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Clara Gimenez
STRETCH Lab,
Department of Biomedical Engineering and Mechanics,
Virginia Tech,
Blacksburg, VA 24061
e-mail: clarajg@vt.edu

Marianna Alperin
Division of Urogynecology and Pelvic Reconstructive Surgery,
Department of Obstetrics, Gynecology, and Reproductive Sciences,
University of California San Diego,
Sanford Consortium for Regenerative Medicine,
La Jolla, CA 92097
e-mail: malperin@health.ucsd.edu

Raffaella De Vita
STRETCH Lab,
Department of Biomedical Engineering and Mechanics,
Virginia Tech,
Blacksburg, VA 24061
e-mail: devita@vt.edu

The Effect of Menopause on Vaginal Tissue Mechanics: A Brief Review

Often called "the change of life," menopause affects every part of a woman's body. As the sex hormones decrease, the reproductive organs experience the most remarkable changes, with the vagina becoming thinner, drier, and less elastic. Despite the important implications of these changes in genitourinary conditions, there are only a few experimental studies that focus on quantifying the effect of menopause on the mechanical properties of the vagina. These studies are mostly conducted using uniaxial tests on strips of vaginal tissues isolated from rats, rabbits, and sheep and, in only a few cases, from humans. The purpose of this article is to present a systematic review of experimental protocols, methods, and results that are currently published on how menopause alters the mechanical behavior of the vagina. This review will enable new investigators in the biomechanics field to identify important gaps and frame research questions that inform the design of new treatment options for menopausal symptoms. [DOI: 10.1115/1.4063101]

1 Introduction

Menopause is typically diagnosed retrospectively after twelve consecutive months of amenorrhea, marking the end of women's reproductive years. The age of menopause is determined by several factors [1], but the natural menopausal transition begins between the ages of 45 and 55. The loss of ovarian function that occurs during menopause leads to a decline in reproductive hormones (e.g., estrogen, androgens, and progesterone). Because these hormones regulate important body functions such as growth, repair, and reproduction, women are at increased risk of developing health conditions such as osteoporosis [2], cardiovascular disease [3], and genitourinary syndrome of menopause [4]. These conditions last for years after menopause and compromise the quality of life of women, with some reporting severe symptoms that disrupt their ability to work, their relationships, or other aspects of their lives [5].

The impact that menopause has on our society is significant. In 2021, there were 167.51 million female residents in the U.S. Roughly, 20 million women were going through menopausal transition and 52 million were in their postmenopausal period (Fig. 1). Moreover, as life expectancy increases due to the advances in medical research and technology, women will spend more than three decades of their life in the postmenopausal period. Thus, the overall health of women during the menopausal years represents a major public health concern that needs to be addressed through sustained interdisciplinary efforts.

Tissue remodeling that takes place during menopause makes women more susceptible to health problems. For example, menopause is marked by a decrease in bone mineral density which results in osteoporosis and fracture susceptibility in postmenopausal women [2]. Similarly, declines in muscle mass and strength occur at an accelerated rate around the time of menopause, causing physical function impairments, and increasing the risk of falls and fractures [7]. The skin becomes thinner, loses its elasticity, and increases its laxity with menopause, producing aging effects such as wrinkling [8]. The normal functions of many other tissues within the body are altered by menopause [9], but vaginal tissue undergoes the most significant changes since this tissue is rich in estrogen receptors and particularly vulnerable to estrogen deficiency.

The vagina is a fibro-muscular canal that connects the uterus and cervix to the outside of the body at the vulva (Figs. 2(*a*) and 2(*b*)). In humans, the diameter of the vagina ranges from 3 to 5 cm near the cervix and from 1.5 to 2.5 cm at the introitus [10,11]. The anterior wall is approximately 6–7 cm long and the posterior wall is about 8–10 cm long [10,12]. The vagina is comprised of four distinct layers: the epithelium, the lamina propria, the muscularis, and the adventitia (Fig. 2(*c*)). The epithelium, the innermost layer of the organ, is primarily made of squamous cells, and it mainly functions to thwart infections. Underneath the epithelium, the lamina propria is a layer of dense connective tissue. The muscularis is made of an inner circumferential layer and outer longitudinal layer of smooth muscle cells. Finally, the adventitia is the outermost layer of the vagina, and it is composed of loose connective tissue [13]. Animal models are commonly used in vaginal research, and there are a few studies comparing the reproductive anatomy of animals to humans [14]. Sheep have been increasingly selected as an animal model because the anatomy of their vagina has many similarities to the anatomy of the human vagina, with a length of 8–11 cm and a width of 1.1–1.5 cm in the narrowest part [15]. In the rat, the vagina is much smaller: 15–20 mm long and 3–5 mm wide [16]. Rabbits have

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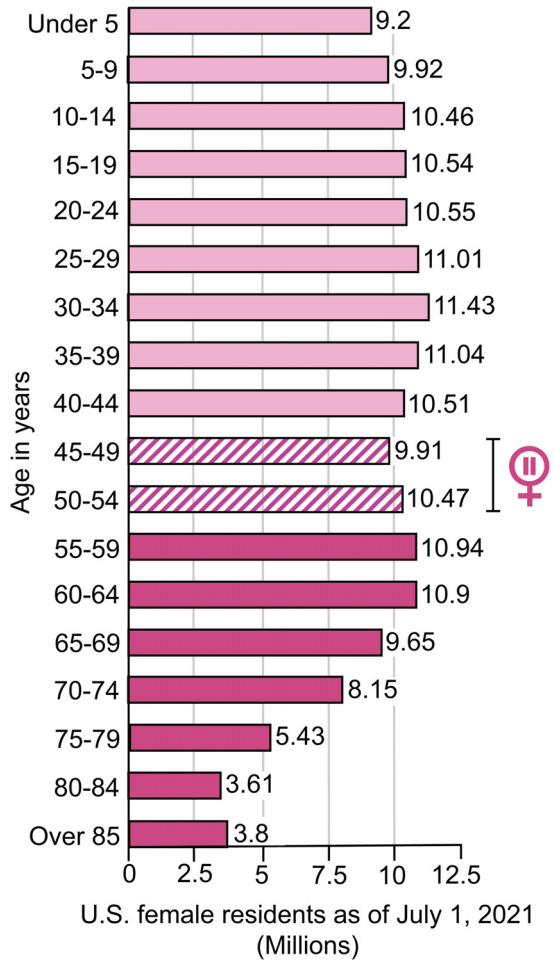

Fig. 1 Female population of the U.S. as of July 1, 2021, according to the U.S. Census Bureau [6]. The estimated menopausal transition begins between ages 45 and 55.

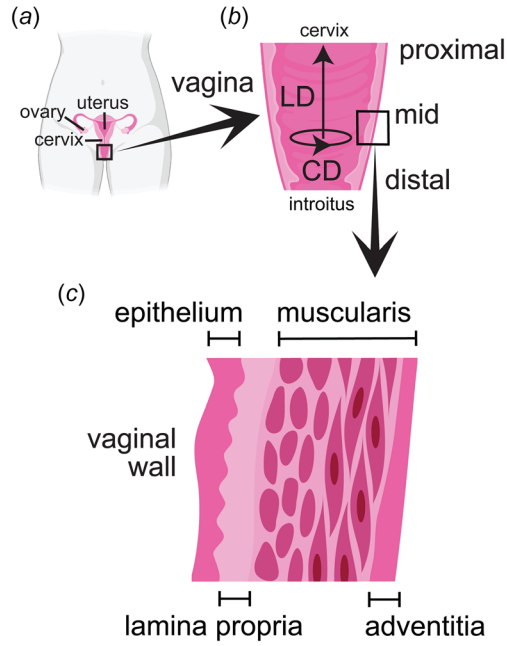

Fig. 2 Schematic of (*a*) the vagina within the female pelvic floor, (*b*) coronal section of the vagina including the proximal (closer to the cervix), mid, and distal (closer to the introitus) regions, and (*c*) the four layers of the vaginal wall. LD: longitudinal direction, CD: circumferential direction.

also been used for research, though their vagina is longer, being about 13–14 cm long [17].

With menopause, the vaginal tissue loses elasticity and becomes less distensible. The vaginal canal shortens and narrows while the rugae, the folds inside the organ, disappear [18]. The organ also loses moisture and becomes drier. These changes likely contribute to sexual dysfunction (e.g., dyspareunia), irritative lower urinary tract symptoms, and other genitourinary conditions which are commonly experienced by women after menopause [19–21]. Given the relationship between sexual function, pelvic floor support, and menopause in women, new research on the structural and mechanical properties of the vagina is needed to investigate the pathophysiology of the menopausal transition.

The layers of the vagina are primarily made of collagen, elastin, and smooth muscle. Collagen confers strength and support, elastin provides extensibility and elastic recoil, and smooth muscle regulates contractions. Quantifying the relative amount of these components in the human vagina is difficult, and this has yet to be done to our knowledge. In swine, the vaginal wall has been found to consist of 58.9 ± 5.6% collagen, 31.3 ± 4.9% smooth muscle, and 1.1 ± 1.5% elastin. [22] The loss of collagen, elastin, and smooth muscle in vaginal tissue following menopause largely goes unquestioned, but contradictory results have been published. Ovariectomized (OVX) animals are commonly used to examine the effects of menopause on women's health since most animals do not experience menopause but only reproductive senescence. In these animals, menopause is surgically induced through the removal of the ovaries. Following ovariectomy, some studies reported a decrease in collagen in rats [23] while others reported an increase in collagen in rats [24], rabbits [25], and sheep [26] or no change in collagen in sheep [27]. Similarly, the density of elastin was found to decrease in the vagina of OVX mice [28], OVX rats [23], and OVX sheep [26] but no significant changes were reported in one recent study using OVX sheep [27]. Vaginal smooth muscle content was observed to decrease in postmenopausal women [29]. These findings, including smaller and disorganized smooth muscle bundles, were confirmed in OVX rodents [24,25,30–32] but, in one study, the smooth muscle content did not change following ovariectomy in sheep [26]. Together with their content, the relative organization of these components of the vaginal tissue appears to be altered at the onset of menopause [33].

Over the past decades, several research efforts in the biomechanics community have focused on addressing women's health disparities [34,35]. The mechanical properties of the reproductive tissues have been identified as key contributors to understanding the physiology and pathophysiology of the reproductive system for the advancement of women's reproductive and sexual health. Some progress has been made in the mechanical characterization of vaginal tissue using rigorous experimental methods [22,36–39], but the effect of menopause has often been overlooked by biomechanists. This is perhaps because menopause signifies the end of women's reproductive life–meaning that the reproductive organs no longer experience the stresses and strains of menstruation, pregnancy, and parturition. However, menopause causes dramatic changes in the reproductive organs that interfere with the well-being of women and hinder their healthy aging.

The goal of this review is to present existing studies that explore how the vagina changes mechanically due to menopause. The published findings on this topic are analyzed and synthesized while gaps or inconsistencies across studies are noted. This paper provides a solid platform for future researchers in biomechanics for starting a debate about women's health during menopause and aging, generating new experimental methods for mechanical testing, and identifying topics requiring more investigation. Improved mechanical characterization of the menopausal vaginal tissue will lead to informed preventive measures and treatment strategies for sexual dysfunction, irritative lower urinary tract symptoms, and pelvic floor disorders, directly impacting women's healthcare for a third of their life.

2 Mechanical Properties of Menopausal Vaginal Tissue

Several experimental studies have been conducted to quantify mechanical changes in vaginal tissue that are induced by menopause (Figs. 3 and 4). In this section, we describe the protocols and results of such studies while similarities and differences among them will be discussed in the Sec. 3 (Fig. 5). All studies were conducted ex vivo using tissue excised from animals such as rats [24,31,32,41–43], rabbits [30], and sheep [26,40,44] or, in a few cases, biopsied from human patients [45,46]. The animals used in these experimental investigations did not undergo natural menopause, as most mammals experience lifelong estrous cycles. Instead, bilateral ovariectomy, which is the surgical removal of both ovaries, was performed to induce menopause [24,26,30–32,40–44]. The majority of mechanical testing was conducted on vaginal tissue from young OVX animals [24,31,40–42].Vaginal tissue was isolated from middle-aged OVX animals only in a few studies [42–44], and from older OVX animals in a single study [26]. Parity varied among studies, nulliparous (never gave birth) virgin animals [24,42], primiparous (gave birth once) [40], and multiparous (gave birth more than once) [26,40,42,44,46] were used for testing. Parity status was often not reported [30–32,41,43]. The time of tissue harvesting following ovariectomy also varied across the published studies: the shortest interval was 3 weeks for the rats [31] and the longest was 22 weeks for the ewe [26]. Uniaxial testing was the most prevalent method used for mechanical testing [24,26,30–32,40–46] and, more recently, ball burst testing was employed [26,40]. Structural and material properties were quantified for vaginal tissues in their passive state [24,26,40–42,44,46] and active state [26,30,31,40,45]. Hereafter, we will report only on experimental studies that quantified the effect of menopause on healthy vaginal tissue, excluding studies where vaginal tissue was collected from diseased humans (e.g., women affected by pelvic organ prolapse). Moreover, we will not present comparisons of different hormonal treatments (e.g., 17β-estradiol, testosterone) on the mechanical properties of vaginal tissue.

2.1 Passive Mechanical Testing. Collagen fibers, the main components of the lamina propria and adventitia layers, play a major role in the mechanical function of the vagina. Together with elastin, collagen determines important passive mechanical properties such as strength and distensibility of the vagina. As estrogen levels drop during menopause, the production of collagen decreases and such decrease has the potential to affect structural properties (e.g., stiffness, ultimate load, ultimate elongation) and mechanical properties (e.g., elastic modulus, ultimate stress, ultimate strain) of the vagina. The effect of menopause on the passive mechanical properties of the human vagina has been studied by Lei et al. [46] who compared data collected from nonprolapsed premenopausal and postmenopausal vaginal tissue. These authors used uniaxial tensile testing to measure elastic modulus, maximum elongation, and maximum fracture of human vaginal tissue oriented in the longitudinal direction (LD). The control (nonprolapsed) group consisted of 14 premenopausal and 8 postmenopausal women, who were undergoing treatment for enlarged uteri with benign diseases. The average age of the premenopausal control was 45.50 $\pm$ 1.56 years and the average age of postmenopausal control was 60.25 $\pm$ 4.53 years. Specimens were obtained from the anterior apical vagina at the colpotomy site, trimmed to 5 mm$\times$25 mm, and loaded at a constant rate of 0.8 mm/s to an elongation of 8 mm. The elastic modulus in postmenopausal women, 10.26 $\pm$ 1.10 MPa, was greater than that of premenopausal women, 6.65 $\pm$ 1.48 MPa indicating that the vaginal tissue stiffened with menopause (Fig. 4(*a*)). Whether this change in elastic modulus was due solely to menopause or also to the age difference between the two control groups remains unclear. There were no changes in the maximum elongation and maximum fracture of premenopausal and postmenopausal vaginal tissue.

The passive structural properties of the vagina in the ewe were measured by Ulrich et al. [44]. Vaginal tissue was harvested from six multiparous postmenopausal sheep, with ages that ranged from 4 to 5 years. Sheep were ovariectomized, and they were euthanized 16 weeks postoperatively. Tissue for uniaxial tensile testing was excised from the proximal, mid, and distal vagina. Specimens measuring 4 mm in width and 34 mm in length were cut with their long axes in the LD (as defined in Fig. 2). They were preloaded at 10 mm/min to 100 mN and preconditioned from 0 to 1 N, 0 to 2 N, and 0 to 3 N each for five cycles at 20 mm/min before being subsequently pulled to failure. The Young's modulus was calculated from the slope of the linear region of the stress-stress curve. This

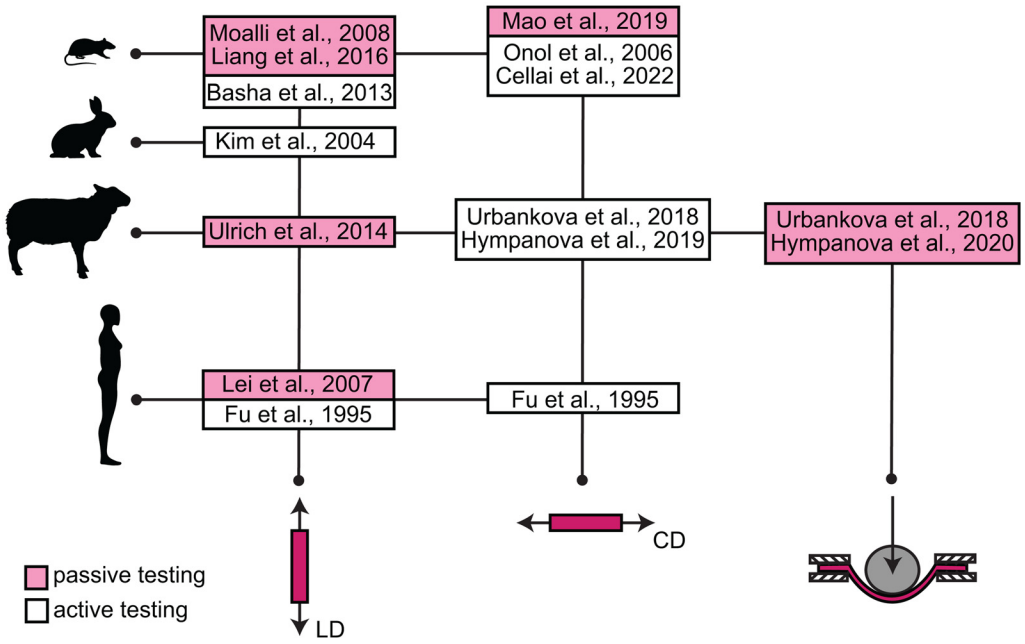

Fig. 3 Summary of published ex vivo mechanical tests (left to right: uniaxial tests in the LD, uniaxial tests in the CD, ball burst tests) conducted on menopausal vaginal tissue from healthy animals and humans (top to bottom: rats, rabbits, sheep, and humans). LD: longitudinal direction, CD: circumferential direction.

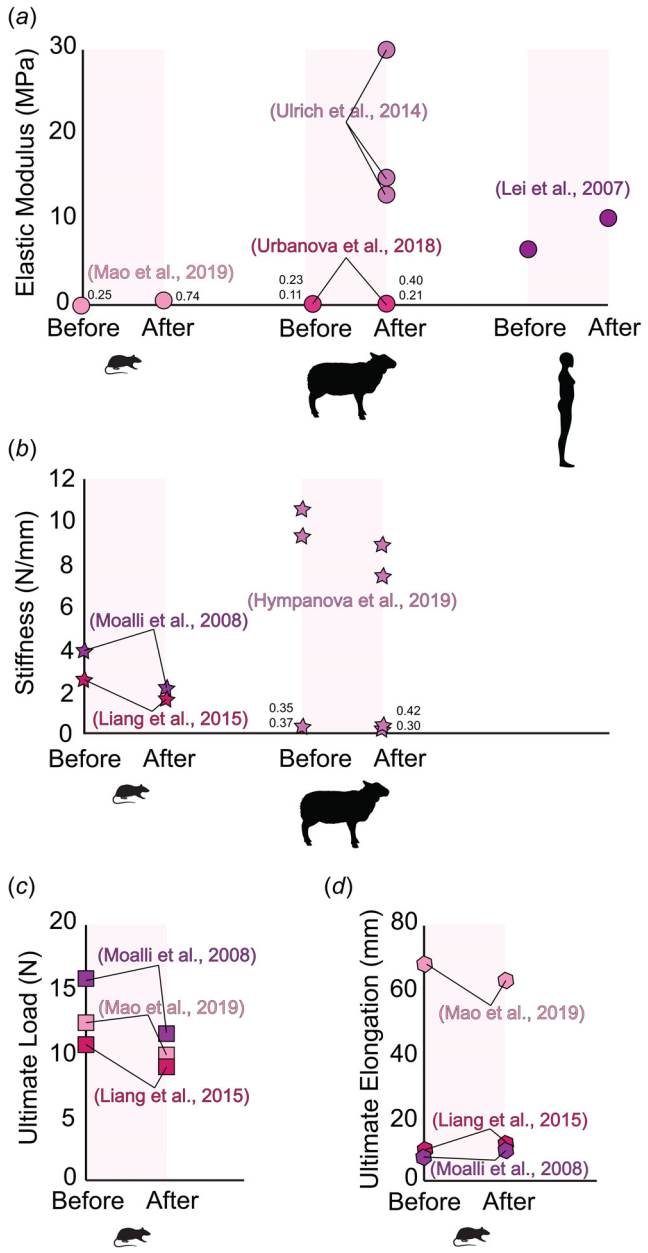

Fig. 4 Mechanical quantities (mean or median) computed for vaginal tissue isolated from different (proximal, mid, or distal, anterior or posterior, and ventral or dorsal) regions, human subjects and animal models (rats and sheep), tested along different anatomical (circumferential and longitudinal) directions using different methods as reported in Fig. 3 and collected "before" and "after" menopause. Data from some studies [24,26,40] were indistinguishable in these plots and, for this reason, numerical values are added to such data.

region was defined as the section of the curve immediately following cyclic loading and before yielding. Young's modulus and maximum stress were found to be significantly higher in the proximal region than in the mid and distal regions (Fig. 4(*a*)).

The effect of menopause on the vagina was mechanically investigated in rodent models [41,42]. Moalli et al. [42] tested the vagina with the attached supporting tissues as a complex. Rats were divided into young (4-month-old) virgin, young (4-month-old) parous, and middle-aged (9-month-old) parous groups. Animals either underwent sham surgery or an ovariectomy and were sacrificed 8 weeks following the procedure. The lower spine of the rats was fixed to the base of a uniaxial testing machine while the distal region of the vagina was attached to the crosshead of the machine and connected to a load-cell. Specimens were preloaded to 0.15 N and then underwent 10 cycles of preconditioning at 25 mm/min between 0 to 2 mm. They were then pulled to failure at the same elongation rate. Ovariectomy induced a decline of biomechanical properties in young rats, but it did not have the same effect for middle-aged rats. Linear stiffness, defined as the steepest positive slope measured over a 1 mm interval of extension, was found to significantly decrease following the ovariectomy in young virgin and parous rats. For the young virgin sham group ($n = 8$ rats), the average linear stiffness was 3.9 ± 0.3 N/mm, while for the young virgin OVX group ($n = 6$ rats) the average linear stiffness decreased to 2.2 ± 0.5 N/mm. The same behavior was observed in young (4-month-old) parous rats: for the sham group ($n = 6$ rats) average stiffness was 4.3 ± 0.8 N/mm and for the OVX group ($n = 5$ rats) the average stiffness was 2.6 ± 0.9 N/mm. It was noted that, when comparing the age-matched virgin and parous rats, parity did not impact biomechanical outcomes. The average ultimate load at failure was significantly lower in the OVX young virgin and OVX young parous rats, 11.5 ± 2.1 N and 13.8 ± 2.3 N, respectively, as compared to their sham surgery counterparts, 15.8 ± 1.9 N and 19.4 ± 2.9 N, respectively. Maximal distension before tissue failure was also measured but was not significantly impacted by ovariectomy in any of the groups. Mean values of the measured mechanical quantities are reported for the young virgin group in Figs. 4(*b*)–4(*d*). The authors concluded that at younger ages ovariectomy leads to weaker tissue that is less resistant to tissue distension. Liang et al. [41] used the same experimental methods presented by Moalli et al. [42] in a follow-up study. Pelvises were dissected from several groups of young (4-month-old) rats that included one sham control group ($n = 8$ rats) and an OVX group ($n = 8$ rats) without drug supplementation. Average stiffness and ultimate load values were 2.6 ± 0.3 N/mm and 10.6 ± 0.6 N for the sham control group, and they were and 1.7 ± 0.5 N/mm and 8.9 ± 1.1 N for the OVX group without drug supplementation (Figs. 4(*b*) and 4(*c*)). These results were consistent with the previous study [42] where OVX rats displayed lower stiffness and ultimate load at failure compared to sham control rats. Similarly, ultimate elongation at failure was not significantly altered (Fig. 4(*d*)).

Uniaxial testing has also been performed on vaginal tissue in the circumferential direction (CD) by Mao et al. [24]. Two groups of young (2-month-old) virgin rats were used for mechanical testing: one control group ($n = 6$) and one OVX group euthanized 16 weeks postop ($n = 6$). The vagina was isolated intact, and the anterior vaginal wall was collected for testing. Specimens were then cut into a dog bone shape with a ratio of 5 (length/width), the ends being 5 mm. They were preloaded to 1 N and then stretched at a constant deformation rate of 12 mm/min with no preconditioning. Load-elongation curves were generated, and from these curves, ultimate load at failure and maximal distension were attained. Ultimate load was defined as the load where tissue failure occurred, and maximal distension as the elongation associated with this load. The stress–strain curves were then derived, and the tangent moduli, specifically the maximum slopes, were computed from the linear regions of these curves. The tangent moduli of the control group and OVX group were 0.25 ± 0.11 MPa and 0.74 ± 0.27 MPa, respectively (Figs. 4(*a*), 4(*c*), and 4(*d*)). The OVX group demonstrated a significant increase in tangent modulus 16 weeks following ovariectomy. There was no significant difference in ultimate load and ultimate elongation at failure 16 weeks after ovariectomy, although the ultimate load at failure showed a declining trend (Figs. 4(*c*) and 4(*d*)).

In addition to uniaxial testing, ball burst testing has been used to quantify the effect of menopause on the multi-axial mechanical properties of the vagina [26,40]. In the study by Urbankova et al. [40], vaginal tissue was isolated from primiparous sheep ($n = 6$) and multiparous OVX sheep ($n = 6$), in addition to other groups. The average age of the primiparous and multiparous groups was 3 years. Tissue specimens were obtained from the distal and mid regions of the vagina, and were trimmed to 30 mm × 30 mm. Each specimen was first preloaded to 0.1 N at a rate of 5 mm/min and then loaded at a

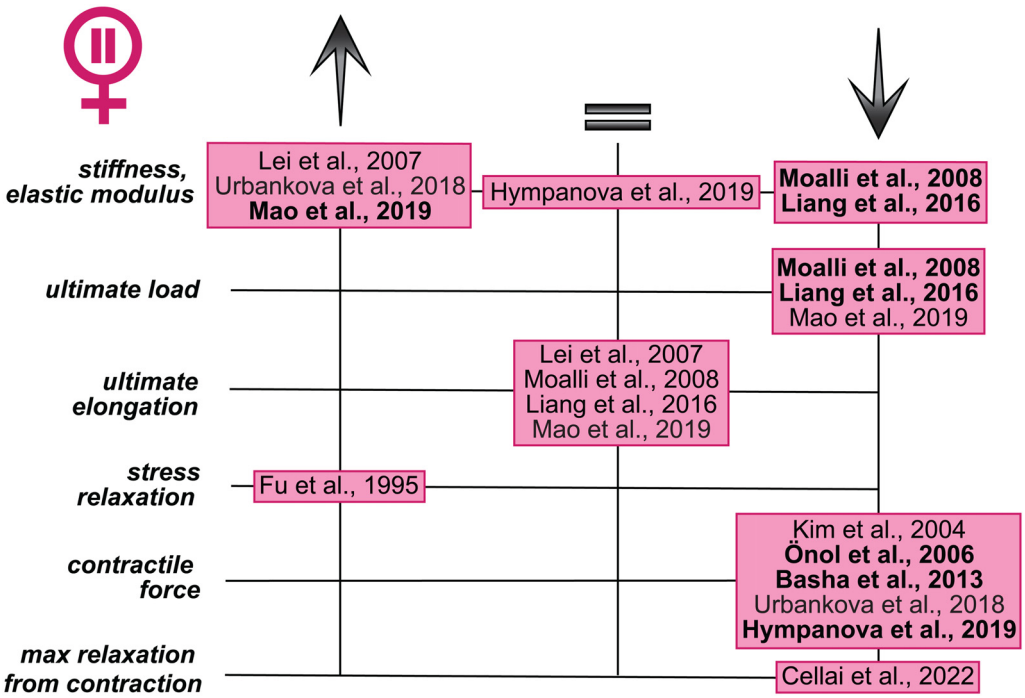

Fig. 5 Summary of trends reported in ex vivo mechanical studies of menopausal vaginal tissue. References in bold case denote studies where statistically significant differences were noted.

rate of 10 mm/min until disruption or until the maximum capacity of the load cell (200 N) was reached. Two regions, a "comfort zone" and a "stress zone," were identified in the load-elongation curves. The comfort zone was defined as the region in which the specimen deformed easily without much stress needed,whereas the stress zone represented the region where higher stresses were needed to deform the specimen [47]. The tangent modulus, used as a measure of the tissue stiffness, was calculated from the comfort zone. In the primiparous group, the tangent moduli (median and interquartile range) in the distal and mid vagina were 0.11 (0.09–0.018) N/mm^2 and 0.23 (0.17–0.32) N/mm^2, respectively. In the multiparous OVX group, the tangent moduli in the distal and mid vagina were 0.21 (0.15–0.28) N/mm^2 and 0.40 (0.29–0.43) N/mm^2 (Fig. 4(*a*)). The authors reported an increase, although not a significant increase, in the tangent modulus of both the distal and mid vaginal tissue following ovariectomy.

In a follow-up study, Hympanova et al. [26] presented mechanical data for vaginal tissue collected from one multiparous group of ewes ($n = 6$) and one multiparous OVX group of ewes ($n = 6$). The multiparous and multiparous OVX sheep were 7 years old. The same testing protocol presented by Urbankova et al. [40] was applied to 35 mm × 35 mm specimens of the distal and mid vagina. The stiffness was reported for both the comfort and stress zones as defined above. No significant differences were found between the stiffness values of the multiparous and multiparous OVX groups. For the multiparous control group, the stiffness of the distal vagina was 0.37 ± 0.10 N/mm in the comfort zone and 9.3 ± 1.63 N/mm in the stress zone. For the same group, the stiffness of the mid vagina was 0.35 ± 0.11 N/mm in the comfort zone and 10.53 ± 4.31 N/mm in the stress zone. For the OVX group, the stiffness of the distal vagina was 0.42 ± 0.15 N/mm in the comfort zone and 7.45 ± 2.64 N/mm in the stress zone. In the mid vagina, the stiffness was 0.30 ± 0.12 N/mm in the comfort zone and 8.94 ± 2.67 N/mm in the stress zone for the OVX group (Fig. 4(*b*)).

2.2 Active Mechanical Testing. The muscularis, which is comprised mainly of smooth muscle cells, is responsible for the active mechanical response of the vaginal tissue. There is a sharp decrease in contractile vaginal smooth muscle at the age of menopause [29]. For this reason, there has been some interest in quantifying the contractions of vaginal tissue following menopause. Contraction of vaginal tissue in pre- and postmenopausal women was first documented by Fu et al. [45]. Vaginal biopsies from the anterior vaginal wall were taken from 10 women: four premenopausal and six postmenopausal women who did not have any urologic diseases or conditions. The age of the donors was not reported. Strips of vaginal tissue used for stress-relaxation tests were dissected along both the LD and CD. These strips were stretched for 20 min intervals while being super-fused with Hepes buffer. After stress relaxation tests, these strips were then depolarized by K^+ at concentrations ranging from 0.01 to 2 M and stimulated by noradrenaline at concentrations ranging from 10^{-6} to 10^{-3} M and prostaglandin GF_{2_α} (PGF_{2_α}) at concentrations ranging from 0.15 to 2.5 μM. Results of the stress-relaxation of tissue from postmenopausal women were compared with those of tissue from premenopausal women. The degree of relaxation was significantly higher in postmenopausal women than in premenopausal women. Relaxation was also significantly higher in the CD compared to the LD. The authors also concluded that K^+, noradrenaline, and prostaglandin evoked tissue contractions that are dose-dependent.

The contractility of rabbit vaginal tissue strips in the LD was tested by Kim et al. [30]. Of forty rabbits included in this study, four were kept intact and seven were ovariectomized and used as control (while the other 29 were treated with steroid hormones). All OVX animals were euthanized 4 weeks following ovariectomy, and longitudinal strips of tissue ranging from 10 to 15 mm in length were isolated from the distal vagina. Strips were progressively stretched and periodically contracted with 2 μM norepinephrine until optimal isometric tension was achieved. The vaginal tissue was then subjected to electrical field stimulation or exposed to increasing concentrations of exogenous contractile and relaxatory agents such as norepinephrine and vasoactive intestinal polypeptide, respectively. Contractile and relaxation responses to electrical field stimulation were not significantly impacted by ovariectomy, though there was a declining trend in the OVX groups. Vaginal tissue from OVX rabbits exhibited significantly attenuated relaxation responses to vasoactive intestinal polypeptide.

Similar decrease in contractility of OVX vaginal tissue was reported for rats by Basha et al. [31]. Animals in this study included a

sham-operated group ($n = 5$) and an OVX group ($n = 5$), and were 4-months-old. Three weeks following the sham surgery or ovariectomy, the rats were euthanized and longitudinal strips, measuring 1.5 mm × 6 mm, were dissected from the proximal vaginas. Strips were equilibrated for 90 min, and stretched uniaxially to a length for maximal active contraction. Stimulation was achieved using 110 mM KCl. The peak force during isometric contractions was recorded together with the maximum velocity of isotonic contractions. The peak force of KCl-induced isometric contractions was significantly lower in the OVX group compared to the sham group but no differences in the velocity of isotonic contraction were detected between the two groups.

Urbankova et al. [40] and Hympanova et al. [26] also tested the contractile properties of vaginal tissue from primiparous and multiparous OVX sheep. They used strips of tissue with their long sides aligned in the CD, trimmed to 10 mm × 8 mm, obtained from the distal and mid regions of the vagina. Specimens were subjected to a 0.1 mN preload and equilibrated for an hour, then subjected to 80 mM KCl. Contractile forces were normalized to the measured tissue volume. Median contractility was 0.065 (0.035–0.084) mN/mm^3 and 0.209 (0.199–0.425) mN/mm^3 for the distal and mid vagina from primiparous sheep, respectively. The multiparous OVX group had median contractility of 0.008 (0.001–0.058) mN/mm^3 and 0.093 (0.038–0.196) mN/mm^3 in distal and mid vagina, respectively. While the observed contractility in the OVX group was smaller in both distal and mid vagina, it is unclear whether such a difference was statistically significant when compared to the primiparous group. In a similar procedure, Hympanova et al. [26] tested circumferential strips from the distal and mid vagina. Specimens were preloaded twice to 0.5 mN and allowed equilibrated for 1 h. Strips were then subjected to increasing doses of potassium, ranging from 15 to 120 mN KCl. Contractile forces were normalized in this case to tissue weight. This study compared vaginal tissue from multiparous OVX sheep to an age-matched multiparous control group. The average contractility was reported to be 239.10 ± 99.19 mN/g in the distal vagina and 444.70 ± 156.60 mN/g in the mid vagina for the multiparous control group. In the OVX group, the average contractility was 167.8 ± 41.91 mN/g for the distal vagina and 119.40 ± 48.52 mN/g for the mid vagina. There was a significant decrease in contractility of the mid vagina following the ovariectomy.

Rats demonstrated a similar decrease in contractility following an ovariectomy. Onol et al. [43] quantified contractile and relaxation responses of 30 mature (6-months-old) rats. The rats were divided into two groups, a control group and a menopausal group which underwent a bilateral ovariectomy. After 6 weeks, animals were euthanized and strips were cut from the proximal and distal vagina along the CD, measuring 7 mm × 3 mm. Contractile responses of the tissue to electrical field stimulation (frequency varied between 1 and 40 Hz), high-potassium solution (120 mM), phenylephrine (10^{-9}–10^{-4} M), carbachol(10^{-9}–10^{-4} M), and the effects of prazosin and yohimbine were studied. Relaxation responses of the precontracted tissue to electrical field stimulation and vardenafil (10^{-9}–10^{-4}M) were recorded. Tension data were then normalized by wet tissue weight and presented as tension generated per gram of tissue weight. Both the distal and proximal strips of the control group gave higher responses to high-potassium solution and electrical field stimulation relative to the OVX group. Phenylephrine-evoked contractile responses were significantly decreased in distal strips in the ovariectomy group. The relaxation responses of distal strips to electrical field stimulation and vardenafil significantly decreased in the ovariectomy group.

More recently, Cellai et al. investigated changes in the relaxation response of vaginal tissue induced by OVX in rats [32]. Three-months-old rats were divided into a control group ($n = 13$) and an OVX group ($n = 11$). Eight weeks following the ovariectomy, all rats were sacrificed. Vaginal strips were cut from the distal vagina and vertically mounted in organ chambers, where they were exposed to a dose of 3 μM noradrenaline for 5 min. Following the contractile response, a relaxant effect was induced using increasing concentrations (1 nM–10 μM) of acetylcholine. The OVX group demonstrated significantly decreased responsiveness to acetylcholine-induced relaxation. Maximum relaxation, measured as percentage of contraction versus maximum contraction, was 73.6 ± 15.7% in the control group and decreased to 27.4 ± 1.6% in the OVX group.

3 Discussion

The vagina undergoes morphological and functional changes over the course of a woman's life, affecting overall health and well-being. During menopause, the decline in reproductive hormones brings on biomechanical changes in the vaginal wall that are still not fully understood. While some preliminary work has been done, a characterization of the organ throughout menopause has yet to be conducted using advanced quantitative methodologies in biomechanics. In this article, we present a review of the published studies evaluating the effect of menopause on the mechanical behavior of vaginal tissue (Fig. 3). Given the differences in animal models, testing methods, and experimental protocols, we are cautious about the conclusions that can be drawn from existing published findings. For example, the vagina is commonly believed to become stiffer with menopause, but such stiffening was not reported in all the reviewed studies. Some authors found a significant increase in stiffness [24], others reported no significant change [26], and others noted a significant decrease [41,42] following ovariectomy or biological menopause (Fig. 5). The study by Lei et al. [46] is frequently cited when reporting increased stiffness of vaginal tissue in postmenopausal women. However, there was a large difference in the age of the premenopausal and postmenopausal study participants which might have contributed to this increase in stiffness since aging appears to increase stiffness of other pelvic tissues [48]. The decrease in stiffness with menopause was observed only in studies by Moalli's group where the vagina and its supportive tissues were mechanically tested together [41,42]. This makes the comparison of these studies to other studies very difficult as the mechanical behavior of the vagina alone in response to ovariectomy was not characterized. Ultimate load at failure significantly decreased [41,42] or showed a declining trend [24] (without meeting statistical significance) following ovariectomy. Maximal distension of vaginal tissue was consistently unaffected by menopausal status [24,41,42,46]. These results indicate that the postmenopausal tissue is weaker, being able to support reduced load before rupture but it deforms as much as the premenopausal tissue. Contractile force following ovariectomy decreased according to several studies [26,31,43], or showed a decreasing trend with no statistically significant difference [30,40]. With such conflicting findings and paucity of mechanical data, how the mechanical properties of the vaginal tissue are altered in response to menopause remains unclear, and further research is needed.

Over the past few years, there has been a surge in engineering research for women's health [34,35,49] including studies measuring the mechanical properties of the vagina. Biaxial testing methods have been adopted since they are more physiologically relevant than the uniaxial testing technique that has been commonly used for measuring the effect of menopause (Fig. 3). In the latest studies, the vagina has been tested either in a planar biaxial configuration, with squared tissue specimens being stretched along the two main anatomical directions of the vagina, the LD and CD, [22,50,51] or in its tubular configuration by slowly increasing the organ's inner volume [36,37,39,52]. Moreover, since vaginal tissue exhibits viscoelastic properties, creep, and relaxation phenomena have also been described [39,50,52]. Given the stark difference between the mechanical behavior of the vagina in the passive (without smooth muscle activation) and active (with smooth muscle activation) states [53], some efforts have been made to analyze the influence of contractions on the deformation mechanisms of these tissues [51,52,54]. As new experimental methods and protocols in biomechanical engineering continue to be developed, researchers will be able to better explore changes to the vagina across the lifespan.

Ovariectomy is the gold standard procedure used to recapitulate menopause in laboratory animals as it gives an acute decline of estrogen, allows for the dissociation between the effects of age and the effects of ovarian hormonal decline, and is associated with no to minimal morbidity [55]. Much of the fundamental research on understanding menopause in humans has been conducted on OVX animal models. In these models, menopause is induced through the surgical removal of ovaries, leading to an abrupt loss of ovarian hormones. This procedure has another advantage: it mirrors oophorectomy, a surgery that is often performed in women. Surgically induced menopause causes more severe symptoms than natural menopause in women. For example, premenopausal oophorectomy has been linked with increased cardiovascular and neurological disease, osteoporosis, and impaired sexual function in addition to menopausal symptoms [56]. Thus, the OVX animal models have the additional potential to advance our understanding of the impact of the common gynecologic procedures affecting woman's physical, mental, and emotional well-being. There are, however, limitations to the OVX animal models. The ovariectomy procedure fails to capture the perimenopause phase, the natural transition to menopause in women. This phase is characterized by disrupted hormone levels and physiological changes, and disorders such as sexual dysfunction can develop. Rodents do experience natural hormonal fluctuations around middle age, sharing multiple traits with humans, such as a decline in follicles, irregular cycling, and steroid hormone levels, and decreased fecundity. However, while rodents go into estropause with low levels of estrogens, they fail to reach the very low levels observed in menopause. Ovotoxins (e.g., 4-vinylcyclohexene diepoxide commonly known as VCD) have been used in rodents to accelerate ovarian failure to mimick the transition from perimenopause to postmenopause while retaining the ovaries [57]. The rodent models with chemically induced menopause serve to discern the effects of age and the effects of ovarian hormone loss. However, since ovotoxins have toxic and carcinogenic effects on several organ systems depending on the dose and duration of exposure, investigators using these menopausal models must consider these limitations in their studies [58].

The biomechanical response of vaginal tissues from OVX animals was found to be dependent on the age of the animals at ovariectomy. Moalli et al. [42] compared the effects of ovariectomy in young and middle-aged rats, finding that ovariectomy had a significant impact on the mechanical properties of the vagina and supportive structures in young rats but not in old rats. This outcome is likely due to the fact that younger animals experience a more rapid decrease in circulating ovarian hormone levels in comparison to older animals [57]. Similarly, young women who have had surgically induced menopause experience more obvious changes in vaginal functionality than older women undergoing natural menopause [59]. Age induces alterations in reproductive tissues that influence how these tissues respond to hormones. Rats are commonly ovariectomized at 12 weeks of age when they become sexually mature, and tissue remodeling due to estrogen deficiency occurs very early in these animals. Thus, although studies of tissue properties in young OVX animals allow for a clear distinction between the effects of menopause versus the effects of age, the translation of findings to humans is questionable since most women experience menopause in later years. Aged female OVX rats appear to exhibit physiological features associated with reproductive aging, and they represent a better model than young OVX rats to study the transition to menopause [55].

Estrogen deprivation has short-term and long-term consequences on the mechanical properties of vaginal tissue. This difference is captured by testing vaginal tissue at different time points following ovariectomy. In several studies, the OVX animals were sacrificed at the shortest time interval that caused significant changes in tissue properties, likely to reduce costs in animal care. Mao et al. [24] investigated both short- and long-term effects of ovariectomy on vaginal tissue in rats, though only performing biomechanical testing at the longest time interval. The histological properties of the rat vagina underwent constant changes after ovariectomy, and the most evident atrophy was observed 16 weeks after surgery. Hympanova et al. tested vaginal tissue from OVX sheep after a recovery time ranging from 60 to 160 days to determine whether the effects of estrogen deprivation persist longer term [26,40]. The increase in vaginal stiffness 60 days following OVX was not observed after 160 days. Several longitudinal studies have been conducted for bone tissue [60–63] to validate the use of the OVX model for testing preventative and treatment methods for osteoporosis. Similar longitudinal studies in OVX animals should be conducted to document changes to vaginal tissue premenopause versus postmenopause as well as compare any short- versus long-term effects of estrogen deprivation.

There is little to no literature reporting on the biomechanical properties of healthy premenopausal and postmenopausal vaginal tissue from women. Rodents, rabbits, and sheep have been used as animal models for menopause in published studies on the mechanical properties of vaginal tissues (Fig. 3). Nonhuman primates appear to be ideal models for studying menopause since they naturally undergo a reproductive aging process that has many similarities to the one experienced by women [64]. In nonhuman primates, menopause causes significant structural and functional changes, including the termination of menstruation (in species that exhibit menstrual bleeding) and atrophy of the vagina [65]. Some toothed whales (i.e., beluga whales, narwhals, and short-finned pilot whales) also cease reproducing before the end of their life [66]. Like humans, these toothed whales have the longest life span after reproductive senescence but how their reproductive tissues change remains unknown. Recently, biomechanical testing of vaginal tissues from dolphins has been conducted to explain copulation mechanisms [67]. Applications of these experimental methods to whales could push forward research on menopause. Comparative studies are still needed to determine the best species for studying the different sequels of menopause on human vaginal tissue.

The pelvis, with its organs and supportive structures, is one of the most complex regions of the human body. Yet, from a biomechanical perspective, the menopausal alterations of the pelvic floor remain understudied. Although some work has been done on quantifying the effects of menopause on the mechanical properties of vaginal tissue, little is known about the impact of menopause on other pelvic organs. The ovaries and uterus become smaller after menopause, and they continue to decrease in volume throughout the postmenopausal stage [18]. Similarly, the cervix shrinks during menopause [68]. Biomechanical testing on the uterus and cervix postmenopause is limited likely because these organs lose their primary reproduction function. However, women who prematurely undergo menopause, due to surgical procedures or chemotherapy, can still become pregnant by in vitro fertilization, thus, understanding how hormone-deprived reproductive organs are impacted is important. Around and after menopause, reduced bladder compliance, urethral shortening, thinning of urethral mucosa, and decreased urinary sphincter contractility occur, as recently reviewed by Alperin et al. [69]. Some studies also reported on the effect of menopause on pelvic ligaments which provide support to the reproductive organs. For example, the resilience of the uterosacral ligaments decreases [70] while the stiffness of the ligaments does not change with menopausal status [71]. Pelvic floor muscles also undergo alterations during menopause that affect their ability to provide support to pelvic organs [72].

Up to 85% of women experience symptoms such as vaginal dryness and dyspareunia associated with the menopausal transition [73], causing poor sexual functioning, reduced quality of life, and poor emotional well-being. In addition to genital and sexual symptoms, women have urinary symptoms such as urgency, dysuria, and recurrent urinary tract infections [74]. Many women are reluctant to discuss their vaginal or sexual discomfort with their physician because of embarrassment or concern about the side effects of existing treatment [75]. Over-the-counter moisturizers and lubricants represent the first lines of therapies, and they function by increasing vaginal hydration to alleviate symptoms. However, these therapies provide only temporary relief and require daily use. Severe

menopausal symptoms are commonly treated with hormone therapy, although this therapy is not recommended for women with gallbladder disease, diabetes, hypoparathyroidism, benign meningioma, high risk of breast cancer, or high risk of heart disease [76]. Vaginal laser therapy has emerged as a nonsurgical thermal treatment that functions by promoting tissue regeneration that ultimately improves the elasticity and strength of the vagina. However, this type of treatment has the potential for harm, burns, or scarring, and the Food and Drug Administration recently put out a warning regarding energy-based devices in the use of vaginal "rejuvenation" [77]. Given the contraindications and ineffectiveness surrounding some of the current treatments, new innovative and safe treatments must be developed to ease menopause. This will require the involvement of experts in biomechanical engineering since the mechanical properties of the vaginal tissue need to be accurately quantified to establish the effectiveness of new treatments that reduce the symptoms and side effects of menopause.

4 Conclusions

With the increase in life expectancy, many women will spend a larger portion of their life in the postmenopausal period. Unfortunately, physiological changes in menopause trigger health problems that severely affect women's quality of life. Vaginal and genitourinary symptoms are uncomfortable topics of conversation not only for women with their partners, family members, and physicians but also within the larger biomechanics community. This review article presents current research on the impact of menopause on the mechanical properties of the vagina with the intent of raising awareness of women's health problems requiring biomechanics-based studies. Paucity of mechanical data, limitations in experimental methods, and conflicting results call for further research. Together with new ex vivo studies, in vivo testing and in silico methods will provide key mechanistic insights for understanding the functional implications of menopause on vaginal tissue. It is our hope that this review article will serve as a stepping stone to new biomechanical studies on vaginal tissue, bolstering the application of the best experimental methods to address important questions related to menopause and women's health.

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Data Availability Statement

No data, models, or code were generated or used for this paper.

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