

# The effect of testosterone treatment on urodynamic findings and histopathomorphology of pelvic floor muscles in female rats with experimentally induced stress urinary incontinence

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## Abstract

**Objective** In recent studies, it has been observed that androgen receptors are densely located in pelvic floor muscles. We aimed to investigate the effect of testosterone on urodynamic findings and histopathomorphology of pelvic floor muscles in rats with experimentally induced stress urinary incontinence.

**Materials and methods** Twenty-eight adult female rats were randomized into four groups. Group I: rats in which SUI was induced and single-dose testosterone was administered 30 days later, group II: rats in which SUI was induced and single-dose testosterone was administered within the same session, group III: rats in which SUI was induced and saline was injected intramuscularly 30 days later, and group IV: the sham group. In order to demonstrate objectively

the curative and preventive role of testosterone in experimental model of SUI, urodynamic examination and histopathomorphological evaluation of levator ani muscle were performed.

**Results** Myofiber areas in groups I and II were detected to be significantly larger than those of the control group ( $P < 0.001$ ). Another parameter was leak point pressure value by urodynamics. Regarding this parameter, LPP values in groups 1, 2 and 4 were observed to be significantly higher than those of group 3 ( $P < 0.001$ ). The results of the comparison among groups 1, 2 and 4 revealed no significance ( $P > 0.05$ ), which indicates that testosterone provides continence in a similar way to the group in which sciatic nerve section was not performed.

**Conclusions** In the present study, it has been demonstrated that testosterone has both preventive and curative effects on rat models of experimental SUI.

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## Introduction

Stress urinary incontinence (SUI) is the condition of involuntary urine leakage from the bladder to the urethra with increased intraabdominal pressure in the absence of a detrusor contraction when intravesical pressure exceeds urethral resistance [1–3]. The prevalence of SUI ranges between 10 and 30% in women aged

between 15 and 64 years [1]. Because of the psychosocial importance of the disease, its pathogenesis and treatment have recently attained great significance.

Androgens are known to exert an anabolic effect on striated muscles [4, 5]. However, testosterone causes an increase in fat-free muscle mass, muscle diameter and maximal muscle strength, whereas it leads to decreased fat deposits [4–6]. Although the anabolic effect of testosterone in males has been comprehensively investigated, there is limited information about the anabolic effect of testosterone in women. Recent studies have shown that androgens may play an important role in the female pelvic floor and lower urinary tract disorders. Muscles in these anatomical structures, particularly levator ani and urethral sphincter, have been observed to be androgen sensitive [7].

In this study, we aimed to investigate the effect of testosterone on urodynamic findings and histopathomorphology of pelvic floor muscles in rats with experimentally induced stress urinary incontinence.

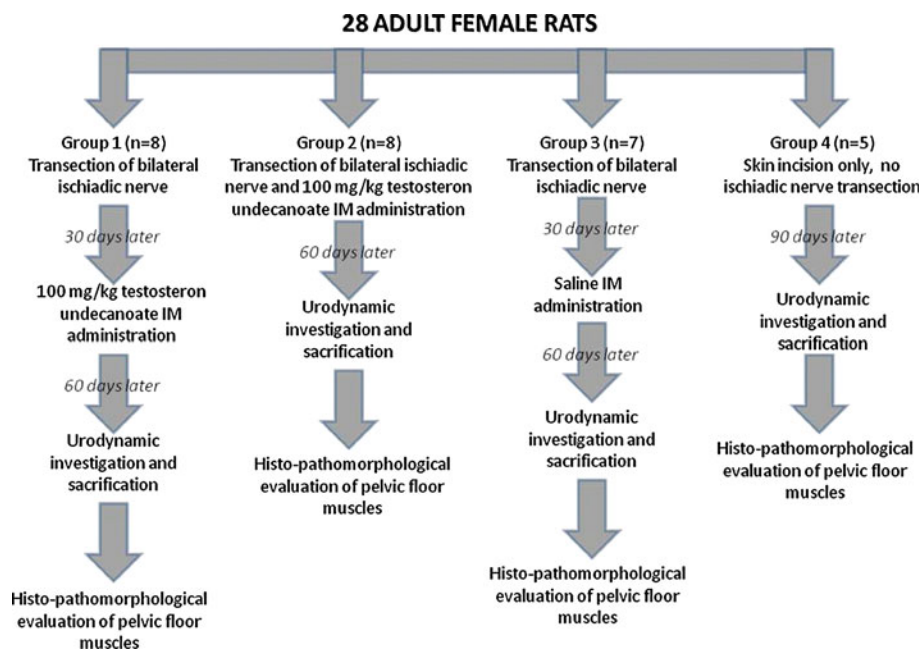
## Materials and methods

Following the approval of the Local Animal Ethics Committee, a total of 28 Sprague–Dawley adult

female rats, weighing between 200–250 g were used in this study. The rats were randomized into four different groups (Fig. 1). Group 1, in which we aimed to show the curative effect of testosterone on denervated levator ani muscle, was called “the curative group”. Group 2, in which we aimed to investigate the preventive effect of testosterone when administered simultaneously with sciatic nerve section, was called “the preventive group”. An experimental model of SUI was developed by the identification and section of the sciatic nerve through bilateral dorsal incisions over the ishiorectal fossa under an operating microscope, as suggested by Lee et al. [8]. The rats were anesthetized with intraperitoneal injection of ketamine HCl (35 mg/kg) in combination with acepromazine maleate (1 mg/kg). The weights of the subjects were measured, and the weight difference was recorded. It was taken into consideration that the time to the occurrence of severe atrophy of muscles was 30 days after sciatic nerve section, and the experiment was planned accordingly [9].

At the end of the experiment, the parameters compared among the groups were as follows:

- The level of free testosterone in the blood.
- The extent of the damage to the caudal part of the sciatic nerve.



**Fig. 1** Experimental flow chart

- Myofiber cross-sectional area of pelvic floor muscles.
- LPP values.
- The weight difference before and after the experiment.

A Biopac MP30 (BIOPAC SYSTEMS Inc., USA) and a pressure transducer were used in urodynamic analysis. A 21-gauge epidural catheter (Braun, Germany) was used for cystometry. The average of three consecutive LPPs was defined as the maximum pressure (P max). After the urodynamic analysis, blood samples were taken from all rats for the measurement of free testosterone levels. The collected blood samples were kept at  $-80^{\circ}\text{C}$  and analyzed by the DSL 4900 Free testosterone Radioimmunoassay (RIA) kit (Diagnostic System Lab, USA). There were no complications during the surgical procedure. The animals were killed by intraperitoneal administration of thiopental sodium (150 mg/kg). The sections were stained with hematoxylin–eosin and covered with Entellan. The tissues were examined under the light microscope (Olympus BX40, Japan). The nuclear count of the levator ani muscle and the measurement of muscle fiber diameter were carried out after the measurement of the images that were randomly selected using a modification of the light microscope, as described by Nnodim et al. [7]. The extent of the damage was scored qualitatively from +1 (very small) to +5 (very large) in a blind fashion based on the degree of edema, inflammation, degeneration and necrosis, and the results were analyzed statistically. All samples were analyzed by the same microscope under the same magnification ( $\times 400$ ). The cross-sectional area of muscle fibers was calculated using digital photomicrographs by multiplying two lengths

perpendicular to each other passing through the midpoint of the muscle fiber. Three separate measurements were made for each image by the same researcher who was blind to the identity of the subject. Then, the average of seven different preparations was calculated and analyzed statistically. One-way ANOVA was used for statistical analysis. A  $P$  value of  $<0.05$  was considered statistically significant.

## Results

Table 1 summarizes the relationship between the investigated parameters and the groups.

The level of free testosterone in the blood

Biochemical measurements at the end of the experiment revealed that free testosterone levels were as follows:  $2.20 \pm 0.60$  pg/ml for group 1,  $3.80 \pm 1.80$  pg/ml for group 2,  $0.60 \pm 0.20$  pg/ml for group 3, and  $0.80 \pm 0.20$  pg/ml for group 4. No statistically significant difference was found in free testosterone levels between group 4 and group 3 ( $P = 0.120$ ). There was a statistically significant increase in free testosterone levels in groups 1 and 2 compared to those in groups 3 and 4 ( $P < 0.001$ ).

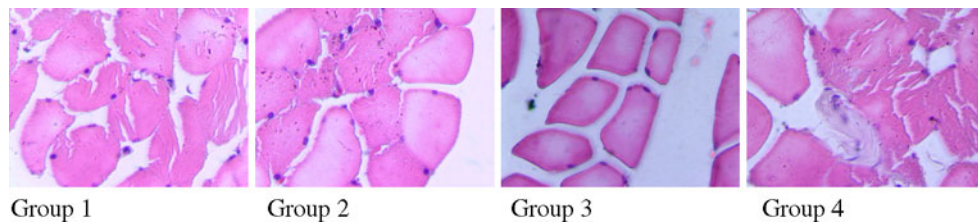
The extent of the damage to the caudal part of the sciatic nerve

Microscopic examination of the caudal part of the sciatic nerve revealed that the epineurium and perineurium were normal; endoneurium and perineural space were normal in appearance in group 4. Sciatic nerve tissue was found to be impaired to varying

**Table 1** Changes and comparison of the mean values of the investigated parameters according to the groups

	Free testosterone levels (pg/ml)	The extent of the damage in nerve	Myofiber cross-sectional areas ( $\mu\text{m}^2$ )	LPP (cmH <sub>2</sub> O)	Weight differences (g)
Group 1	$2.20 \pm 0.6$ <b><math>P = 0.046</math></b>	$86 \pm 0.90$ $P = 0.320$	$719 \pm 75$ <b><math>P = 0.042</math></b>	$15.4 \pm 4.9$ $P = 0.768$	$38.7 \pm 39.4$ $P = 0.420$
Group 2	$3.80 \pm 1.8$ <b><math>P &lt; 0.001</math></b>	$2.71 \pm 0.76$ $P = 0.080$	$867 \pm 115$ <b><math>P &lt; 0.001</math></b>	$15.8 \pm 5.1$ <b><math>P &lt; 0.001</math></b>	$50.2 \pm 34.2$ $P = 0.110$
Group 3	$0.60 \pm 0.20$ $P = 0.120$	$3.43 \pm 0.53$ <b><math>P &lt; 0.001</math></b>	$376 \pm 58$ <b><math>P &lt; 0.001</math></b>	$5.7 \pm 2.1$ <b><math>P &lt; 0.001</math></b>	$18.2 \pm 13.4$ $P = 0.820$
Group 4	$0.80 \pm 0.20$	$1.29 \pm 0.49$	$1,201 \pm 122$	$14.5 \pm 2.1$	$17.6 \pm 5.0$

Bold numbers mean statistical significant



**Fig. 2** Images of myofiber cross-sectional areas

degrees in groups 1, 2, and 3. In addition, we observed degeneration of transverse myelinated fibers, configurations of structural defects and abnormal bulges or protrusions. There was edema in endoneurium and perineurium and disintegration of axoplasm. Morphometric examination revealed that the mean value for the extent of the damage was  $2.86 \pm 0.90$  in group 1,  $2.71 \pm 0.76$  in group 2,  $3.43 \pm 0.53$  in group 3, and  $1.29 \pm 0.49$  in group 4. A statistically significantly higher level of damage was found in groups 1, 2, and 3 when compared to group 4. Although testosterone was found to have no positive effect on the damage to the sciatic nerve, all groups but the sham group (group 4) exhibited severe damage, which may be considered as an evidence showing that nerve section was performed.

#### Histological examination of the levator ani muscle and myofiber cross-sectional area

Microscopic examination of levator ani muscle showed that group 4 had fibrils of normal size and minimum necrosis, phagocytosis, regeneration, vacuoles, inclusions, central nucleolizations, fibrosis, vascular anomalies and inflammation. It was found that all parameters investigated for the levator ani muscle were impaired to varying degrees in groups 1, 2 and 3. Morphometric examination revealed that the mean myofiber cross-sectional area was  $719 \pm 75$  in group 1 and  $867 \pm 115$  in group 2,  $376 \pm 58$  in group 3 and  $1,201 \pm 122 \mu\text{m}^2$  in group 4. Myofiber cross-sectional areas were found to be statistically significantly diminished in all groups compared to those in the sham group. There was a statistically significant increase in myofiber cross-sectional area in groups 1 and 2 compared to group 3 ( $P < 0.001$ ), whereas the values for myofiber cross-sectional areas were found to be statistically significantly higher in group 2 than those in group 1 ( $P = 0.042$ ). This finding indicates that both curative and preventive

administration of testosterone prevent atrophy of pelvic floor muscles and the timing of testosterone administration makes a difference in favor of the preventive group (group 2) in which testosterone was administered 30 days earlier (Fig. 2).

#### Leak point pressure

The mean LPP was found to be  $15.4 \pm 4.9$  cmH<sub>2</sub>O in group 1,  $15.8 \pm 5.1$  cmH<sub>2</sub>O in group 2,  $5.7 \pm 3.2$  cmH<sub>2</sub>O in group 3 and  $14.5 \pm 2.1$  cmH<sub>2</sub>O in group 4. The mean LPP value was found to be statistically significantly higher in groups 1, 2 and 4 compared to that in group 3 ( $P < 0.001$ ). There was no statistically significant difference in LPP values between groups 1, 2, and 4. After the administration of testosterone, LPP values were significantly increased than those of the control group (Group 3) and similar to those of the sham group (group 4), which is indicative of the positive effect of this agent in the treatment and prevention of SUI.

Another important finding is that the results of the comparison of curative (group 1) and preventive (group 2) effects of testosterone did not reach statistical significance ( $P = 0.768$ ).

#### The weight difference before and after the experiment

The weight of the subjects was measured before and after the experiment, and the obtained difference was defined as the weight increase. The mean weight increase was  $38.7 \pm 39.4$  g in group 1,  $50.2 \pm 34.2$  g in group 2,  $18.2 \pm 13.4$  g in group 3, and  $17.6 \pm 5.0$  g in group 4. The weight increase was higher in groups 1 and 2 compared to that in groups 3 and 4, which however did not reach statistical significance between the groups.

## Discussion

Levator ani muscle is regarded as the main supporting structure of the pelvic floor and plays an important role in the continence mechanism [10–12]. Being cheap, non-invasive, simple and effective, exercises to strengthen pelvic floor muscles are frequently recommended as the first choice in studies investigating treatment options for SUI [13–16]. Matsuura et al. demonstrated that the method of anesthesia we used had no effect on the micturition reflex [17].

There is limited data in the literature about the morphology of the levator ani muscle in women with SUI. A study by Hanzal et al. demonstrated degenerated striated muscles in 63% of biopsy samples of levator ani muscle obtained from women with SUI, more than half of whom had relapsing urinary incontinence later on [18]. In a similar study by Zhu et al., degenerated striated muscles were found in 74% of biopsy samples of pubococcygeus muscle obtained from women who had undergone surgery for SUI, whereas this rate was 0% in the control group [19]. As indicated by these two studies, the morphological features of the levator ani muscle in SUI are of great importance.

Nnodim et al. were the first to experimentally investigate the relationship between androgens and the pelvic floor. Their study demonstrated that myofiber cross-sectional area was larger in the levator ani muscle of non-castrated rats than that of castrated rats and androgens had a positive effect on pelvic floor muscles [7]. However, Bhasin et al. in their study reported that androgens had an effect on almost all striated muscles, increasing muscle mass and contraction strength [20]. Based on this finding, in this study, myofiber cross-sectional area was observed to be statistically significantly larger in the sham group (group 4) than in the other groups and significantly higher in two testosterone-administered groups than in the control group, which indicates that not only endogenous androgens, as suggested by Nnodim et al. but also exogenous androgens have an anabolic effect on the pelvic floor. The results obtained from the comparison between groups 1 and 2 indicated that impairment of the pelvic floor muscles was statistically significantly higher in group 1. Accordingly, we consider that the preventive effect of testosterone is stronger than its curative effect in SUI.

Urodynamic examination was performed in subjects for the comparison and objective evaluation of the efficacy of the treatment. Given that pelvic floor muscles play a major role in all hypotheses aiming at elucidating the physiopathology of SUI, it is important to keep these muscles strong with testosterone for the treatment and prevention of the disease. Our study demonstrated that the administration of testosterone for both curative and preventive purposes was considerably effective, with no significant difference noted between them. Although it does not seem reasonable to take a drug for preventive purposes for a disease with an unknown date of onset, the absence of a significant difference between the preventive and curative effects of this drug is promising.

Other than the two main parameters mentioned earlier, the other parameters compared in the present study included the weight difference between the groups before and after the experiment, the level of free testosterone in the blood and the extent of the damage to the caudal part of the sciatic nerve. The weight difference before and after the experiment was defined as the weight increase. The weight increase was higher in the testosterone-administered groups compared to the other groups, which however did not reach statistical significance. We consider that the higher weight increase in group 1 and group 2 may be explained by the increased muscle mass due to the anabolic effect of testosterone.

The finding that the level of free testosterone in the blood at the end of the experiment was statistically significantly higher in groups 1 and 2 compared to non-testosterone groups was included in this study to further strengthen our hypothesis within the context of evidence-based medicine.

The severe damage to the caudal part of the sciatic nerve and statistically significantly higher damage in the nerve section groups than in the sham group indicate that complete nerve section was performed, a parameter that was also included in our study as evidence supporting our hypothesis.

## Conclusion

The major role of pelvic floor muscles in the pathogenesis of stress urinary incontinence is beyond doubt. Intramuscular injection of testosterone undecanoate for both curative and preventive purposes has

proven to have a high success rate in rats with experimentally induced stress urinary incontinence, which has been demonstrated to result from the anabolic effect of testosterone on pelvic floor muscles. However, larger experimental study groups and comparative clinical studies are required to use testosterone in the treatment of stress urinary incontinence.

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