GYNECOLOGY

Pregnancy-induced adaptations in intramuscular extracellular matrix of rat pelvic floor muscles

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BACKGROUND: Birth trauma to pelvic floor muscles is a major risk factor for pelvic floor disorders. Intramuscular extracellular matrix determines muscle stiffness, supports contractile component, and shields myofibers from mechanical strain.

OBJECTIVE: Our goal was to determine whether pregnancy alters extracellular matrix mechanical and biochemical properties in a rat model, which may provide insights into the pathogenesis of pelvic floor muscle birth injury. To examine whether pregnancy effects were unique to pelvic floor muscles, we also studied a hind limb muscle.

STUDY DESIGN: Passive mechanical properties of coccygeus, iliocaudalis, pubocaudalis, and tibialis anterior were compared among 3-month old Sprague–Dawley virgin, late-pregnant, and postpartum rats. Muscle tangent stiffness was calculated as the slope of the stress–sarcomere length curve between 2.5 and 4.0 μm, obtained from a stress-relaxation protocol at a bundle level. Elastin and collagen isoform concentrations were quantified by the use of enzyme-linked immunosorbent assay. Enzymatic and glycosylated collagen crosslinks were determined by high-performance liquid chromatography. Data were compared by the use of repeated-measures, 2-way analysis of variance with Tukey post-hoc testing. Correlations between mechanical and biochemical parameters were assessed by linear regressions. Significance was set to $P < .05$. Results are reported as mean ± SEM.

RESULTS: Pregnancy significantly increased stiffness in coccygeus ($P < .05$) and pubocaudalis ($P < .0001$) relative to virgin controls, with no change in iliocaudalis. Postpartum, pelvic floor muscle stiffness did not differ from virgins ($P > .3$). A substantial increase in collagen V in coccygeus and pubocaudalis was observed in late-pregnant, compared with virgin, animals, ($P < .001$). Enzymatic crosslinks decreased in coccygeus ($P < .0001$) and pubocaudalis ($P < .02$) in pregnancy, whereas glycosylated crosslinks were significantly elevated in late-pregnant rats in all pelvic floor muscles ($P < .05$). Correlations between muscle stiffness and biochemical parameters were inconsistent. In contrast to the changes observed in pelvic floor muscles, the tibialis anterior was unaltered by pregnancy.

CONCLUSIONS: In contrast to other pelvic tissues, pelvic floor muscle stiffness increased in pregnancy, returning to prepregnancy state postpartum. This adaptation may shield myofibers from excessive mechanical strain during parturition. Biochemical alterations in pelvic floor muscle extracellular matrix due to pregnancy include increase in collagen V and a differential response in enzymatic vs glycosylated collagen crosslinks. The relationships between pelvic floor muscle biochemical and mechanical parameters remain unclear.

Key words: passive mechanics, pelvic floor muscles, pregnancy, rat

Pelvic floor disorders include pelvic organ prolapse, urinary, and fecal incontinence. Collectively, they represent a major public health problem, given their high prevalence, negative impact on quality of life, lack of ability to predict who is at risk, lack of preventive measures, high failure rate of available treatments, and the associated economic burden.[1-3] Clinical studies provide ample evidence that pelvic floor skeletal muscles (PFMs) are major contributors to proper female pelvic floor function. Radiologically detected defects and dysfunction of the PFMs are associated with a significantly increased risk of pelvic floor disorders, as well as recurrence of pelvic organ prolapse after surgical treatment.[4] PFMs are composed of coccygeus (C) and the levator ani muscle complex; however, most published investigations use PFMs and levator ani muscle interchangeably and do not include C.

Childbirth is identified as the leading cause of PFM trauma. The reason for the trauma, suggested by modeling and imaging studies, is excessive PFM strain that occurs during vaginal delivery.[5-7] Surprisingly, despite these predictions, many parous women do not sustain PFM injury.[8] It is therefore likely that, to achieve extraphysiologic strain without injury, pregnancy-induced adaptations take place within these muscles. Previously we showed that PFMs adjust their contractile architecture to increase excursion, or range of motion, in preparation for delivery.[9] Another major skeletal muscle component is the extracellular matrix (ECM) network, which consists of the endomysium that envelops individual muscle fibers, the perimysium, which connects adjacent fibers and surrounds muscle bundles and fascicles, and the epimysium, which ensheathes the whole muscle.[10] Intramuscular ECM bears the majority of passive load, provides support to the myofibers, and is the main determinant of muscle stiffness.[10,11] Human and animal studies of ligaments, symphysis pubis, cervix, and vaginal connective tissue demonstrate dramatic changes in the mechanical properties of these structures during pregnancy.[12-17] Biochemical alterations and remodeling of ECM are thought to account for the decreased stiffness and increased distensibility of these tissues, which

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facilitates delivery of the fetus and protects against maternal birth injury.[18,19]

Taken together, these findings motivate our thesis that pregnancy-induced protective adaptations also take place in the PFM ECM. One of the main functions of intramuscular ECM is to shield myofibers from mechanical stresses and strains by increasing passive tension, which is tension generated when muscle is stretched independently of muscle electrical activation.[11] Thus, we hypothesized that, in contrast to other pelvic tissues, PFM stiffness would increase in pregnancy. We postulated that such adaptation will protect the contractile component of the PFMs from injury caused by excessive mechanical strain during parturition.

Given the obvious ethical constraints associated with procurement of sufficient human PFM tissue from pregnant women to perform such a study, we used a rat model to determine PFM ECM alterations during gestation. Comparison of PFM structural parameters between human and rat demonstrated architectural similarity, suggesting that they have comparable functional design.[20] The rat model also has proven valuable in many studies of pregnancy-related changes in other pelvic structures.[17,21,22] Furthermore, we recently reported that adaptations during pregnancy occur in the myofibers of rat PFMs, which increase their length by adding sarcomeres in series.[9] As a complement, the current investigation primarily focuses on the biomechanical properties of PFM ECM to provide further insight into potential pregnancy-induced functional changes of human PFMs and pathogenesis of PFMs maternal birth injury.

The secondary objectives of this study were to explore pregnancy-induced biochemical alterations in the PFM ECM that could potentially account for the changes in muscle mechanical properties. Pregnancy induces marked alterations in collagen isoform ratios and increases elastin in vaginal connective tissue.[23] In this study, we analyzed the content of pertinent to skeletal muscle collagen isoforms and elastin, as well as collagen crosslinks, because previous research has shown that increased collagen crosslinks are associated with greater skeletal and cardiac muscle stiffness.[24,25] Consistent with our hypothesis that PFM stiffness will increase in pregnancy, we speculated that pregnancy does not lead to a decrease in collagen I content or an increase in elastin of the intramuscular ECM. We further proposed that PFM collagen crosslinks would increase in the pregnant group. To determine whether the results were specific to PFMs and not a general skeletal muscle response to an altered hormonal environment, we examined the tibialis anterior (TA) hind limb muscle as well.

Materials and Methods

The University of California Institutional Animal Care Committee approved all procedures performed. Seven virgin and 7 late-pregnant (19−21 days) 3-month-old Sprague−Dawley rats (*Rattus norvegicus*) were euthanized, and samples of C, iliocaudalis (IC), pubocaudalis (PC), and TA were obtained immediately and placed in storage solution at −20°C, as previously described, to prevent hyperpolarization and destruction of muscle tissue.[26] PFMs from 7 four-week postpartum rats were used to determine recovery of the passive mechanical properties after delivery. Virgin and postpartum animals were in similar parts of the estrus cycle, as determined by vaginal smears.

Passive mechanics

All muscles were tested within 2 weeks of harvest in a relaxing solution to ensure muscle tissue integrity. Muscle stiffness was determined at the bundle level, which reflects mechanical behavior predominantly due to intramuscular ECM.[11] To avoid compromising the structural proteins, which can affect the elastic modulus, tissue digestion, often used to facilitate dissection, was not performed.[11] Three fiber bundles, composed of ~30 individual muscle cells or fibers and the associated endo- and perimysium, were tested from each muscle via the use of a custom apparatus, as previously described.[27] To summarize in brief, muscle bundles were placed into a testing chamber and secured between a force transducer (405 A; Aurora Scientific Aurora, ON, Canada) and a fixed pin connected to a rotational bearing (MT-RS; Newport Irvine, CA). Sarcomere length (L_s) provided objective quantification of muscle strain and myofibrillar array quality control and was measured throughout the experiments by laser diffraction.[28] Bundle length was set to the minimum length that produced measurable forces, and baseline force and L_s were determined. Baseline sample diameter was measured optically with a cross-hair reticle mounted on a dissecting microscope and micromanipulators on an x−y mobile stage to determine cross-sectional area.

Force−L_s data were generated for each bundle subjected to a stress-relaxation protocol. Bundles were elongated until failure or to a L_s of 4.0 μm in 10% strain increments followed by 3-minute stress−relaxation periods after which further stress decline is insignificant.[11] Force was converted to stress by dividing force by baseline cross-sectional area, assuming isovolumetric cylindrical shape of fiber bundles.[29] Muscle passive tension varies depending on starting L_s, with increasing stiffness occurring at longer L_s. To assure proper comparison of passive mechanical properties among PFMs, we used *tangent stiffness*, which quantifies stress at a given L_s, instead of *tangent modulus*, which defines stress required to achieve a particular percent strain and loses L_s as a relevant parameter. Tangent stiffness was quantified as the slope of the stress-L_s curve between 2.5 and 4.0 μm.

Biochemical studies

To associate biochemical with mechanical results, samples were derived from adjacent portions of the same muscle used for mechanical testing. Tissue samples were immediately placed in complete mini protease inhibitor/phosphate-buffered saline solution (Roche, Indianapolis, IN) to prevent collagen degradation, snap frozen in liquid nitrogen, and stored at −80°C. Previously, we found that total collagen content of PFM ECM substantially increases in pregnancy[9]; however, quantitative changes in total collagen have not