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PARITY IS NOT RELATED TO MYELINATED AXON CONTENT OF LEVATOR ANI NERVE WHILE PARTURITION TRANSIENTLY AFFECTS VOLUME AND MAGNETIC RESONANCE CONTRAST INTENSITY OF PELVIC FLOOR MUSCLES IN THE SQUIRREL MONKEY.

Hypothesis / aims of study: Pelvic organ prolapse (POP) in squirrel monkeys is associated with aging and parity as in women. POP has been postulated to result from nerve and muscle injury related to parturition. Altered support function of the levator ani (LA) muscles is implicated in both squirrel monkeys and women. To test this hypothesis, we compare the number of myelinated axons in the levator ani nerve (LAN) in parous and non-parous animals and in three age groups (pubertal, adolescent, adult). To more directly examine the hypothesis that parturition affects pelvic floor muscles, we compared the volume and contrast intensity of three muscles before, immediately after, and remote from vagina parturition.

Study design, materials and methods: Nerve specimens from 19 animals without vaginal prolapse between 2.5 and 25 years of age were obtained from the section of LAN beginning medial to the posterior division of the hypogastric artery and ending distal to the bifurcation of the nerve before penetration of the iliocaudalis muscle. Myelinated axons were counted by an observer blinded to animal characteristics including age and parity. Paired Student's *t* tests were used to assess the differences in large and small myelinated axons related to site of the samples (three sites within each nerve were examined) and side (left and right) within animals. Analysis of variance was used to examine effects of age and parity. In a second group of 6 females, LA, obturator internus (OI), and coccygeus (COC) muscle volumes were measured using 3-D Doctor software to trace axial images obtained as T1-weighted scans using Siemens MR with 4 channel wrist coil in animals following administration of gadolinium contrast that distributed into the extra-cellular space. Females were imaged prior to or within the first month of a five month pregnancy, on the third postpartum day, and 2 months following delivery. Muscle volumes and contrast-to-noise intensity measurements were compared within animals using analysis of variance.

Results: LAN contains on the average 279 ± 18 (SE) myelinated axons that are distributed in size as a bimodal distribution of 168 ± 15 small axons (minor diameter < 8 microns) and 111 ± 6 large axons (minor diameter > 8 microns). No significant differences of large and small myelinated axons were found related to sites within nerves ($p > 0.3$) or between left and right sides within animals ($p > 0.2$). No differences related to three age groups ($p = 0.44$ with 8 pubertal, 6 adolescent, and 5 adults) or parity ($p=0.19$ with 8 non-parous and 8 parous) were found in the number of small and large myelinated axons. While nerve crush artefacts were noted in regions of the nerve segment grasped during the collection process, these did not extend to other portions of the nerve segments and no other histopathology was noted. Muscle edema was identified as an increase in contrast-to-noise intensity relative to a control muscle (caudal flexor) within the same image. Muscles on left and right sides did not differ in volume for LA, OI, and COC ($p=0.50$, $p=0.17$, $p=0.18$, respectively). Muscle volumes differed ($p < 0.01$) between the three observation times for all 3 sets of muscles. LA volumes decreased ($p = 0.001$) from 422 mm^3 prepregnancy to 347 mm^3 immediately after delivery and returned to 389 mm^3 ($p = 0.06$ vs. prepregnancy) by 2 months postpartum. Similarly, OI volumes decreased ($p = 0.01$), then returned to prepregnancy levels ($p = 0.09$). COC volumes increased ($p = 0.001$) from 184 mm^3 to 229 mm^3 immediately after delivery and returned to 173 mm^3 ($p = 0.27$ vs. prepregnancy) 2 months postpartum. COC volume increase was reflected as an increase ($p = 0.017$) in contrast-to-noise ratios 3 days postpartum with a return to prepregnancy levels ($p = 0.52$ vs. prepregnancy) by 2 months postpartum. No changes in intensity were noted for LA and OI ($p > 0.8$ and $p > 0.5$, respectively). Defects and disruptions in the attachments of muscles to the bony pelvis were evident in LA and COC muscles for some animals.

Interpretation of results: The observations that myelinated axons of the LAN do not vary with age or parity and the previously reported observation that POP increases with age and parity suggest that permanent nerve damage with a loss of nerve fibers or with sustained peripheral nerve histopathology of this nerve may not be an intervening mechanism for POP in this species. This report of the presence of minimal affects of parturition on LA muscle (a transient decrease in volume) in the squirrel monkey including a lack of increase in contrast suggest along with our recent report of an increase in LA volume for animals with POP, that LA muscle injury plays less of a role in this species. However, the profound changes in the COC muscle that plays a central role in interconnecting muscles and connecting bony elements of the pelvis and tail suggest that muscle injury may be a common feature of parturition initiated POP in both women and squirrel monkeys, although different muscles may be involved. It is important to further appreciate that magnetic resonance images obtained in this study do not demonstrate functional properties that could be altered.

Concluding message: These data describing the LAN in the female squirrel monkey are the first reported for primates and demonstrate the lack of effect of aging and parity on myelinated axon numbers. These observations do not rule out the potential for short-term effects of childbirth on nerves of the pelvic floor muscles, but suggest that such changes, if present, can be repaired. COC volume and intensity increase three days following delivery in the squirrel monkey suggesting that the COC is affected by parturition in this species, likely due to edema at a specific region of the muscle that responds to excessive stretch during delivery of the fetal head through this narrow portion of the pelvis.

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