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Structure–function relationship of the human external anal sphincter

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Abstract

Introduction and hypothesis—Obstetrical external anal sphincter (EAS) injury and subsequent dysfunction are leading risk factors for female fecal incontinence (FI). Limited knowledge of the EAS structure–function relationship hinders treatment optimization. We directly measured functionally relevant intrinsic parameters of human EAS and tested whether vaginal delivery alters the EAS structure–function relationship.

Methods—Major predictors of in vivo EAS function were compared between specimens procured from vaginally nulliparous (VN, $n = 5$) and vaginally parous (VP, $n = 7$) cadaveric donors: operational sarcomere length (L_s), which dictates force–length relationship; physiological cross-sectional area (PCSA), which determines isometric force-generating capacity; fiber length (L_{fn}), responsible for muscle excursion and contractile velocity; and muscle stiffness. Data were analyzed using unpaired and paired t tests, $\alpha < 0.05$. Results are presented as mean $\pm$ SEM.

Results—The VN and VP (median parity 3) groups were similar in age and BMI. No gross anatomical defects were identified. EAS L_s ($2.36 \pm 0.05 \mu\text{m}$) was shorter than the optimal L_{so} ($2.7 \mu\text{m}$), at which contractile force is maximal, $P = 0.0001$. Stiffness was lower at L_s than L_{so} ($5.4 \pm 14 \text{ kPa}/\mu\text{m}$ vs $35.3 \pm 12 \text{ kPa}/\mu\text{m}$, $P < 0.0001$). This structural design allows active and passive tension to increase with EAS stretching. EAS relatively long L_{fn} ($106 \pm 24.8 \text{ mm}$) permits rapid contraction without decreased force, whereas intermediate PCSA ($1.3 \pm 0.3 \text{ cm}^2$) is conducive to maintaining resting tone. All parameters were similar between groups.

Conclusions—This first direct examination of human EAS underscores how EAS intrinsic design matches its intended function. Knowledge of the EAS structure–function relationship is important for understanding the pathogenesis of FI and the optimization of treatments for EAS dysfunction.

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Compliance with ethical standards

Conflicts of interest None.

Keywords

External anal sphincter; Muscle architecture; Fecal incontinence

Introduction

Fecal incontinence (FI) is a morbid condition that affects 9–15% of women in the USA [1]. Although the underlying etiology is multifactorial, it is clear that external anal sphincter (EAS) dysfunction plays a major role in the pathogenesis of FI, as EAS is critical in the maintenance of anal continence [2]. Obstetrical anal sphincter injury (OASI) correlates strongly with EAS dysfunction and the resultant FI in women [2, 3]. Despite a decline in the use of routine episiotomies, OASI continues to complicate close to half of all vaginal deliveries, with 11% diagnosed at the time of delivery and an additional 35% diagnosed postpartum [4, 5].

Sphincteroplasty is used world-wide for the repair of damaged EAS; however, this therapy has failed to produce lasting satisfactory results. Furthermore, there is a lack of consensus regarding the best approach to sphincter repair, with published studies yielding conflicting results for overlapping vs end-to-end techniques [6, 7]. One of the main barriers hindering the ability to optimize the effectiveness of the current treatments, and to design more successful ones, stems from our limited understanding of the EAS structure–function relationship and pathological changes in the intrinsic muscle components that lead to EAS dysfunction subsequent to trauma during parturition.

Skeletal muscle physiological properties that best predict active muscle function include the length–tension relationship, force-generating and excursions capacity, and contractile velocity. These important functional parameters are determined by the length of muscle's "building blocks," known as sarcomeres, physiological cross-sectional area (PCSA), and normalized fiber length (L_{fn}). In addition, muscle passive mechanical properties, dominated by intramuscular extracellular matrix, determine muscle stiffness and load-bearing capacity [8, 9].

One of the fundamental properties of skeletal muscle is that its contractile force depends on the sarcomere length (L_s), with maximal force occurring at optimal L_s . Optimal L_s , which is determined by the length of the thick and thin myofilaments, myosin and actin, is species-specific because of the differences in actin length between species. Optimal L_s of human skeletal muscles is 2.7 μm [10, 11]. However, the operational L_s of some striated muscles differs from optimal L_s in a way that is advantageous to their in vivo function. The Frank–Starling relationship in the heart exemplifies this point. As preload increases, the cardiac myofibers stretch, leading to sarcomere elongation. Because the resting L_s of the cardiac muscle is less than the optimal L_s , sarcomere elongation brings its operational L_s closer to optimal L_s . This results in a greater contractile force, allowing a larger ejection volume when the preload is increased [12, 13]. Analogous to the cardiac muscle, skeletal wrist flexor muscles operate at $L_s < \text{optimal } L_s$, so that L_s and the accompanying contractile force are increased when these muscles are stretched. This architectural design ensures that the wrist remains stable during hand extension [10]. Studies utilizing a rabbit model indicate that,

similar to the muscles described above, the EAS operates at $L_s < \text{optimal } L_s$ [8]. However, rabbit models have not been validated for human EAS and it has previously been shown that rabbit pelvic floor muscles' architecture is the least similar to humans among experimental animals commonly used for pelvic floor disorder research [14, 15]. The same investigators used imaging combined with anal manometry to demonstrate that EAS contractile force rises with increasing distension in human clinical studies [16]. From the above, the authors deduced that EAS operational L_s in women is also likely to be shorter than optimal L_s . However, to our knowledge, L_s of human EAS has never been measured directly. Even though the above studies provide the framework for the current investigation, validation of the derived measures by primary experimental data is of outmost importance. For example, indirect clinical assessments led to the notion that the human puborectalis muscle operates at a short L_s [17]. In contrast, our previous direct tissue level assessments of the human pelvic floor muscles demonstrate that the puborectalis operates at optimal L_s [15]. Furthermore, direct anatomical studies enable the assessment of the individual components of the anal sphincter complex, providing a critical advantage over the indirect techniques that simultaneously measure the output of the entire complex.

Thus, the primary objective of this study was to empirically test the hypothesis that the operational L_s of human EAS is shorter than optimal L_s by directly measuring L_s in cadaveric specimens, which represents muscle in vivo L_s . Second, we aimed to determine the other major parameters, predictive of EAS isometric force generating an excursionsal capacity, and passive mechanical properties, responsible for muscle stiffness. Last, we sought to assess the impact of vaginal delivery, a leading risk factor for FI associated with pathological changes in EAS muscle, on these parameters.

Materials and methods

Human cadaveric EAS specimens ($n = 6$) were fixed in situ and harvested from vaginally nulliparous (VN) and vaginally parous (VP) female donors, whose medical records were available for review, through collaboration with The Bequest Body Donation Program at the University of Minnesota. Donors with a history of sphincteroplasty, rectal prolapse repair, or hemorrhoidectomy were excluded. In addition, specimens were excluded if the rectum was distended with a large caliber stool at the time of fixation. To ensure unbiased analysis, investigators were blinded to donor group assignments. The study was exempt from Institutional Review Board approval, as it did not include living human subjects.

External anal sphincter width in a coronal plane and thickness in a transverse plane were measured at 12, 3, 6, and 9 o'clock in each specimen. Muscles were weighed. Despite the common notion that EAS is comprised of three portions, we found neither the definitive connective tissue planes segregating EAS into separate compartments nor any specific anatomical variation, i.e. clearly delineated differential orientation of the myofibers, within any of the en bloc specimens examined. To ensure thorough sampling, each specimen was divided into thirds, as illustrated in Fig. 1. Fiber bundles, consisting of 10–20 myofibers, were dissected from each region, maintaining in vivo orientation, and fiber length was measured using digital calipers. Myofibers were microdissected from each bundle and a minimum of 3 L_s measurements were obtained by laser diffraction at each (12, 3, 6, and 9

o'clock) position [18]. L_s was compared with human optimal L_s , which is 2.7 μm [19]. Fiber length was normalized using well-established methods [20, 21] to account for potential differences at the time of death and/or fixation. The PCSA was calculated as previously described [14, 19].

Even though fixation has been shown to shorten L_s by a maximum of 10% and measurements obtained from fixed muscle tissue have been widely accepted to represent in vivo L_s [18], we wanted to ensure that this, however small, difference is not biasing our results. To this effect, we also determined L_s in additional fresh post-mortem EAS specimens ($n = 6$), procured from unpreserved VN and VP donors, using the exclusion criteria listed above. Muscle biopsies were obtained from anterior and posterior portions of the EAS with a specialized custom clamp, developed in our laboratory and validated as a reliable method for in vivo L_s measurements by comparison with the intraoperative L_s measurements of in situ muscles. [22].

Using a well-established in our laboratory protocol, the fresh post-mortem specimens were also utilized to determine EAS stiffness, the force required to achieve a particular extent of tissue elongation, which reflects passive mechanical muscle properties, dominated by intramuscular extracellular matrix. [23]. Briefly, three bundles from the anterior and posterior portions of each specimen were tested using a custom apparatus, equipped with a motor and a force-transducer [24]. L_s , measured throughout experiments by laser diffraction, provided objective quantification of muscle strain and myofibrillar array quality control. Baseline force, L_s , and sample cross-sectional area were obtained at slack length, the starting length before initiation of elongation. Force - L_s data were generated for each bundle subjected to a stress-relaxation protocol, consisting of bundle elongation until failure, or to an L_s of 4.0 μm in 10% strain increments followed by 3-min stress-relaxation periods. Force was converted to stress by dividing force by baseline cross-sectional area, assuming isovolumetric cylindrical shape of fiber bundles [24]. The slopes of the stress- L_s curves between 2.0 and 4.0 μm were used to calculate tangent stiffness, which quantifies stiffness at a given L_s .

Comparisons between operational L_s and a known human optimal L_s value were performed using a one-sample Student's t test. Based on the experimental EAS L_s variability (standard deviation 0.22), 6 samples were needed to achieve 80% power at a significance level set at 0.05. Data of fixed and fresh post-mortem specimens and of VN and VP groups were compared using an unpaired Student's t test (coefficient of variance 9%). Five samples in each group were needed to detect a 15% difference with 80% power and a significance level set at 0.05. Tangent stiffness at operational vs optimal L_s was compared using a paired Student's t test. Width and thickness of the four locations were compared using one-way repeated measures analysis of variance with Tukey's range test pairwise comparisons, as appropriate. Data were screened for normality and skew to satisfy the assumptions of the parametric tests used. Results are presented as mean $\pm$ standard error of the mean (SEM), unless otherwise noted. All statistical analyses were performed using GraphPad Prism version 6.00 GraphPad Software, San Diego, CA, USA.

Results

VN and VP donors did not differ with regard to age (63.2 ± 12.3 years vs 66.7 ± 7.5 years, $P = 0.8$) or body mass index (21.3 ± 2.1 kg/m² vs 19.1 ± 1.4 kg/m², $P = 0.4$). Median parity was 3 (range 1–7) in the VP group. On macroscopic examination, there were no anterior or posterior defects in either group (Fig. 1), although microdissection of fiber bundles and the individual myofibers revealed discontinuity of fibers anteriorly in 33% of EAS specimens. Notably, anterior fiber discontinuity was present in the EAS specimens in both groups. The average EAS mass was 11.4 ± 2.1 g. The average width was similar at all measured positions (12 o'clock: 14.8 ± 3.5 mm; 3 o'clock: 16.4 ± 3.5 mm; 6 o'clock: 14.8 ± 2.9 mm; and 9 o'clock: 17.5 ± 3.7 mm; $P > 0.2$), as was average thickness (12 o'clock: 8.2 ± 1.4 mm; 3 o'clock: 7.5 ± 1.5 mm; 6 o'clock: 9.9 ± 1.3 mm; and 9 o'clock: 6.3 ± 0.5 mm; $P > 0.1$).

External anal sphincter L_s measured in the fixed specimens ($n = 6$, 2.37 ± 0.07 μ m) was within 1% of the L_s determined in the fresh post-mortem specimens ($n = 6$, 2.34 ± 0.08 μ m), $P = 0.7$. Because the fixation process did not alter EAS L_s , the data from fixed and fresh specimens were combined. EAS operational L_s (2.36 ± 0.05 μ m) was significantly shorter than the human optimal L_s (2.7 μ m), $P < 0.001$. Passive tension of the EAS at the operational L_s was significantly lower than at optimal L_s , with 5.4 ± 1.4 kPa/ μ m vs 35.3 ± 12 kPa/ μ m respectively, $P < 0.0001$ (Fig. 2).

Vaginal parity did not significantly affect EAS L_s , with 2.39 ± 0.07 μ m in VN vs 2.33 ± 0.07 μ m in VP specimens, $P = 0.06$. Comparably, there were no significant differences between the groups with regard to other EAS structural parameters, namely normalized fiber length, 112 ± 31 mm in VN vs 126 ± 41 mm in VP, $P = 0.8$, and the PCSA, 1.3 ± 0.51 cm² in VN vs 1.4 ± 0.30 cm² in VP, $P = 0.9$ (Fig. 3).

Discussion

The findings of this first direct examination of the structure–function relationship of the human EAS provide empirical evidence for the following:

1. The EAS has a short in vivo L_s , which indicates that it operates on the ascending limb of a length–tension curve (Fig. 2), confirming our hypothesis
2. The EAS has relatively long fibers, which increases muscle excursion and contractile velocity
3. The EAS has an intermediate PCSA, predictive of relatively large isometric force-generating capacity

So how does this structural design facilitate EAS function as a fecal continence mechanism?

When defecation is undesirable, the action of the EAS in response to rectal distention with stool represents an eccentric contraction, defined as muscle contraction during elongation, similar to an arm extension during a bicep curl. As the myofibers elongate, an increase in the L_s of muscles operating on the ascending limb of the length–tension curve, such as the EAS, leads to a rise in active and passive tension (Fig. 2). Thus, the physiological force–length relationship of the EAS enables the generation of higher forces with contraction during

muscle elongation, likely facilitating fecal continence. The importance of the length–tension relationship for the function of the EAS in vivo has been highlighted in clinical studies, demonstrating that the increase in force with distention of the anal canal is either absent or substantially diminished in patients with FI, compared with women without FI [17].

Muscles with longer fibers have a larger range of motion, or excursion, higher contractile velocity, and less force decline during dynamic contraction. A large PCSA is found in stabilizer muscles that are tonically active. Thus, sizeable EAS fiber length appears conducive to a rapid contraction, without a significant loss of force, when the EAS is activated to prevent loss of gas and/or stool, whereas an intermediate PCSA is well suited to its contribution to the resting tone of the anal sphincter complex. Overall, the structural design of the EAS appears to be well aligned with its sphincteric function. Furthermore, our data suggest that in the absence of gross muscle defects, the functionally important structural parameters of the EAS are unaltered by vaginal delivery. Although the difference in operational L_s between VN and VP groups approached statistical significance, our overall results are consistent with the indirectly derived findings of normal length–tension curves in parous women without FI [17].

Interestingly, in contrast to the gross macroscopic appearance of the circumferential arrangement of the EAS fascicles, we identified discontinuity of the contractile muscle components anteriorly, with the myofibers terminating lateral to the 12 o'clock position in 33% of specimens during microdissection. This was observed in both VP and VN groups. Although the physiological anterior fiber discontinuity has been acknowledged in the historic and more recent literature, the prevailing and often dogmatic notion is that of a circular arrangement of the EAS fibers. Consequently, contemporary publications continue to propagate the idea that any anterior discontinuity represents an EAS tear and, therefore, is pathological. Our findings of anterior fiber discontinuity on direct anatomical examination of EAS from donors with no history of vaginal delivery or colorectal surgery (VN group) are consistent with the results of imaging studies [25] and lend further support to the presence of physiological variance in the arrangement of EAS fiber bundles anteriorly. Together with the other related studies, these data have important implications for establishing clinically relevant diagnostic parameters for pathological anterior defects of the EAS.

The strengths of this study include direct tissue-level outcome measures and the use of experimental methods that have been extensively validated in other skeletal muscles. Importantly, laser diffraction enables simultaneous measurement of ~300 individual sarcomeres. Based on the sampling strategy utilized in this study, our results are drawn from more than 100,000 sarcomeres per EAS. In addition, this proven method of L_s measurement has been shown to accurately represent in vivo L_s and to reflect whole muscle L_s , as the effects of fixation are negligible [18, 22]. The limitations of the current study are related to the use of cadaveric specimens. However, we have previously shown that cadaveric tissue can be used for passive mechanics measurements, as these muscle properties are unaffected by post-mortem time up to 7 days [26]. To ensure that the fixation process did not have an impact on our results, we explicitly demonstrated that L_s of fixed and fresh post-mortem EAS muscles did not differ. In addition, our findings in cadaveric specimens are consistent

with the conclusions of published studies that directly measured EAS L_s in animal models [8] and previously deduced EAS length–tension relationship in women [16].

The surgical repair of injured EAS to prevent or treat FI currently offers marginal promise at best, with disappointing long-term functional outcomes [6, 7]. Despite the emergence of new therapies for FI, such as sacral nerve modulation and sphincter magnetic augmentation, the associated high cost, significant complication rate, and multiple contraindications substantially limit their use [3]. In addition, we are currently faced with a threat of a continual decrease in device availability for the treatment of pelvic floor disorders. Thus, there is an urgent need to improve therapies that do not rely on implants.

Knowledge of the structure–function relationship is crucial for the development of scientifically rational therapeutic approaches. For example, important advances in the field of orthopedics became possible once the structural design of normal and injured hand muscles was elucidated. The above allowed the discerning surgeon to make an informed, rather than an arbitrary, choice during tendon transfer, which is based on a muscle's force generating and excursions capacity best suited to restoring the lost function. These surgical modifications, guided by the fundamental knowledge of the structure–function relationship of the hand muscles, lead to substantial improvements in postoperative function [10, 11, 27]. We believe that similar to the progress in other fields, exemplified above, better outcomes of primary OASI repair and sphincteroplasty are possible once the EAS structure is elucidated. This knowledge can also help obstetricians, urogynecologists, and colorectal surgeons to identify the potential causes behind the conflicting results of studies comparing overlapping with end-to-end EAS repairs [6, 7].

The discrepancy in the published literature possibly stems from the fact that neither technique is the “right” answer. Optimal outcomes can be potentially achieved by individualizing the surgical approach based on specific alterations in the structural components of the EAS. Analogous to other skeletal muscles [28, 29], acute vs chronic injury, and the variability in the extent of muscle damage likely lead to differential changes in the EAS structural parameters. Such differences necessitate an individualized approach to choosing the most suitable operative technique.

Previous studies have shown that the limb muscles maintain the L_s that is set during orthopedic surgeries, and that muscle overstretching decreases contractile force [30]. Similarly, the overlapping sphincteroplasty is likely to result in a myofiber stretch, in turn, leading to the increased L_s . Thus, the amount of overlap, which dictates the extent of fiber elongation, can be selected based on the starting and the desired L_s . New tools for the intraoperative assessment of muscles' L_s are rapidly developing. The currently available methods include a portable laser diffraction device and second harmonic generation microscopy. These approaches are limited by a small depth of penetration, but could be utilized in a setting in which the muscle is exposed. Even though these techniques have not yet been adopted into clinical practice by obstetricians or urogynecologists, their application can guide how sphincteroplasty and primary repair are performed. Investigating whether individualizing EAS surgical repairs based on intraoperative L_s measurements has an impact on postoperative patient outcome is a fruitful avenue for future studies. In addition, it is

possible that setting the EAS sarcomeres to the optimal length would allow a greater force generation in response to nonsurgical treatments, such as muscle training or neuromodulation, in turn, leading to an improved clinical response.

In conclusion, understanding the EAS structure–function relationship and the effects of injury is essential to enable scientifically rational approaches for the optimization and individualization of EAS repairs.

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Fig. 1.
Human cadaveric en bloc external anal sphincter with *arrows* indicating divisions used for thorough sampling

Fig. 2.

Human skeletal muscle active length–tension curve and external anal sphincterpassive tension curve, with sarcomere length in micrometers (μm) on the x-axis, percentage of maximum active muscle tension on the left y-axis, and stress in kilopascals (kPa) on the right y-axis. Maximum contractile force is produced at the optimal sarcomere length, 2.7 μm , corresponding to the plateau region of the active length–tension curve

Fig. 3.

Scatterplot of external anal sphincter normalized fiber length in millimeters (mm), which determines excursion and contractile velocity, vs physiological cross-sectional area (*PCSA*), a predictor of isometric force-generating capacity, comparing vaginally nulliparous (*VN*) and vaginally parous (*VP*) groups. Individual pelvic floor muscles, coccygeus (*C*), iliococcygeus (*IC*), and pubovisceralis (*PV*) are shown for comparison [21]