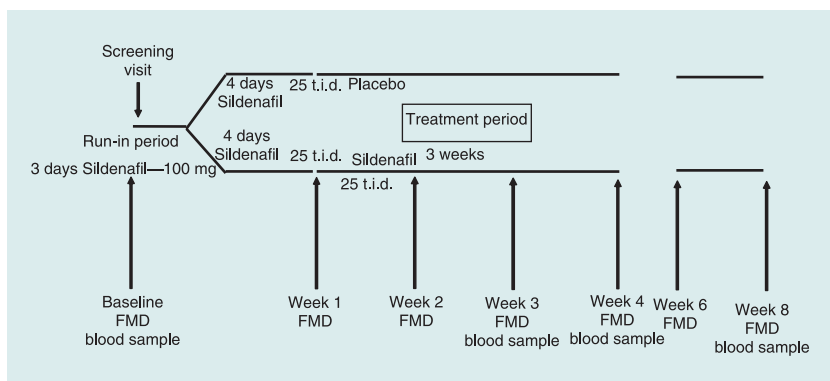


FIGURE 1 Study design. Flow-mediated dilation (FMD) and RH-PAT.

hyperaemia, a procedure that increases blood flow and shear stress through the vessel, and also by measuring reactive hyperaemia by peripheral arterial tonometry (RH-PAT). Brachial artery flow mediated dilatation (FMD) was measured with high-resolution Doppler ultrasound, while RH-PAT was assessed by peripheral arterial tonometry (Endopath; Itamar Medical Ltd, Cesarea, Israel). The same trained investigator carried out studies on each patient in order to avoid interobserver variability.

Studies on brachial artery reactivity were conducted according to a previously reported protocol [5]. In brief, all patients were studied in a quiet, temperature-controlled room (22° to 23°C). Participants were asked to avoid drinking beverages containing caffeine and to refrain from smoking for 6 h prior to the study procedures. The right brachial artery was visualized by using an Acuson Sequoia C256 high-resolution ultrasound machine (Acuson Corporation, Mountain View, CA, USA) equipped with a 7.5 to 12.5-MHz linear-array transducer. The diameter of the right brachial artery was measured four times: at rest, during reactive hyperaemia and after a 10-min recovery period; afterwards, in order to assess endothelium-independent vasodilatation, sublingual nitroglycerin (0.4 mg) was administered, and a fourth scan was recorded for 5 min.

Studies on peripheral arterial tonometry were performed contemporarily along with the FMD studies. Peripheral arterial tonometry probes were placed on one finger of each hand for continuous recording of the PAT signal. The RH-PAT data were analysed by a computer in an operator-independent manner. As a measure of reactive hyperaemia, the RH-PAT index was calculated as the ratio of the average amplitude of the PAT signal over a 1-min time interval, starting 1 min after cuff deflation, divided by the average amplitude of the PAT signal of a 3.5-min time period before cuff inflation (baseline). Subsequently, RH-PAT index values from the study arm were normalized with those of the control arm.

Image analysis

The ultrasound images were recorded directly onto the hard disk of the ultrasound machine, as well as transferred to an external hard drive. Image analysis was performed using semi-automated edge detection software (Crisell Instruments, Roma, Italy). The diameter of the brachial artery was measured from the anterior to the posterior interface. For each measurement, the mean arterial diameter was calculated from four cardiac cycles. All measurements were made at the end of the diastolic phase. The diameter change was expressed as the per cent change compared with baseline diameter.

Fifteen studies of brachial artery reactivity were randomly selected for a second analysis in order to calculate intraobserver variation. This intraobserver variation was $0.38 \pm 0.26\%$ (range 0.1–1.2%), yielding a coefficient of variation of 1.26% and a coefficient of repeatability of 0.5%. Accordingly, a significant response in flow-mediated vasodilatation was defined as an improvement > 20%.

Biochemical analyses

Venous blood samples were taken with a Vacutainer system (Becton-Dickinson, Franklin Lakes, NJ, USA), after at least 10-

h fasting, in the supine position and after 20 min of rest. Blood samples were collected in tubes containing EDTA or trisodium citrate (1 : 9 v/v) and were immediately placed on ice and centrifuged within 1 h of collection. Plasma was divided into aliquots and stored at -80°C until laboratory analysis. All sera were assessed in duplicate. Plasma was thawed and assayed for CRP by use of a high-sensitivity assay with a coefficient of variation < 5.0% (Dade Behring, Deerfield, IL, USA). An ELISA method was also used to measure IL-6, ICAM-1, VCAM-1 (R & D Systems Inc., Minneapolis, MN, USA). The lower limit for the detection of IL-6 was 0.2 ng/l; the blood sedimentation rate was measured according to the method of Wintrobe [28].

Cholesterol, triglyceride and HbA_{1c} concentrations were determined on the same day of the vascular reactivity studies. Total plasma cholesterol and triglycerides were measured on a dimension analyser (Dade Behring) using enzymatic methods. Intra-assay coefficients of variation for total cholesterol and triglyceride levels were < 2.0%. HbA_{1c} was assayed by a routine HPLC method. At each visit, 10 ml of blood were withdrawn at the end of each test and were assayed for plasma nitrite and nitrate levels and endothelin-1. Blood was collected in 5-ml tubes with 2% EDTA and 500 IU aprotinin. Serum endothelin-1 was extracted through absorption column cartridge (Sep-pack C18; Waters, MA, USA) and measured by RIA; intra- and interassay coefficient of variation were 3.0 and 6.0%, respectively.

Statistical analysis

Data are expressed as mean \pm SD. After testing data for normality, Student's paired *t*-test or Wilcoxon Signed Rank test were used to compare values before and after each therapy and the relative changes in values in response to each therapy. The effects of the different therapy regimens on vascular function and plasma levels of endothelin-1 relative to respective pretreatment values were analysed by one-way repeated measures ANOVA or Friedman Repeated ANOVA on Ranks. The analyses were considered to show a significant difference when $P < 0.05$.

Results

The baseline clinical characteristics of study patients are shown in Table 1. The mean duration of diabetes was 6.3 ± 3.2 years and none of the patients had diabetic neuropathy or diabetic foot disease. Safety analyses included all enrolled subjects. The most frequent adverse events in sildenafil-treated subjects were headache, dyspepsia and nasal congestion which were present in three out of 20 patients (15%). These adverse events were typically reported as mild, and tended to disappear over time, with no subject discontinuing the study medication because of them. There were no reports of abnormal vision, prolonged erections or priapism in either group, and no clinically significant changes attributable to sildenafil in vital signs, i.e. orthostatic hypotension or abnormal laboratory tests. No drop in blood pressure was found in either treatment groups (data not shown).

Baseline brachial artery diameter remained unchanged after the loading dose of sildenafil, as well as after daily sildenafil or matching placebo (Table 2). After 1 week of therapy, a significant

Table 1 Baseline clinical features of study patients

	All patients (n = 20)	Sildenafil (n = 10)	Placebo (n = 10)
Mean age (years)	63 ± 6.0	64.2 ± 5.4	63.6 ± 5.7
Body mass index (kg/m ²)	28.4 ± 3.6	28.9 ± 3.1	27.6 ± 2.9
Risk factors for CAD			
Arterial hypertension	14	7	7
Dyslipidaemia	11	6	5
Cigarette smoking	6	2	4
Family history of CAD	8	5	3
Glucose (mmol/l)	6.3 ± 0.8	6.5 ± 0.6	6.2 ± 0.9
Cholesterol (mmol/l)	5.1 ± 0.6	5.1 ± 0.7	4.9 ± 0.8
HDL cholesterol (mmol/l)	1.01 ± 0.10	1.00 ± 0.11	1.02 ± 0.11
LDL cholesterol (mmol/l)	3.1 ± 0.3	3.1 ± 0.3	3.0 ± 0.4
Tryglicerides (mmol/l)	2.1 ± 0.3	2.1 ± 0.3	2.1 ± 0.2
HbA _{1c} (%)	6.4 ± 1.3	6.5 ± 1.8	6.5 ± 0.9
Concurrent therapy			
Metformin	15	7	8
Glicazide	9	5	4
Glibenclamide	5	3	2
Anti-platelet agent	18	8	10
Diuretics	5	3	2
β-blockers	6	3	3
Ca-antagonists	8	5	3
ACE-inhibitors	10	5	5
Angiotensin II receptor blockers	6	3	3
Statins	20	10	10

Mean ± SD or number.

ACE, angiotensin-converting enzyme; CAD, coronary artery disease; HbA_{1c}, glycated haemoglobin; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

Table 2 Effect of Sildenafil and Placebo on Endothelial Function at 4 weeks

	Sildenafil		Placebo	
	Baseline	After therapy	Baseline	After therapy
Serum nitrogen oxides (μmol/l)	39.1 ± 11.4	46.4 ± 7.3*	40.6 ± 12.1	39.8 ± 7.8
Endothelin-1 (pg/ml)	3.6 ± 0.5	2.8 ± 0.7*	3.5 ± 0.7	3.4 ± 0.8
Brachial artery diameter (mm)				
Basal-1	4.57 ± 0.32	4.58 ± 0.29	4.57 ± 0.32	4.56 ± 0.34
Hyperaemia	4.88 ± 0.65	5.15 ± 0.45†	4.88 ± 0.47	4.87 ± 0.45
FMD, %	6.8 ± 0.5	12.5 ± 0.7†	6.7 ± 0.7	6.5 ± 1.2
RH-PATH	1.33 ± 0.08	1.98 ± 0.05	1.31 ± 0.07	1.33 ± 0.1
Correlation coefficient FMD/RH-PAT (r)	0.67†	0.69†	0.65†	0.71†

Data are expressed as means ± SD.

**P* < 0.05 and †*P* < 0.01 vs. respective baseline values.

FMD, flow-mediated dilatation; RH-PAT, reactive hyperaemia by peripheral arterial tonometry.

increase in FMD was observed in both groups of patients compared with baseline. Also, at the same time points, the number of patients with an improvement in flow-mediated dilation compared with baseline was similar in both groups of patients (40 vs. 50% and 60 vs. 70%, respectively). At week 4, hyperaemic diameters increased significantly in patients randomized to daily sildenafil, but not in those receiving placebo from

week 2 onwards (Table 2). At the end of the study, a significant improvement in FMD compared with baseline was noted after chronic sildenafil (FMD from 6.8 ± 0.5 to 12.5 ± 0.7%, *P* = 0.01), while the hyperaemic response was not different from baseline in patients randomized to placebo. Similarly, a significant increase in RH-PAT normalized index was noted after 1 week of sildenafil in both groups; at week 4, the RH-PAT

FIGURE 2 Per cent change compared with baseline in endothelial function (RH-PATH) in patients treated with sildenafil (25 mg t.d.s., ■) or placebo (□) for 4 weeks. *P*-values refer to comparison between sildenafil vs. placebo.

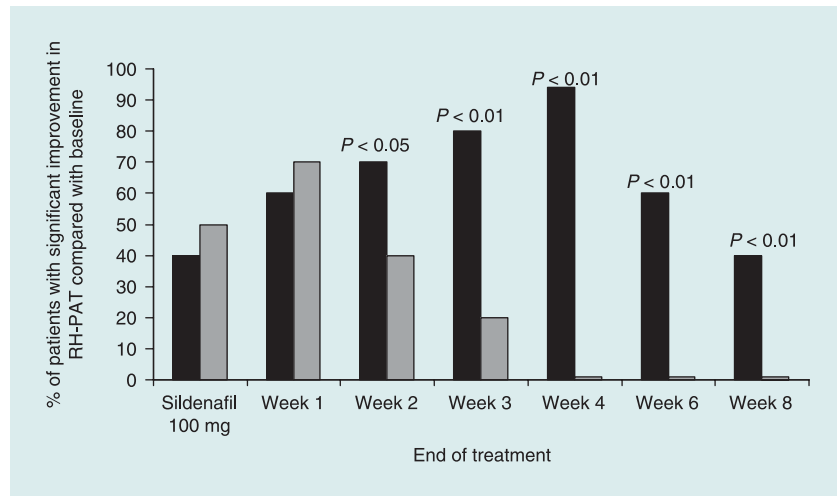
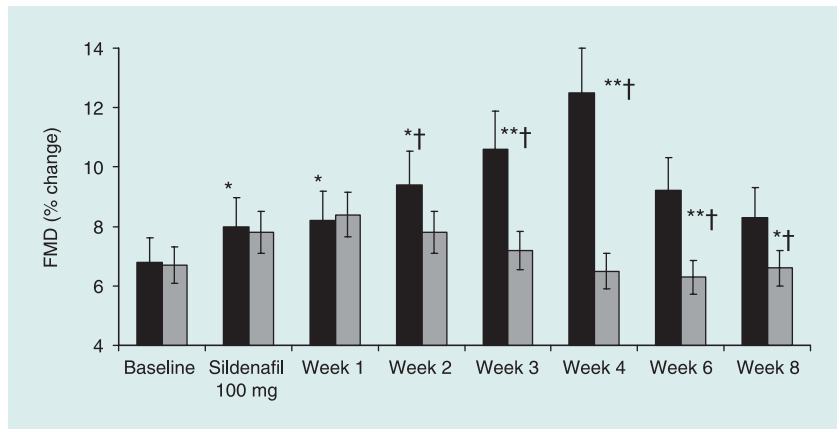


FIGURE 3 Changes in flow-mediated dilatation (FMD) in patients allocated to sildenafil (■) or placebo (□) throughout the study. **P* < 0.05 compared with baseline study; ***P* < 0.01 compared with baseline study; †*P* < 0.01 sildenafil compared with placebo.



normalized index was significantly different in patients allocated to sildenafil compared with placebo (Table 2). Furthermore, a significant positive correlation was found between FMD and RH-PAT normalized index at each study time point (Table 2).

The number of patients with a significant increase in FMD compared with baseline progressively increased in those patients receiving daily sildenafil (70, 80 and 100% at 2, 3 and 4 weeks, respectively) while a progressive decrease in hyperaemic response was detected in the placebo group (40, 20 and 0% at 2, 3 and 4 weeks, respectively; Fig. 2). Significant differences in percentage changes in FMD between baseline and respective post-dose levels at each time point in the sildenafil group occurred, but no significant differences were observed in the placebo group (Fig. 4). A difference in endothelial function compared with baseline persisted in patients allocated to sildenafil after 2 and 4 weeks after discontinuation of the study drug, while no changes were noted in patients allocated to placebo. After 4 weeks' drug withdrawal, the number of patients with a significant increase in FMD and RH-PATH was greater in patients allocated to sildenafil (40 vs. 0%, *P* < 0.01; Fig. 2). No significant changes of the FMD following

sublingual glyceryl trinitrate (GTN) were found in either group (data not shown).

Significant differences in plasma levels of biomarkers of endothelial function between the treatment groups at different time points were found. At the end of the treatment period, a significant decrease in all measured markers of endothelial function compared with baseline was noted in patients allocated to sildenafil (CRP = -22 ± 6 vs. $-5 \pm 3\%$; IL-6 = -29 ± 7 vs. $6 \pm 3\%$; ICAM = -18 ± 8 vs. $7 \pm 6\%$; VCAM = -32 ± 9 vs. $-4 \pm 2\%$, *P* < 0.05 for all comparisons; Fig. 3) while no changes in any marker was detected in patients allocated to the placebo arm. Also, significant increases from baseline in nitrite and nitrate levels were found in patients allocated to sildenafil, but not in those receiving placebo (15.7 vs. 0%, *P* < 0.05). Endothelin-1 levels decreased in patients receiving daily sildenafil, but not in those receiving placebo (-22 vs. $+20\%$, *P* < 0.05; Table 2).

Discussion

This study shows for the first time that in men with DM2, daily sildenafil administration progressively improves endothelial

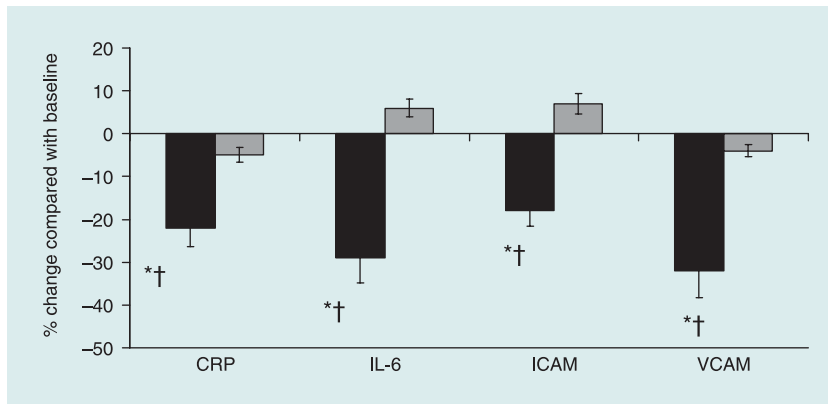


FIGURE 4 Plasma levels of markers of endothelial function in patients treated with sildenafil (■) or placebo (□) after 4 weeks of treatment. * $P < 0.05$ between groups; † $P < 0.02$ sildenafil compared with baseline. CRP, C-reactive protein; ICAM, intercellular adhesion molecule; IL-6, interleukin 6; VCAM, vascular adhesion molecule.

function and increases the percentage of patients with a significant improvement of endothelial function. This effect may be relevant for those DM2 patients affected by erectile dysfunction. The study also shows that chronic sildenafil administration is associated with an increase in nitrite/nitrate levels, an improvement in markers of vascular inflammation and with a concurrent decrease in endothelin-1. The study strongly confirms previous observations suggesting that acute and short-term sildenafil administration improves endothelial function in men with DM [16]. The vascular effects found in the present study were obtained independently from the presence of ED, as we had previously reported with another PDE5 inhibitor [13]. Although a non-diabetic control group was not included in this study, significant changes in markers of endothelial function in non-diabetic subjects with ED during chronic PDE5-i therapy had been reported elsewhere [15]. Another important finding of the present study is that the effects of short-term daily sildenafil administration were sustained after 1-month withdrawal of the study drug. Of note, 4 weeks after discontinuation, the number of patients with a significant increase in forearm FMD and RH-PAT was similar to that observed after a single loading dose of 100 mg of sildenafil. Our data are in agreement with a preliminary report from other authors who demonstrated that daily sildenafil administration at bedtime for 12 months led to a significant improvement in penile arterial blood flow [17]. Also, the direct improvement of surrogate markers of endothelial function in our study augments the knowledge regarding the putative effect on both erectile and endothelial recovery induced by chronic PDE5 inhibition.

It is likely that the results of the present study are dependent on the inhibitory effect of sildenafil on cGMP degradation at the endothelial level and are consistent with previous reports showing that chronic PDE5 inhibition may also improve endothelial function in men at increased cardiovascular risk [13]. Our findings are in agreement with those of Behr-Roussel *et al.*, who suggested that chronic treatment with sildenafil potentiates acetylcholine-induced endothelium-dependent cavernosal responses and enhances erectile responses in rats [12]; and with those of Fisher *et al.* who demonstrated that, in

mice, sildenafil administration stimulates the synthesis and transcription of inducible nitric oxide synthase (iNOS) and eNOS mRNA in cardiac myocytes [18]. This action might be mediated by a sildenafil-induced direct stimulation of NOS via protein kinase C (PKC) and/or extracellular signal-regulated kinase (ERK) pathways, resulting in increased NO generation, guanylate cyclase activation and enhanced formation of cGMP [19]. Even although we studied a small number of diabetic patients over a period of time shorter than the conventional 12-week controlled studies, our results are in keeping with experimental findings suggesting that muscarinic receptors and/or the transduction mechanisms leading to the activation of eNOS are stimulated by chronic sildenafil treatment [20]. Indeed, the activation of the PI3-kinase/Akt/eNOS phosphorylation cascade could be involved, thereby causing more sustained NO release and vascular relaxation [21]. As reduced endothelial function as a result of inadequate NO release is of critical importance in the development and progression of atherosclerosis and ED [4,5,22], chronic therapy with sildenafil may represent an important therapeutic strategy for the improvement of endothelial health in individuals with diabetes. Another possible explanation for this mechanism has been provided by the pioneering work of Ayala and co-workers, who have shown that chronic inhibition of PDE5 with sildenafil results in improved energy balance and enhanced insulin action in a mouse model of diet-induced obesity and insulin resistance [23]. In that study, chronic sildenafil treatment had no effect on PI3-kinase binding to tyrosine-phosphorylated IRS-1 or Akt activation in muscle, thus suggesting that enhanced insulin action resulting from long-term PDE5 inhibition occurs by a mechanism other than insulin signalling itself. Furthermore, in an animal model of cardiac chronic pressure overload exposure, chronic inhibition of cGMP/PDE5 by sildenafil reverses pre-established hypertrophy, thus restoring cardiac chamber size, and cellular and molecular remodelling [24]. In this view, data from our study indirectly suggest that daily sildenafil may have added potential benefits on vascular wall remodelling compared with those exerted by concomitant therapies (i.e. statins, angiotensin-converting enzyme inhibitors, angiotensin-II receptor blockers, calcium-channel blockers or β -blockers), as

patients who received placebo showed a progressive decrease of NO-induced vasodilatation throughout the study, despite the use of vascular agents for their co-morbidities.

Recently, chronic therapy with high-dose sildenafil has been approved for the treatment of patients with pulmonary hypertension of various aetiologies [25]. In these patients, treatment with sildenafil is effective and improves the efficacy of inhalative agents, leading to site-specific, additive effects on pulmonary ventilation and perfusion [26]. In this view, the results of our study may have important clinical implications for the treatment of patients with either DM2 and endothelial dysfunction or those with concomitant ED, who are known to have a blunted clinical response to PDE5-i compared with the general population. In addition to modulating vascular tone, cGMP signalling can also regulate muscle glucose uptake [27]. Our study was also designated to assess a possible sildenafil action in countering the effects of high trygliceride-induced endothelial dysfunction, but not on insulin resistance and glucose utilization. Because of the beneficial effect on endothelial function and vascular inflammation obtained, our data may suggest a putative effect of daily sildenafil on the endothelial pathway triggering the atherogenic process; these findings need further validation from larger studies, taking into account the evidence-based risk reduction.

In conclusion, daily sildenafil improves endothelial function in men with DM2. It is important to note that the prolonged use of sildenafil progressively improves endothelial responsiveness of these patients and that the effects are sustained long term. Further studies are needed to determine whether chronic treatment with sildenafil or other PDE5 inhibitors prevents or slows the development of atherosclerosis in diabetic patients, and for how long the beneficial effects are maintained after discontinuation of therapy.

Competing interests

GS has received consultancy fees from Pfizer and Eli-Lilly. AF has been paid by Pfizer and Lilly for running educational programmes and his research registrar has also received an unrestricted grant from Pfizer for studies regarding PDE5 (MURST-PRIN 40%, 2002); he has been reimbursed by Pfizer, the manufacturer of Viagra, for attending several conferences. AA has received occasional grants from Pfizer, and by Eli-Lilly, the manufacturer of Cialis, for attending several conferences. GMCR and MF have received research grants from Pfizer and Eli-Lilly; these grants co-financed a research programme in conjunction with the Italian Ministry of Health (Ricerca Finalizzata 2005).

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