

## ORIGINAL RESEARCH

# Ultrastructural Study of Clitoral Cavernous Tissue and Clitoral Blood Flow From Type 1 Diabetic Premenopausal Women on Phosphodiesterase-5 Inhibitor

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## ABSTRACT

**Background:** The effects of phosphodiesterase-type 5 (PDE5) inhibitors on the in vivo clitoral structure of women with diabetes have never been investigated.

**Aim:** To study the in vivo structural and hemodynamic changes of the clitoris in premenopausal women with type 1 diabetes on PDE5 inhibitors.

**Methods:** 38 premenopausal women with type 1 diabetes aged 36–46 years. A randomized 1:1 study design was used: Study Group (group A) on Tadalafil 5 mg daily, and control group (group B). Blood samples were taken from each woman to measure HbA1c, testosterone, and Free Androgen Index. The women underwent microbiopsy of the clitoral body by means of semiautomatic gun during total anesthesia for surgery therapy of a benign gynecological pathology. The tissue removed was processed for electron microscopy. Translabial color Doppler ultrasound was used to measure the peak systolic velocity (PSV), the end diastolic velocity (EDV), and the pulsatility index (PI) of clitoral arteries.

**Main Outcome Measures:** Micro-ultrastructure observation of clitoral tissue and color Doppler sonography of clitoral blood flow.

**Results:** Of the 38 women, 13 (68.4%) of group A and 15 (78.9%) of group B completed the study. Group A showed a mean PSV and EDV increase, and a mean PI decrease with respect to baseline ( $P < .001$ ). Group B did not show any change in both the parameters ( $P = \text{NS}$ ). By a quantitative study in both groups a variable degree of ultrastructural abnormalities of smooth muscle cells (SMCs) was observed, consisting in increased glycogen and lipoic deposits, cytoplasmic vacuoles, and focal increase of electron density of SMCs. Moreover, the mean SMC thickness of group A ( $1.83 \pm 0.68 \mu\text{m}$ ) was larger than that of group B ( $1.3 \pm 0.41 \mu\text{m}$ ) ( $P = .02$ ).

**Clinical Implications:** PDE5 inhibitors could be used to treat diabetic women with genital arousal disorder.

**Strengths & Limitations:** The study shows a clear effect of PDE5 inhibitors on clitoral SMCs. However, a limit was to not have investigated the sexual function/behavior of women of both groups, this was because of the short time of the study.

**Conclusion:** This study could help to understand in what way PDE5 inhibitors act on the ultrastructural pathophysiological clitoral cavernous tissue of women with diabetes. It could support PDE5 inhibitor usage in women with genital sexual arousal disorder due to metabolic diseases. **Caruso S, Cianci A, Cianci S, et al. Ultrastructural Study of Clitoral Cavernous Tissue and Clitoral Blood Flow From Type 1 Diabetic Premenopausal Women on Phosphodiesterase-5 Inhibitor. J Sex Med 2019;XX:XXX–XXX.**

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## INTRODUCTION

Diabetic women are receiving more attention than in the past with regard to the increased risk of female sexual dysfunction.<sup>1</sup> Diabetes can contribute to female sexual dysfunction by causing psychological, cardiovascular, and neurophysiologic events.<sup>2</sup> Although coexisting depression can negatively affect sexuality,<sup>3</sup> neurovascular organic factors, due to diabetes, can impair vulvar clitoral vaginal blood flow.<sup>4</sup>

Generally, diabetic women are affected by sexual arousal disorder (SAD).<sup>5</sup> Physiologically, female genital sexual arousal consists of clitoral and vaginal engorgement due to genital vasocongestion and vaginal swelling; the clitoral tumescence is due to the relaxation of smooth muscle.<sup>6</sup> Diabetes is usually associated with atherosclerosis in large arteries and with microangiopathy. Consequently, chronic ischemia of the clitoral cavernous vessels could cause fibrosis of the clitoral cavernous tissue and loss of smooth muscle in human women<sup>6</sup> and animals.<sup>7,8</sup>

Recently, age-related ultrastructural changes of the smooth muscle cells (SMCs), vascular spaces, and vascular lacunae of the clitoral cavernosal tissue in women affected by metabolic pathologies have been investigated. Diabetic premenopausal women had more ultrastructural abnormalities of SMCs than healthy women.<sup>9</sup>

Previous studies suggested that phosphodiesterase type 5 (PDE5) inhibitors, inhibiting cyclic guanosine monophosphate-specific PDE5, may improve clitoral blood flow in healthy women<sup>10</sup> and in those women affected by SAD<sup>11</sup> or with type 1 diabetes.<sup>12–14</sup>

The objective of this investigation was to study the effects of PDE5 inhibitors on the *in vivo* histomorphologic structure of the clitoral cavernosal tissue of premenopausal type 1 diabetic women. Based on our previous study,<sup>14</sup> we chose to use Tadalafil, 5 mg daily, because of its prolonged half-life and its pharmacokinetic properties.<sup>15</sup>

## MATERIALS AND METHODS

### Setting and Sample

A prospective, randomized study was performed at the Centre of the Research Group for Sexology of the Gynecological Clinic, Department of General Surgery and Medical Surgical Specialties, School of Medicine, University of Catania, Italy. 38 consecutive volunteer type 1 diabetic premenopausal women, aged 36–46 years, with a mean age ( $\pm$ SD) of 39 ( $\pm$ 3.6) and a body mass index of  $24.6 \pm 2.7$ , were invited to participate in the study. They were placed on the waiting list of the gynecologic clinic to undergo surgical therapy for benign gynecologic diseases. Of these women, 11 were affected by urinary incontinence, 3 by endometrial hypertrophy, and 9 and 15 by endometrial polyps and uterine myoma, respectively.

All subjects gave their written informed consent before entering the study, which was conducted in accordance with the 1975 Helsinki Declaration. However, the subjects could terminate participation at any time. The study was not advertised, and no remuneration was offered. The following women were excluded from the study: those with a history of hypertension, coronary artery disease, or thromboembolic disorder; those with impaired hepatic or renal function or neoplasia; those who were taking hormone therapy or oral contraceptives; or those with a history of smoking or alcohol abuse.

### Procedures

During enrollment, all inclusion and exclusion criteria were adopted. Women underwent a physical examination, including assessment of vital signs, and electrocardiography, mainly for the surgical treatment.

All women admitted to the screening phase had regular menstrual cycles (mean cycle length  $26.9 \pm 4.5$  days) with ovulation. To confirm the ovulatory cycle, ultrasound scanning was performed on days 10, 12, and 15 of the cycle, and serum progesterone (P) concentrations were measured on days 21 and 23 of the cycle. Menstrual cycle was defined as ovulatory when serum P was  $>18$  IU/mL. The P level was measured using commercially available enzyme-linked immunosorbent assay kits (Elecys System 2010; Roche, Monza, Italy).

Data on use of medications and adverse events were recorded. Hemoglobin glycosylate (HbA1c) and hormonal levels were measured on the day of enrollment by collecting a venous blood sample. To determine HbA1c, the Cobas Integra assay (Roche, Basel, Switzerland) was used (normal range 4.0–6.0%). Subjects with HbA1c  $\geq 10\%$  were excluded from the study because they were considered to be affected by diabetes that was not well controlled. In addition, testosterone (TT, ng/dL, normal range 0.3–1.2 ng/mL), and sex hormone binding globulin (nmol/L) were measured by enzyme-linked immunosorbent assay (Elecys Systems 2010, Roche, Monza, Italy). The free androgen index (FAI) was calculated by using  $FAI = [TT/SHBG \text{ (nmol/L)}] \times 100$ .

At the start of the study (T0), according to a computer-generated list of random number groups, each woman was randomly allocated into 1 of the 2 possible groups: study group (group A) on Tadalafil 5 mg/d, and control group (group B) without PDE5 inhibitor intake. Both groups underwent micro-biopsy of the clitoral body 4–5 weeks after the start of the study (T1). The micro ultrastructure observation was performed by V.C. Figure 1 shows the flowchart of the study.

### Instruments

At enrollment, color-flow Doppler sonography was performed to measure the clitoral blood flow by using a Voluson E6 (GE Healthcare, Solingen, Germany) with a 7.5MHz linear transducer. Each woman was scanned in the gynecologic position. The Doppler translabial probe was placed sagittally on the clitoris at an angle of  $\leq 20^\circ$ , without any significant pressure exerted on the tissues. After identifying the clitoral artery using color-flow mapping, the Doppler probe was positioned over the vessel, and at least 3 sequential Doppler waveforms were obtained. Consequently, the peak systolic velocity (PSV) and the end diastolic velocity (EDV) were measured, and the pulsatility index (PI) was automatically calculated by the software of the Voluson E6 ( $PI = PSV - EDV/\text{mean maximum flow velocity of clitoral artery}$ ).<sup>13</sup> Color-flow Doppler sonography was performed on each woman by 1 independent investigator (V.F.), who was blinded to the drug or control status of subject. The 2

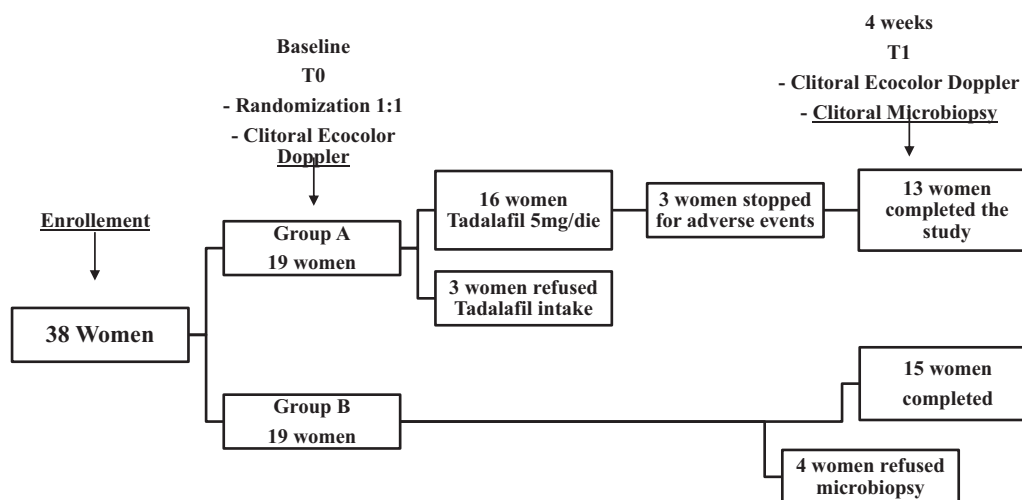


Figure 1. Flowchart.

parameters, nominally PSV and EDV, were chosen because they indicate the quality of the arterial and venous function, respectively. In fact, low PSV means arterial insufficiency, whereas high EDV means venous obstruction.<sup>16</sup> Moreover, PI reflects resistance to blood flow, which is correlated with microvascular lesions.<sup>17</sup> Sonography was repeated 1 day before surgery (T1).

A semiautomatic gun having a fine needle of 18G diameter (Precisa; HSHospital Service, Aprilia, Latina, Italy) was used to perform microbiopsy of the clitoral body of each woman during total anesthesia before the start of her main surgery; the microbiopsy had been planned to take place during the periovulatory phase of the cycle. This timing was chosen to make use of endocrine effects of ovarian steroid peaks, nominally estrogens and androgens, on the clitoral body volume. Because of the invasive methodology, it was possible to obtain only 1 tissue fragment from each woman. However, 4 women underwent biopsy twice during the same procedure because of the insufficient tissue obtained by the first biopsy. No post-biopsy complications were observed.

Specimens consisting of 1 fragment measuring approximately  $3 \times 0.5$  mm were immediately immersed in Karnowsky's fluid, containing 2% glutaraldehyde and 3% paraformaldehyde, and fixed at 4°C for 2 hours. Tissue fragments were then post-fixed with 1% buffered osmium tetroxide, dehydrated in graded ethanol, cleared with propylene oxide, and embedded in epoxy resin. 1  $\mu\text{m}$ -thick sections were obtained from the plastic-embedded blocks, taking care to sample the largest tissue surface possible, stained with toluidine blue, and observed by light microscopy to assess the adequacy of the sampling. Thin (400–500 Å) sections were collected on copper grids, stained with uranyl acetate and lead citrate, and observed with an electron microscope operating at 80 KVs. Standard specimens (Balzers Union cross grating; Oerlikon Balzers, Balzers, Liechtenstein) were used to assess the actual magnification on photographs and digital images. 10–20 photographs were recorded in each case at a constant magnification of 6,000 $\times$ , using a standard systematic sampling method. The negative

photographic plates were digitized using a high-resolution camera mounted on a diaphanoscope especially designed for this purpose. The images were processed using Jack Paint Shop with a standardized sequence of operations: gray scale, negative image, and brightness/contrast adjustment.

### Quantitative Analysis

To describe the SMC population in the small-needle biopsy fragments, we used SMC mean thickness. The choice of this quantitative descriptor is necessary because of the random orientation of the SMC profiles in reference spaces; area fractions of the SMCs were calculated for each group and compared. The measurements were performed on individual cell profiles and were available for statistical analysis. An interactive routine was developed by V.C., providing the mean thickness derivation by outlining 2 opposite sides on the SMC profile. This method allows for accurate measurements in randomly oriented SMCs, using the only bias of considering the long axis of the profile. In circular or polygonal profiles, outlining is conventionally performed on 2 random-selected opposite sides, and the calculated mean thickness corresponded to the profile diameter with a good approximation. In detail, a program for the quantitative evaluation of ultrastructure on digital electron microscopic images was developed in Visual. The program calculates the linear distances between the coordinates of each discrete point of the 2 lines and derives the mean linear distance between the 2 opposite sides of the cell profile (mean thickness) with the standard deviation (SD).<sup>18</sup> The ultrastructure evaluation was performed by 1 independent investigator (V.C.) blinded to the study and the control group.

### Statistical Analysis

Using the sample size calculation, 15 women in each group would be the minimum number required for the study to have 95% power. This number was obtained on the basis of the previous results of 2.5 PSV (cm/sec) differences in clitoral arterial blood flow from baseline to 12 week of daily Tadalafil 5 mg

intake,<sup>14</sup> with 1.8 SD, considering  $\alpha$  ( $P$  value) = .05. A 2-sided  $t$  test for independent samples by ANOVA was used to compare the demographic and clinical data between the 2 groups.

The SMC mean thickness between the 2 groups was compared by ANOVA; the Bonferroni method of correction for multiple comparisons was used if a significant  $F$  ratio was found. Statistical results are given in terms of  $F$ -values, and  $F$ -tail probability differences were statistically significant when  $P \leq .05$ .

Paired Student's  $t$ -test was used to compare the changes within the groups between baseline (T0) clitoral arterial blood flow values and the values after Tadalafil usage (T1). The scores are presented as the mean  $\pm$ SD. The result was statistically significant at  $P \leq .05$ . Statistical analysis was performed using a software package for Windows 95 (Grantz SA, Primer of Biostatistics, McGraw-Hill, New York, NY, USA).

## RESULTS

Table 1 shows the demographic and metabolic characteristics at baseline of both groups. The mean duration of diabetes of group A and group B was  $8.4 \pm 2.7$  and  $8.1 \pm 2.5$  years,

**Table 1.** Demographic characteristics

	Study group A (n = 19)	Control group B (n = 19)	$P^*$
Age range, y	34–46	34–46	1
Mean age, SD	$38.1 \pm 3.7$	$37.5 \pm 4.1$	.63
BMI, kg/m <sup>2</sup>	$23.8 \pm 3.8$	$24.5 \pm 3.5$	.55
Age at menarche, y	$12.1 \pm 2.3$	$12.4 \pm 2.1$	.67
Menstrual cycle length, d	25–32	26–33	.92
Duration of menses, d	$4.6 \pm 2.2$	$4.8 \pm 2.5$	.79
Onset of diabetes, y	$8.4 \pm 2.7$	$8.1 \pm 2.5$	.72
HbA1c, %	$8.0 \pm 1.8$	$7.5 \pm 1.9$	.41
Testosterone, ng/dL	$0.4 \pm 0.6$	$0.6 \pm 0.7$	.35
SHBG, nmol/L	$38 \pm 11$	$41 \pm 12$	.42
FAI, %	1.05	1.46	.21
Pregnancies, no (%)			
Nulliparous	4 (21.1)	5 (26.3)	.89
One or more children	15 (78.9)	14 (73.7)	.96
Cigarette smokers			
Nonsmokers	19 (100%)	19 (100%)	1
Current coffee drinker	19 (100%)	19 (100%)	1
Daily cups of coffee	$2.6 \pm 1.4$	$2.5 \pm 1.7$	.84
Systolic pressure, (mm Hg)	$135.5 \pm 12.5$	$137.8 \pm 10.8$	.54
Diastolic pressure, (mm Hg)	$81.4 \pm 6.8$	$82.9 \pm 8.9$	.56
Heart rate, (x/min)	$65.7 \pm 10.5$	$67.8 \pm 9.8$	.52

BMI = body mass index; FAI = free androgen index; SHBG = sex hormone binding globulin.

\*A 2-sided  $t$ -test for independent samples by ANOVA.

respectively; the mean HbA1c value of group A and group B was  $8.0 \pm 1.8$  and  $7.5 \pm 1.9$  years, respectively ( $P = \text{NS}$ ). TT and FAI were in the normal range, and similar values were observed in both groups ( $P = \text{NS}$ ).

Of the 19 women randomly allocated to group A, 3 (15.8%) were excluded after assessment at T0 because they refused Tadalafil usage; and 3 (15.8%) did not complete the study because of adverse events; in fact, 2 (12.4%) had headache and nausea at the 12th and 16th day of treatment; and 1 (6.2%) had headache at the 9th day of treatment. 2 women (10.5%) had headache and nausea that were transient and mild in nature during the first 14 days of drug intake; however, none of them discontinued the drug. The frequency of forgetting to take Tadalafil over the study period was 2, 3, and 4 times in 5 (31.2%), 3 (18.7%), and 2 (12.4%) women, respectively. Moreover, 4 (21.1%) women allocated to group B refused clitoral microbiopsy. Therefore, 13 (68.4%) women in group A and 15 (78.9%) in group B completed the study. The range during which the biopsy was performed was after 32–37 days from the beginning of Tadalafil usage.

Table 2 shows the results obtained by color-flow Doppler sonography at T0 and at the day before surgery (31–36 days from drug initiation). Group A showed a mean PSV and EDV increase with respect to T0 ( $P < .001$ ). On the other hand, group B did not show any change in both parameters ( $P = \text{NS}$ ). Moreover, the PI decreased in group A ( $P < .004$ ); however, no change was observed in group B ( $P = \text{NS}$ ).

Figure 2 shows the ultrastructure images of the clitoral cavernous tissue of women of group A (A and B) and of group B (C and D). Thinning and irregular contours of the SMCs were observed, mainly, in the specimens of women in group B; the SMCs were focally thinned and anisometric; moreover, an increased intercellular matrix contained abundant packed collagen, and infolded cell borders were observed. Basement membranes were thin or slightly thickened. A variable degree of ultrastructural abnormalities of SMCs was observed, consisting of increased glycogen and lipoic deposits, cytoplasmic vacuoles, and focal increase of electron density of SMCs. However, in women in group A, SMCs were regularly distributed and better preserve than in those of in group B. Importantly, no vascular spaces but only infrequent vessels were observed in both groups, and they were larger in the women in group A than in those in group B. Moreover, 168 and 193 SMC profiles were measure in group A and group B, respectively. The mean thickness (in  $\mu\text{m}$ ) of the SMCs of the women in group A was larger than that of women in group B,  $1.83 \pm 0.68 \mu\text{m}$  and  $1.32 \pm 0.41 \mu\text{m}$  ( $t = 2.441$ ; 95% confidence interval, 0.08059 to 0.9394; 26 degrees of freedom;  $P < .02$ ), respectively (Figure 3).

## DISCUSSION

To our knowledge, this is the first study evaluating in vivo ultrastructural clitoral cavernous tissue in premenopausal type 1 diabetic women on PDE5-inhibitor, namely Tadalafil, 5 mg daily.



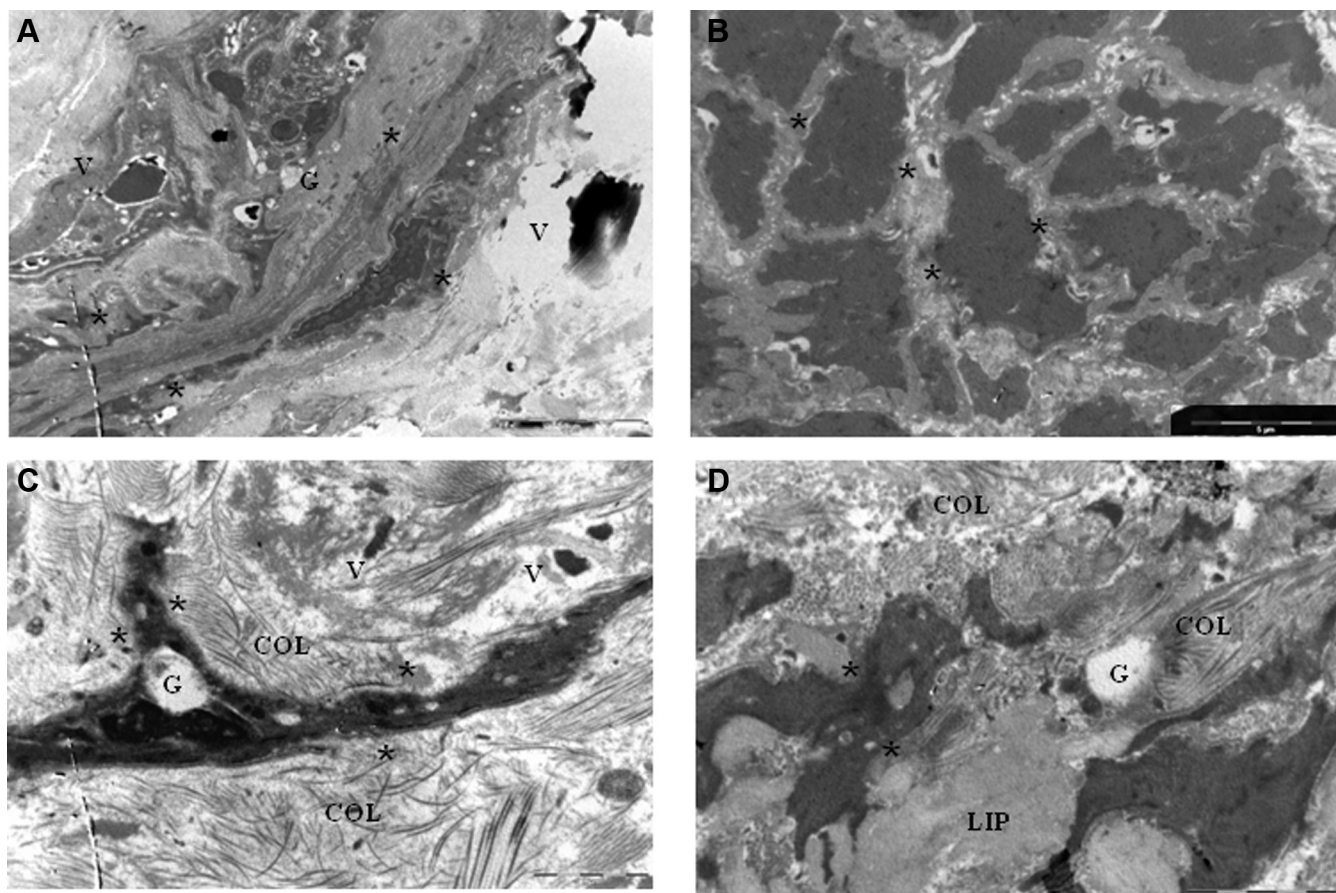
**Table 2.** Color-flow Doppler ultrasonography of clitoral arterial blood flow measurements of type 1 diabetic premenopausal women, at baseline (T0) and after 4 weeks (T1) of Tadalafil 5 mg/d intake (group A) or not on Tadalafil intake (group B)

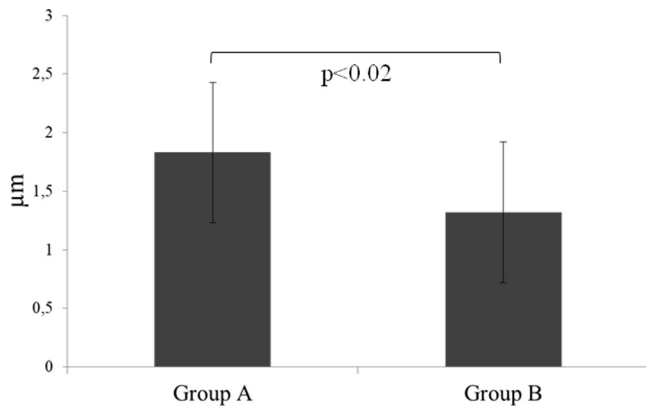
Parameter	Group A			Group B			Group A vs B	
	T0 (n.19)	T1 (n.13)	P	T0 (n.19)	T1 (n.15)	P	T0	T1
EDV (cm/sec)	0.9 ± 1.6	2.5 ± 1.2	<.001	0.8 ± 1.7	0.5 ± 1.5	.59	.85	<.001
PSV (cm/sec)	7.1 ± 1.8	10.3 ± 1.5	<.001	7.4 ± 1.9	8.1 ± 1.1	.25	.62	<.001
PI	1.5 ± 0.3	1.2 ± 0.2	<.004	1.6 ± 0.5	1.7 ± 0.4	.53	.46	<.001

EDV = end-diastolic velocity; PI = pulsatility index; PSV = peak systolic velocity.  
Data are presented as mean values ± SD.

First, this investigation showed that diabetic women had ultrastructural abnormalities of SMCs, consisting of the increase of glycogen deposits, infolding cell borders, and cytoplasmic vacuoles, in agreement with our previous data<sup>9</sup>. However, women on PDE5 inhibitor had a larger mean SMC thickness than women without drug intake. These changes could depend on increased endothelial guanosine causing relaxation of clitoral smooth muscle. The ultrastructural evidence correlated with color-flow Doppler sonography, by which a better clitoral blood flow was recorded in women on PDE5 inhibitor rather than in women not on the drug; a significantly increased systole and diastole, as well as PI reduction

corresponding to the beginning of a filling phase of the clitoral artery, were recorded. The blood flow changes could be the direct vasodilator effect of the PDE5 inhibitor on female genital tissues. Because the aim of this study was not to measure the clitoral blood flow during sexual arousal, nor to evaluate changes in the sexual function of the participants, but only to investigate the structural and hemodynamic changes of the clitoris, the clitoral tumescent phase was not recorded in each woman. Usually, in the resting phase, only monophasic systole with a low-velocity, without diastole, waveforms are recognized in the clitoral artery. The clitoral artery gives off many helicine arteries, which supply the

**Figure 2.** Ultrastructure aspects of clitoral cavernous tissue of type 1 diabetic premenopausal women on Tadalafil (A and B) and not on Tadalafil (C and D). Thinning and irregular contours of SMCs (\*) with glycogen deposits (G) in both specimens. Vessels (V) are larger in A than those in C. SMC thickness from 168 SMC profiles (A),  $1.83 \pm 0.68 \mu\text{m}$ . The SMCs of women on PDE5 inhibitor (B) had a greater thickness ( $1.83 \pm 0.68 \mu\text{m}$ ) than women of the control group (C) ( $1.32 \pm 0.41 \mu\text{m}$ ). Bar =  $2 \mu\text{m}$ .



**Figure 3.** SMC mean thickness derivation ( $\mu\text{m}$ ) by outlining 2 opposite sides on the SMC profile of clitoral cavernous tissue in type 1 diabetic premenopausal women on Tadalafil 5 mg/die (group A) and not on Tadalafil (group B). Data are presented as mean  $\pm$  SD. SMC = smooth muscle cell.

trabecular tumescent tissue and the sinusoids. The helicine arteries are contracted and tortuous in the resting state and become dilated and straight during tumescence. Moreover, the absence of the venous plexus in the clitoris suggests that this organ achieves tumescence but not rigidity during sexual arousal.<sup>19</sup> In this study, only the filling phase was recorded, because women were not engaged in sexual activities. Interestingly, during sexual arousal, intracavernosal pressure begins to rise, a diastolic notch appears at the end of systole, and, progressively a decreased diastole is observed. As the intracavernosal pressure equals the systemic diastolic pressure, EDV disappears. Then, diastole is reversed because of the increased intracavernosal pressure, reflecting the full engorgement phase.

The complexity of the SMC contour was a consistent finding in our observations, and it was different in the ultrastructural histologic samples of the 2 groups. An increasing degree of “infolding” was observed in the diabetic women, but an infolding contour was also related, in dynamic SMCs, to contraction, which is in the resting phase of sinusoidal cavernous bodies; on the other hand, relaxed cells exhibited regular cellular borders, and the cell contour is smooth.<sup>9</sup> In this study, a larger number of the SMCs in women on PDE5 inhibitor showed a greater thickness than in women of the control group. This evidence could lead to the hypothesis that the PDE5 inhibitor promotes muscle relaxation.

Furthermore, in women on PDE5, we also observed no responder cells to the drug, particularly in SMCs having numerous abnormalities such as an increase in cytoplasmic vacuoles, or with a numerical decrease, even severe, of the SMCs, associated or not with an increase in the connective membrane and a thinning of the basement membrane. Interestingly, it was not possible to highlight vascular lacunae, probably due to the biopsy sampling of clitoral cavernous tissue; in fact, its timing presumably coincided with the filling phase of the corpora

cavernosa rather than with the tumescence phase. Moreover, myelinated and unmyelinated nerve fibers were inconsistently found in the small biopsy specimens.

In this study, Tadalafil was chosen because of its prolonged half-life of 17.5 hours.<sup>15</sup> This pharmacokinetic property is able to produce a constant stretching of the cavernous tissue, different from that of Sildenafil<sup>20</sup> and Vardenafil<sup>21</sup> that have a mean elimination half-life of 4–5 hours. Tadalafil 5 mg daily has been shown to be effective in modifying clitoral blood flow in premenopausal women with type 1 diabetes.<sup>14</sup>

The PDE5 inhibitors were shown to be able to inhibit cyclic guanosine monophosphate hydrolysis by high-affinity selective PDE5 inhibition in intact cells and in soluble extracts of human clitoral corpus cavernosum SMCs. The finding of immunohistochemical NOs and the PDE5 expression in clitoral and vaginal tissue<sup>22–24</sup> could sustain the activity of Tadalafil on female peripheral genital targets.<sup>25,26</sup> Because androgens modulate the physiological mechanisms of both genital tissue and sexual arousal in women by improving NO synthase activity,<sup>27</sup> only women with normal total T and FAI levels were included in our study. We could calculate the FAI, even if the T measurement by mass spectroscopy is the “gold standard.”<sup>28</sup> Unfortunately, this was not feasible in our laboratory.

To date, there are discrepancies in the efficacy of PDE5-inhibitors adopted to treat women with SAD. This could depend on the difficulty of correctly diagnosing female SAD where the biologic pathophysiology is usually due to neurovascular events.<sup>29</sup> Previously, several authors reported that PDE5 inhibitors were ineffective for women with SAD. Moreover, most of them had used single or very short administration of PDE5 inhibitors to study genital modifications, by using dynamic MRI<sup>30</sup> or vaginal pulse amplitude responses by photoplethysmography.<sup>31</sup> Moreover, the low, moderate, or high efficacy of the drug could depend on the various PDE isoforms expressed in sexual genital tissue. Consequently, when PDE5 expression is low, the PDE5 inhibitors used to treat SAD could be ineffective.<sup>32</sup> This will be an objective of our future study. On the other hand, authors adopting study designs involving the long-term use of the drug reported significant improvements of genital arousal and lubrication of women affected by SAD.<sup>33,34</sup>

Probably, a therapeutic pharmacologic treatment, and not an on-demand regimen, should be adopted in women affected by sexual dysfunction because of chronic metabolic or organic disorders. Moreover, an integrated treatment, based on both psychosexual and drug treatment, could be more effective than a single treatment in women with sexual dysfunction, depending on the biologic disorder.<sup>34,35</sup>

Our study had some limits. One consisted in not being able to perform clitoral microbiopsy before and after the women took the PDE5 inhibitors. In fact, given the highly invasive method, ethically we chose the comparison with a control group, choosing a randomized study.

Moreover, because of the difficulties of obtaining an inert drug similar in appearance to Tadalafil, we could not adopt a controlled placebo design; although this could be considered a limitation, it depended on the short time-window of 4–5 weeks between the start and the end of the study. We considered it sufficient for this study but inadequate for a drug/placebo study. A placebo-controlled study could be a future investigation, adopting a period of time greater than that of this study.

Previously, we investigated the effect of a PDE5 inhibitor, namely Sildenafil, on SAD but not on biologic targets of diabetic women, using a placebo-controlled study.<sup>13</sup> We observed that women on Sildenafil experienced an improvement in sexuality, as well as of the hemodynamic events of clitoral blood flow, compared with women on placebo. These previous results cannot justify the lack of a placebo group in this study, because the pharmacologic properties of Tadalafil appear to be different from those of Sildenafil, and a placebo effect cannot be excluded.

Finally, another limit was to not have investigated the sexual function/behavior of women of both groups; this was due to the short time of the study (4–5 weeks) and to the time of biopsy; in fact, it was performed on each woman during total anesthesia for surgical therapy of a benign gynecologic pathology. Previously, we observed a significant improvement of sexual life of diabetic women during PDE5 inhibitor intake.<sup>12–14</sup>

## CONCLUSION

Our in vivo study could help to understand in what way PDE5 inhibitors act on the ultrastructural pathophysiological clitoral cavernous tissue of diabetic women. It could support PDE5 inhibitor usage in women with genital sexual arousal disorder due to metabolic diseases. Apart from the evidence obtained from studying premenopausal diabetic women, future investigations will be aimed at studying subgroups of otherwise naturally and surgically postmenopausal women using systemic or local hormone therapy, or affected by metabolic disease, during PDE5 inhibitor intake.

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### Category 3

- (a) **Final Approval of the Completed Article**  
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