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Alteration of vaginal elastin metabolism in women with pelvic organ prolapse

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Abstract

Objective—This study's goal was to compare elastin metabolism in the vagina of women with and without pelvic organ prolapse and to define the regulation of this process by hormone therapy (HT).

Methods—Eighty-seven histologically confirmed full-thickness vaginal biopsies were procured from study subjects at time of surgery. Premenopausal women with no prolapse served as controls. Women with prolapse were divided into 3 groups – premenopausal, postmenopausal not on HT and postmenopausal on HT. The epithelium was excised leaving the subepithelium, muscularis and adventitia for analyses. The elastin precursor, tropoelastin, was measured by immunoblotting and mature elastin protein via a desmosine crosslink radioimmunoassay. Matrix metalloproteinases (MMPs) -2, -9 were quantitated by gelatin zymography. Data were analyzed using Kruskal-Wallis test and post-hoc analysis using the Mann-Whitney U test.

Results—Tropoelastin (432%), mature elastin (55%), proMMP-9 (90%) and active MMP-9 (106%) were increased in women with prolapse relative to controls while active MMP-2 (41%) was decreased. Comparison of tropoelastin and mature elastin values obtained from the same women showed them to be independently regulated ($r = 0.19$). Interestingly, the highest amount of both proteins occurred in postmenopausal patients not on HT.

Conclusion—Elastin metabolism is altered in the vagina of women with prolapse relative to controls suggesting that vaginal tissue is rapidly remodeling in response to mechanical stretch. The finding that elastin levels are highest in the absence of hormones warrants further investigation.

Introduction

Over the last two decades, the number of surgeries to treat pelvic organ prolapse (POP) in older women has increased and the demand for medical attention addressing POP is estimated to increase by 45% in the next 30 years (1,2). This has generated a significant social and economic burden on society (3). An understanding of factors that lead to both the

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onset and progression of POP will not only help improve treatment, but may also contribute to the development of prevention strategies.

Elastin is an extracellular matrix protein that confers the biomechanical properties of extension and recoil to tissues. Physiologically, elastin is one of the most stable proteins in the body over an individual's lifetime (4). However, the female reproductive tract is unique in that elastin does turnover throughout the reproductive stage of the female life cycle. For example, in the uterus, elastin content dramatically increases during pregnancy and drops back to pre-gravid level post-partum (5). Although elastin likely also turns over in the vagina, the mechanism by which it does this is unknown (6). Elastin plays a critical role in providing support to the pelvic organs as demonstrated in mouse elastinopathy models (7) and women with genetic disorders of elastin metabolism, such as cutis laxa, have increased risk of developing POP (8).

Mature, insoluble elastin is degraded by a limited number of proteinases referred to as elastases, two of which are matrix metalloproteinases (MMPs)-2, -9 (4). The end result of MMP-mediated degradation of elastin is a change in tissue structure leading to altered biomechanical properties and presumably, altered supportive function (9).

To date, little is known of the quantitative differences in elastin in vagina of women with and without prolapse. In this study, we hypothesized that vaginal elastin homeostasis is disrupted in women with prolapse secondary to accelerated tissue remodeling associated with increased loading (resulting from increased stretch) of the vagina in women with POP. We therefore sought to compare vaginal elastin metabolism in women with and without POP. As hormones have previously been shown to regulate elastin metabolism in pelvic connective tissues (10), we also sought to determine the impact of menopausal status on elastin content and degradation. To this end, we measured the amount of the mature elastin, its precursor tropoelastin, and the elastin degrading enzymes, MMPs -2, and -9, in full thickness vaginal biopsies of women with and without POP divided according to their menopausal status.

Material and Methods

Human subjects and tissue procurement

Following the approval by the Institutional Review Board of Magee-Womens Hospital and informed consent, 87 biopsies of full-thickness vagina were procured from the upper 1/3 of the vagina in women undergoing surgery in the University Gynecology and gynecology private practices at Magee-Womens Hospital of the University of Pittsburgh between March of 2004 and June of 2007. Our rationale for choosing the apex was to standardize the biopsies and to use a part of the vagina that in most cases would be relatively shielded from confounding secondary effects of prolapse; particularly, ulceration and thickening due to contact with undergarments. The eligible patients were divided into 4 groups strictly following specific criteria developed in our lab as described in details in our previous publication (11). Briefly, the control group (N=20) consisted of premenopausal women aged > 24 years and < 55 years without prolapse (defined as < stage II) or incontinence, and regular periods over the preceding 12 months. The women with prolapse were divided according to menopausal status. Similar to controls, premenopausal women with prolapse (N=20) were aged > 24 years and < 55 years and regular periods over the preceding 12 months but had $\geq$ stage II prolapse. Postmenopausal status was defined as no menses over the previous 12 months. Postmenopausal women with prolapse were $\geq$ 50 years with $\geq$ stage II prolapse and not on hormone therapy (N=29) or currently on hormone therapy (N=18) for > 12 months. Women with urinary incontinence (stress, urge or mixed type) in addition to prolapse were included. Exclusion criteria were as previously described (11).

The specimens were immediately processed by a trained technician. A small portion was imbedded for histological confirmation that a full thickness biopsy had been obtained and immunofluorescence (see below for a description of this assay). The remaining tissue was dissected under a dissecting microscope and the epithelium was excised leaving the subepithelium, muscularis and adventitia for biochemical analyses. This tissue was then frozen in liquid nitrogen and stored at -80°C until use.

Subject demographic variables were collected by certified staff onto a data collection sheet and entered into a study database. Variables included age, race, ethnicity, body mass index (BMI), gravidity and parity, POP Q points, presence of urinary incontinence, type of menopause (surgical or natural), date of last menstrual period, stage of menstrual cycle, previous hysterectomy, previous surgery for incontinence or prolapse, type of hormone therapy (estrogen or estrogen and progesterone), and smoking status (never, current, former).

Western immunoblotting

The protein expression of tropoelastin in the tissue extracts of the biopsies was quantitated via Western immunoblotting following a protocol previously detailed (12). In brief, tissue proteins were extracted in the buffer consisting of 50mmol/L Tris (hydroxymethyl) aminomethane, pH 7.5, 150mmol/L sodium chloride, 0.1% Tween-20, 1mmol/L phenylmethylsulfonyl fluoride (PMSF), 10mmol/L ethylenediaminetetraacetic acid (EDTA) and protease inhibitor cocktail (Pierce, Rockford, IL). Thirty-five micrograms of proteins of total tissue extracts were separated by 10% SDS-gel electrophoresis under reducing conditions. The primary antibody was rabbit anti-human tropoelastin (against amino acids 431-730, 1:500, Santa Cruz Biotechnology, Inc., Santa Cruz, CA) and the secondary antibody was goat anti-rabbit IgG (Santa Cruz). Purified human lung tropoelastin (EMD Biosciences Inc, San Diego, CA) was employed as positive control and BSA (Sigma-Aldrich) as negative control. Experiments were performed at least in duplicate.

Measurement of desmosine

Mature elastin content was measured via a desmosine crosslink radioimmunoassay (crosslinks / total protein). The assay was carried in collaboration with Dr. Barry Starcher as previously described (13).

Immunofluorescent staining

A limitation of the above biochemical assays is their failure to afford a site-specific analysis of elastin which can be from both a vascular and nonvascular source. Indeed, conceivably, an alteration in total elastin could simply reflect a change in vascularization of a tissue. Thus, we performed an immunofluorescent analysis of elastin and smooth muscle myosin in 40 samples, with 10 samples randomly selected from each group. We followed a protocol developed in our lab as described in detail previously (11,14). Briefly, sections were simultaneously incubated with primary antibodies for both elastin (guinea pig 1:1000, Dako, Carpinteria, CA) and myosin (mouse antihuman 1:1000, Novotec, Leno, France) followed by the secondary antibodies (donkey anti-guinea pig Cy3 from Jackson Immuno and donkey anti-mouse 488 from Molecular Probes, respectively). Two individuals blinded to the identities of the specimens analyzed the samples. Eight sites were randomly selected within each sample. Blood vessels were identified by their characteristic circular shape and the simultaneous labeling with both myosin and elastin. Nonvascular elastin was counted as elastin labeling in areas not simultaneously labeling with myosin. The average area of elastin in blood vessels versus connective tissue was then compared using the quantitative analysis software Metamorph (Molecular Devices).

Gelatin zymography

The expression of elastin degrading enzymes, MMPs-2 and -9, in 20 micrograms of tissue proteins per sample was concurrently evaluated via gelatin zymography. The experiment was carried out in duplicate with a pre-cast 10% gelatin gel system (Invitrogen, Carlsbad, CA) by an individual blinded to sample group identifiers following a protocol detailed in our previous publication (11).

Statistical analysis

The primary outcome of the study was to detect a difference in the amount of immature elastin (tropoelastin) or mature elastin (desmosine crosslinks) in women with and without prolapse divided by menopausal status. From a previous study, the median (range) of elastin detected by immunofluorescence was 986 (84-2099), 859 (261- 2702), and 1321 (148-1797) voxel intensity per area squared for premenopausal women, post-menopausal women not on HT, and postmenopausal women on HT, respectively (14). Based on these data, 15 women were required in each menopausal status group in order to detect a difference of at least 50% in the amount of immature or mature elastin, and at least a 25% difference between women with and without prolapse for a power of 80% at the 0.05 significance level.

All statistical analyses in this study were performed by a statistician (L.A.M) who was blinded to the study aims, using SPSS 17.0.1 statistical software (SPSS Inc., Chicago, IL). Statistical tests were evaluated at the 0.05 significance level. Normality of the data was assessed using the Shapiro-Wilk test which indicated that while the MMP data followed a normal distribution, the elastin data did not. Therefore, elastin data were analyzed using Mann-Whitney U and Kruskal-Wallis tests, where appropriate and post-hoc comparisons were made using Mann-Whitney U tests evaluated at the 0.017 significance level. Pearson's correlation coefficient (r) was used to assess the linear association between desmosine and tropoelastin. MMP data were analyzed using Student's t-tests and analysis of variance, where appropriate, and post-hoc comparisons were made using Dunnett's t-tests. Multivariable linear regression was used to evaluate the impact of age, body mass index, parity, and stage of prolapse on elastin and MMP levels. Models were developed using a forward stepwise selection procedure.

Results

In the present study, a total of the 87 patients were recruited, of whom 90% were Hispanic, 10% were non-Hispanic (8% African American, 1% Asian and 1% American Indian). In regards to the surgical procedures, 38% of the subjects underwent an abdominal hysterectomy, 32% a vaginal hysterectomy. Twenty-three percent of the subjects had a colporrhaphy, 32% a vaginal vault suspension (native tissue or mesh procedure), and 11% an obliterative procedure. Among the women with pelvic organ prolapse (POP, N=67), 40% had POP without urinary incontinence (UI) while 60% had both. Of the 18 patients on hormone therapy (HT), 89% were on estrogen only, and the remaining 11% were on estrogen plus a progestin. The postmenopausal women were older (median age: 70, $P<0.001$) and had higher parity (median parity: 3, $P=0.033$). However, premenopausal women with and without POP had similar age, gravidity, parity and body mass index (BMI). These parameters were also similar in the postmenopausal women with POP on and off HT. Among the women with POP, the median stage of prolapse was stage III (See Supplemental Digital Content 1).

Western immunoblotting was used to analyze the expression of the elastin precursor – tropoelastin in vaginal tissues of women with and without POP. We first evaluated the impact of stage of menstrual cycle on tropoelastin expression in the premenopausal women

regardless of POP status and found that the expression of the protein was lower in the follicular phase than in the luteal phase ($P = 0.03$), however, after controlling for prolapse status, menstrual phase was no longer a significant factor affecting the expression of tropoelastin (P s=0.08, 0.36, respectively. See Supplemental Digital Content 2). With regards to the possible impact of urinary incontinence (UI), we found no difference in tropoelastin expression in women with prolapse with and without UI ($P=0.32$, see Supplemental Digital Content 3).

As shown in Figure 1 (panel A) and Figure 2, relative to controls, the amount of tropoelastin was considerably increased in women with POP (by 432%, $P<0.001$). Among women with prolapse, tropoelastin was higher in all groups relative to controls; however, the percent increase in tropoelastin was the highest in menopausal women with POP not on HT (754% increase, $P<0.001$). Hormones suppressed tropoelastin expression in both postmenopausal women with prolapse and premenopausal women with prolapse but not to the level of controls (Table 1).

Since a change of the precursor form of a protein does not necessarily correlate with a change of its mature form, we concurrently measured the amount of mature elastin in vaginal tissues via a desmosine crosslink radioimmunoassay. As demonstrated in Figure 1 (panel B) and Table 2, the overall pattern of change of desmosine (mature elastin) was similar to that of tropoelastin. Desmosine was significantly increased in women with POP (not divided by hormonal status) compared to controls (55% increase, $P=0.019$). Pair-wise comparisons between prolapse groups and controls, however, showed that desmosine was increased only in postmenopausal women with POP not on HT ($P=0.011$). In premenopausal and postmenopausal women on hormones, there was no difference in the amount of desmosine in women with POP relative to controls ($P=0.19$, 0.07, respectively). Interestingly, further analysis of 52 women in the study in whom both tropoelastin and desmosine were measured showed an overall lack of correlation between tropoelastin and desmosine levels ($r = 0.19$, $P = 0.18$) suggesting a role of tropoelastin beyond simply acting as a precursor to the mature protein. Although postmenopausal women were older and had higher parity, we found, using multivariable regression modeling, that age and parity were not independent predictors of the amount of tropoelastin or desmosine. Postmenopausal status was identified as an independent predictor for tropoelastin expression but not for desmosine.

One may argue that the vascularity of prolapsed tissue could be increased due to increased loading of the vagina as may occur with advanced prolapse. Thus, the amount of elastin in blood vessels in prolapsed vs control vagina was compared using a quantitative immunofluorescence. Blood vessels were easily identified by their characteristic tubular shape and simultaneously labeling with antibodies to both smooth muscle myosin and elastin. In contrast, the dense connective tissue of the vagina was amorphous and labeled with anti-elastin antibody alone. By this method, we found no overall difference in vascular elastin of prolapsed tissues relative to controls or according to menopausal status ($P=0.28$, Figure 3) indicating that the overall changes in the amount of tropoelastin and elastin in vagina were not contributed by the change of vascularity.

To determine whether the change of elastin was associated with altered elastin degradation, we assayed the amount of both the proenzyme and active forms of the key elastin degraders, matrix metalloproteinases – 2 and -9 (MMP-2 and MMP-9), via quantitative gelatin zymography. As shown in Figure 1 (panels C, D), quantitative analysis of zymograms showed that, mirrored with the increases in tropoelastin and desmosine, both proMMP-9 and active MMP-9 were increased in prolapsed vaginal tissues relative to controls (90%, 106%, P s<0.001, respectively). In contrast, the amount of active MMP-2 was decreased (41%

decrease, $P<0.001$) with an unchanged quantity of proMMP-2 ($P=0.43$). The altered expression of active MMP-2 ($P=0.06$), proMMP-9 ($P=0.29$) and active MMP-9 ($P=0.65$) among women with POP did not vary according to hormonal status (Table 2, Figure 4). Using multivariable regression modeling, age was not found to be an independent predictor of the amount of MMP-2 or MMP-9 (both proenzyme and active forms). However, the expression of active MMP-2 was significantly inversely related to the presence of prolapse ($P<0.001$).

Discussion

The vagina and its supportive connective tissues provide one of the primary mechanisms of support to the pelvic organs. Functional failure of this support apparatus results in the descent of the pelvic organs into the vagina and the development of pelvic organ prolapse (POP) (15). Together with interstitial collagens, elastin comprises one of the key important structural constituents in the vaginal connective tissues (7). The most important findings of this study were that, relative to controls, both immature and mature elastin and the elastin degrading enzyme MMP-9 were increased in the vagina of women with POP. In contrast, active MMP-2 was decreased. Interestingly, although tropoelastin and desmosine behaved independently of each other in the prolapsed vagina, the highest levels of both proteins occurred in postmenopausal women not on hormone therapy.

Clinically, chronic conditions that increase intra-abdominal pressures are associated the development and progression of POP, including obesity, chronic cough and occupations associated with heavy lifting, etc. (16). It is likely that the increased intra-abdominal pressure is directly transmitted onto the vaginal wall (17) increasing its mechanical load and causing tissue stretch (18). The mechanical load has been shown to alter the synthesis of extracellular matrix components including elastin. At mRNA and protein levels, mechanical stretch up-regulates the elastin precursor - tropoelastin in rat lung cells and in smooth muscle cells derived from rat aorta (19,20). In this study, the patients with POP had advanced prolapse (median stage: III). In this way, our finding that the amount of elastin was increased with advanced prolapse is not surprising. Indeed it is likely that this represents a secondary response to prolapse such that as prolapse progresses, the vagina remodels to accommodate the altered loads. Together with our previous data demonstrating an increase in collagen III in the prolapsed vagina (11), the present data provide additional evidence to the likelihood that, with advancing prolapse, the structural components of connective tissue increase because the tissue is rapidly remodeling under the biomechanical stresses of prolapse. Importantly, in our study, we excluded the possibility that the increase of elastin in the prolapsed vagina was attributed to an increase of elastin in blood vessels associated with the prolapse, consistent with the findings in a recent study demonstrating that the number of blood vessels was similar in prolapsed vs normal vaginas (21).

Prior publications on elastin expression in human prolapsed vagina are limited and somewhat contradictory. Karam *et al.* (22) used immunohistochemistry to compare elastin expression in women with and without prolapse. Specifically within the vaginal muscularis of 33 postmenopausal women with vaginal prolapse vs 10 postmenopausal control women, the authors found that elastin expression in the prolapsed vagina was decreased. However, the authors used a technique which fails to distinguish tropoelastin from mature elastin. In addition, the control group was comprised of patients with bladder cancer which may alter elastin expression and menopausal status was not strictly defined, for example, the prolapse group contained a higher percentage of patients on hormone therapy (HT) (51% vs 30% of control, $P = 0.14$). In a second study employing a similar technique to analyze elastin, Lin *et al.* (23) found that the elastin expression in women with vaginal wall prolapse was significantly increased relative to controls (by Student t-test); however, the amount of elastin

did not vary between prolapse vs controls by multivariate analysis controlling for age, menopausal status, parity and vaginal childbirth. The absence of significance may be due to the failure to use an antibody that distinguishes between immature (tropoelastin) and mature elastin and to the small sample size.

We found that, in spite of the lack of correlation between tropoelastin and elastin in the vaginal wall, the amount of both proteins was the highest in postmenopausal women with POP not on hormone therapy. Only tropoelastin appeared to be specifically regulated by hormones. Although the data are difficult to reconcile, it is possible that tropoelastin is elevated to provide a steady pool of precursor protein for synthesis of the mature form and or may be independently increased in response to mechanical stretch. In women with prolapse, in the presence of hormones, mature elastin levels remain the same as controls. In the absence of hormones, however, remodeling appears to be accelerated with a simultaneous increase in the mature form of elastin. To date, there has been limited research defining the interplay between mechanical stretch and hormones on the expression of elastin in human tissues. Large arteries, like the aorta, in vivo are physiologically under high mechanical loads. With several different animal models, it has been demonstrated that supplementation of estrogen with or without progesterone, decreases the content / deposition of elastin in the aortic connective tissues (24-26). It is possible that the inhibition of elastin by hormones in the prolapsed vaginal connective tissues may be through a similar mechanism. However, a detailed mechanism remains to be elucidated.

Because the destruction of elastin fibers may result from an increase in the elastin degrading enzymes, MMP-2 and MMP-9 (27), it is reasonable to assume that, with increased amounts of elastin in the vagina of women with advanced POP in this study, both MMP-2 and MMP-9 would be decreased correspondingly. However, we found that in the prolapsed tissue, relative to control tissue, only active MMP-2 was decreased while both proMMP-9 and active MMP-9 were increased. Furthermore, the changes in the levels of MMP-2 and MMP-9 in the prolapsed vaginal tissue occurred independently of hormonal status. Importantly, the decrease in active MMP-2 was associated with the presence of prolapse while the increase in MMP-9 was not. Although the mechanism is still unclear, previous literature has supported such an inverse relationship between MMP-2 and MMP-9 in the presence of increasing mechanical loads (28,29). Thus, similar to these systems, it is likely that in the vagina the inverse relationship between MMP-2 and MMP-9 is being driven by the presence of increasing mechanical loads.

While it is known that both MMP-2 and MMP-9 are actively involved in degrading mature elastin in vitro and in vivo (30,31), it is not clear which one plays a more prominent role in elastin digestion. It has been proposed that a concurrence of both MMP-2 and MMP-9 is required in the initiation and progression of elastin degradation (32) since disruption of either can lead to a resistance to elastin digestion in vivo (33). The events leading to the altered synchronization of MMP-2 and MMP-9 in the prolapsed vagina are not clear from the results of this study; however, it is likely that the disruption in the balance between elastin synthesis and degradation is an indicator of an unstable tissue that is remodeling with prolapse progression.

The strengths of our study include the large sample size; the standardized fashion in which all tissue samples were collected and processed; the multiple parameters used to analyze elastin metabolism in parallel; and the blinding of the individuals performing the assays to the sample identities. The shortcomings include the absence of a proper control group for postmenopausal women with POP. However, as it is rare for postmenopausal women to undergo a hysterectomy for a benign indication other than prolapse, it is virtually impossible to collect tissue from this group of women. We have overcome this shortcoming by first

analyzing the impact of prolapse by comparing premenopausal women with and without prolapse and then analyzing the impact of hormones by comparing women with prolapse on and off HT.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.

The amount of tropoelastin and desmosine and MMPs -2, -9 in vaginal tissue in controls and all women with prolapse combined independent of menopausal status. MMP: matrix metalloproteinase. Panels A and B: P -value from Mann-Whitney U test; Panels C and D: P -value from Student t-test.

Figure 2.

A representative Western blot of expression of tropoelastin in different groups of women with prolapse. Control: premenopausal women with no prolapse. HT: hormone therapy.

Figure 3.

Comparison of expression of elastin in blood vessels in different groups of women with prolapse. Control: premenopausal women with no prolapse. HT: hormone therapy. Overall P value was from ANOVA.

Figure 4.

A representative gelatin gel of the various forms of MMP-2 and MMP-9 in different groups of women with prolapse. MMP: matrix metalloproteinase. Control: premenopausal women with no prolapse. HT: hormone therapy.

Table 1
Expression of tropoelastin and desmosine in vaginal tissue in controls and all women with prolapse divided according to menopause status

	N	Median	Overall <i>P</i>	Pos hoc <i>P</i> [†]
		(Interquartile range)		
Tropoelastin (arbitrary unit)			< 0.001 [*]	
Control	15	0.84 (0.74, 1.43)	0.01 [§]	
^a Premenopausal	14	1.73 (1.06, 2.28)	< 0.001 (a vs b)	0.001
^b Postmenopausal, no HT	14	7.17 (5.40, 13.75)	< 0.001 (a vs c)	< 0.001
^c Postmenopausal, HT	15	4.47 (2.96, 7.76)	0.004 (b vs c)	< 0.001
Desmosine (pmol/mg)			0.10 [*]	
Control	15	386 (319, 609)		
Premenopausal	18	533 (319, 1160)		0.19
Postmenopausal, no HT	28	635 (464, 898)		0.011
Postmenopausal, HT	17	511 (412, 972)		0.07

HT: hormone therapy;

^{*} overall *P*-value from Kruskal-Wallis test;

[§] overall *P*-value of difference among groups of prolapse;

[†] *P*-value from post-hoc analysis using Mann-Whitney U test comparing control group and the groups of prolapse, *P*-values were evaluated at the 0.017 significance level, using the Bonferroni method.

Table 2
Expression of MMP-2 and MMP-9 in vaginal tissue in controls and all women with prolapse divided according to menopause status

	N	Expression [‡]	Overall P	Pos hoc P [‡]
ProMMP-2			0.79 [*]	
Control	19	0.39 ± 0.11		
Premenopausal	15	0.42 ± 0.07	0.93 [§]	
Postmenopausal, no HT	17	0.41 ± 0.08		
Postmenopausal, HT	16	0.41 ± 0.07		
Active MMP-2			< 0.001 [*]	
Control	19	0.63 ± 0.15		
Premenopausal	15	0.39 ± 0.07	0.06 [§]	<0.001
Postmenopausal, no HT	17	0.36 ± 0.05		< 0.001
Postmenopausal, HT	16	0.35 ± 0.04		< 0.001
ProMMP-9			<0.001 [*]	
Control	19	0.31 ± 0.06		
Premenopausal	15	0.60 ± 0.12	0.29 [§]	<0.001
Postmenopausal, no HT	17	0.56 ± 0.08		<0.001
Postmenopausal, HT	16	0.61 ± 0.09		<0.001
Active MMP-9			<0.001 [*]	
Control	19	0.35 ± 0.06		
Premenopausal	15	0.72 ± 0.14	0.65 [§]	<0.001
Postmenopausal, no HT	17	0.70 ± 0.13		<0.001
Postmenopausal, HT	16	0.74 ± 0.16		<0.001

MMP: matrix metalloproteinase; HT: hormone therapy;

[‡] data presented as mean ± standard deviation;

^{*} overall P-value from ANOVA;

[§] overall P-value of difference among groups of prolapse from ANOVA;

[‡] P-value from post-hoc Dunnett's comparing control group and the groups of prolapse.