

🏆 Best in Category Prize – Pelvic Organ Prolapse

45

Catanzarite T¹, Bou-Malham L², Esparza M³, O'Connor S³, Alperin M¹

1. Division of Female Pelvic Medicine and Reconstructive Surgery, University of California, San Diego, 2. Department of Biological Sciences, University of California, San Diego, 3. Department of Orthopaedic Surgery, University of California, San Diego, CA

PELVIC FLOOR MUSCLE RESPONSE TO MECHANICAL STRAINS ASSOCIATED WITH PARTURITION

Hypothesis / aims of study

Birth trauma to the pelvic floor muscles (PFMs) is thought to result from mechanical demands that exceed muscle physiological limits. Previous work demonstrates that rat PFMs, including coccygeus (C), iliocaudalis (IC), and pubocaudalis (PC), undergo differential alterations in pregnancy [1, 2]. We sought to determine how pregnancy-induced adaptations modulate individual PFM response to strain produced by vaginal distention (simulating parturition) in a rat model.

Study design, materials and methods

Three-month-old Sprague-Dawley primigravid late pregnant (P) (N=13) and virgin nonpregnant (NP) rats (N=15) underwent vaginal distention (VD), replicating fetal crowning, with balloon volumes ranging from 1-5 millilitres (mL). Because differences in distensibility of other pelvic tissues in P and NP animals could impact strain effect on the PFMs, intrapelvic pressure (IP) generated during vaginal distention was measured with a pressure gauge contiguous with the balloon. To match a full spectrum of IPs observed in the NP group, an additional 11 P rats underwent VD with 6-10 mL volumes. Age-matched P and NP rats (N=10/group) that did not undergo vaginal distention served as respective controls. Animals were anesthetized using isoflurane gas mixed with oxygen delivered via nose cone. P and NP rats were sacrificed and PFMs, which include coccygeus (C), iliocaudalis (IC), and pubocaudalis (PC), were fixed *in situ* to preserve in vivo architecture, harvested, and divided into 3 regions. Fiber bundles, consisting of 10-20 fibers, were dissected from each region of each muscle and fiber length (Lf) was measured. Bundles were microdissected into fibers for sarcomere length (Ls) determination by laser diffraction. Comparisons were performed by repeated measures and two-way analysis of variance (ANOVA) followed by multiple comparisons with Šidák or Dunnett's test, as appropriate. Significance level (α) was set to 5%. Results are presented as means \pm standard error of the mean (SEM), except where noted.

Results

Gross anatomic changes were observed in the PFMs following vaginal distention (VD). In particular, PC was extremely attenuated, with an almost translucent appearance of its enthesial region at distention volumes > 2 mL. Similar distortion was noted in C, while alterations in IC were less prominent. We believe these gross changes are due to myofiber stretch, as evidenced by significant increases in PFM fiber length (Lf) after VD compared to controls. As expected, PFM stretch ratios increased progressively with increase in strain volume. The largest stretch ratios were observed in C (1.7-2 in NP and 1.4-1.8 in P), compared to PC (1.4-1.8 in NP and 1.3-1.5 in P) and IC (1.3-1.4 in NP and 1.2-1.3 in P). Paralleling gross anatomic and macroscopic findings, VD resulted in progressive increase in Ls with rising distention volume. The magnitude of strain effect varied by muscle, with the greatest Ls change observed in C, followed by PC, and a smaller rise in IC, observed only at higher distention volumes (Table). We next compared PFM Ls of NP and P rats at each distention volume between 1-5 mL. Ls were significantly longer in NP compared to P animals at all volumes above 1 mL in both C ($P<0.0001$ for all except 5mL, $P=0.009$) and PC ($P<0.0001$ for all except 5mL, $P=0.007$). In contrast, in IC, there was no significant difference in Ls between NP and P rats at any volume besides 4 mL ($P=0.01$). The pattern of Ls changes in the individual PFMs in NP and P animals relative to respective controls and to each other was similar when the groups were matched either by strain volumes or intrapelvic pressures (IP).

Interpretation of results

To our knowledge, this is the first study to examine PFMs' response to strains associated with simulated parturition on a microscopic level. Our data demonstrate that in PFMs, mechanical strain results in acute sarcomere elongation, a well-established cause of mechanical injury in other skeletal muscles. Pregnancy-induced adaptations in PFMs appear to attenuate sarcomere hyperelongation in response to strain. The limitations of our study are inherent to use of animal models to represent human conditions. Given the ethical impossibility of directly probing PFMs in women during pregnancy and childbirth, animal models are essential to expand our understanding of the impact of pregnancy and vaginal delivery on the PFMs. The rat has been shown to have similar PFM architecture to humans, exhibits PFM adaptations in pregnancy, and is a useful model for the study of pregnancy and delivery-induced changes in the pelvic floor. In our protocol, since each animal must be euthanized in order to harvest PFMs, 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. Although this was a necessary constraint of our study design, Sprague-Dawley rats are widely used in biomedical research in general and, specifically, in studies of simulated birth injury. Furthermore, rats were obtained from a single vendor, were age-matched, were often from the same litter, and were housed together to minimize environmental variability and to assure concordant estrous cycle stage in NP animals.

Concluding message

PFM sarcomere hyperelongation, caused by mechanical strain during simulated parturition with vaginal distention, is attenuated by pregnancy-induced adaptations. Remarkably, the extent of adaptation in the individual PFMs appears to be proportional to the degree of strain experienced by each muscle during simulated parturition.

Table. Mechanical impact of pelvic floor muscle strain induced by vaginal distention with 1-5 mL balloon volumes, in nonpregnant and pregnant animals, represented as sarcomere length (L_s) in coccygeus, pubocaudalis, and iliocaudalis versus controls (0 mL). Measurements in micrometers (μm) are expressed as mean \pm standard error of the mean (SEM).

Distention Volume (mL)	Nonpregnant (L_s , μm)	N	*P value	Pregnant (L_s , μm)	N	*P value
Coccygeus						
0	2.3 \pm 0.06	10	--	2.25 \pm 0.06	10	--
1	2.62 \pm 0.06	3	0.004	2.33 \pm 0.02	2	0.9
2	3.53 \pm 0.03	3	< 0.0001	2.43 \pm 0.02	2	0.3
3	4.06 \pm 0.1	3	< 0.0001	2.68 \pm 0.04	3	< 0.0001
4	4.09 \pm 0.09	3	< 0.0001	2.82 \pm 0.03	3	< 0.0001
5	4.34 \pm 0.06	3	< 0.0001	3.81 \pm 0.07	3	< 0.0001
Pubocaudalis						
0	2.25 \pm 0.04	10	--	2.3 \pm 0.04	10	--
1	2.33 \pm 0.01	3	0.8	2.31 \pm 0.03	2	0.9
2	2.95 \pm 0.05	3	< 0.0001	2.34 \pm 0.03	2	0.9
3	3.47 \pm 0.01	3	< 0.0001	2.55 \pm 0.08	3	0.03
4	3.68 \pm 0.01	3	< 0.0001	2.63 \pm 0.08	3	0.002
5	3.7 \pm 0.11	3	< 0.0001	3.35 \pm 0.11	3	< 0.0001
Iliocaudalis						
0	2.36 \pm 0.04	10	--	2.39 \pm 0.04	10	--
1	2.35 \pm 0.06	3	0.9	2.34 \pm 0.02	2	0.9
2	2.45 \pm 0.03	3	0.8	2.35 \pm 0.06	2	0.9
3	2.71 \pm 0.1	3	0.001	2.43 \pm 0.09	3	0.9
4	2.86 \pm 0.1	3	< 0.0001	2.51 \pm 0.04	3	0.5
5	2.99 \pm 0.12	3	< 0.0001	2.72 \pm 0.07	3	0.002

References

1. Alperin M, Lawley DM, Esparza MC, Lieber RL. Pregnancy-induced adaptations in the intrinsic structure of rat pelvic floor muscles. *Am J Obstet Gynecol.* 2015;213(2):191.e1-e191.e7.
2. Alperin M, Feola A, Duerr R, Moalli P, Abramowitch S. Pregnancy and delivery-induced biomechanical changes in rat vagina persist postpartum. *Int Urogynecol J.* 2010;21(9):1169-1174.

Disclosures

Funding: K12 HD001259 **Clinical Trial:** No **Subjects:** ANIMAL **Species:** Rat **Ethics Committee:** University of California San Diego Institutional Animal Care and Use Committee