GYNECOLOGY

Impact of vaginal parity and aging on the architectural design of pelvic floor muscles

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BACKGROUND: Vaginal delivery and aging are key risk factors for pelvic floor muscle dysfunction, which is a critical component of pelvic floor disorders. However, alterations in the pelvic floor muscle intrinsic structure that lead to muscle dysfunction because of childbirth and aging remain elusive.

OBJECTIVES: The purpose of this study was to determine the impact of vaginal deliveries and aging on human cadaveric pelvic floor muscle architecture, which is the strongest predictor of active muscle function.

STUDY DESIGN: Coccygeus, iliococcygeus, and pubovisceralis were obtained from younger donors who were ≤51 years old, vaginally nulliparous (n = 5) and vaginally parous (n = 6) and older donors who were >51 years old, vaginally nulliparous (n = 6) and vaginally parous (n = 6), all of whom had no history of pelvic floor disorders. Architectural parameters, which are predictive of muscle's excursion and force-generating capacity, were determined with the use of validated methods. Intramuscular collagen content was quantified by hydroxyproline assay. Main effects of parity and aging and the interactions were determined with the use of 2-way analysis of variance, with Tukey's post-hoc testing and a significance level of .05.

RESULTS: The mean age of younger and older donors differed by approximately 40 years ($P = .001$) but was similar between nulliparous and parous donors within each age group ($P > .9$). The median parity was 2 (range, 1-3) in younger and older vaginally parous groups ($P = .7$). The main impact of parity was increased fiber length in the more proximal coccygeus ($P = .03$) and iliococcygeus ($P = .04$). Aging changes manifested as decreased physiologic cross-sectional area across all pelvic floor muscles ($P < .05$), which substantially exceeded the age-related decline in muscle mass. The physiologic cross-sectional area was lower in younger vaginally parous, compared with younger vaginally nulliparous, pelvic floor muscles; however, the differences did not reach statistical significance. Pelvic floor muscle collagen content was not altered by parity but increased dramatically with aging ($P < .05$).

CONCLUSIONS: Increased fiber length in more proximal pelvic floor muscles likely represents an adaptive response to the chronically increased load placed on these muscles by the displaced apical structures, presumably as a consequence of vaginal delivery. In younger specimens, a consistent trend towards decrease in force-generating capacity of all pelvic floor muscles in the parous group suggests a potential mechanism for clinically identified pelvic floor muscle weakness in vaginally parous women. The substantial decrease in predicted muscle force production and fibrosis with aging represent likely mechanisms for the pelvic floor muscle dysfunction in older women.

Key words: aging, architecture, pelvic floor muscle, vaginal delivery

Pelvic floor disorders (PFDs) include pelvic organ prolapse and urinary and fecal incontinence. Collectively, they represent a major public health problem because of their high prevalence, negative impact on quality of life, lack of preventive measures, high failure rate of available treatments, and associated economic burden.[1-3] The cause of these complex disorders is not well understood but is thought to be multifactorial, with vaginal delivery and aging identified as the major inciting and promoting events.[4-6] These risk factors are thought to destabilize structural components that are integral to the proper function of female pelvic floor, including pelvic floor skeletal muscles (PFMs).

PFMs are comprised of the levator ani complex and coccygeus, which makes up the dorsal part of the pelvic floor and is associated intimately with the posterior portion of the levator ani. PFMs counteract gravitational force and resist intraabdominal pressure, provide support to pelvic and abdominal viscera, and aid in urinary and fecal continence. Despite this, current knowledge pertaining to structure, function, and pathologic transformations of human PFMs is limited because directly probing these muscles in vivo is not feasible due to their location deep in the pelvis. Most research that has focused on PFMs relies on imaging, computational modeling, or indirect clinical assessment. Although such investigations have been invaluable in establishing a framework for the role of PFM dysfunction in the pathogenesis of PFDs,[7,8] significant knowledge gaps remain. Importantly, decreased PFM strength precedes the onset of symptomatic PFDs.[9,10] Furthermore, one-third of women with pelvic organ prolapse do not exhibit radiologic defects in PFMs[8]; and stress urinary incontinence is more frequent in women with minimal, compared with major, levator ani defects on magnetic resonance imaging.[11] Prolapse and urinary incontinence can result from a compromise in the integrity of the striated urethral sphincter or pelvic connective tissue supportive structures. Alternatively, alterations in the intrinsic PFM structure, which are not detectable by traditional imaging modalities, may impair muscle function in the absence of radiologically evident defects.

Skeletal muscle architecture governs the magnitude of force that a muscle

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generates, how fast it contracts (velocity), and its range of contraction (excursion).[12] A shift in architectural design directly impacts muscle functional capacity. Therefore, measuring alterations in architecture provides insight into the mechanics of PFM dysfunction and allows us to overcome the limitations that are inherent to the radiologic investigations, which invoke an anatomic cross-sectional area that previously has been described to be a poor proxy measure for muscle force generating capacity.[12] The aforementioned is especially pertinent to the examination of aging effects on skeletal muscles, because the loss of muscle strength with age exceeds that of muscle mass.[13,14] Marked alterations in muscle architecture that are caused by aging and result in loss of sarcomeres, both in parallel and in series, partially explain this finding.[14-16] Furthermore, radiologic studies did not identify age-related atrophy as an essential component of PFM dysfunction in older women.[17] We believe that standard imaging techniques are limited because of their inability to distinguish among and identify changes in the contractile and intramuscular extracellular (ECM) components of PFM, which are remarkably thin, compared with other human skeletal muscles.[18] Studies that probe muscle architecture are highly invasive and not feasible in living women, thus human cadaveric specimens serve as an invaluable source of tissue[19]. A number of transformative concepts in the field of musculoskeletal medicine have resulted from investigations of cadaveric limb muscles.[20-22]

The goal of this study was to determine the independent and combined effects of vaginal childbirth and aging, which are the leading epidemiologic risk factors for PFM dysfunction, on muscle architecture in the absence of significant PFDs by directly examining human cadaveric PFM specimens. We tested the following hypotheses: (1) because of their distinct proximal-distal spatial distribution, the architecture of individual PFMs is variably, but permanently, altered by vaginal delivery; (2) similar to other human skeletal muscles, age-related changes in the PFM architectural parameters deleteriously impact muscle predicted force production.

Materials and Methods

Performing this study required unique access to well-defined cadaveric specimens in which age, parity status, delivery mode and a history of symptomatic PFDs could be determined unambiguously. Thus, we partnered with the University of Minnesota Bequest Body Donation Program, which provides access to donors' medical records. The study was exempt from institutional review board approval because of the exclusion of living human subjects. Specimens were obtained from donors who had no history of PFDs or rectal prolapse to eliminate potential confounding effects of disease on architecture. Donors with a history of pelvic radiation, gynecologic or colorectal malignancy, pelvic metastasis, connective tissue disorder, myopathy, colectomy, or proctectomy were also excluded. In contrast to vaginal delivery, PFM injury is rarely observed after cesarean childbirth,[23,24] and PFM strength is unchanged by abdominal deliveries.[9] Thus, nulliparous donors (n = 8) and donors with a history of cesarean deliveries only (n = 3) were designated as *vaginally nulliparous*. Over a 2.5-year period, we accumulated 23 specimens that enabled statistical comparison among the following 4 groups: younger vaginally nulliparous (YVN; n = 5), younger vaginally parous (YVP; n = 6), older vaginally nulliparous (OVN; n = 6), and older vaginally parous (OVP; n = 6). Based on epidemiologic studies, age surpasses parity as a risk factor for PFDs after menopause,[25] which is a marker of biologic senescence in women.[26] Using statistics from the National Institute of Aging, the average age of menopause in the United States is 51 years. Thus, we defined *younger* as $\leq$51 years and *older* as >51 years, which is consistent with previously reported onset of age-related sarcopenia in appendicular muscles in the sixth decade of life.[27,28]

Muscle architecture

To maintain in vivo architectural structure, coccygeus (C), iliococcygeus (IC), and pubovisceralis (PV), consisting of pubococcygeus and puborectalis, were perfusion fixed in situ (ie, attached to the skeleton) via injection of formaldehyde through the common carotid artery, in contrast to most studies that immersion fix tissues. PFMs were harvested en block and separated into individual muscles, identified by tracking each muscle along its length, weighed, and divided into cephalad, middle, and caudate regions, as previously described.[18] Muscle thickness was measured with electronic calipers to the nearest 0.01 mm in each region. The following architectural parameters were determined with the use of validated methods[29]: muscle mass; physiologic cross-sectional area (PCSA), which is a predictor of isometric force generation capacity; fiber length, which is a predictor of muscle excursion and contractile velocity; and sarcomere length (L_s), which determines the force that is produced when muscle is stimulated. Three fiber bundles were dissected from each region, and fiber length was measured with electronic calipers to the nearest 0.01 mm.

Myofibers were isolated from each bundle under a dissecting microscope with a 6X objective and an overall magnification of 60X (Leica MZ16; Meyer Instruments Inc, Houston, TX) and mounted on a slide for L_s determination by laser diffraction.[30] The fixation process shortens L_s by approximately 10% of its in vivo length; thus, L_s provides information regarding a muscle's in vivo sarcomere length. L_s is also used to calculate the number of sarcomeres in series within fibers to normalize fiber length (L_{fn}) and correct for potential differences in length among specimens at the time of fixation. PCSA was calculated as previously described, with the use of an optimal human sarcomere length of 2.7μm.[31]

Intramuscular ECM

PFMs have a composite structure that consists of the contractile myofibers embedded within a large connective tissue network of ECM, which primarily consists of collagen.[32] Muscle