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URETHRAL CIRCULAR SMOOTH MUSCLE IN YOUNG AND OLD WOMEN

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Abstract

Objective—To determine if thickness, fiber count, and density of urethral circular smooth muscle (CSM) differ in young and old women.

Study Design—Mid-urethral hemi-axial sections from female cadavers aged 20–39 (n=12) and 70–89 (n=16) were stained for smooth muscle alpha actin. The CSM was studied at 0 degrees (pubic bone side), 180 degrees (vaginal side) and in between at 45, 90, and 135 degrees. CSM layer width was measured; fibers were counted; density was calculated (fiber count/layer width)

Results—Density of urethral CSM was 25–50% higher in specimens aged 20–39 compared to those aged 70–89. Differences were observed at 0, 135, and 180 degrees. In the younger group, higher fiber counts were observed at 135 and 180 degrees, and the CSM layer was thinner but not significantly so.

Conclusion—The density of circular smooth muscle was lower among older women, possibly accounting for the age related decline in urethral closure pressure.

Keywords

urethral smooth muscle; urethral striated muscle; urinary incontinence; histology

Introduction

Aging is a well known risk factor for urinary incontinence (1,2). The mechanism underlying the pathophysiology of age and urinary incontinence remains only partially explained. Maximum urethral pressure, a physiologic measure of urethral competence, decreases with age (3) even in the absence of vaginal parity (4) and is associated with the occurrence and severity of stress urinary incontinence (5). The number of striated muscle fibers in the urethra is known to decrease with age (6,7). A 1% loss per year of striated muscle fibers and a decrease in that layer's thickness has been observed (8). The circular smooth muscle (CSM) layer of the urethra, which is medial to the striated muscle layer and closer to the urethral lumen, could exhibit changes further accounting for the decrease in maximum urethral pressure. A previous study reported that while there was an increasing amount of connective tissue, the total number of smooth muscle fibers in the urethra did not differ in women across the age span (6). Morphologic characteristics such as CSM thickness and density in discrete areas have not been studied.

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Condensation Urethral circular smooth muscle density is lower in older women and may in part account for the age related decrease in urethral closure pressure.

The objectives of this study are: 1) to determine if there are differences at the mid-urethra in urethral CSM morphology—e.g. fiber count, layer thickness, density, and percent smooth muscle, and 2) to evaluate if there is an association between the morphologic changes in striated urethral sphincter muscles and circular smooth muscle.

Materials and Methods

Inclusion & Exclusion criteria

Between 1995 and 2005, a convenience sample of 109 urethras was collected at autopsy at the University of Michigan Hospital. Patient age was recorded and presumptive parity (nulliparous versus parous) was established by perineal and/or cervical examination and review of medical records. Specimens were fixed and stored in 10% formalin. Permission for removal of tissues for research purposes is a provision of the autopsy consent. Autopsy studies are exempt from Institutional Review Board review as post mortem issues are outside of the purview of the IRB.

The urethra was divided in the mid-sagittal plane into two halves. One half of each specimen was divided into 5 mm hemiaxial sections and embedded in paraffin. A midurethral section was chosen for study while the other half was retained as a sagittal specimen for future analysis. The mid-urethral axial section was stained with Masson's trichrome stain to identify striated urethral muscle and an adjacent section processed with alpha-actin immunohistochemical stain to identify smooth muscle. To assess age-related differences, age extremes were chosen. All complete specimens from women aged 20–39 years (n=12) and 70–89 years (n=16) for whom a specimen was collected were used for this analysis. Specimens that had been fixed with a Foley catheter in place were not included because this could affect muscle layer diameter.

Specimen orientation

The axial hemi-urethral sections were oriented as shown in Figure 1 for analysis. The anterior urethra near the pubic bone was defined as 0 degrees and the posterior urethra on the vaginal side as 180 degrees. Intervening equidistant sites between the anterior and posterior urethra at 45, 90, and 135 degrees were marked on the slide. At the intersection of each radial line with CSM and striated sphincter, the morphological measures (i.e. layer width, fiber count, and density) were evaluated using images captured at 100x magnification. An example of these measures is shown in Figure 2 and explained further in the methods below.

Specimen measurements

All measurements were made by AC and reviewed by DMM and both authors were blinded to the subject's age. If there was a discrepancy between these two authors with respect to a measurement, the specimen was reviewed and the decision adjudicated by JOLD who was also blinded to the subject's age.

Muscle layer width measurement

The inner and outer edge of the layer was identified, and the layer width was measured using a digital caliper system (SPOT Advanced software, Diagnostic Instruments, Inc). The calipers were placed on the photomicrograph perpendicular to the fiber direction rather than along the radial line that might be oblique to the CSM. Only fibers in the body of the CSM were considered and fibers diverging from the circular orientation were not included in the count. In regions where the circular smooth muscle was missing or not clearly visible, it was not possible to measure muscle layer width. This often occurs in the posterior aspect of the urethra where the fibers of the trigonal plate are interposed between the ends of the smooth muscle.

Fiber count

Fibers were counted as they intersected a line placed perpendicular to the sphincter fiber direction near each radial line at 0, 45, 90, 135, and 180 degrees. When the CSM was not clearly visible, a fiber count was not done.

Fiber density

Fiber density was calculated by dividing the fiber count by muscle layer width. Density could not be calculated when a layer width measurement was not possible.

Percent smooth muscle

The percent tissue occupied by of the circular smooth muscle cells was evaluated stereologically at each location. (Note: this was not calculated for striated muscle because it was the subject of a previous study by Perucchini et al). A rectilinear grid was digitally superimposed on each image. It consisted of lines equidistantly placed across the width and height of each specimen's smooth muscle layer. Each crosshatch of the grid falling on circular smooth muscle was scored 2 while vascular smooth muscle and other connective tissue was scored zero. The percent smooth muscle was calculated by summing the number of points of a specimen and dividing that by the total number of points possible.

Statistics

T-tests were used to compare the mean width, fiber count, density and percent smooth muscle of the two groups. Because our major findings were with respect to density, we did a post hoc power analysis to evaluate how many more individuals would be required to demonstrate a difference in density at the 90 degree site where there was a marginal difference. This study had 34% power to detect a 25% difference in the density of the CSM. To detect this difference with 80% power, a sample size of 31 in each group would be needed. Associations between smooth and striated muscle measures were tested with a Spearman's correlation coefficient.

Results

There were 28 specimens that met the inclusion and exclusion criteria and that were processed for analysis in this study. One specimen in the 20–39 year old group could not be analyzed due to extensive edema and tissue fragmentation which destroyed the tissue architecture. Patient demographics for the two age groups are summarized in Table 1. The groups did not differ in the frequency of nulliparity, BMI, or time from death to autopsy. The percentage of non white individuals in the 20–39 year old group was higher but this difference was not statistically significant.

CSM measures are presented in Table 2. Several significant differences were found, mostly in the posterior urethra. Density between the groups differed at 0, 135, and 180 degrees and mean smooth muscle percent differed at 90, 135, and 180 degrees. There were marginal differences in density and smooth muscle percent at the other sites examined. The differences observed in density are accounted for by two trends in the measures of muscle layers thickness and fiber counts. Fiber counts tended to be higher in the young specimens, especially in the posterior urethra, and the CSM tended to be thinner or more compact. These trends were not individually statistically significant but the two considered together in the calculation of density did result in a statistical difference.

The corresponding striated urethral muscle layer morphometry is described in Table 3. The mean number of muscle fibers differed at all five sites examined. The mean thickness was greater at three of the five sites at 90, 135, and 180 degrees. The density of the striated muscle layer was higher in the young with significant differences at four of five sites analyzed. With

the loss of almost all striated muscle at 180 degrees in specimens from the group aged 70–89 years, we were only able to compare the proportion with visible muscle (70–89 v 20–39 years: 25% v 93.8%, $p < 0.001$).

The morphometry of the smooth and striated muscle layers were analyzed for associations (see Table 4). Loss of muscle density was correlated at 0 and 45 degrees but not in the posterior sites (i.e. 90, 135, and 180 degrees). There were no associations between fiber counts of the smooth and striated muscle layers or layer diameter.

Comment

This study demonstrates that the density of urethral circular smooth muscle decreases with aging. Loss of circular smooth muscle is most prominent in the posterior urethra and is seen to a lesser extent in the anterior urethra. This finding may help to explain the mechanism behind the decrease in urethral closure pressure and the increased prevalence of urinary incontinence with aging.

The findings of this study contribute to our understanding of changes in urethral smooth muscle anatomy with aging. Carlile et al evaluated 26 female cadaveric specimens and reported that fiber counts of smooth muscle did not differ with aging (6). The difference between our findings and those of Carlile et al may be due to methodology. Carlile et al used a microscopic graticule to assess the relative composition of connective tissue and striated, non vascular smooth, and vascular smooth muscle in entire hemi-urethral specimens. This method provided total fiber counts but did not account for differences in discrete regions of the urethra. Using a systematic sampling approach, we compared five discrete anatomical regions. Fiber counts in our study did not differ in the anterior and lateral urethra (i.e. sites at 0, 45 and 90 degrees) but those in the posterior urethra (sites at 135 and 180 degrees) did. When considering the calculation of density, it should be remembered that the CSM layer was 11 to 33% thinner in the 20–39 year old group and that the fiber counts of this group were higher, leading to significant or marginal differences in layer density at all sites. The multiple parameters of morphometry in this study provide a more thorough assessment of changes in CSM morphology in the urethra with aging. The loss of circular smooth muscle in the urethra may begin to account for the increased prevalence of urinary incontinence with aging. It is interesting to consider that aging is more commonly associated with smooth muscle abnormalities such as hypertrophy (e.g. uterine fibroids, benign prostatic hypertrophy) and unregulated growth (e.g. malignancies) than loss of muscle.

The differences we found in striated muscle morphology are consistent with previous studies (6,7,8,9). Striated muscle fiber counts were significantly higher in women aged 20–39 years compared to those 70–89 years at all sites analyzed. Perucchini et al and Carlile et al have reported similar findings of decreasing striated muscle fiber counts with aging (6,7). Striated muscle layer thickness in our analysis was significantly decreased in the posterior urethra—an observation also reported by Perucchini et al (8).

The differences in CSM and striated muscle morphometry were correlated with each other in the anterior but not posterior urethra. We suspect that we were unable to find a correlation in the posterior region of the urethra due to a loss of sample size. The percentage difference in most measures between the groups was highest in the posterior urethra, but there are many more specimens in this area that lacked any visible muscle to analyze. At 180 degrees, striated muscle fibers were not seen in 75% of the specimens from the group aged 70–89 years and 25% of the group aged 20–39. This resulting lack of variation in the data of both groups makes it difficult to find a correlation between loss of striated and smooth muscle fibers. The anterior region of the urethra has more subtle loss of muscle but the muscle is visible in almost all

specimens from both groups. Thus, even with this small sample size, we were able to find evidence that loss of smooth muscle is associated with loss of striated muscle.

An anatomical study of autopsy specimens has limitations. The continence status of the subjects is not known and cannot be discerned in enough patients for a meaningful analysis. This study could also benefit from a larger sample size. The lack of differences in density of the anterior regions (i.e. 0 and 45 degrees) suggests that a larger sample size may be required. A post hoc analysis suggests that to detect 25% difference with 80% power, 31 specimens would be required in each group. This would require us to double our sample size. This is a relatively modest sample size and is worthy of future study. This percentage difference in the CSM could plausibly lead to a clinical difference if one considers that there is a 37% decrease in maximum urethral closure pressure between women aged 20–39 and women older than age 71 (3). Another area of the urethra that deserves study is the longitudinal smooth muscle layer. Its borders are difficult to distinguish in comparison to the circular smooth muscle, but developing an image analysis schema to evaluate this area is worthy of study.

The loss of circular smooth muscle and striated muscle adds to our understanding of how aging leads to a higher prevalence of urinary incontinence. The average loss of maximum urethral closure pressure of women at the extremes of age has ranged from 37–58% in samples of continent and incontinent women (3,4). The loss of circular smooth muscle in this study ranges from 17–50% and the loss of striated muscle from 29–47%. It is possible that these anatomic differences in smooth and striated muscle might account for these physiologic differences noted in other studies. An improved understanding of urethral tissue composition of the urethra may eventually have important implications for efforts of tissue modeling and regeneration.

Acknowledgments

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Figure 1. Orientation of a midurethral specimen. The anterior portion of the urethra nearer the pubic bone is defined as 0 degrees and the posterior urethra nearer the vagina as 180 degrees. At equidistant sites between the anterior and posterior urethra there are lines to define 45, 90, and 135 degrees.

Figure 2.
Photomicrograph of the circular smooth muscle demonstrating the technique for layer width measurement, fiber count, and the density calculation.

Table 1

Patient demographics

	Young: 20–39 (n)	Old: 70–89 (n)	P Value
Age (mean years $\pm$ SD)	30.2 $\pm$ 6.5 (12)	77.2 $\pm$ 4.6 (16)	<0.001
Parous (%)	75.0 (12)	68.8 (16)	0.407
Non-white (%)	33.3 (12)	18.8 (16)	0.378
BMI(kg/m ² $\pm$ SD)	27.7 $\pm$ 2.8 (11)	29.3 $\pm$ 1.5 (15)	0.617
Time to autopsy (mean hours $\pm$ SD)	18.0 $\pm$ 4.9 (11)	17.1 $\pm$ 9.3 (16)	0.779

Table 2
Smooth Muscle layer thickness, fiber counts and fiber density & percent smooth muscle

	Orientation within Urethra	20–39 years	70–89 years	Percent Difference	P value
Thickness (Mean mm ± SD) (n)	0 (n=25)	1.41 ± 0.91 (10)	1.88 ± 0.94 (15)	33.3	0.229
	45 (n=25)	1.27 ± 0.94 (11)	1.47 ± 0.72 (14)	15.7	0.539
	90 (n=21)	1.09 ± 0.63 (10)	1.23 ± 0.59 (11)	12.8	0.604
	135 (n=17)	1.36 ± 0.64 (9)	1.51 ± 0.51 (8)	11.0	0.607
	180 (n=10)	1.84 ± 0.19 (3)	1.45 ± 0.54 (7)	21.2	0.278
Fiber Count (mean ± SD) (n)	0 (n=25)	22.4 ± 11.9 (10)	21.3 ± 12.0 (15)	4.9	0.819
	45 (n=25)	19.2 ± 11.0 (11)	18.1 ± 9.4 (14)	5.7	0.788
	90 (n=21)	17.3 ± 8.5 (10)	15.0 ± 7.8 (11)	13.3	0.525
	135 (n=17)	27.0 ± 1.8 (9)	15.4 ± 8.6 (8)	43.0	0.037
	180 (n=10)	25.7 ± 6.4 (3)	13.3 ± 7.2 (7)	48.2	0.033
Fiber Density (Mean fibers/mm ± SD) (n)	0 (n=25)	17.1 ± 4.9 (10)	11.7 ± 4.2 (15)	31.6	0.007
	45 (n=25)	16.9 ± 7.1 (11)	12.7 ± 3.5 (14)	24.9	0.066
	90 (n=21)	17.9 ± 6.7 (10)	13.4 ± 4.7 (11)	25.1	0.087
	135 (n=17)	20.6 ± 4.0 (9)	10.2 ± 4.2 (8)	50.5	<0.001
	180 (n=10)	14.0 ± 3.3 (3)	9.0 ± 3.1 (7)	35.7	0.052
Percent Smooth Muscle (Mean % ± SD) (n)	0 (n=25)	24.0 ± 8.2 (10)	19.7 ± 6.3 (14)	17.9	0.154
	45 (n=25)	25.4 ± 7.2 (11)	20.4 ± 6.8 (13)	19.7	0.089
	90 (n=21)	26.6 ± 8.8 (10)	17.2 ± 6.9 (11)	35.3	0.014
	135 (n=17)	26.7 ± 6.7 (9)	17.0 ± 5.5 (8)	36.3	0.006
	180 (n=10)	28.3 ± 0.6 (3)	14.1 ± 5.3 (7)	50.2	0.002

Table 3
Striated muscle layer thickness, fiber counts and fiber density

	Orientation within Urethra	20–39 years	70–89 years	Percent Difference	P value
Thickness (Mean mm ± SD) (n)	0 (n=28)	3.44 ± 1.1 (12)	2.62 ± 1.3 (16)	23.8	0.092
	45 (n=28)	3.75 ± 1.0 (12)	2.84 ± 1.8 (16)	24.3	0.128
	90 (n=28)	3.41 ± 1.4 (12)	1.63 ± 1.7 (16)	52.2	0.006
	135 (n=28)	1.86 ± 1.2 (12)	0.52 ± 1.0 (16)	72.0	0.004
	180 (n=28)	0.76 ± 0.8 (12)	0.02 ± 0.1 (16)	97.4	0.001
Fiber Count (mean ± SD) (n)	0 (n=28)	35.1 ± 16.3 (12)	17.7 ± 11.1 (16)	49.6	0.002
	45 (n=28)	36.3 ± 13.5 (12)	17.3 ± 11.7 (16)	52.3	<0.001
	90 (n=28)	26.5 ± 12.8 (12)	6.6 ± 6.4 (16)	75.1	<0.001
	135 (n=28)	16.3 ± 11.8 (12)	2.4 ± 5.3 (16)	85.3	<0.001
	180 (n=28)	6.8 ± 7.8 (12)	0.1 ± 0.5 (16)	98.5	0.002
Fiber Density (Mean fibers/mm ± SD) (n)	0 (n=28)	10.0 ± 3.8 (12)	7.1 ± 2.3 (16)	29.0	0.019 (28)
	45 (n=28)	9.8 ± 3.2 (12)	6.4 ± 2.6 (15)	34.7	0.005 (27)
	90 (n=28)	7.7 ± 2.7 (12)	4.3 ± 1.5 (11)	44.2	0.001 (23)
	135 (n=28)	9.4 ± 1.1 (10)	5.0 ± 2.8 (4)	46.8	0.050 (14)
	180 (n=28)	12.5 ± 10.3 (9)	8.0 (1)	36.0	-

Note: The number of specimens for density changes because some specimens had no visible muscle layer to measure and division by zero is not possible.

Table 4
Correlations between loss of urethral striated and circular muscle

	0 degrees	45 degrees	90 degrees	135 degrees	180 degrees
Muscle layer thicknesses	r=-0.252, p=0.205	r=-0.036, p=0.857	r=-0.04, p=0.843	r=-0.053, p=0.792	r=-0.174, p=0.385
Fiber counts	r=-0.095, p=0.637	r=0.059, p=0.767	r=-0.014, p=0.945	r=0.053, p=0.792	r=-0.329, p=0.093
Fiber densities	r=-0.423, p=0.028	r=0.516, p=0.007	r=0.01 p=0.637	r=0.473, p=0.075	r=-0.091, p=0.803