Histomorphometric study of the inferior urinary tract of adult female rats during the interval between castration and hormonal replacement

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Summary

Objective: To evaluate the epithelial thickness, number of vessels, amount of collagen and muscular fibers of the bladder and urethra of castrated adult female rats during the time between castration and the beginning of the administration of synthetic conjugated estrogen.

Method: 118 adult female rats were divided into four groups: Group I (n = 30): noncastrated female rats; group II (n = 30): female rats treated with synthetic conjugated estrogen in the dose of 50 µg/animal/day for 28 days, beginning immediately after castration; group III (n = 28): female rats treated with synthetic conjugated estrogen, 50 µg/animal/day for 28 days, beginning 30 days after castration; group IV (n = 30): female rats sacrificed after 30 days of castration. The histology of the bladder wall and the medium-third of the urethra wall were evaluated after flushing with hematoxylin-eosin and picrosirius for morphometric analysis.

Results: It was verified that the epithelial thickness in groups II and III were similar whereas in groups I, II and III the thickness of the bladder and also the urethra were larger than in group IV. Concerning the bladder groups I and II were similar. In group I the urethra was superior than in groups II and III. In relation to the number of vessels and muscular fibers, groups I, II and III were similar to each other and superior to group IV in the bladder and urethra. The amount of collagen was similar in groups I, II and III and inferior in group IV in the bladder and in the urethra.

Conclusion: Independent of the time of estrogen administration (immediate or within 30 days) after castration, the thickness of the epithelium, the number of vessels, amount of collagen and muscular fibers were similar. The female rats with estrogen replacement presented significantly larger thickness of the epithelium, number of vessels and muscular fibers, and a smaller amount of collagen in the bladder and urethra in relation to the castrated group. Finally, estrogen therapy (immediate and 30 days after castration) reverted the effects of the estrogen deficiency in the vessels, collagen and muscular fibers, the bladder and of the urethra when compared to the group of castrated female rats, thus becoming similar to noncastrated animals.

Key word: Estrogen; Climacteric; Lower urinary tract.

Introduction

Due to the improvement of social and economic conditions, and the access to medical care, a larger number of women reach the climacteric and postmenopause period, thus being exposed to the consequences of chronic estrogen deficiency [1]. Considering that life expectations have increased and that the age of incidence of menopause has not changed over time (about 50 years), an important part of a women's life is spared after postmenopause, which brings about the very important clinical aspects of estrogen deficiency [2].

The climacteric is characterized by a deficiency of ovarian hormones, when a woman starts to suffer from several metabolic alterations which affect different organs and systems, causing vasomotor instability, and disturbances of the bones, cardiovascular system, and genitourinary tract, as well as psychological instability [3-5].

When the etiology of a woman's dysfunctions in postmenopause is observed, it is difficult to separate the dysfunctions that come from age and those from hypoestrogenism.

However, hormonal replacement has been successful after menopause, particularly in women with genitourinary tract alterations [6-8], especially vaginal dryness, dyspareunia, pain, discharge, infection of the urinary tract, urgency and urinary incontinence [9].

It is understood that urinary incontinence is a condition in which the involuntary loss of urine, objectively demonstrable, causes social or hygienic problems. Stress urinary incontinence is defined as the involuntary loss of urine (extraurethral), where intravesical pressure exceeds intraurethral pressure, in the absence of detrusor activity [10]. Urinary incontinence rebounds with a great social, economic impact and a negative influence on the quality of a woman's life [11-13].

The embryologic development of the genital tract is intimately related to the urinary tract because they originally came from the same embryonic area (urogenital sinus) [14].

Revised manuscript accepted for publication August 27, 2002

In fact, estrogen receptors have been identified in the bladder, in the urethra and in the musculature of the pelvic floor [15, 16]. Progestogen receptors have also been found but with antagonistic effects [17]. Several authors have verified that estrogen therapy can influence the alpha-adrenergic receptor of the urethral musculature, thus increasing its activity and sensibility [18, 19].

Although urinary incontinence is common in the post-menopausal years, the real role of estrogen deficiency has not been established.

Thomas *et al.* [20] studied 10,000 British women and they noticed an increased prevalence of urinary incontinence with age, but not specifically with the time of menopause.

Maintenance of urinary continence is determined by several factors such as the urethral sphincter system, the intra-abdominal topography of the bladder neck, the urethral mucosa, the peri-urethral vessels, and the musculature of the pelvic and urogenital diaphragms [21-24].

The pelvic floor musculature is an important factor for continence maintenance. The function of these muscles deteriorates after menopause, and there is increased risk of urgency, urge-incontinence, urinary incontinence and prolapse [25].

Trophism of the urethral mucosa also aids the mechanism of urinary continence due to be influence of estrogens [26].

The peri-urethral vessels promote efficient mechanical support that propitiates the transmission of intra-abdominal pressure to the proximal urethra [1].

Eika *et al.* [27] noticed that the bladders of ovariectomized female rats were smaller and had a larger concentration of collagen when compared with bladders of intact female rats; estrogen replacement reverted these parameters.

In 2001, Sartori *et al.* [28], evaluating the collagen of the bladder muscular layer and of the urethra layer of castrated female rats, noticed that isolated estrogen replacement decreased the amount of collagen fibers significantly, increased muscular fibers and reduced the collagen/muscle relationship significantly.

The physiologic role of estrogens in the maintenance of continence is still debated. In fact, it is difficult to have definitive conclusions. Most of the exhibit research subjective results or great variability of technical urodynamics. Moreover, the inclusion of approaches, results, the limited numbers of controlled studies and the variety and dose of estrogen applied hinder the analysis of the results.

Although there is evidence that estrogen replacement acts on the urogenital tissue, there are no studies showing if the time elapsing between menopause and the beginning of hormonal replacement influences those effects. Thus, we were interested in studying if the time to the beginning of hormonal replacement with synthetic conjugated estrogen would influence the epithelial thickness, the number of vessels and the amount of muscular and collagen fibers of the bladder and urethra of adult female rats after castration.

Material and Methods

This study was developed in the Section of Urogynecology and Vaginal Surgery of the Gynecology Department and in the Department of Histology and Structural Biology of the Morphology Department of the Federal University of São Paulo - São Paulo College of Medicine (UNIFESP-EPM).

One hundred and eighteen virgin adult female rats were used (Rattus norvergicus allbinus, Rodentia, Mammalia), of the colony EPM-1 Wistar, with approximately 120 days of life, weighing about 210 grams, originating from of the Center of Experimental Models Development in Medicine and Biology (CEDEME) of UNIFESP-EPM.

The animals were distributed in four groups:

Group I - composed of 30 noncastrated adult female rats.

Group II - constituted by 30 female rats that received synthetic conjugated estrogen in a dose of 50 μg/animal/day orally for 28 days immediately after castration [29].

Group III – included 28 female rats that were administered synthetic conjugated estrogen in a dose of 50 μg/animal/day for 28 days beginning after 30 days of castration [29].

Group IV - formed by 30 female rats that were sacrificed after 30 days of castration.

After the acclimatization phase, the rats were ovariectomized under anesthesia with ethyl ether (groups II, III and IV).

The administration of the hormones was done by gavage over 28 days with a metallic standardized probe at the Pharmacology Department of UNIFESP-EPM, always at the same time of day (9:00-11:00 a.m.). We used synthetic conjugated estrogen diluted in 0.5 ml propilenoglycol.

The animals were manipulated so that at the end of the experiment all were the same age, around 178 days.

The obtained material was divided into two representative fragments of the different anatomic areas: bladder and medial urethra. The samples were flushed by hematoxylin-eosin and picrosirius [30].

The morphometric analysis was performed in the Laboratory of Experimental Gynecology of the Gynecology Department of UNIFESP-EPM. Technique of image representation was used, with quantification by means of a computerized system, constituted by light microscope (CARL ZEISS) with objective between 10 and 40x, color video camera (SONY, Hyper HAD), Pentium computer 200 mHZ, graphic plate for image acquisition 640 x 480 pixels and 24 bits (16 million colors) and processing and analysis of image software - IMAGELAB (SOFTIUM Informatica LTDA).

Determination of the bladder and urethal epithelial thickness was accomplished by means of four linear measures in chosen areas, always in 0slim areas, to avoid mistakes of sample interpretation.

Ten reading fields were selected for the vessel count of the own blade. Digital imaging was used with increases 400x, to mark the counted vessels and avoid counting again.

For the quantification of collagen and muscular fibers an increase of 400x was also used. On the computer screen, reticule was coupled contends 25 points geometrically distributed. The reticule projected on the samples allowed the count of the points occurring on the collagen and muscular fibers. Twenty fields were counted totaling 500 points for structure (bladder and uretra) per animal.

Statistical method

The calculation of measures summary was used (average and standard error), (Tables 1 and 2), to observe the behavior of the groups in a previous evaluation to the inferential analysis.

For the inferential analysis, parametric tests were used taking into consideration the nature of the studied variables:

- the analysis of variance (ANOVA) was used with the objective of verifying the equality of number of vessel averages, of epithelium thickness, and of the number of collagen and muscular fibers in the bladder and urethra, in the four groups;
- it was complemented with Tukey's test of multiple comparisons for the analysis of the variables to observe the significant differences among the groups;
- trust intervals were built (g = 95%) for the averages of the studied variables in the several groups.

The significancy level is 0.05 or 5% ($\alpha \leq 0.05$); significant values are marked with an asterisk.

Results

The results of the bladder epithelial thickness are shown in Table 1. It was observed that groups I, II and III presented larger bladder epithelial thickness than group IV (Figure 1).

In relation to the thickness of the urethra epithelium (Table 2), group I presented higher averages than the other groups. Groups II and III were similar and they had higher values than group IV (Figure 2).

Table 1 shows the number of bladder vessels. It was observed that groups I, II and III did not differ from each other, and they presented a higher number of vessels than did group IV (Figure 3).

The urethral vessel numbers are shown in Table 2. It was verified that, as in the bladder, groups I, II and III were similar and superior to group IV (Figure 4).

The results of the collagen count in the muscular layer of the bladder are also shown in Table 1. It can be noticed that groups I, II and III were similar to each other, and with an inferior amount of collagen than that observed in group IV (Figure 5).

The amount of collagen in the muscular layer of the

Table 1. — *Epithelial thickness, vessel numbers, number of collagen fibers and number of muscle fibers in the detrusor muscle of the bladder according to the studied groups.*

		Groups			
		I	II	III	IV
Epithelium	Mean	34.24	33.02	31.55	27.07
	SD	0.429	0.565	0.842	0.385
Vessels	Mean	22.53	21.10	20.60	16.16
	SD	0.641	0.566	0.661	0.553
Collagen	Mean	160.27	168.93	173.96	203.10
	SD	3.25	3.35	4.13	5.36
Muscle Fibers	Mean	265.63	263.20	250.71	194.57
	SD	3.09	3.48	4.39	5.17

Variance analysis (I x II x III x IV): $p < 0.001$* - Tukey's test:

Epithelium	I = II	(–0.872; 3.321)	II = III	(–0.665; 3.602)	I > III (0.560; 4.827)*
	II > IV	(3.848; 8.041)*	I > IV	(5.073; 9.266)*	III > IV (2.343; 6.610)*
Vessels	I = II	(–0.782; 3.649)	II = III	(–0.762; 2.748)	I = III (–0.329; 4.181)
	II > IV	(2.718; 7.149)*	I > IV	(4.151; 8.582)*	III > IV (2.186; 6.695)*
Collagen	I = II	(–23.72; 6.39)	II = III	(–20.35; 10.29)	I = III (–29.02; 1.62)
	I < IV	(–49.92; –19.11)*	I < IV	(–57.89; –27.78)*	III < IV (–44.45; –13.82)*
Muscle Fibers	I = II	(–12.58; 17.45)	II = III	(–2.79; 27.77)	I = III (–0.36; 30.20)
	II > IV	(53.62; 83.65)*	I > IV	(56.05; 86.08)*	III > IV (40.87; 71.43)*

Table 2. — *Epithelial thickness, vessel numbers, number of collagen fibers and number of muscle fibers in the middle third muscle layer of the urethra according to the studied groups.*

		Groups			
		I	II	III	IV
Epithelium	Mean	35.14	33.08	32.06	24.14
	SD	0.386	0.606	0.724	0.354
Vessels	Mean	17.56	19.16	18.60	10.93
	SD	0.602	0.498	0.510	0.643
Collagen	Mean	182.70	188.27	188.54	218.87
	SD	6.57	5.04	3.93	5.13
Muscle Fibers	Mean	200.33	197.57	206.07	11.87
	SD	6.16	5.33	5.01	5.56

Variance analysis (I x II x III x IV): $p < 0.001$* - Tukey's test:

Epithelium	I > II	(0.112; 4.011)*	II = III	(–0.962; 3.006)	I > III (1.099; 5.068)*
	II > IV	(6.991; 10.890)*	I > IV	(9.052; 12.951)*	III > IV (5.934; 9.903)*
Vessels	I = II	(–3.681; 0.481)	II = III	(–1.559; 2.678)	I = III (–3.159; 1.078)
	II > IV	(6.152; 10.315)*	I > IV	(4.452; 8.715)*	III > IV (5.556; 9.792)*
Collagen	I = II	(–24.94; 13.81)	II = III	(–19.99; 19.45)	I = III (–25.56; 13.89)
	II < IV	(–49.98; –11.22)*	I < IV	(–55.54; –16.79)*	III < IV (–50.05; –10.61)*
Muscle Fibers	I = II	(–17.54; 23.08)	II = III	(–29.17; 12.17)	I = III (–26.41; 14.93)
	II > IV	(65.39; 106.01)*	I > IV	(68.16; 108.78)*	III > IV (73.58; 114.87)*

urethra is given in Table 2. It was also verified that groups I, II and III were similar to each other, and with smaller amounts than in group IV (Figure 6). Concerning the musculature it was observed that the number of muscular fibers in the bladder (Table 1, Figure 7) and in the urethra (Table 2, Figure 8) were similar in groups I, II and III, and in larger number than obtained in group IV.

Discussion

Several urinary alterations begin or become worse in the climacteric, constituting great importance for psychological and social aspects [11, 12].

The physiopathology of urinary disturbances has been studied, especially during the climacteric, being related to estrogen deficiency and to aging tissue [11, 16].

Studies show that hormonal replacement acts positively, improving urinary symptoms [31-34], however, it is not specified if the beginning of hormonal therapy in the climacteric causes different effects in the urogenital tissues. It interested us to know if the time elapsing from castration to the beginning of hormonal replacement with synthetic conjugated estrogen would influence the epithelial thickness, the number of vessels, the collagen and the muscular fibers of the bladder and urethra of adult female rats.

With the presence of hormonal receptors in the urogenital tissues [15] and the known influence of estrogen in the receptors [18, 19], we hoped to find differences in the effects of the estrogen replacement in agreement with the time of starting the treatment.

The alterations provoked by castration and the consequent estrogen deficiency on such receptors could alter them, thus, we would not obtain the same results with the replacement that began even after 30 days.

We verified that independently of the time at the beginning of the estrogen administration, the epithelial thickness, the vessel numbers, the collagen and the muscular fibers in the bladder and urethra were similar. This research also confirms that estrogen replacement increases the epithelial thickness of the bladder and the urethra in castrated female rats.

The epithelial thickness of the urethral mucosa produces a seal effect that aids the mechanisms of urinary continence which are modulated by the estrogen action [35].

With the start of estrogen deficiency in the climacterium, such mechanisms stop working correctly and with the decreased vascular tissue, there is a decrease in the intra-urethral pressure causing urinary incontinence. Several authors have observed that the peri-urethral and peri-

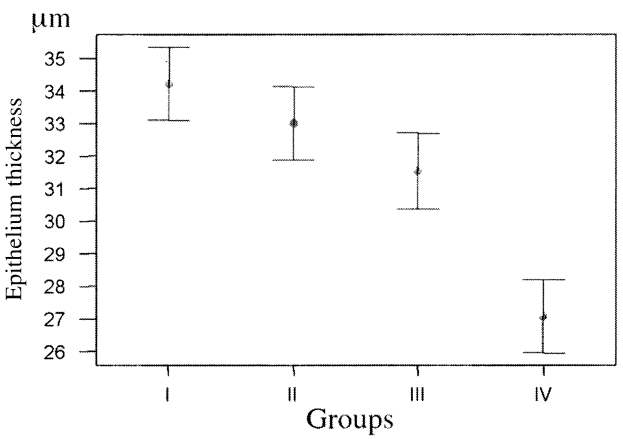

Figure 1. — Epithelial thickness of the bladder in the studied groups.

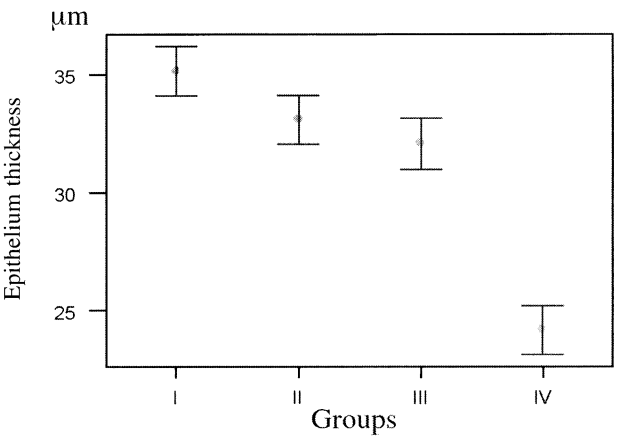

Figure 2. — Epithelial thickness of the urethra in the studied groups

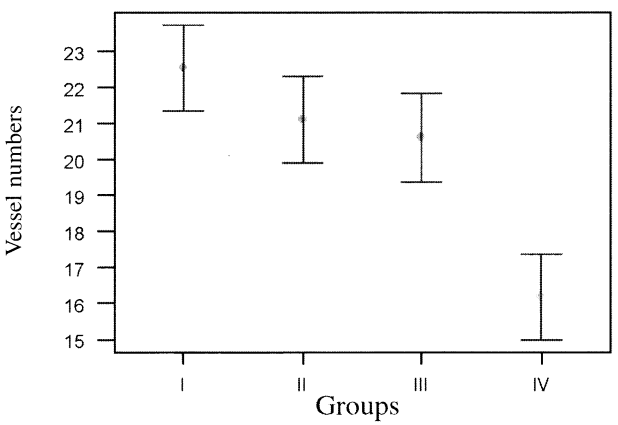

Figure 3. — Vessel numbers of the bladder in the studied groups.

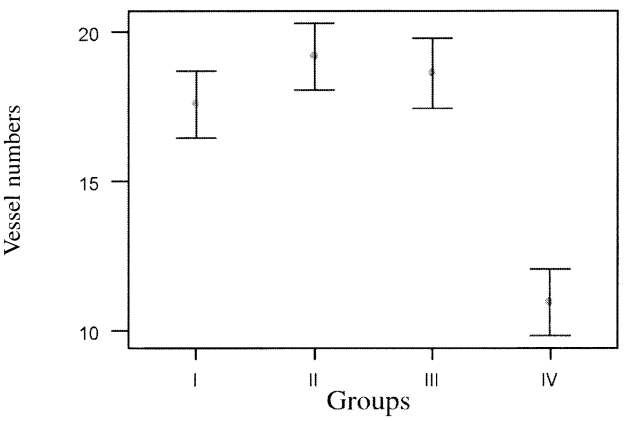

Figure 4. — Vessel numbers of the urethra in the studied groups.

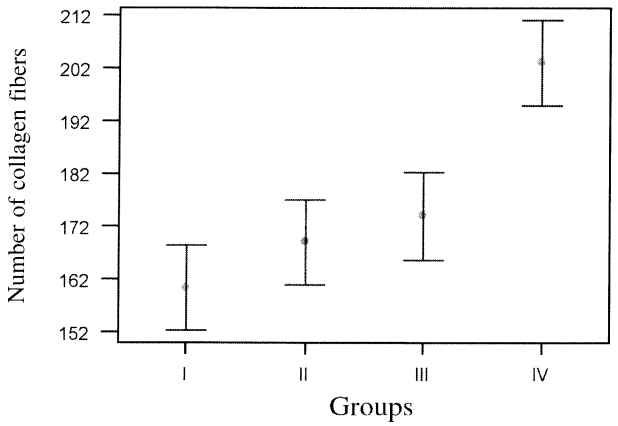

Figure 5. — Number of collagen fibers of the bladder in the studied groups.

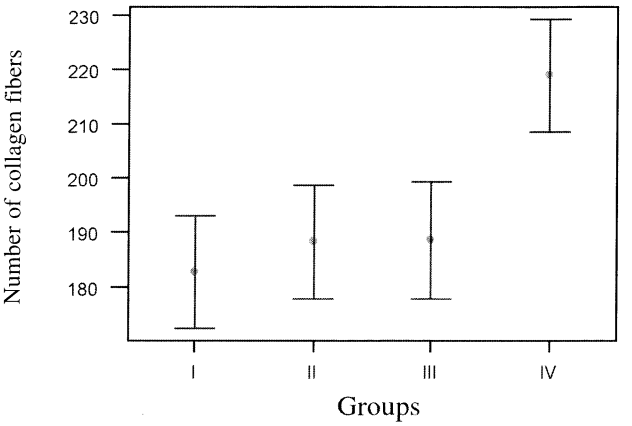

Figure 6. — Number of collagen fibers of the urethra in the studied groups.

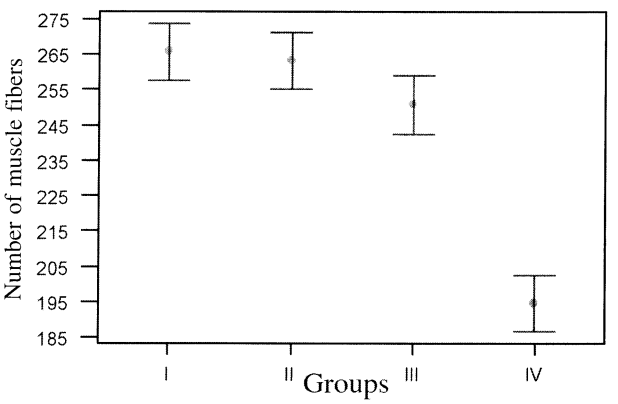

Figure 7. — Number of muscle fibers of the bladder in the studied groups.

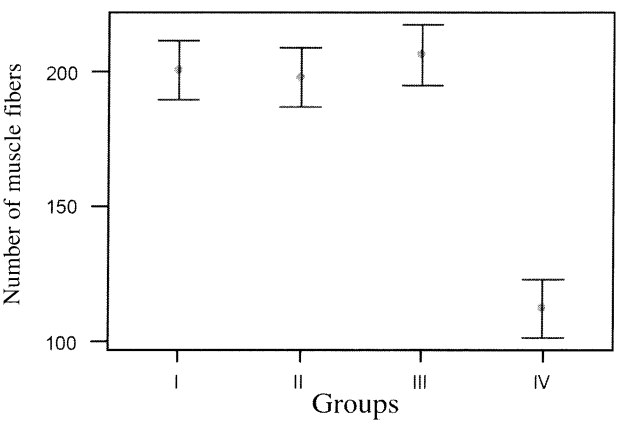

Figure 8. — Number of muscle fibers of the urethra in the studied groups.

bladder vessels are influenced by the estrogen action [36].

Rud *et al.* [37] demonstrated that the vascular component is responsible for a third of the intra-urethral pressure.

Carlile *et al.* [35] noticed that with advanced age a relative decrease of the human urethra vessels occurs.

We observed that estrogen administration totally reverted the effects of the estrogen deficiency caused by the castration, reducing the bladder and urethral collagen significantly. Such findings are similar to the ones reported by Eika *et al.* [27].

Longhurst *et al.* [38] when comparing the bladder of female rats of six and 24 months, concluded that the concentrations of collagen were significantly smaller in the bladders of young females.

Falconer *et al.* [39] noticed that the concentration of para-urethral collagen was almost half in postmenopausal women when compared to premenopausal women.

Together with collagen, we verified a significant increase of the muscular fibers in the bladder and urethra with hormonal replacement in the group with immediate replacement and in the group after 30 days.

These results are in agreement with those of Sartori *et al.* [28] who found an increased number of muscular fibers and decreased collagen in the bladder and in the urethra of castrated adult female rats treated with estrogen.

In agreement with Susset *et al.* [40] we believe that increased collagen concentrations in the bladder and urethra and the decrease of muscular fibers reduce the elastic capacity causing alterations, thus producing the typical complaints of postmenopausal women. Moreover, the decrease of muscular fibers found in the climacterium alters the contractility of the detrusor, and consequently the residual volume increases, which favors urinary infections and affects the emptying vesical and the continence mechanisms.

By virtue of the tendency of the obtained data, we could hypothesize that estrogen replacement, independent of the time of menopause, could reverse the main decisive factors of urinary dysfunctions in climacteric and postmenopausal patients.

However, we can not transfer the results from experimental research to human beings. Additionally, we do not know if we had prolonged the time to begin hormonal replacement if we would have obtained the same results, and, perhaps, such effects would be reversed, because we just evaluated the effect of estrogen deficiency without appreciating the effect of the aging process.

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