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The penis in diabetes: structural analysis of connective tissue and smooth muscle alterations in a rabbit model

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OBJECTIVE

- To assess the volumetric density of collagen, elastic system fibres and smooth muscle cells in the corpus cavernosum (CC), corpus spongiosum (CS) and tunica albuginea (TA) in the penis of diabetic rabbits.

MATERIALS AND METHODS

- Twenty-six New Zealand white rabbits were used. Diabetes was induced at 8 weeks of age in 13 rabbits by i.v. injection of 100 mg/kg of alloxan. The remaining 13 rabbits served as a control group. After 10 weeks, the rabbits were killed using sodium thiopenthal.
- Midshaft penile fragments were obtained and processed by routine histological techniques. Stereological analysis of collagen, elastic system fibres and smooth muscle was performed in 5-μm sections by using a M42 test grid system.
- Data were expressed as volumetric density (Vv; %). Collagen organization was evaluated by Picrosirius red staining under polarization.

RESULTS

- In the TA of diabetic rabbits, thickness increased by 88% ($P < 0.001$) with an enhanced collagen turnover. Moreover, the elastic fibre content was 34% higher ($P < 0.001$). In the CC of diabetics, collagen was diminished by 45% ($P < 0.001$) with a more organized collagen.
- The elastic fibres were decreased by 46% ($P < 0.001$). Diabetes induced a 11% increase in CS collagen ($P < 0.024$) with an enhanced collagen turnover.
- Smooth muscle in the CC of diabetic rabbits was increased by 40% ($P < 0.001$), whereas, in the CS, it was decreased by a similar amount ($P < 0.001$).

CONCLUSIONS

- Penile tissues were affected differently by diabetes, possibly as a result of cellular heterogeneity.
- These changes could have an impact on blood flow and tissue resistance, and therefore might adversely affect erection.

KEYWORDS

penis, rabbit, stereology, histology, diabetes, erectile dysfunction

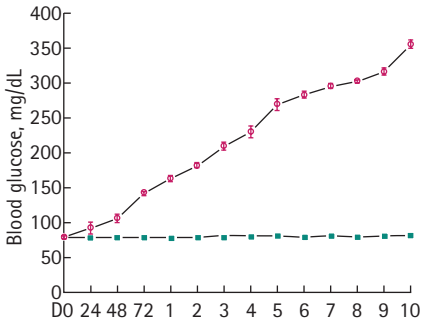
INTRODUCTION

Diabetes is a condition that is associated with a reduced life expectancy and a diminished health-related quality of life [1]. Recent estimates indicate that, in 2000, there were 171 million people worldwide with diabetes and this is projected to increase to 366 million by 2030 [1].

Although diabetes is involved in a number of complications such as microvascular damage , until recently, the frequency of urological complications of diabetes were less commonly appreciated [2]. Indeed, erectile dysfunction (ED) is a common complication of diabetes, as shown in different studies [3–5]. Additionally, experimental evidence from animal models and diabetic patients indicates that neural and vascular alterations in the penis are major causes of ED [4,5]. These changes could affect smooth muscle cells and different components of the extracellular matrix (ECM), including collagen and elastic fibres. These are important penile components that maintain penile structure during erection, allowing adequate resistance during the return to the flaccid state [6–8]. However, the way in which these various elements are affected is not yet well established, and precise quantitative data regarding smooth muscle and fibrous elements of the penis in diabetes have not been reported in the literature.

Changes in the morphology and physiology of the penis have been well documented in several animal models [9–11], although morphological data on the ECM of rabbit penis are still scarce [12]. The rabbit has a vascular penis that contains two erectile structures: the dorsolateral corpus cavernosum (CC) and the ventral corpus spongiosum (CS) that surrounds the penile urethra. Its vascular penis as well as the lack of a penile bone are features that make it more similar to the human penis, and therefore a suitable animal model for studying penile structure and erectile dysfunction [10]. These erectile structures in the rabbit penis are also covered by a dense connective tissue, the tunica albuginea (TA), which projects intracavernosal pillars or septa, mainly in the CC [12]. As a result of its anatomical characteristics, the rabbit penis is

FIG. 1. Fasting blood glucose levels in controls (squares) and alloxan-treated (circles) rabbits during the period of the present study. Glucose was assayed on capillary blood samples shortly before (D0) and hours (24, 48, 72 h) or weeks (1–10 weeks) after alloxan treatment. Each point represents the mean of 13 animals in each group.



one of the best models for studies on the effects of diabetes mellitus (DM) on erection.

The present study aimed to evaluate the diabetes-induced structural changes in the ECM and smooth muscle of the TA and in the erectile tissues (CC and CS) of the rabbit penis.

MATERIALS AND METHODS

Twenty-six white New Zealand rabbits were obtained from the Institute of Zootechny of Rural Federal University of Rio de Janeiro. The research protocol was approved by the Institutional Ethics and Research Committee.

The animals were divided into two groups of 13 rabbits each: a control group (C) and a diabetic group (DM). The diabetic condition was induced, at 8 weeks of age, by a single i.v. injection of 100 mg/kg of alloxan monohydrate (Sigma Chemical Co., St Louis, MO, USA) [11] Serum glucose was assayed on capillary blood samples (One Touch Ultra, Johnson & Johnson Co., Rio de Janeiro, Brazil) from fasting animals and monitored at 24, 48 and 72 h after injection, and once a week from then onward. Rabbits were considered diabetic when the glucose serum level was higher than 126 mg/dL.

Both groups were fed with the same standard pellets for rabbits and, 10 weeks after alloxan injection, the rabbits were killed by an overdose of sodium thiopental.

The penises were dissected and a fragment of the middle shaft was removed and fixed in a 10% buffered formalin solution. Fragments of CC, CS and TA were obtained and an 'ortrip' cleavage was performed for stereology. This method consisted of three random slice sections, with the second section being orthogonal to the first, and the third section also being orthogonal to the second. Thus, isotropically uniform random sections were obtained [13].

The samples were routinely processed for embedding in paraffin and 5-μm thick sections were obtained. To highlight the elastic system fibres, the sections were stained with Weigert's resorsin fuchsin technique with previous oxidation [9,12]. Masson's trichrome was used to detect the collagen fibres, whereas the smooth muscle was evidenced by immunohistochemical analysis using a rabbit anti-α-actin antibody [9].

From each penis, five different sections were selected from five fragments. From each section, five random fields were analyzed, resulting in 25 fields (test areas) in total for each penis. The data were expressed as volumetric density (Vv; %). To enable the qualitative analysis of collagen, the sections were stained with Picrosirius red and observed under polarized light.

The sections were observed under ×400 magnification using an Olympus light microscope (Olympus, Tokyo, Japan) coupled with a Sony CCD video camera (Sony Corp., Tokyo, Japan), and the images transferred to a Sony monitor KX14-CP1. The selected histological areas were then quantified using a M42 test grid system on the digitized fields. All numerical results are presented as the mean ± SD.

The data were analyzed with the GraphPad software (GraphPad Software Inc., San Diego, CA, USA). To compare the quantitative data of CC, CS and TA in both groups, Student's *t*-test was used ($P < 0.05$ was considered statistically significant).

	Albuginea	
	Control group	Diabetic
Vv of elastic system fibres (%)	11.10 ± 1.62	14.88 ± 1.17
		$P < 0.0001$
Thickness (mm)	0.26 ± 0.06	0.49 ± 0.15
		$P < 0.0003$

TABLE 1 Volumetric density (Vv) of elastic system fibres and thickness in the tunica albuginea of controls and diabetic rabbits

RESULTS

GLUCOSE CONCENTRATION

Blood glucose concentration 72 h after alloxan treatment had already approached a mean value of 150 mg/dL (Fig. 1). Subsequently, this value increased steadily, with little variability among animals, whereas, in controls, the blood glucose had a constant mean value of ≈79 mg/dL throughout the duration of the experiment.

When all animals were sacrificed, 10 weeks after treatment, blood glucose was ≈350 mg/dL. Therefore, alloxan-treated rabbits remained in a diabetic condition for at least 10 weeks.

MORPHOLOGICAL ANALYSIS

Tunica albuginea

TA is a dense fibrous tissue, and its overall thickness was significantly increased by 88% ($P < 0.001$) in diabetic rabbits compared to controls (Table 1). Furthermore, the organization of the fibrous collagen components in the TA was greatly altered in diabetic animals as revealed by the Picrosirius red staining under polarization (Fig. 2). Thus, in tissue sections from diabetic rabbits, there was a marked shift towards green birefringence (Fig. 2B) as opposed to the mostly orange – reddish colour in controls (Fig. 2A). In this same tissue, there was a 34% increase ($P < 0.001$) in the relative content of elastic system fibres (Table 1).

Erectile tissue

CC and CS were differently affected by diabetes with regard to the contents and structural organization of the major connective tissue components. Thus, in the CC of diabetic rabbits, the collagen content was diminished by 45% ($P < 0.001$) compared to controls (Table 2). This change was accompanied by a slight predominance of

*FIG. 2. Collagen arrangement in the tunica albuginea of controls (**A**) and diabetic rabbits (**B**). Note that, in the section from diabetic rabbits (**B**), there was a marked shift towards green birefringence as opposed to the mostly orange – reddish colour in controls (**A**). Picrosirius red under polarization, ×400.*

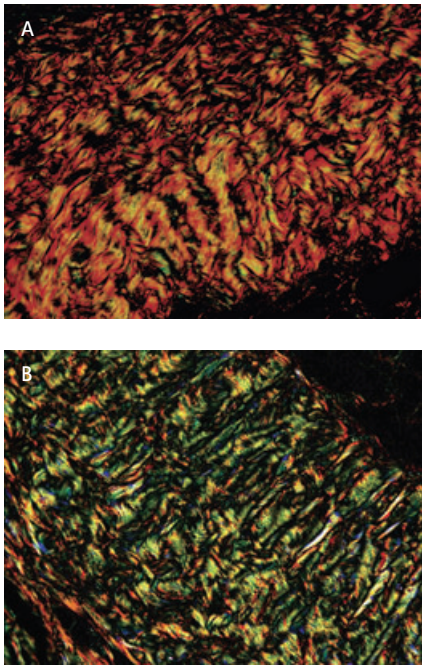


TABLE 2 Volumetric density (%) of smooth muscle cells, collagen and elastic system fibers in corpus cavernosum and corpus spongiosum in controls and diabetic rabbits

	Corpus cavernosum		Corpus spongiosum	
	Control group	Diabetic	Control group	Diabetic
Smooth muscle cells (%)	49.28 ± 2.14	68.77 ± 1.89 $P < 0.0001$	50.62 ± 1.91	31.24 ± 1.77 $P < 0.0001$
Collagen (%)	25.79 ± 1.69	14.31 ± 1.54 $P < 0.0001$	32.06 ± 1.81	35.59 ± 1.70 $P < 0.0235$
Elastic system fibres (%)	15.63 ± 2.33	8.46 ± 2.22 $P < 0.0001$	22.69 ± 1.29	24.40 ± 1.62 $P < 0.0025$

*FIG. 3. Collagen arrangement in the corpus cavernosum of controls (**A**) and diabetic rabbits (**B**). Note that, in the section from diabetic rabbits, there is a slight predominance of greenish birefringence (**B**), whereas, in controls, there are mostly orange – reddish signals (**A**). Picrosirius red under polarization, ×400.*

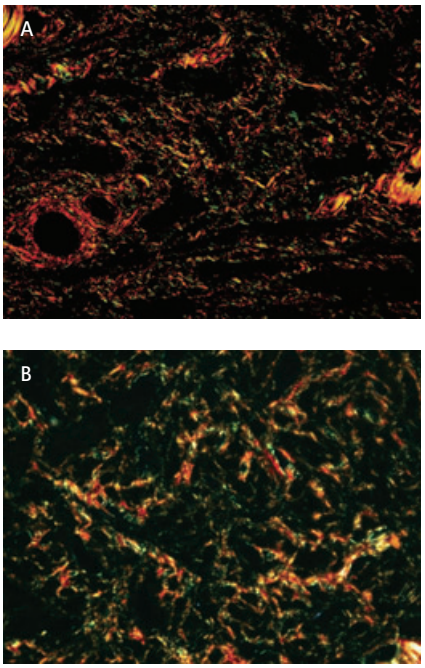
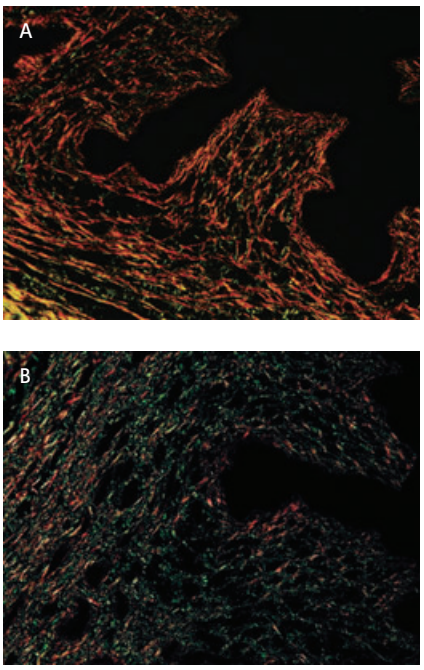


*FIG. 4. Collagen arrangement in the corpus spongiosum of control (**A**) and diabetic rabbits (**B**). Note that, in diabetic rabbits (**B**), there is a marked enhancement of the green birefringence, whereas, in controls, there are mostly orange – reddish signals (**A**). Picrosirius red under polarization, ×400.*



greenish birefringence as seen in the Picrosirius red stained sections under polarization (Fig. 3B), whereas, in controls, there were mostly orange – reddish signals (Fig. 3A).

By contrast, in the CS from diabetic rabbits, collagen was increased by 11% ($P < 0.024$) and there was a marked enhancement of the green birefringence (Fig. 4).

The diabetic condition changed the smooth muscle content in CC and CS by equivalent amounts, although in opposite directions. Accordingly, there was a 40% increase in the CC ($P < 0.001$), whereas, in the CS, it was decreased by 38% ($P < 0.001$) (Table 2). Similarly, whereas, in the CC, the elastic fibre content was decreased by 46% in diabetic animals ($P < 0.001$), in the CS, this condition led to an 8% increase ($P < 0.003$) for this same component (Table 2).

DISCUSSION

Approximately 50% of diabetic men experience problems of ED [2–4]. Changes observed in the penis of diabetic animals in both the CC and CS imply an association with ED.

Diabetes (i.e. chemically induced in animals) has been widely used to assess the changes caused by this disease in different organs. The present study aimed to investigate the effects of diabetes on the penis of rabbit, which is of the vascular type, and similar to that of humans [12]. In the case of ED, diabetes is known to be primarily associated with endothelial changes [4]. Moreover, several factors have been implicated with the onset of ED, including changes in smooth muscle cells, collagen and elastic fibres, which are major penile structural components responsible for erection. However, these factors remain poorly understood. In the present study, we analyzed the changes that occur in these structural components of the diabetic rabbit penis, using qualitative and quantitative methods.

One of the characteristics of the ECM and of its components is its adaptability in response to a changing environment and different stimuli [11,14]. The characterization and quantification of fibrous elements of the ECM have been shown to comprise an effective method for the evaluation of morphological and functional changes associated with pathological conditions in humans and several animal models [6,12,15]. In the specific case of the penis, a change in any one of its components can affect the response and normal functioning of the erectile tissue [7]. The collagen and elastic fibres are the two main structures of the erectile tissue of the penis that allow an increase in circumference and length during tumescence, at the same time as providing adequate recovery to return quickly to the flaccid state during detumescence [8,11].

The results obtained in the present study show that, in the penis of diabetic rabbits, changes in collagen in response to disease depended on the penile region (i.e. CC and CS). Regarding the CC, a moderate decrease in the amount of collagen diabetics was observed, which could be associated with the appearance of ED. In the CS, however, there was a slight increase in collagen content. The appearance of different changes in different regions of the penis, mediated by the same cause, also occurs in other situations, as in the case of priapism, where the glans penis, which is essentially CS, is differently affected with regards to the CC [16]. The changes caused by diabetes affected the content of collagen as well as its structural organization. Sections stained by Sirius red and observed under polarized light showed that, in diabetic animals, there was a change in collagen turnover and/or organization, including a possible shift in the ratio of collagen types. A change in the relative content of collagen types also occurs in other organs in diabetes. A previous study in diabetic mice [17], and also using Sirius red stain, showed that there was a predominance of green colour in the mesenteric artery, which was suggested to be the result of a higher content of collagen type III. However, investigations using post-partum uterus [18] and advanced atheromatous plaque [19] have shown that greenish birefringence is more of a consequence of higher collagen turnover, when fibrils are disrupted or degraded, rather than a change in collagen types.

The location and arrangement of elastic fibres are related to functionality and reflect the local mechanical properties of tissue [7–9,12]. Tissues that are constantly under stretching forces are rich in elastic system fibres [9,12] and loss of elastic-fibre architecture and function is a pathological feature of a number of degenerative and inflammatory diseases [20]. Despite the importance of elastic system fibres in the penis, there are few studies that have accurately characterized this component of ECM and its possible alteration in diabetes. In young adult rabbits, the volumetric density of elastic system fibres in the CC is ≈15%, whereas values for humans are 9% [6] and, for young adult rats, are 5% [9]. These data suggests that elastic fibres play a particularly important functional role in the rabbit penis. In the diabetic rabbits in the present study, there was a significant decrease of ≈7% in the CC and an increase of 9% in the CS.

Impaired relaxation of the cavernous smooth muscle cells or an alteration in its density may be a factor for ED [7]. In DM, cavernous smooth muscle relaxation is impaired and this has been associated with erectile dysfunction [5]. Smooth muscle increased significantly, by ≈40%, in the CC of diabetic rabbits. This increase has been observed in other organs of the urogenital tract, including the bladder, in which smooth muscle undergoes hypertrophy [21], as well as in the vessels [19]. This significant increase in smooth muscle in the CC of the penis could lead to a decrease in sinusoidal vascular spaces and hence to diminished blood flow, which may also contribute to ED.

Smooth muscle cells present high phenotypic variability [22], and cells from different tissues may have different synthetic profiles and may even respond differently to the same stimulatory or inhibitory factor. For example, hypercholesterolaemia induces a marked decrease in the amount of smooth muscle cells in the CC of the rat [23], yet this same condition in arteries stimulates smooth muscle cell proliferation [24]. Thus, this phenotypic variability of smooth muscle cells may explain, at least in part, the opposite effects diabetes has had on these cells in the CC and CS, as shown by the results obtained in the present study. Because smooth muscle cells are the main source of ECM molecules in erectile tissues, the heterogeneity of these cells may also underlie the different responses of the CC and CS with regard to collagen and elastic fibres in diabetic animals.

The TA is a fibroelastic sheath surrounding the trabeculae of the CC and is mainly composed of thick collagen bundles and elastic fibres [8]. There are few reports dealing with the morphological alterations of TA in diabetes, and the TA involvement in DM-induced ED remains unclear [25]. Salama *et al.* [26] evaluated the ultrastructural changes of penile TA in Zucker Diabetic Fatty rats (type 2 diabetes) under scanning electron microscopy and found that, as the disease progressed to a 40-week duration, an increase in the thickness and a loss of undulation of collagen bundles in penile TA occurred. Wei and Chang [27], via the induction of DM in mice using streptozotocin, observed a decrease in the density of elastic fibres in the TA. By contrast, in the present study, we showed a significant increase of 34% in the volumetric density of elastic system fibres in the TA of diabetic rabbits in addition to a significant increase in the thickness of the TA and in collagen turnover. The response of TA to diabetes in rabbits may therefore be different from that of mice. Lu *et al.* [25] induced diabetes in rats using streptozotocin and found that TA thickness was decreased. However, these measurements were performed on dehydrated tissue preparations, so that TA thickness might be artifactually diminished.

In conclusion, the results obtained in the present study show that alloxan-induced experimental diabetes causes profound changes in the various elements of the rabbit penis. These changes directly affect the normal functioning of erectile tissues and associated structures, and might therefore have an adverse effect on erection.

CONFLICT OF INTEREST

None declared.

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Abbreviations: **CC**, corpus cavernosum; **CS**, corpus spongiosum; **ECM**, extracellular matrix; **ED**, erectile dysfunction; **TA**, tunica albuginea.