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Alterations in pelvic floor muscles and pelvic organ support by pregnancy and vaginal delivery in squirrel monkeys

Jessica N. Bracken,

Departments of Obstetrics & Gynecology, Scott and White Memorial Hospital and Clinic, 2401 South 31st Street, Temple, TX 76508, USA, Texas A&M Health Science Center College of Medicine, Temple, TX, USA

Michelle Reyes,

Departments of Obstetrics & Gynecology, Scott and White Memorial Hospital and Clinic, 2401 South 31st Street, Temple, TX 76508, USA, Texas A&M Health Science Center College of Medicine, Temple, TX, USA

Jilene M. Gendron,

Department of Radiology, Scott and White Memorial Hospital and Clinic, Temple, TX, USA, Texas A&M Health Science Center College of Medicine, Temple, TX, USA

Lisa M. Pierce,

Departments of Obstetrics & Gynecology, Scott and White Memorial Hospital and Clinic, 2401 South 31st Street, Temple, TX 76508, USA, Texas A&M Health Science Center College of Medicine, Temple, TX, USA

Val M. Runge, and

Department of Radiology, Scott and White Memorial Hospital and Clinic, Temple, TX, USA, Texas A&M Health Science Center College of Medicine, Temple, TX, USA

Thomas J. Kuehl

Departments of Obstetrics & Gynecology, Scott and White Memorial Hospital and Clinic, 2401 South 31st Street, Temple, TX 76508, USA, Department of Pathology, Scott and White Memorial Hospital and Clinic, Temple, TX, USA, Departments of Molecular & Cellular Medicine, Scott and White Memorial Hospital and Clinic, Temple, TX, USA, Texas A&M Health Science Center College of Medicine, Temple, TX, USA

Jessica N. Bracken: jbracken@swmail.sw.org; Thomas J. Kuehl: tkuehl@swmail.sw.org

Abstract

Introduction and hypothesis—The objective of this study was to measure the effects of pregnancy and parturition on pelvic floor muscles and pelvic organ support.

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Correspondence to: Jessica N. Bracken, jbracken@swmail.sw.org.

Present Address: V. M. Runge, Department of Radiology, The University of Texas Medical Branch, 2.103 University Hospital Clinics, 301 University Boulevard, Galveston, TX, USA

Conflicts of interest None.

Methods—Levator ani, obturator internus, and coccygeus (COC) muscle volumes and contrast uptake were assessed by MRI of seven females prior to pregnancy, 3 days, and 4 months postpartum. Bladder neck and cervix position were measured dynamically with abdominal squeezing.

Results—The sides of three paired muscles were similar ($p>0.66$). COC volumes were greater ($p<0.004$) after parturition than before pregnancy or after recovery. COC contrast uptake increased ($p<0.02$) immediately after delivery. Bladder neck position both in the relaxed state and abdominal pressure descended ($p<0.04$) after delivery and descended further ($p<0.001$) after recovery. Cervical position in the relaxed state before delivery was higher ($p<0.001$) than postpartum but was unchanged ($p=0.50$) with abdominal pressure relative to delivery.

Conclusion—In squirrel monkeys, coccygeus muscles demonstrate the greatest change related to parturition, and parturition-related bladder neck descent seems permanent.

Keywords

MRI; Pelvic floor muscles; Pelvic organ prolapse; Parturition; Squirrel monkey

Introduction

Pelvic floor dysfunction including stress urinary incontinence, fecal incontinence, and pelvic organ prolapse (POP) affects many women worldwide. Pelvic floor disorders have significant negative social and economic impacts. There are multiple risks for the development of POP including female gender, increasing age, parity, and mode of delivery [1–3]. POP is understood to result from a defect in muscle and/or connective tissue support which may be mediated by nerve injury or physical detachment of the muscle or its connective tissue from bony structures. Many investigators have used magnetic resonance imaging (MRI) to evaluate the pelvic floors in both asymptomatic and symptomatic patients and in both nulliparous and parous women after vaginal delivery and in subjects after the development of POP [4–11]. The relationship of pelvic organ positions to a pelvic floor reference line has also been reported [9]. MRI is a noninvasive means of evaluating muscle morphology and dimensions. Images in 2D and 3D format have been utilized in humans to determine total muscle volumes comparing asymptomatic women to women who experience pelvic floor dysfunction [4]. However, using a PUBMED search of the interval from November 2001 to November 2010 with key words “MRI, pelvic floor muscles, pelvic organ prolapse, and parturition”, we found no studies that have followed a cohort serially from a time before symptoms of POP developed through to a point after pelvic support defects were manifested. Women are not the only upright species of primate to spontaneously develop pelvic support defects with age and parity as risk factors. Squirrel monkeys also show this tendency. In a population of squirrel monkeys, nearly half of the older animals have presence of pelvic support defects [12]. In a larger breeding cohort of younger animals, pelvic organ defects are associated with childbirth and age [13]. Kramer et al. used MRI images to demonstrate that levator ani volumes in parous squirrel monkeys with POP were not reduced in volume compared to those without POP [14]. This study was undertaken to follow a cohort of nulliparous squirrel monkeys that were to be imaged prior

to pregnancy, a few days after spontaneous parturition, and several months postpartum, to compare changes in pelvic floor muscles and pelvic organ support through this life event.

Methods

Squirrel monkeys

Squirrel monkeys were obtained from the National Squirrel Monkey Breeding and Research Resource. They are housed at the Scott & White Memorial Hospital Animal Facility in Temple, TX. Squirrel monkeys are seasonal breeders with peak breeding activity between December and March in northern latitudes. Females undergo puberty between 2.5 and 3.5 years of age. At the time of conception, a cohort of eight nulliparous females was between 4 and 5 years of age when their body size had reached adult levels. Pregnancies were dated and monitored using ultrasound in awake animals.

MRI of the pelvic floor in squirrel monkeys

MRI was performed using a validated procedure as previously described by Kramer et al. [14]. Monkeys were sedated with a ketamine solution consisting of 100 mg ketamine/10 mg xylazine/1 mg acepromazine/mL. Contrast medium [gadolinium chelate (ProHance, Bracco Diagnostics, Inc, Princeton, NJ) yielding a dose of 0.25 mmol/kg] was given IV into a peripheral vein solely to increase signal-to-noise ratios and thus increase image quality due to normal enhancement of muscle tissues. Each anesthetized animal was brought to the MRI unit (3T Siemens Trio) and placed on its back with legs and body supported in a natural position inside of an 8-channel wrist coil (InVivo Technologies). Two localizer sequences were obtained to set the region of anatomic interest to produce serial images of the pelvic floor from L7 to C4. Using a 3D FLASH with TR 7.38, TE 2.71, flip angle of 22° gradient echo sequence, 224 sagittal images were acquired lasting 4.63 min with 0.3×0.3×0.3 mm voxels. After the procedure, a neonatal blood pressure cuff was placed around the animal's abdomen to provide transabdominal pressure. A dynamic series (using 2D FLASH with TR 6.22, TE 2.16, and flip angle of 10° gradient echo) of images was obtained during 25 s while inflating the cuff with a sufficient volume of air to reproduce prolapse as evaluated by perineal exam, if present, or voiding of bladder contents [15]. During this dynamic series, 20 sagittal images along the midline were obtained to evaluate the change of position of the bladder neck and cervix relative to bony landmarks as previously reported [15]. Animals were visually observed for motion of arms, feet, and tail as a monitor of sedation status during the entire MRI process. The high resolution T1-weighted gradient echo sagittal views were reformatted as 224 axial views for muscle volume and contrast intensity measurements. Animals were returned to the animal facility for monitored recovery.

The DICOM image files were processed using 3D-Doctor software (Able Software Corporation, Lexington, MA) for review, manipulation, measurement, and analysis. The left and right sides of levator ani, obturator internus, and coccygeus muscles were traced by authors (JNB and TJK). The volumes were calculated by the software algorithm from the surfaces and thicknesses of the image series. The total volume for both sides of each paired muscle is reported. For all of the muscles, we traced high contrast areas separately. Recording and analysis of images using 3D-Doctor software has been established in our

laboratory with good inter-observer correlations for pelvic floor muscle volume measures [14]. A bony reference line was created from the anterior aspect of the pubic symphysis to the anterior aspect of the first tail vertebra [15]. The position of the bladder neck and cervix relative to this reference line in millimeter was measured as an objective description of the degree of prolapse of these pelvic structures.

Females were examined during the interval from before conception to less than 1 month gestation for their first MRI. The second MRI was performed between 2 and 4 days (mean of 2.9 days) postpartum in females delivering offspring spontaneously. One of eight animals in this cohort had a fetal malpresentation requiring surgical delivery. This animal was excluded from the analyses. The third MRI was performed between 114 and 137 days (mean of 127 days, about 4 months) postpartum.

Statistical methods

Demographic characteristics of the females were presented as means with standard deviations. Analysis of variance was utilized for parametric measures of muscle volumes and descent of the bladder neck and cervix with a repeated measures design (Statistica software from StatSoft, Tulsa, OK). Post hoc testing of means was performed using Duncan's test with $p < 0.05$ taken as significant.

Results

Results are based on seven animals with spontaneous vaginal deliveries. Females were similar in age (5.4 ± 0.6 years with a range of 4.2 to 5.8 years) at the time of delivery. Their body weight increased by about 5% from 738 ± 83 g (range of 612 to 862 g) prior to pregnancy to 776 ± 74 g (range 697 to 868 g) 3 days postpartum. Body weight returned to 727 ± 59 g (range 656 to 803 g) by the time of recovery at 4 months postpartum. Five infants were born alive and two were stillborn via spontaneous vaginal delivery at term which was 139 ± 3 (range of 135 to 143) days of gestation in this series based on ultrasound dating of gestational sacs. The birth weights averaged 124 ± 9 g (range of 115 to 136 g) or 16% of their mothers' body weight prior to pregnancy. All five females with live babies nursed their infants during this interval.

The left and right sides of each muscle were consistently similar throughout serial imaging ($p < 0.66$) so that an analysis was performed using total muscle volumes. Levator ani and obturator internus muscle volumes did not change ($p = 0.37$ and 0.06 , respectively) during the three observations. However, the volume of coccygeus muscles was greater ($p < 0.027$) immediately after parturition than either before pregnancy or after recovery (Fig. 1). While contrast levels in the muscles were not seen to change for levator ani and obturator internus muscles (Fig. 2), there were significant ($p < 0.02$) changes in the percent of high contrast regions within the coccygeus muscles immediately after delivery that were only partially corrected by 4 months after delivery. In assessing the percentage of coccygeus muscle volume affected, the volume affected by this increase in contrast differed ($p < 0.001$) between the three imaging times (Table 1). The region of high contrast was less than 1% prior to the first vaginal delivery, increased, on average, to 47% of the volume of the coccygeus muscles at 3 days postpartum and decreased to 12% by the fourth month postpartum. The region of

this high contrast is in the section of coccygeus muscle toward the animal's head which represents a region of contacts with bony elements of the pelvis and vertebra.

Evidence of pelvic organ descent was noted on comparison of images before and after delivery. A bony reference line was created from the anterior aspect of the pubic symphysis to the anterior aspect of the first tail vertebra [15]. The bladder neck position in the relaxed state and with abdominal pressure descended ($p<0.04$) relative to the reference line following vaginal delivery (Fig. 3). The bladder neck descended further ($p<0.001$) by 4 months postpartum. Abdominal pressure also produced bladder neck descent ($p=0.008$) relative to the relaxed state. The position of the cervix in the relaxed state before delivery was higher ($p<0.001$) than immediately postpartum or after 4 months recovery (Fig. 4). However, the position of the cervix with abdominal pressure was unchanged ($p=0.50$) between different assessment times.

Finally, the distance between the interior aspects of the posterior pelvic bones were measured. As the pubic symphysis was not seen to be expanded shortly after delivery, it seemed reasonable that the pelvic bones might separate along the posterior edge to accommodate the fetal head as if hinged at the front. The measurement of this distance as demonstrated in Fig. 5 was increased ($p=0.020$) at 3 days postpartum. By 4 months postpartum, this dimension was no longer different ($p=0.27$) from the pre-pregnancy measurement.

Discussion

Pelvic organ prolapse results from an injury to the pelvic floor muscles and/or the nerves. Vaginal delivery is felt to be a significant risk factor [16]. Squirrel monkeys similarly develop pelvic support defects with parity and age as risk factors [12, 13]. Previous studies in this species have utilized dissections and histological methods developed as terminal endpoints [17–20]. These reports demonstrated that similar structural organization of the pelvic floor muscles, levator ani innervation, and endopelvic fascia was found in human and squirrel monkey females. However, the squirrel monkey does not have a distinct puborectalis muscle. Myogenic and neurogenic changes similar to those seen in women occurred in the squirrel monkey levator ani muscle and were associated with aging and parity. Three-dimensional models created with MRI demonstrated that levator muscle volumes differed between multiparous monkey females with and without POP matched in age, parity, and weight. Vaginal biopsies in women and monkeys contained distinct epithelial, mucosal, and muscularis layers interspersed with elastin and collagen; histology did not vary with POP status in either species. Cellularity (fibroblasts) in the vaginal mucosa was not reduced in monkeys or women with POP but did decrease with age. Elastin content increased with age in monkeys. From these observations first reported in 2005 [21], squirrel monkeys and women share many pelvic structural and histological similarities. Because the squirrel monkey is unique among nonhuman primates in that parturition is often traumatic, resulting in significant maternal and fetal morbidity and mortality, and that a high frequency of older, parous females develop spontaneous POP, it seems an appropriate species for comparison to women in advanced pelvic floor research.

This study was performed with a serial assessment design using an animal species previously shown to develop the same type of POP reported in women. MRI for anatomic assessment and a serial design was anticipated to be the most sensitive for detection of variations related to pregnancy and parturition. The focus of this study was to detect changes in muscle volume and contrast agent uptake shortly after delivery as an indication of transient muscle injury or trauma associated with pregnancy and delivery. In this sample of intact nulliparous females, the levator ani and obturator internus muscles were unchanged, while coccygeus muscles both increased in size and in accumulation of contrast. These results suggest that there is injury to the coccygeus muscles in the squirrel monkey after pregnancy and parturition. The coccygeus muscle connects the spine to the edge of the bony pelvis. While the coccygeus muscle terminates on either side at the base of the tail, a structure not found in great apes and women, the squirrel monkey does not move its tail laterally. The bulk of the muscle is cephalic of this point and connects to multiple pelvic structures. Unlike in women, the pubic symphysis in the squirrel monkey does not separate during vaginal delivery. Thus, the pelvis must widen toward the posterior rim of the pelvis resulting in forces that might produce excessive stretch to the cephalic end of the coccygeus muscles. The squirrel monkey delivers in the mentum anterior position. These same forces of labor can cause damage to the anterior compartment. As seen in the serial axial sections, the ligamentous connection at the pubic symphysis is not seen to open in images a few days postpartum. However, the region of the pelvis between the posterior aspects of the pelvic bones and the fused vertebra of the sacrum may be subject to expansion during the delivery of the fetal head as demonstrated by dimension change in Fig. 5. The muscle connections at this point may be stretched to the point of injury leading to edema or bruising. The extracellular fluid associated with this acute injury would be expected to accumulate contrast material. Later, at 4 months postpartum, portions of this injury may be repaired and portions may be modified with a high content of fat or connective tissue leading to MR changes. We have previously reported an evidence for this effect using histological evaluations in animals euthanized following MR where longer term injury of the levator ani muscles had been induced by nerve injury [15]. In this cohort of animal, no evidence of pelvic organ prolapse was observed during perineal inspection under anesthesia at the time of the MRI examination. However, there was descent of the bladder neck and cervix relative to a reference line that suggests the beginnings of pelvic support loss, not unlike asymptomatic minimal changes that are seen with some patients. The changes in bladder support and in coccygeus muscle appearance may be unrelated. Further work will be needed to evaluate these novel observations.

There are limitations to the interpretations of MRI images including an inability to compare subtle changes in contrast to actual observations of tissue histology in the living animal. The uptake of contrast that we termed edema could be from scar tissue formation, fat deposit, or true edema of tissue injury. As animals were not assessed immediately prior to delivery, it is not possible in this study to claim that these changes are solely due to parturition events as some effects could be the result of the burden of carrying a large uterus for the later portion of a 20-week gestation.

Variation in muscle measurements between the sides of paired muscles was not noticed within subjects. However, muscle volumes did vary up to 25% between the monkeys which

also varied in body size. The advantage of performing serial measurement is the reduction of the impact of variation between animals as a component of the measurement. Others have suggested that the variation in levator ani muscle shape and certain length and width measurements may be relatively uniform in asymptomatic women [4, 10]. On the contrary, Rizk et al. found that most dimensions of the pelvic floor and bony pelvis were different in healthy, nulliparous white and non-white women [11]. These authors reported on small sample groups of ten or less. With limited ranges of body mass indices, age, and race, we caution concluding that there is uniformity among all women.

Of course, the squirrel monkey pelvic floor muscular anatomy differs somewhat from that of human females. It is interesting to note that Handa et al. in a recent report using serial MRI evaluation in 246 postpartum patients after their first delivery noted no differences in soft tissue dimensions in women with sphincter lacerations at vaginal delivery compared to those without a clinically diagnosed sphincter laceration [22]. The results of this study support the concept that pelvic floor muscle volumes are not systematically permanently altered by a single vaginal delivery. However, we have demonstrated an evidence of morphologic changes in muscle related to the accumulation of high contrast regions in an area of the muscle that spans a location likely to experience trauma from stretch injury delivery. Hoyte et al. reported significant changes to levator ani volumes in women who were asymptomatic, had genuine stress incontinence, and pelvic organ prolapse albeit not through serial measurements over time [8]. In a previous report using this methodology in squirrel monkeys, we also found variations in levator ani volumes associated with prolapse [14]. However, we were concerned that covariates related to group composition might confound such analysis approaches. Therefore, designs with serial assessments would be needed to document and dissect anatomic changes associated with life events that challenge the pelvic floor. These patient observations and our observations in the squirrel monkey support the need for additional series studies in women using imaging methods for both detailed anatomic endpoints and dynamic assessments of subtle support changes.

Our study is the first evidence in the squirrel monkey, a species that develops POP similar to women, of a change in pelvic floor muscles associated with pregnancy and parturition. As squirrel monkeys do develop spontaneous pelvic organ prolapse with increasing age and parity, the role of these muscle changes in the evolution of prolapse is of interest. The observation that the first pregnancy and delivery leads to descent of the bladder neck demonstrates the support begins to alter in this species before any external clinical manifestations are observed, similar to women.

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Fig. 1.

Plot of total muscle volumes for each of three pelvic floor muscle groups demonstrating the lack of change for levator ani and obturator internus ($p=0.37$ and 0.06 , respectively, using analysis of variance with repeated measures) and change for coccygeus among measurements obtained prior to pregnancy, 3 days postpartum, and 4 months postpartum

Fig. 2. Serial axial images of one of the seven animals demonstrating variation in contrast for coccygeus muscles related to vaginal delivery. The reference line in this sagittal section is the point where the axial images were taken on this female at the three study times

Fig. 3.

Bladder neck position at rest and with abdominal pressure pre-pregnancy, 3 days postpartum, and 4 months postpartum. The *bar* on the MR image is 10 mm in length

Fig. 4. Cervix position at rest and with abdominal pressure pre-pregnancy, 3 days postpartum, and 4 months postpartum. The *bar* on the MR image is 10 mm in length

Fig. 5.
Distance changes between pelvic bones in relation to vaginal delivery

Table 1

Changes in the region of high contrast of coccygeus muscles as a percentage with SE of the total volume related to pregnancy and parturition in seven squirrel monkey females

	Prior to pregnancy	Three days postpartum	Four months postpartum	<i>p</i> value
Left side	0	46±6	10±4	<0.001 ^a
Right side	0	49±6	14±3	<0.001 ^a
<i>p</i> value	0.97	0.34	0.18	

Comparisons made using analysis of variance with repeated measures design

^aThe percent of muscle volume with high contrast in MR images differs between times with each time differing from the other two