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PELVIC MUSCLES' MECHANICAL RESPONSE TO STRAINS IN THE ABSENCE AND PRESENCE OF PREGNANCY-INDUCED ADAPTATIONS IN A RAT MODEL

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Abstract

BACKGROUND—Maternal birth trauma to the pelvic floor muscles is thought to be consequent to mechanical demands placed on these muscles during fetal delivery that exceed muscle physiological limits. The above is consistent with studies of striated limb muscles that identify hyperelongation of sarcomeres, the functional muscle units, as the primary cause of mechanical muscle injury and resultant muscle dysfunction. However, pelvic floor muscles' mechanical response to strains have not been examined at a tissue level. Furthermore, we have previously demonstrated that during pregnancy, rat pelvic floor muscles acquire structural and functional adaptations in preparation for delivery, which likely protect against mechanical muscle injury by attenuating the strain effect.

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OBJECTIVES—1. To determine the mechanical impact of parturition-related strains on pelvic floor muscles' microstructure. 2. To test the hypothesis that pregnancy-induced adaptations modulate muscle response to strains associated with vaginal delivery.

STUDY DESIGN—3-month-old Sprague-Dawley late-pregnant (N=20) and non-pregnant (N=22) rats underwent vaginal distention, replicating fetal crowning, with variable distention volumes. Age-matched uninjured pregnant and non-pregnant rats served as respective controls. After sacrifice, pelvic floor muscles, which include coccygeus, iliocaudalis, and pubocaudalis, were fixed *in situ* and harvested for fiber and sarcomere length measurements. To ascertain the extent of physiological strains during spontaneous vaginal delivery, analogous measurements were obtained in the intrapartum rats (N=4), sacrificed during fetal delivery. Data were compared with repeated measures and two-way analysis of variance, followed by pairwise comparisons, with significance set at $P<0.05$.

RESULTS—Gross anatomic changes were observed in the pelvic floor muscles following vaginal distention, particularly in the enthesal region of pubocaudalis, which appeared translucent. The above appearance resulted from dramatic stretch of the myofibers, as indicated by significantly longer fiber length compared to controls. Stretch ratios, calculated as fiber length after vaginal distention divided by baseline fiber length, increased gradually with increasing distention volume. Paralleling these macroscopic changes, vaginal distention resulted in acute and progressive increase in sarcomere length with rising distention volume. The magnitude of strain effect varied by muscle, with the greatest sarcomere elongation observed in coccygeus, followed by pubocaudalis, and a smaller increase in iliocaudalis, observed only at higher distention volumes.

The average fetal rat volume approximated 3 mL. Pelvic floor muscle sarcomere lengths in pregnant animals undergoing vaginal distention with 3 mL were similar to the intrapartum sarcomere lengths in all muscles ($P>0.4$), supporting the validity of our experimental approach. Vaginal distention resulted in dramatically longer sarcomere lengths in non-pregnant compared to pregnant animals, especially in coccygeus and pubocaudalis ($P<0.0001$), indicating significant attenuation of sarcomere elongation in the presence of pregnancy-induced adaptations in pelvic floor muscles.

CONCLUSIONS—Delivery-related strains lead to acute sarcomere elongation, a well-established cause of mechanical injury in skeletal muscles. Sarcomere hyperelongation resultant from mechanical strains is attenuated by pregnancy-induced adaptations acquired by the pelvic floor muscles prior to parturition.

Keywords

pelvic floor muscles; birth injury; pregnancy adaptations; rat

INTRODUCTION

The pathways by which vaginal delivery leads to pelvic floor disorders (PFDs) are believed to be multifactorial, involving damage to nerves,^{1–3} connective tissue,^{4–7} and pelvic smooth and striated muscles.^{8–10} Clinical studies suggest that maternal trauma to the pelvic floor muscles (PFMs) results from mechanical demands imposed on these muscles during parturition that exceed skeletal muscle physiological limits.⁹ Hyperelongation of

sarcomeres, muscle functional units, has been shown to be a primary cause of mechanical muscle injury and resultant muscle dysfunction in the other striated muscles.^{11–16} Prior studies have shown that significant limb muscle injury reliably occurs when muscle stretch exceeds 60% of resting muscle length.¹⁷ Such strains result in supraphysiologic sarcomere lengths at which actin and myosin filaments no longer overlap, in turn leading to myofibrillar disruption acutely and muscle dysfunction in the long-term.^{18,19}

Computational models of human parturition demonstrate that PFMs' elongation up to 300% of resting muscle length is necessary to achieve fetal delivery.^{20,21} Thus, muscle injury would be expected to occur in most, if not all, vaginal deliveries; however, the majority of women do not exhibit radiologically-detected PFM avulsion injuries after vaginal childbirth.²² Furthermore, despite a strong association between PFDs and postpartum PFM avulsions observed on imaging, a large subset of women with pelvic floor dysfunction exhibit decreased PFM strength but do not demonstrate this type of defect.^{8,22–26} These findings suggest that muscle injury other than avulsion tears may contribute to postpartum PFM dysfunction.

Our group previously identified a constellation of changes in rat PFMs during pregnancy, including fiber elongation via serial addition of sarcomeres, or sarcomerogenesis, and increase in intramuscular extracellular matrix collagen content and muscle stiffness.^{27,28} We hypothesized that these antepartum alterations enable PFMs to withstand deformations during parturition without excessive sarcomere hyperelongation, in turn, decreasing PFMs' susceptibility to mechanical birth injury. Taken together, the above suggests that structural and functional adaptations, acquired by the PFMs during pregnancy, may account for the ability of these muscles to withstand "supraphysiologic" strains during parturition without injury. Thus, the objectives of the current study were twofold: 1) to determine the mechanical impact of delivery-related strains on PFM microstructure and 2) to test the hypothesis that pregnancy-induced adaptations of the PFMs modulate muscle response to strains associated with parturition by attenuating sarcomere elongation, the primary cause of mechanical injury in skeletal muscles.

MATERIALS AND METHODS

Given technical and ethical constraints associated with directly probing PFMs in living women, animal models are essential to study the mechanisms of PFMs' birth injury at the tissue level. We chose to utilize the rat model for the following three reasons: 1) rat PFMs' anatomy and architecture, the strongest predictor of *in vivo* active muscle function, are similar to those of humans;^{29,30} 2) rat has proven to be a reliable exemplar to study tissue changes due to pregnancy;^{31–33} 3) pregnancy induces unique adaptations in the intrinsic structural components of rat PFMs.^{27,28} The University of California San Diego Institutional Animal Care and Use Committee approved all study procedures. Three-month-old Sprague-Dawley rats were utilized to determine the mechanical impact of delivery-related strains on the PFMs' microstructure and to assess the effect of pregnancy-induced adaptations, acquired by the PFMs antepartum, on muscle response to mechanical strains. Primigravid gestational day 20–21 late-pregnant (P) (N=20) and nulligravid non-pregnant (NP) (N=22)

animals underwent vaginal distention (VD), replicating fetal crowning, with balloon volumes ranging from 1–5 milliliters (mL).

To ascertain physiological strain during vaginal delivery, neonatal rat (N=13) bi-parietal and abdominal diameters were measured and spherical volumes were calculated using $V = (4/3) \cdot \pi r^3$ equation, with $\pi = 3.14$, r – radius, V – volume. To determine the impact of the strain imposed on the PFMs during spontaneous parturition and to confirm validity of our experimental approach, intrapartum rats (N=4) were sacrificed during vaginal delivery and the outcomes of interest were compared to pregnant animals undergoing vaginal distention with the 3 mL balloon volume, analogous to that of a neonatal rat. Age-matched P and NP rats (N=10/group) that did not undergo vaginal distention served as respective controls. NP animals were in similar parts of estrous cycle, as determined by vaginal smears.

Vaginal distention procedure

Animals were weighed and anesthetized using isoflurane gas mixed with oxygen delivered via nose cone for the duration of the procedure. Genital hiatus (GH; base of the external urethral meatus to posterior fourchette), total vaginal length (TVL), and perineal body (PB; posterior fourchette to mid-anus) were measured using digital calipers. Vaginal distention was performed utilizing 12 French transurethral balloon catheters (Bard Medical, Covington, GA) with 130 gram weight attached to the end of the catheter to simulate circumferential and downward distention. The catheter was placed intravaginally, inflated to varying volumes, and left in place for 1–2 hours, as previously described.³⁴

Fiber length and sarcomere length measurements

Pregnant (P), non-pregnant (NP), and intrapartum rats were sacrificed and PFMs, including coccygeus (C), and pubocaudalis (PCa) and iliocaudalis (ICa), which comprise the levator ani muscle in rats,³⁵ were fixed *in situ* to preserve *in vivo* architecture, harvested, and divided into three regions. Small fiber bundles, consisting of either 10–20 or 3–5 fibers, were microdissected from each region for fiber length (L_f) and sarcomere length (L_s) measurements, respectively, using previously described methods.^{27,36} Muscle stretch ratios were calculated by dividing fiber length after vaginal distention at each distention volume by the fiber length in the control groups.

Assessment of muscle ultrastructure

In addition to the assessment of gross anatomy and sarcomere length measurements, PFMs' ultrastructure was examined with transmission electron microscopy (TEM). Muscle specimens were prepared for TEM using a standard protocol that combines chemical fixation with embedding in Durcupan. Briefly, PFMs, harvested from perfusion-fixed animals, were immersed in modified Karnovsky's fixative (2.5% glutaraldehyde and 2% paraformaldehyde in 0.15 M sodium cacodylate buffer, pH 7.4) for at least 4 hours, postfixed in 1% osmium tetroxide in 0.15 M cacodylate buffer for 1 hour and stained en bloc in 2% uranyl acetate for 1 hour. Samples were dehydrated in ethanol, embedded in Durcupan epoxy resin (Sigma-Aldrich), sectioned at 60 nm on a Leica UCT ultramicrotome, and picked up on Formvar and carbon-coated copper grids. Sections were stained with 2% uranyl acetate for 5 minutes and Sato's lead stain for 1 minute. Grids were viewed using a

JEOL 1200EX II (JEOL, Peabody, MA) transmission electron microscope and photographed using a Gatan digital camera (Gatan, Pleasanton, CA).

Statistical considerations

Baseline characteristics were compared between P and NP animals using independent Student t-test. Comparisons between muscles and conditions were performed by repeated measures and two-way analysis of variance (ANOVA) followed by pairwise comparisons with Šidák or Dunnett's tests, as appropriate. Significance level (α) was set to 5%. To obtain 80% power ($1-\beta$) to detect a 15% difference, the sample size of 4/group/distention volume was calculated utilizing the equation: $n=16*((CV)^2/(\ln(1-\delta))^2)$ using experimental variability in the control groups, with variables n =sample size; δ =difference desired (15%); CV =coefficient of variation (8%). Results are presented as means $\pm$ standard error of the mean (SEM), except where noted. All statistical analyses were performed using GraphPad Prism version 6.00, GraphPad Software, San Diego, CA, USA.

RESULTS

As expected, the baseline characteristics differed between the groups. Specifically, body weight of non-pregnant rats (209.3 ± 2.3 g) was lower than that of pregnant animals (284.9 ± 4.7 g), $P<0.0001$. Genital hiatus (NP: 3.9 ± 0.1 mm vs. P: 4.3 ± 0.1 mm, $P=0.01$); perineal body (NP: 7.6 ± 0.2 mm vs. P: 8.8 ± 0.3 mm, $P=0.002$); and total vaginal length (NP: 20.4 ± 0.3 mm vs. P: 24.4 ± 0.5 mm, $P<0.0001$) were also smaller in non-pregnant, compared to pregnant animals. GH increased dramatically during vaginal distention in both, NP (from 7.8 ± 0.3 mm at 1 mL to 16.3 ± 0.6 mm at 5 mL) and P (from 6.6 ± 0.4 mm at 1 mL to 14.4 ± 1.5 mm at 5 mL), groups. Interestingly, GH was substantially increased during spontaneous vaginal delivery, ranging from 10.7 to 13.6 mm in the intrapartum rats (median 13.1; mean 12.6 ± 0.7 , $P<0.0001$ relative to the antepartum values (Figure 1)).

Departure from normal muscle structural organization in response to vaginal distention was evident from the dramatic changes in the PFMs' gross appearance, as well as the distortion of Z-lines and misalignment of adjacent sarcomeres, visualized by TEM (Figure 2). These changes were mainly observed in the PCa and C, but were much less pronounced in ICa. PCa was noted to be extremely attenuated, with an almost translucent appearance of its enthesal region at distention volumes > 2 mL (Figure 2A). Similar distortion was noted in C, while alterations in ICa appearance were less prominent, indicating variability in the magnitude of strain impact across the individual components of the PFM complex. These gross changes corresponded to a dramatic stretch of the myofibers, as evident by significant increase in PFM fiber length with VD compared to controls (Table 1). As expected, PFM stretch ratios increased progressively with the increase in strain. The largest stretch ratios across distention volumes were observed in C, which has the shortest resting fiber length fibers relative to the other PFMs (1.4–1.8 in NP and 1.2–1.7 in P). Analogous to the pubococcygeus component of the levator ani complex that is predicted to experience the highest strains during human vaginal delivery,²⁰ PCa stretch ratios (1.2–1.7 in NP and 1.1–1.5 in P) exceeded those in ICa (1.2–1.4 in NP and 1.1–1.3 in P), Table 1.

Paralleling gross anatomic and macroscopic findings, VD resulted in acute and progressive increase in L_s with rising distention volume. The magnitude of strain effect on L_s also varied by muscle, with the greatest change observed in C, followed by PCa, and a smaller increase in ICa, observed only at higher distention volumes (Table 2).

To confirm that L_s increase in response to strains induced by VD is representative of the mechanical impact of physiological strains placed on the PFMs during spontaneous vaginal delivery, we compared PFM L_s during fetal delivery to antepartum L_s values in the respective muscles of P animals. Analogous to our findings in P rats undergoing VD, L_s increased in all PFMs during vaginal delivery compared to antepartum values. Moreover, the extent of intrapartum L_s elongation exhibited similar variation across the individual PFMs. The largest L_s increase (24.4%) occurred in C: $2.25 \pm 0.06 \mu\text{m}$ antepartum vs. $2.8 \pm 0.04 \mu\text{m}$ intrapartum, $P < 0.0001$. In PCa, L_s increased by 15.2%, from $2.3 \pm 0.04 \mu\text{m}$ antepartum to $2.65 \pm 0.04 \mu\text{m}$ intrapartum, $P = 0.0001$. In contrast, the 5% increase in ICa L_s was not significantly different from the antepartum length of $2.39 \pm 0.04 \mu\text{m}$, compared to $2.51 \pm 0.04 \mu\text{m}$ intrapartum, $P = 0.3$.

Using the equations outlined above, we calculated fetal rat volume, which approximated 3 mL ($2.93 \pm 0.1 \text{ mL}$). Importantly, L_s resultant from PFM strains induced by VD with 3 mL balloon volume did not differ from the intrapartum values in any of the muscles ($P > 0.4$), Figure 3. The above findings support the validity of our experimental approach in replicating physiologic PFM strain during vaginal delivery.

To determine if structural and functional alterations acquired by the PFMs during pregnancy^{27,28} modulate PFM response to strain, we compared PFM L_s of NP and P rats at each distention volume between 1–5 mL (Figure 3). VD with 3 mL volume, analogous to physiologic intrapartum strain, resulted in dramatically longer C and PCa L_s in NP compared to P animals, $P < 0.0001$ (Table 3). In contrast, the difference in ICa L_s between NP and P rats was not significant ($P = 0.07$). When evaluating the pattern of sarcomere lengthening across strain levels, C and PCa L_s were significantly longer in NP compared to P animals at all volumes above 1 mL. In contrast, there was no significance difference in ICa L_s between NP and P rats with smaller strains. However, VD with volumes $> 3 \text{ mL}$, which approximates average fetal rat volume, resulted in significantly increased L_s in NP compared to P animals ($P < 0.01$) in all muscles.

COMMENT

To our knowledge, this is the first study to examine PFMs' response to strains associated with parturition on a microscopic level. The main findings of the current study demonstrate that delivery-related strains result in acute sarcomere elongation, a well-established cause of mechanical injury in other skeletal muscles.^{11–16} Although directly probing PFMs is not possible in pregnant women, published studies utilizing computational modeling provide invaluable data on differential strains placed on the individual components of the human levator ani complex during vaginal delivery, with the largest stretch ratios occurring in the enthesal region of the pubococcygeus, at its origin on the inner surface of the pubic ramus.²⁰ This enthesal region has also been identified in imaging studies as a characteristic

“injury zone” for avulsion-type injuries of the levator ani at childbirth.^{37,38} This region corresponds to the exact location at which the greatest degree of macroscopic and microstructural damage among the muscles comprising rat levator ani (PCa and ICa) was noted in our study. Furthermore, prior studies demonstrate a direct relationship between infant head size and degree of muscle stretch,²⁰ concordant with our findings of increased sarcomere elongation with higher distention volumes.

Importantly, the current experimental results support our hypothesis that pregnancy-induced adaptations of the PFMs modulate muscle response to strains associated with parturition by attenuating sarcomere hyperelongation and, thus, appear to be protective. Interestingly, the differential extent of sarcomere lengthening in response to strain between pregnant and non-pregnant animals was most profound at the distention volume corresponding to the physiological strains placed on the PFMs during vaginal delivery (3 mL, analogous to average pup size). This may represent the “tipping point” beyond which the adaptations acquired by the PFMs during pregnancy are insufficient to protect these muscles against the mechanical impact of strain, as evident from smaller differences in sarcomere length between P and NP groups at higher distention volumes. Based on these findings, we propose that mechanical strains that exceed pregnancy-induced adaptations cause pathologic sarcomere lengthening, thus contributing to PFM injury and postpartum muscle dysfunction.

We have previously shown that structural and functional alterations acquired by different PFMs during pregnancy are not uniform; namely, C undergoes the greatest degree of fiber length elongation (via addition of sarcomeres in series), while PCa has the largest increase in stiffness, which is unaltered in ICa.^{27,28} Another interesting finding of the current investigation is that each individual PFM – C, PCa, and ICa – is exposed to a different degree of mechanical strain during both spontaneous vaginal delivery and the experimental vaginal distention procedure, with C and PCa subjected to larger strains compared to ICa.

The limitations of our study are inherent to the use of animal models to represent human conditions. As such, it remains unknown whether mechanical PFM injury due to excessive sarcomere hyperelongation is one of the PFM birth injury mechanisms in women. However, given the obvious ethical constraints associated with directly probing PFMs in pregnant women, animal models are essential to expand our understanding of the impact of pregnancy and vaginal delivery on the PFMs’ structural components.

In the current study, vaginal distention in the rat model did not lead to PFM avulsions. However, our findings of near-translucent appearance of certain parts of the PFMs – particularly the PCa enthesal region – support the notion that variable phenotypes of maternal PFM trauma likely exist.^{8,39} It is conceivable that if analogous PFM myofiber stretch occurs during vaginal delivery in some women, the resultant “see-through” appearance would likely be indistinguishable from muscle avulsion injuries on imaging. The above may explain the recently published findings that a large proportion of levator ani avulsions observed shortly after childbirth were no longer evident at 1 year postpartum.⁴⁰ It appears that variable phenotypes of the PFMs’ birth injury possibly exist. For instance, in some cases, irreversible damage to the myofibers may occur due to myofibrillar disruption associated with sarcomere hyperelongation, while in other cases, the regions of the pelvic

floor muscles most affected by strains could avulse. The rat has proven to be a useful model for studies of pregnancy and delivery-induced changes in the pelvic floor.^{28,32–34} Furthermore, the architectural design of rat PFMs closely approximates human PFM architecture.^{29,30} Importantly, the rat PFMs acquire structural and functional adaptations during pregnancy.^{27,28} The plasticity of rat PFMs allows the investigations of the protective effects of pregnancy-induced muscle adaptations.

The current experimental approach necessitates euthanasia of each animal at the time of vaginal distention. Thus, we were not able to follow the same animal longitudinally. Instead, we had to rely on cross-sectional examination of animals from each group and assumed homogeneity among individual animals. Although this was a necessary constraint of our study design, Sprague-Dawley rats are widely used in many domains of biomedical research in general and, specifically, in studies of simulated birth injury.^{41–43} Furthermore, the rats utilized in this study were obtained from a single vendor, were age-matched, and in many cases were from the same litter. In addition, the animals were housed together (5/cage) to minimize potential impact of environmental variables and to assure concordant estrous cycle stage in non-pregnant animals. Finally, our study was adequately powered to detect a 15% difference in L_s , which was achieved in the C and PCa muscles. However, substantially smaller L_s changes occurred in the ICa. Our post-hoc analysis of power to detect difference in ICa L_s between the groups is as follows: 62% at 2 mL (effect size 6.8%); 76% at 3mL (effect size 7.5%); 96% at 4mL (effect size 15%), and 44% at 5 mL (effect size of 8%). Thus, it is possible that the difference in ICa L_s with 2, 3 and 5 mL distention volumes between the groups may have become significant with larger sample size.

In conclusion, excessive mechanical strain during parturition results in sarcomere hyperelongation, a well-established cause of mechanical skeletal muscle injury. The findings of the current study support our hypothesis that adaptations acquired by rat PFMs during pregnancy modulate muscle response to strains by attenuating the extent of sarcomere elongation. Our future studies will focus on confirming that acute sarcomere hyperelongation at larger strains leads to pathological alterations of the pelvic floor muscles long-term.

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ABBREVIATIONS

PFDs	pelvic floor disorders
PFMs	pelvic floor muscles
P	pregnant
NP	non-pregnant
VD	vaginal distention

GH	genital hiatus
TVL	total vaginal length
PB	perineal body
C	coccygeus
ICa	iliocaudalis
PCa	pubocaudalis
L_f	muscle fiber length
L_s	sarcomere length

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Condensation

Rat pelvic floor muscles' mechanical response to parturition-related strains, resultant in sarcomere hyperelongation, is modulated by pregnancy-induced adaptations, acquired by these muscles prior to delivery.

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Implications and Contributions

- We demonstrated that mechanical strains related to parturition result in acute sarcomere hyperelongation of rat pelvic floor muscles. This well-established cause of mechanical injury in the limb muscles, represents a novel mechanism of birth trauma to the pelvic floor muscles, complementing previously studied avulsion-type injuries.
- Pregnancy-induced adaptations in the contractile and extracellular matrix pelvic floor muscle components, previously identified by our group, appear to prepare these muscles to withstand “supraphysiological” strains during delivery by attenuating sarcomere hyperelongation and protecting against mechanical muscle injury.
- The protective effect of pregnancy is greatest at the distention volume corresponding to average fetal size. At higher volumes, this protective effect is diminished, potentially indicating a “tipping point” beyond which pregnancy-induced adaptations are no longer sufficient to protect against maternal pelvic floor muscle injury.

Figure 1. Rat genital hiatus (GH) before delivery (A) is markedly increased during fetal delivery (B, C) and vaginal distention procedure (D), demonstrating similarity to the human parturition (E).

Figure 2.

(A) Photograph of left pubocaudalis fixed *in situ* during vaginal distention procedure. The instrument is visible through the translucent enthesal region (asterisk). Analogous appearance of pubocaudalis was noted during spontaneous fetal delivery. *SP*: symphysis pubis has been disarticulated to aid with visualization of the pelvic floor muscles. Transmission electron microscopy images of pubocaudalis muscles, procured from control (B) and vaginal distention with 5 mL volume (C) groups. Significant departure from normal muscle microstructure, specifically distortion and smearing of Z-lines and misalignment of adjacent sarcomeres, is observed in response to parturition-related strains (C).

Figure 3.

Vaginal distention (VD) with 1–5 mL volumes resulted in progressive sarcomere elongation (strain effect). Increase in sarcomere length was attenuated in pregnant, compared to non-pregnant rats (protective effect due to pregnancy-induced adaptations), especially in coccygeus and pubocaudalis. Distention with 3 mL in pregnant rats resulted in sarcomere length analogous to the intrapartum values.

Table 1

Changes in pelvic floor muscles' fiber length (L_f) in response to vaginal distention (VD) in non-pregnant (NP) and pregnant (P) groups. Stretch ratios are calculated as fiber length after vaginal distention divided by fiber length in respective controls (0 mL). Measurements in millimeters (mm) are presented as mean $\pm$ standard error of the mean.

Distention volume (mL)	Non-pregnant (L_{f0} , mm)	N	Stretch Ratio	P-value ^a NP+VD vs NP control	Pregnant (L_p , mm)	N	Stretch Ratio	P-value ^a P+VD vs P control
Coccygeus								
0	7.7 $\pm$ 0.3	10	–	–	10.5 $\pm$ 0.3	10	–	–
1	10.8 $\pm$ 0.2	4	1.4	0.01	12.9 $\pm$ 0.8	4	1.2	0.3
2	11.9 $\pm$ 1.2	4	1.5	0.0005	13.2 $\pm$ 0.3	4	1.3	0.02
3	13.1 $\pm$ 1.2	5	1.7	<0.0001	13.4 $\pm$ 0.3	4	1.3	0.0006
4	13.5 $\pm$ 1.1	4	1.7	<0.0001	14.6 $\pm$ 0.3	4	1.4	<0.0001
5	14.1 $\pm$ 0.8	5	1.8	<0.0001	17.3 $\pm$ 0.3	4	1.7	<0.0001
Pubocaudalis								
0	16.2 $\pm$ 0.4	10	–	–	19.6 $\pm$ 0.4	10	–	–
1	19.4 $\pm$ 0.9	4	1.2	0.01	21.3 $\pm$ 0.3	4	1.1	0.2
2	22.0 $\pm$ 0.1	4	1.4	<0.0001	23.2 $\pm$ 0.3	4	1.2	0.001
3	24.6 $\pm$ 1.1	5	1.5	<0.0001	25.4 $\pm$ 0.3	4	1.3	<0.0001
4	26.5 $\pm$ 1.6	4	1.6	<0.0001	26.3 $\pm$ 0.3	4	1.3	<0.0001
5	27.9 $\pm$ 0.7	5	1.7	<0.0001	28.7 $\pm$ 0.3	4	1.5	<0.0001
Iliocaudalis								
0	14.8 $\pm$ 0.2	10	–	–	18.0 $\pm$ 0.5	10	–	–
1	17.2 $\pm$ 0.9	4	1.2	0.08	20.3 $\pm$ 0.3	4	1.1	0.07
2	18.0 $\pm$ 0.7	4	1.2	0.01	20.2 $\pm$ 0.7	4	1.1	0.08
3	20.6 $\pm$ 0.8	5	1.4	<0.0001	21.9 $\pm$ 0.7	4	1.2	0.0005
4	20.8 $\pm$ 0.9	4	1.4	<0.0001	23.2 $\pm$ 0.3	4	1.3	<0.0001
5	21.2 $\pm$ 0.9	5	1.4	<0.0001	23.9 $\pm$ 0.4	4	1.3	<0.0001

^a P-values derived from repeated measures 2-way analysis of variance, followed by pairwise comparisons with respective controls using Dunnett's test.

Table 2

Within-group comparisons of the pelvic floor muscles' sarcomere length (L_s) in response to mechanical strains induced by vaginal distention with varying volumes (1–5mL) in non-pregnant and pregnant animals, relative to respective controls. Measurements in micrometers (μm) are presented as mean $\pm$ standard error of the mean.

Distention volume (mL)	Non-pregnant (L_s , μm)	N	<i>aP</i> -value	Pregnant (L_s , μm)	N	<i>aP</i> -value
Coccygeus						
Control	2.3 $\pm$ 0.06	10	–	2.25 $\pm$ 0.06	10	–
1	2.61 $\pm$ 0.07	4	0.04	2.4 $\pm$ 0.03	4	0.27
2	3.53 $\pm$ 0.03	4	< 0.0001	2.57 $\pm$ 0.09	4	0.001
3	3.95 $\pm$ 0.11	5	< 0.0001	2.68 $\pm$ 0.07	4	< 0.0001
4	4.07 $\pm$ 0.11	4	< 0.0001	2.8 $\pm$ 0.08	4	< 0.0001
5	4.49 $\pm$ 0.19	5	< 0.0001	3.65 $\pm$ 0.08	4	< 0.0001
Pubocaudalis						
Control	2.25 $\pm$ 0.04	10	–	2.3 $\pm$ 0.04	10	–
1	2.42 $\pm$ 0.07	4	0.45	2.31 $\pm$ 0.01	4	0.99
2	2.92 $\pm$ 0.07	4	< 0.0001	2.47 $\pm$ 0.08	4	0.17
3	3.5 $\pm$ 0.05	5	< 0.0001	2.6 $\pm$ 0.03	4	0.003
4	3.69 $\pm$ 0.01	4	< 0.0001	2.72 $\pm$ 0.08	4	< 0.0001
5	3.84 $\pm$ 0.13	5	< 0.0001	3.32 $\pm$ 0.12	4	< 0.0001
Iliocaudalis						
Control	2.36 $\pm$ 0.04	10	–	2.39 $\pm$ 0.04	10	–
1	2.37 $\pm$ 0.08	4	0.99	2.36 $\pm$ 0.02	4	0.99
2	2.53 $\pm$ 0.07	4	0.45	2.37 $\pm$ 0.04	4	0.99
3	2.64 $\pm$ 0.09	5	0.046	2.46 $\pm$ 0.05	4	0.87
4	2.86 $\pm$ 0.12	4	0.0002	2.49 $\pm$ 0.02	4	0.64
5	3.02 $\pm$ 0.11	5	< 0.0001	2.79 $\pm$ 0.1	4	< 0.0001

^a *P*-values derived from repeated measures 2-way (muscle $\times$ distention volume) analysis of variance, followed by pairwise comparisons with the respective controls using Dunnett's test.

Table 3

Comparisons *between* non-pregnant and pregnant groups, demonstrating a differential response of the pelvic floor muscles' sarcomere lengths (L_s) to vaginal distention with varying volumes. Distention volume of 0 milliliters (mL) corresponds to control animals. Measurements in micrometers (μm) are presented as mean $\pm$ standard error of the mean.

Distention volume (mL)	Non-pregnant (L_s , μm)	N	Pregnant (L_s , μm)	N	αP -value
Coccygeus					
0	2.3 $\pm$ 0.06	10	2.25 $\pm$ 0.06	10	0.99
1	2.61 $\pm$ 0.07	4	2.4 $\pm$ 0.03	4	0.64
2	3.53 $\pm$ 0.03	4	2.57 $\pm$ 0.09	4	< 0.0001
3	3.95 $\pm$ 0.11	5	2.68 $\pm$ 0.07	4	< 0.0001
4	4.07 $\pm$ 0.11	4	2.8 $\pm$ 0.08	4	< 0.0001
5	4.49 $\pm$ 0.19	5	3.65 $\pm$ 0.08	4	< 0.0001
Pubocaudalis					
0	2.25 $\pm$ 0.04	10	2.3 $\pm$ 0.04	10	0.99
1	2.42 $\pm$ 0.07	4	2.31 $\pm$ 0.01	4	0.9
2	2.92 $\pm$ 0.07	4	2.47 $\pm$ 0.08	4	0.0006
3	3.5 $\pm$ 0.05	5	2.6 $\pm$ 0.03	4	< 0.0001
4	3.69 $\pm$ 0.01	4	2.72 $\pm$ 0.08	4	< 0.0001
5	3.84 $\pm$ 0.13	5	3.32 $\pm$ 0.12	4	< 0.0001
Iliocaudalis					
0	2.36 $\pm$ 0.04	10	2.39 $\pm$ 0.04	10	0.99
1	2.37 $\pm$ 0.08	4	2.36 $\pm$ 0.02	4	0.99
2	2.53 $\pm$ 0.07	4	2.37 $\pm$ 0.04	4	0.61
3	2.64 $\pm$ 0.09	5	2.46 $\pm$ 0.05	4	0.42
4	2.86 $\pm$ 0.12	4	2.49 $\pm$ 0.02	4	0.008
5	3.02 $\pm$ 0.11	5	2.79 $\pm$ 0.1	4	0.17

^a P -values derived from two-way (group $\times$ distention volume) analysis of variance, followed by pairwise comparisons using Šidák correction.