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ORIGINAL ARTICLE

Mechanical properties of pelvic soft tissue of young women and impact of aging

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Abstract
Introduction and hypothesis The female pelvic floor is a complex network of ligaments and muscles whose mechanical properties have not been completely understood. The goal of this study is to understand the biomechanical properties of the pelvic floor tissues of young women and the impact of aging.
Methods Biomechanical uniaxial tension tests were performed on pelvic floor tissues (ligaments and organs) of six young female cadavers (average 29 years old). Results have been analyzed in order to define the characteristics of the mechanical properties of young pelvic soft tissues. Results have been compared with those in the literature in order to understand the similarities and discrepancies between young and old patients.
Results Damageable, nonlinear elastic biomechanical behavior is observed. The variation in stiffness among the pelvic floor organs could be shown. Ligaments and the vaginal wall are the most rigid organs, whereas the rectum and bladder tend to be less rigid (approximately two times less rigid for small deformations and three times less rigid for large deformations). This study shows that ligaments and the vaginal wall of young women have similar mechanical behavior while those of older women differ. Furthermore, young women's tissues differ slightly from older women's tissues.
Conclusions Results show that aging and possibly diverse "trauma" have an impact on modifying the mechanical behavior of pelvic floor tissues. Over time pelvic floor ligaments and vaginal tissues will differentiate and acquire different mechanical behavior, as seen within the literature in older cadavers.

Keywords Biomechanics · Pelvic floor · Pelvic organ prolapse · Aging

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Introduction

Pelvic organ prolapse (POP) is a disease with which many women are confronted at some point during their life [1–4]. Around 50 % of parous women suffer from some degree of prolapse. Even though physiopathology is not clearly identified, pelvic organs play an important role in the suspension of the complete system [5–7]. The organs' biomechanical properties are critical to understanding the static of the pelvic system and to further developing accurate surgical techniques and physiological prosthetic materials. Several studies have looked at the biomechanical properties of pelvic organs and ligaments [5–10], their properties are well understood. The biomechanical properties of the pelvic organs have been compared in order to propose a classification of tissue compliance.

All tissues have nonlinear, visco-elastic mechanical behavior [5–9]. The bladder is the most stretchable organ of the pelvic floor, followed by the rectum and vagina [5]. The utero-sacral ligament appears to be the most rigid entity of the pelvic floor [6]. However, to our knowledge, no complete comparison of the different organs and ligaments at different ages has been performed. This study compared the biomechanical properties of different components of the pelvic floor at different ages.

Few biomechanical tests have been performed on premenopausal cadavers, which could lead to a better understanding of mechanical properties [10]. Some interesting work has been done on the mechanical behavior of prolapsed vaginas [11] showing a stiffening of pathological tissues. With our global study on different age classes we will try to enrich and understand these results and mechanisms.

This study compares the biomechanical properties of the vagina, bladder, rectum, and pelvic ligaments of young cadavers with published data of older cadavers. Biomechanical testing was performed using cyclic and noncyclic uniaxial tension tests on pelvic floor tissues harvested from fresh young female cadavers. To be able to compare these data with those of former publications, an validated protocol proposed by Rubod et al. was used [12].

Materials and methods

Tissue samples were obtained from 24 fresh female cadavers with no history of pelvic reconstructive surgery and with no clinically relevant POP, which was excluded by gynecological examination and the use of a Pozzi clamp to pull on the cervix. Cadavers were frozen for conservation and unfrozen just before the dissection.

Six of these cadavers were of young women (mean 29 years old; 16 to 40 years old), which were used to perform tests on organs (bladder, vagina, and rectum) and uterine ligaments. These pelvic floor samples were provided to us by the Universität Klinikum Eppendorf (UKE) in accordance with German laws. Five were older women (mean 75 years range), which were used to perform tests on organs (bladder, vagina, and rectum). Thirteen were older women (mean 83.5±12.3 (range, 61–100) years), which were used to perform tests on uterine ligaments.

The cadavers of older women were dissected and studied at the Anatomical Institute of the Faculty of Medicine of the CHRU Lille, France. Each patient had agreed before death to have their cadaver used for medical purposes according to the legislation in force. Fundamental research not involving living patients does not need Institutional Board Approval.

The results of the young cadavers are new data never published whereas those from the older cadavers have already been identified by Rubod et al. and Rivaux et al. [5, 6]

Having no uniaxial tension machine available in the operating room in the morgue, a portative uniaxial tension machine was developed to allow immediate mechanical testing. This is composed of a linear actuator and a 100 N force gage. The control of the linear actuator and data acquisition were performed using LabView®.

The surgeon checked for abnormalities or tissue damage. The organs were identified: vagina, bladder, rectum, utero-sacral ligaments, round ligaments, and the broad ligament. If the organs were intact and of sufficient size, samples were excised in the longitudinal axis in accordance with the protocol proposed by Rubod et al. [12]. Samples were then stored in saline solution between punching and testing. The thickness of the punched tissue was measured with a caliper. The width of the samples is defined by the punch, meaning that the initial cross-section (S_0), of samples in the center zone is known.

Samples were placed and clamped between the grips of the uniaxial machine, without stretching the tissue. Once clamped and extended to a distance where the sample is no longer compressed, the length was measured.

In accordance with the cited protocol, the stretching was driven at 1 mm/s. Different stretching schemes were performed. Either the sample was stretched directly until rupture; otherwise, subsequent cycles between 0 N force and a defined stretch (10 %, 20 %, etc.) were conducted until tissue rupture. During the test, displacement was recorded thanks to a video extensometer and force was recorded thanks to a force gage.

At that point, the displacement was in millimeters and the force in Newtons. Strain and nominal stress (MPa) are required to analyze and compare the data.

Strain (ε) is defined as: $\frac{displacement}{initial\ length}\frac{(mm)}{(mm)}$ while nominal stress (σ) is defined as: $\frac{Force}{initial\ crosssection\ S0}\frac{(N)}{(mm^2)}$

Regarding biomechanical testing, only the part within a physiological range is of interest and studied, even if the test results in rupture of the tissue (Fig. 1). This range is defined as 100 % of elongation (arbitrary).

In order to perform a comparative statistical analysis of experimental data, a behavior model had to be defined taking into account nonlinear elasticity phenomena during major deformation. The model used is a Mooney–Rivlin type model [5]. Two parameters, C0 and C1, characterizing the biomechanical behavior, were identified on curves using a least square roots method. C0 characterizes the asymptotic mechanical behavior at small deformations and C1 at large deformations (Fig. 1).

Statistical calculations have been carried out using the Minitab 15 statistical software. As data are not normally distributed, a Mood's median test has been used. The *p* values of <0.05 were considered to be statistically significant.

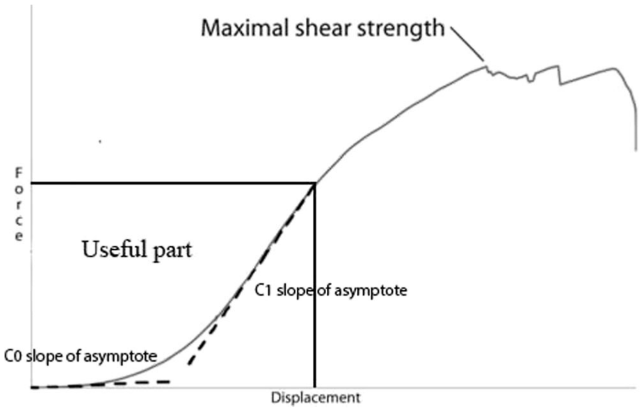


Fig. 1 Example of a nominal-stress/strain curve of uniaxial load

Results

Tissue samples from the utero-sacral, broad, round ligaments, vagina, bladder, and rectum were collected from 24 fresh young and older female cadavers without prolapses. The mean age was 29 years old (range, 16–40 years old) for the group qualified as "young" and 75 years old and 83.5 years old for respectively the "old" organ group and the "old" ligament group.

Two hundred and seventy-seven samples were harvested and studied all together, i.e., 30 utero-sacral ligaments from old cadavers, 11 utero-sacral ligaments from young cadavers, 45 round ligaments from old cadavers, 11 round ligaments from young cadavers, 33 broad ligaments from old cadavers, 4 broad ligaments from young cadavers, 32 vaginas from old cadavers, 21 vaginas from young cadavers, 36 bladders from old cadavers, 18 bladders from old cadavers, 30 rectums from old cadavers and 6 rectums from young cadavers.

Two different tests were performed, the first was cyclic, with load and unload phases increasing the maximum stretch level at each cycle, and the second was pure stretching up to rupture. In order to study the nonlinear elastic behavior, neglecting viscosity and damage, we focused on the monotonic load up to rupture, as proposed by Clay et al. [11]. This technique allows us to compare the mechanical behavior of pelvic floor tissues in their initial state and to perform statistical calculations.

According to the method described above C0 and C1 were calculated for each sample. As values were not normally distributed the median and interquartile range (Q3-Q1) were compared.

We will first look at the young patients because those are new data.

C0 and C1 are used as defining parameters of the mechanical behavior of tissues (Fig. 2). Statistical tests were run to define if the different organs had various mechanical behaviors.

No statistical difference was found among the different groups of ligaments collected from young pelvic tissues; thus, the results will not be differentiated (Table 1). Nor was any difference seen according to vaginal explantation site (anterior or posterior wall; upper or lower vagina).

For young cadavers no statistical difference was noted between vagina and ligaments for both small and large strain (respectively $p=0.131$ and $p=0.365$).

Again for the young cadavers no statistical difference was noted between rectum and the other organs, but a strong trend is observed for the large deformations (for C1 $p=0.08$ with vagina, $p=0.059$ with bladder and $p=0.082$ with ligaments; for C0 $p=0.41$, $p=0.059$, $p=0.139$ respectively).

The bladder shows a statistical difference with vagina and ligaments for both small and large deformations (vagina $p=0.002$ and $p<0.001$; ligaments $p=0.017$ and $p<0.001$). Although the vagina and the ligaments are more rigid than the bladder, the rectum tends to lie in between the two groups. Slightly different mechanical behaviors of ligaments from left and right side of the same patient were observed; however, this was not statistically significant.

We now compare the C0 and C1 between age groups (Table 1). For the uterine ligaments and vagina there is a statistical difference between young and old tissues for C0 and C1, except for the broad ligament. For the bladder and rectum there is a significant statistical difference only for C0.

Discussion

In this study, the biomechanical properties of the pelvic floor tissue of young cadavers were studied and compared with those of older female cadavers in order to better understand the pelvic static, the impact of aging, and the mechanical contribution of tissue stiffness.

It is challenging to obtain young deceased tissue; therefore, the number of tests performed was limited. It has also been almost impossible to obtain information on the parity of women or any other medical history related to the patient (cause of death, for example), which obliged us to direct our discussion at the age factor only. Because of these limitations some absolute comparisons are hard to perform; nonetheless, we will still try to understand what mechanisms are at stake. A similar study with more patients and a complete set of data concerning parity would be very fruitful. Owing to the relatively small size of organs compared with the size of tested samples, it has not been feasible to perform a proper intra-individual study. We also acknowledge that biaxial testing might have been valuable; unfortunately, the size of the samples did not allow us to perform such testing. Moreover the mere use of deceased tissues is inaccurate, but for such destructive testing no other options are available so far.

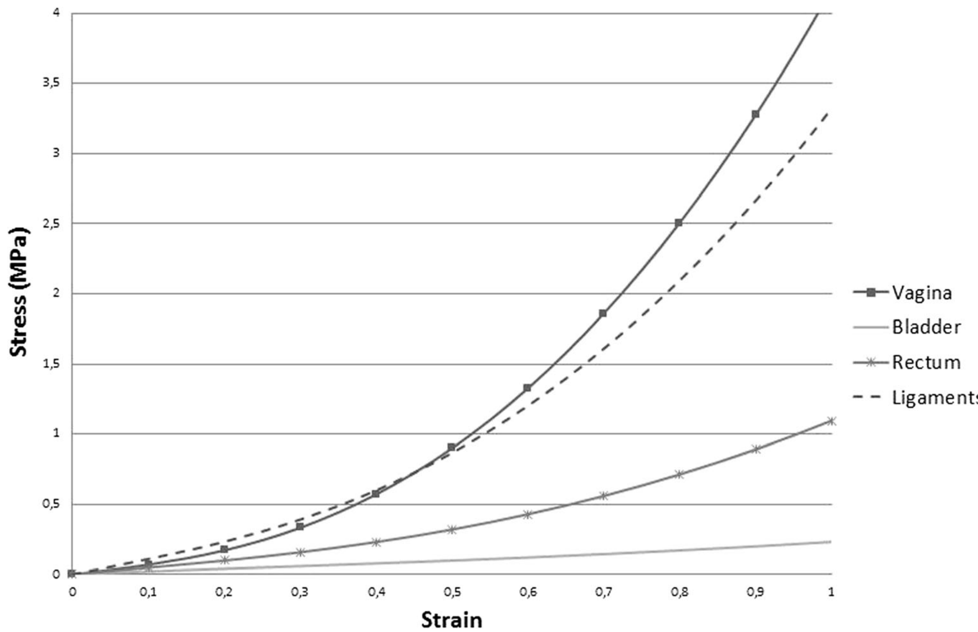


Fig. 2 Median behavior of each young pelvic tissue

However, despite these limitations, the study leads to some interesting results.

We first looked only at the results of young patients. On Fig. 3 an example of cyclic loads can be observed (the different grayscale straight lines represent the different cycles, going from darker to lighter with the first, second, and third loads). An analysis of data resulting from cyclic loads reveals a nonlinear relation between strain and stress. It also shows a hysteresis between the load and unload phases; this means that the nominal stress/strain relationship is not the same between loads and unloads. These two points reveal a nonlinear visco hyperelastic behavior as already mentioned by Rubod et al. [12] for old female tissues. For two consecutive loads, tests also showed that the nominal stress at a prescribed strain reached during the second load is always smaller than that during the first load. This softening shows that pelvic soft tissues can be damaged and that this damage is proportional to the prescribed strain [11, 12]. Performing two consecutive loads, the curve of the second load follows the trend that was expected for the first curve. This phenomenon is also referred to as “Mullins type damage” or “Mullins effect” and is well known for hyperelastic material [13–15].

Table 1 Results of young and old (C0, C1)

		Young	Old	*p*
Ligaments (all)	C0	0.19 (0.3)	Not available	Not available
	C1	0.19 (0.23)	Not available	Not available
Utero-sacral ligament	C0	0.13 (0.33)	0.83 (1.42)	0.005
	C1	0.2 (0.7)	5.7 (16.1)	< 0.001
Round ligament	C0	0.22 (0.18)	0.7 (0.7)	< 0.001
	C1	0.19 (0.19)	4.62 (5.18)	< 0.001
Broad ligament	C0	0.37 (0.75)	0.42 (0.46)	1
	C1	0.2 (0.68)	1.32 (3.14)	0.277
Vagina	C0	0.11 (0.37)	0.39 (0.361)	0.003
	C1	0.27 (0.58)	1.49 (2.44)	< 0.001
Rectum	C0	0.09 (0.11)	0.35 (0.36)	0.007
	C1	0.06 (0.01)	0.07 (0.27)	0.371
Bladder	C0	0.04 (0.25)	0.09 (0.21)	0.004
	C1	0.007 (0.07)	0.007 (0.11)	0.847

We have demonstrated that ligaments, vagina, bladder, and rectum have a nonlinear, visco-elastic mechanical behavior. This is in accordance with our works performed on older women’s cadavers [5, 6]. It has also been shown that young pelvic soft tissue was damaged by a Mullins effect. This may indicate that young pelvic floor tissues, if subjected to specific conditions inducing great strain (i.e., birth, trauma, overweight), may undergo Mullins effect damage. Consequences of the Mullins effect could be the following: rigidity increases after deformations, as Clay et al. observed for prolapsed tissues [11]; and a permanent deformation comparable to plasticity could arise. We can suppose that tissues have an anisotropic mechanical behavior. During the process of aging, naturally, with or without trauma and birth, pelvic floor tissues may become longer, stiffer, and anisotropic. According to the recurrence and intensity of loadings, the consequences will be more or less noticeable. This may explain why women with a greater number of natural births have statistically more prolapse symptoms than women after only one birth [1, 2]. After damage and even if tissues are reconstructed over time, reconstruction may be partial and pelvic tissue may no longer stabilize the pelvic floor as it used to, mainly because all entities became longer.

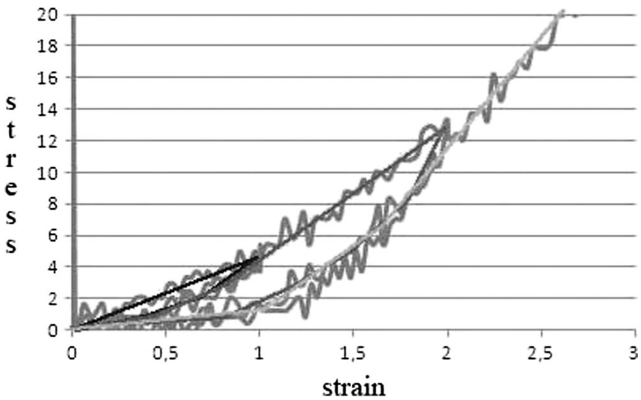


Fig. 3 Example of cyclic loads

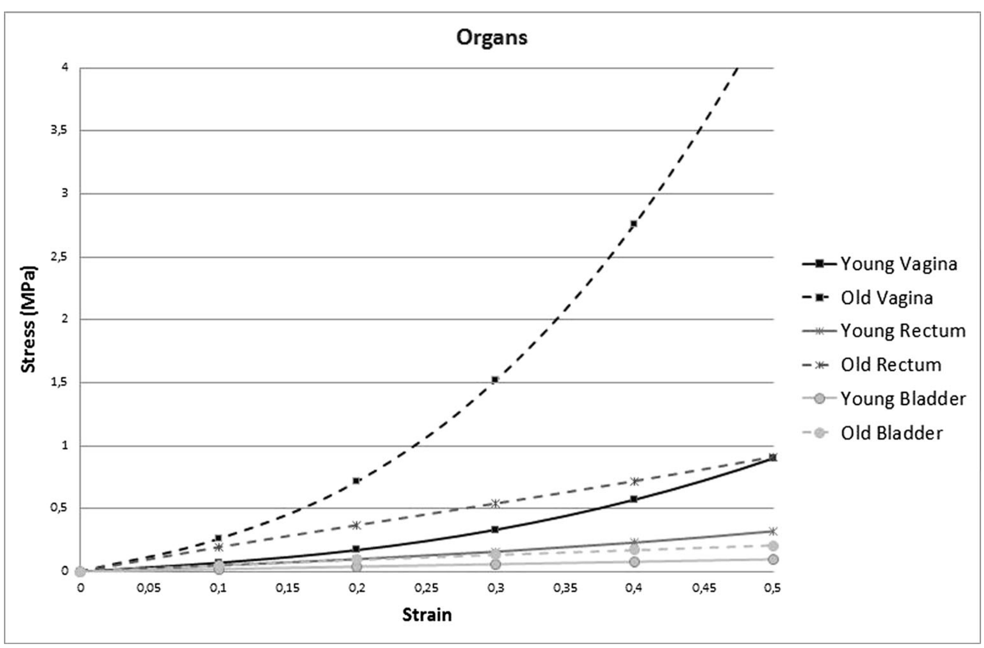
We then compared our results on young cadavers by organs. We showed that the bladder is significantly less rigid than the vagina and ligaments and tends to be less rigid than the rectum. No statistical difference was found for the rectum, which could be due to the limited number of specimens (n=6). We could not differentiate ligaments from one another. Vagina and ligaments also have comparable mechanical behavior; thus, we propose organization of the mechanical behavior of the pelvic tissues of young women, the bladder being the most compliant, followed by the rectum, and then the vagina with all the ligaments.

We can now compare these data with those from the older cadavers. The mechanical organization of the pelvic tissues has already been studied by Rivaux et al. and Rubod et al. [5, 6], which is where our data on old patients come from. Combining these data we can propose an organization of the pelvic tissues for older patients, the bladder being the most compliant, followed by the rectum, followed by the vagina, and then the broad ligament, the large ligament ,and the utero-sacral ligament respectively. There is then a differentiation of the tissues while ageing. Using the data of Table 1 we will try to better understand this process (Figs. 4, 5).

As discussed previously, C0 and C1 are two parameters that are used to characterize the behavior at small and large strains respectively. For uterine ligaments and the vagina there is a statistical difference between young and old tissues for C0 and C1, except for the broad ligament. However, the small population of young tissues for the broad ligament (n=4) does not allow us to make further comments. For the bladder and rectum there is a significant statistical difference only for C0. This suggests that both curves might still have the same shape and the same trend. With a different C0 the tissues will react differently to small displacements (everyday movements), but will then react quite similarly for exceptional stretching (trauma) because they have a similar C1. This is not the case for the vagina and ligaments, which have different C0 and C1. It is therefore interesting to further investigate this area of research. To illustrate this, we compared curves of the utero-sacral ligaments and rectum (Fig. 6). It illustrates the different ways of aging that a tissue can undergo depending on its location, history, and original mechanical behavior.

In both cases, young and old tissues have different mechanical behaviors, but for the rectum the trend of the curve remains the same for large deformation. We think that this is due to the different kind of damage, growth, and aging of these tissues. Ligaments and vagina undergo different kinds of stress throughout a person's life and at different intensities than the rectum and bladder. Pregnancy and natural birth, for example, from the mechanical viewpoint, induce strain that

Fig. 4 Median behavior of young and old pelvic organs



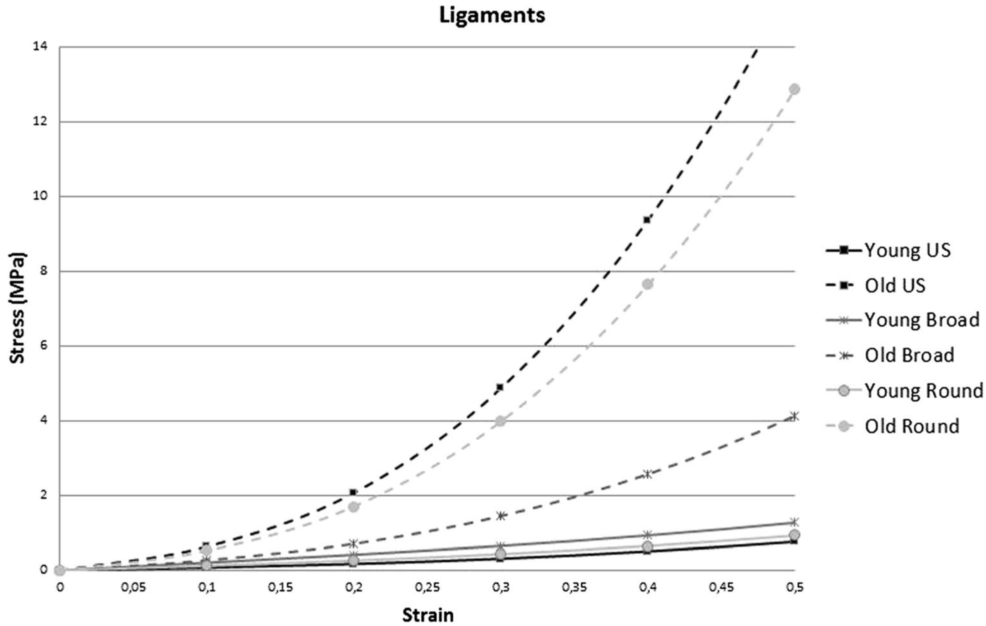


Fig. 5 Median behavior of young and old pelvic ligaments. *US* utero-sacral

the bladder and rectum do not have to undergo or at least not to such an extreme extent. With the same idea, the standing position and physical exercises of daily activities induce moderate but repeated trauma, mainly on the vagina and uterine ligaments. The type of curve that we have for the rectum and bladder would then describe a "natural" aging of tissues, introducing a moderate stiffening of the tissues all over the curve. The type of curve that we have for the ligaments and vagina would describe a "traumatic" aging of tissues, introducing increasing stiffening of the tissues over the curve and a strong elongation over time. This is also in accordance with the data collected on POP patients who most likely had undergone trauma at some point during their lives [11].

One could also argue that it is dependent on the original mechanical behavior and that the aging of different tissues is different because they are different tissues. However, ligaments are a great illustration that similar types of tissues can age differently because of the different loadings they have to undergo over time. Indeed, as explained previously, we have demonstrated that there is no statistical difference between the uterine ligaments of young women; while, there is a statistical difference between those tissues in older women. We may assume that these tissues are exposed to different stresses during a lifetime, inducing different responses and then modification of the mechanical behavior of these tissues. Therefore, beginning with three "mechanically similar" tissues, three mechanically different tissues are obtained. This suggests that the ligaments of a young woman, being all the same tissue, have no reason to have different mechanical behaviors. Nonetheless, after many years, these tissues have had the opportunity to dissociate in groups of different stiffness in order to resist the loads they have encountered.

The same kind of "traumatic" aging is observed for the vagina, even though, unfortunately, it has not been possible to obtain such strong comparisons as for ligaments. A complete study comparing two kinds of patients may offer this

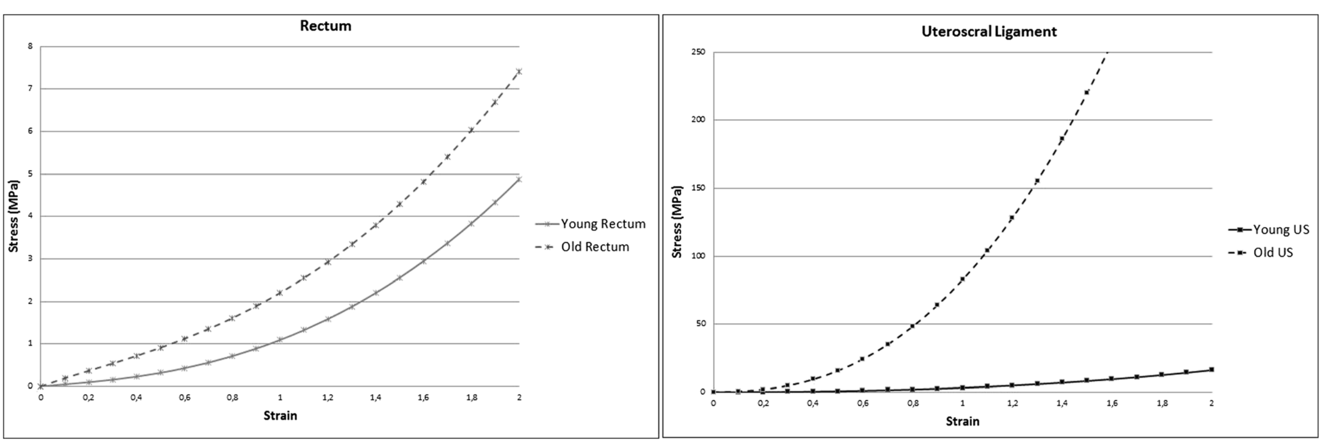


Fig. 6 Median behavior of young and old utero-sacral ligaments compared with young and old rectum

comparison. For example, studying the mechanical behavior of the tissue of thin, nonparous old women compared with overweight, multiparous old women would be very interesting. Obviously, conducting this kind of study is a challenge because of the difficulty in obtaining enough tissue and proper history information on the body donors.

We could hypothesize that for the young patient most of the suspension is due to cohesive and tissues and fascia that are not very differentiated. With ageing, daily standing position and even more in the case of obstetrical trauma these tissues will progress differently depending on their different mechanical trauma. These evolutions will result on the progressive “creation” of stiff, rigid, and strong tissues, especially the uterine ligaments and vagina fascia in parallel with the weakening of the pelvic cohesive fascias. The ligaments will in parallel show an increase in length leading in some pathological cases to pelvic floor prolapse.

An overall analysis of the histological composition of all pelvic floor tissues compared with their mechanical behavior would also be very interesting to see whether the mechanical evolution does fit the histological evolution. More mechanical data on more patients would also allow a better understanding of the stress-induced aging process. More data might allow use of more age groups and differentiating parous and nonparous women.

Even though there is a lot to explore, this study has been able to open the door of stress-induced aging in the pelvic floor.

The mechanical behaviors of pelvic floor organs of young and old cadavers were studied. We observed a hyperelastic biomechanical behavior of the different organs and uterine ligaments. We demonstrated a statistical difference in the mechanical behavior of organs and uterine ligaments between young and old tissues. The effect of aging through pelvic floor tissues could be underlined. The rectum and bladder undergo a “natural” aging of tissues, introducing a moderate stiffening of the tissues for every strain. On the other hand, the ligaments and vagina undergo significant aging of tissues, introducing increasing stiffening of tissues for every strain. The exact origin of this traumatic “aging” should be further discussed, in addition to our proposed explanation.

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Conflicts of interest None.

References

1. Samuelsson EC, Arne Victor FT, Tibblin G et al (1997) Signs of genital prolapse in a Swedish population of women 20 to 59 years of age and possible related factors. Am J Obstet Gynecol 89:501–506
2. Olsen AL, Smith VJ, Bergstrom JO et al (1997) Epidemiology of surgically managed pelvic organ prolapse and urinary incontinence. Obstet Gynecol 89:501–506
3. Bump RC, Norton PA (1998) Epidemiology and natural history of pelvic floor dysfunction. Obstet Gynecol Clin North Am 25:723–746
4. Luber KM, Boero S, Choe JY (2001) The demographics of pelvic floor disorders: current observations and future projections. Am J Obstet Gynecol 184:1496–1503
5. Rubod C, Brieu M, Cosson M, et al. Biomechanical properties of human pelvic organs. Int Urogynecol J 79(4):968.e17–968.e22. doi: 10.1007/s00192-010-1237-7
6. Rivaux G, Rubod C, Dedet B et al. Uterine ligaments: are they stronger than vaginal tissue? A biomechanics study. Int Urogynecol J (in press)
7. Epstein LB, Graham CA, Heit MH (2007) Systemic and vaginal biomechanical properties of women with normal vaginal support and pelvic organ prolapse. Am J Obstet Gynecol 197:165.e1–165.e6
8. Lei L, Song Y, Chen R (2007) Biomechanical properties of prolapsed vaginal tissue in pre- and postmenopausal women. Int Urogynecol J 18:603–607
9. Qiao Y, Pan E, Chakravarthula SS (2005) Measurement of mechanical properties of rectal wall. J Mater Sci 16:183–188
10. Clobes A, DeLancey JOL, Morgan DM (2008) Urethral circular smooth muscle in young and old women. Am J Obstet Gynecol 198:587.e1–587.e5
11. Clay JC, Rubod C, Brieu M et al (2010) Biomechanical properties of prolapsed or non-prolapsed vaginal tissue: impact on prolapse surgery. Int Urogynecol J 21:1535–1538
12. Rubod C, Boukerrou M, Brieu M et al (2007) Biomechanical properties of vaginal tissue. Part 1: new experimental protocol. J Urol 178: 320–325
13. Mullins L (1947) Effect of stretching on the properties of rubber. J Rubber Res 19:275–289
14. Merckel Y, Diani J, Brieu M (2011) Experimental characterization and modelling of the cyclic softening of carbon-black filled rubbers. Mater Sci Eng A 528:8651–8659
15. Merckel Y, Diani J, Brieu M (2011) A Mullins softening criterion for general loading conditions. Mater Sci Eng A 528:8651–8659