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Characterizing the Muscle Architecture
in Cadaveric Female Pelvic Floor Muscles

A thesis submitted in partial satisfaction of the requirements for the degree of Master of Science

in

Bioengineering

by

Olivia Thuy-Minh Nguyen

Committee in charge:

Richard L. Lieber, Chair
Samuel R. Ward
Charles Nager
John Watson

2012

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University of California, San Diego

2012

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NOMENCLATURE

PF	Pelvic floor
LA	Levator ani
CM	Coccygeus muscle
ICM	Iliococcygeus muscle
FL	Fiber length
SL	Sarcomere length
PCSA	Physiological Cross-Sectional Area
m	Mass
ρ	Density
Dt	Diameter transversa
Dc	Diameter conjugata
PC/RM	Pubococcygeus/rectalis muscle
PBS	Phosphate buffer solution
n	Sample size
CV	Coefficient of variation
δ	Expected treatment effect
SD	Standard deviation
SEM	Standard error of the mean

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ABSTRACT OF THE THESIS

Characterizing the Muscle Architecture
in Cadaveric Female Pelvic Floor Muscles

by

Olivia Thuy-Minh Nguyen

Master of Science in Bioengineering

University of California, San Diego, 2012

Richard L. Lieber, Chair

It's estimated that up to 37% of women are affected by a pelvic floor (PF) disorder and that at least 11% of women will require a surgical PF repair in their lifetime (Lukacz et al., 2006). Despite the prevalence of PF dysfunction in women, there's limited knowledge on PF skeletal muscle architecture. To understand muscle design and functional performance, we need to have an accurate knowledge of its architecture which refers to the muscle's macroscopic arrangement of fibers (Gans, 1982; Sacks and Roy, 1982). Architectural parameters include sarcomere length (SL), fiber length (FL), and physiological cross-sectional area (PCSA). By studying architecture, we can gain insight on the muscles' functional properties. The purpose of this project was to quantify female PF muscle architecture and better understand their function within the human body.

Muscle architecture was characterized according to the method previously described by Lieber et al., 1990. Pelvic floors were harvested from 5 formaldehyde-fixed human female cadavers without known PF dysfunction. PF muscles (coccygeus, iliococcygeus, and combined pubococcygeus and puborectalis).were bilaterally dissected (n=10) and used to determine

architectural properties. SL was relatively short for all three muscles. FL was shortest in the coccygeus, intermediate in the iliococcygeus, and longest in the pubococcygeus/rectalis. PCSA was highest in the coccygeus, moderate in the iliococcygeus, and lowest in the pubococcygeus/rectalis. The coccygeus had the lowest FL coefficient of variation. Architectural data suggests that the coccygeus is designed for force production (\propto PCSA) while the pubococcygeus/rectalis is designed for excursion (\propto FL).

CHAPTER I

INTRODUCTION

1.1 The Pelvic Floor

The pelvic floor (PF) is a complex of connective tissue, smooth and skeletal muscle that provides support for pelvic organs. The bladder, uterus, and rectum do not have an inherent shape so their structure and position is essentially determined by their muscular and connective tissue attachments to the pubic bones (Pemberton et al., 2002). When properly functioning, the pelvic floor allows voluntary evacuation of urine and fecal matter. Damage or injury to the pelvic floor can cause incontinence, pain, and organ prolapse. There are several main functions of the pelvic floor. It acts as a supportive structure, counteracting gravity and intra-abdominal pressure (Gray et al., 1995). It is also sphincteric, aiding in the closure of the urethra, vagina, and rectum by controlling the width of the levator hiatus (Hjartardóttir et al., 1997). Additionally, the pelvic floor is dilative, able to elongate during childbirth (Levangie and Norkin, 2001). The muscles of the pelvic floor lie deep within the pelvis and include the levator ani (LA) muscles and the coccygeus muscle (CM) (Figure 1.1). The levator ani complex is composed of the puborectalis, pubococcygeus, and iliococcygeus muscles (ICM). On the inferior and superior surfaces, the LA muscles are covered by connective tissue to form the respective inferior and superior fascia of the levator ani. The levator ani muscles coordinate to stretch and orient the pelvic organs so that they are compressed by intra-abdominal pressure which prevents prolapse and aids in the closure of the urethra and anus (Petros, 2007). Although the functional roles of pelvic floor muscles have been investigated, these muscles still remain poorly characterized within the human body as there are limited studies that have evaluated its muscle architecture.

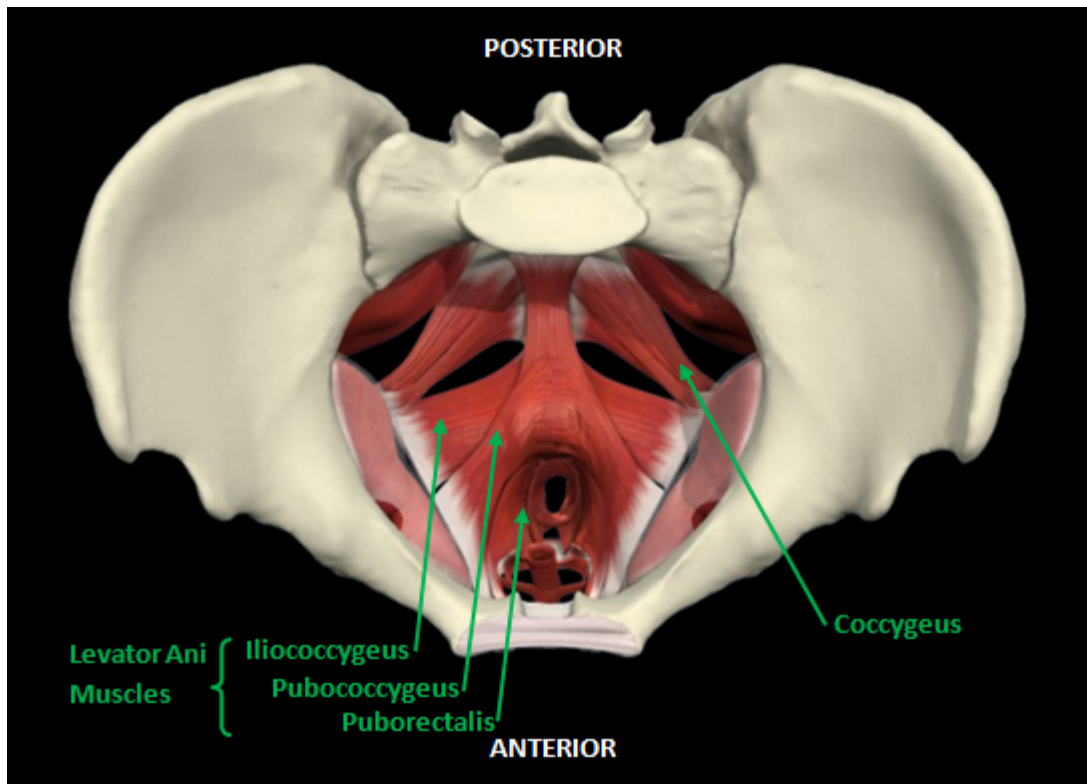


Figure 1.1. 3D generated image showing the pelvic floor muscles lying deep within the pelvis, the pelvic floor muscles. The PF muscles include the coccygeus and the muscles of the levator ani (iliococcygeus, pubococcygeus, and the puborectalis). (Original unlabeled 3D image generated by ©Primal Pictures)

1.2 Muscle Architecture

It is well known that skeletal muscle is highly organized at the microscopic level, but there is also organization at the macroscopic level of fiber arrangement. Skeletal muscle architecture refers to the muscle's macroscopic arrangement of fibers and more specifically, its arrangement relative to the axis of force generation (Gans, 1982; Lieber, 2010). While fiber type distribution has been emphasized as an important factor in muscle function, it has also been found that architectural properties influence the contractile properties of whole muscle (Close, 1972; Lieber, 2010). Previous muscle architectural studies that have been done in humans, cats, and rabbits suggest that skeletal muscle architecture is related to the functional requirements of the specific muscle (Lieber et al., 1990).

Muscle architectural parameters include orientation of fibers relative to axis of force generation which is characterized by the pennation angle, muscle length, fiber length (FL), sarcomere length (SL), and physiological cross-sectional area (PCSA). Pennation angle is typically measured by determining the average angle of fiber orientation relative to the axis of force generation on the superficial muscle surface. Muscle length is defined as the origin of the most proximal muscle fibers to the insertion of the most distal fibers and is generally not the same as fiber length since most muscle fibers do not extend to the entire muscle (Lieber, 2010). Fiber length is determined by microdissection of individual fibers from fixed tissues. When most studies report fiber lengths, unless they specifically state that they are performing single fiber measurements, it is likely that the fiber lengths actually represent fiber bundle lengths since an individual fiber is difficult to dissect from tissue. Sarcomere length is determined within isolated fiber bundles by various methods such as using a light microscope with a calibrated eyepiece micrometer or by laser diffraction (Saks and Roy, 1982; Lieber et al., 1984). The laser diffraction technique works through a mechanism exploiting the natural striation patterns on skeletal muscle. The striations are due to the actin and myosin contractile elements that form the alternating light I and dark A bands in a sarcomere. The resulting striation pattern acts as a diffraction grating to incident laser light. The laser constructively interferes with the skeletal muscle striations to form a pattern with different diffraction orders (Figure 1.2).

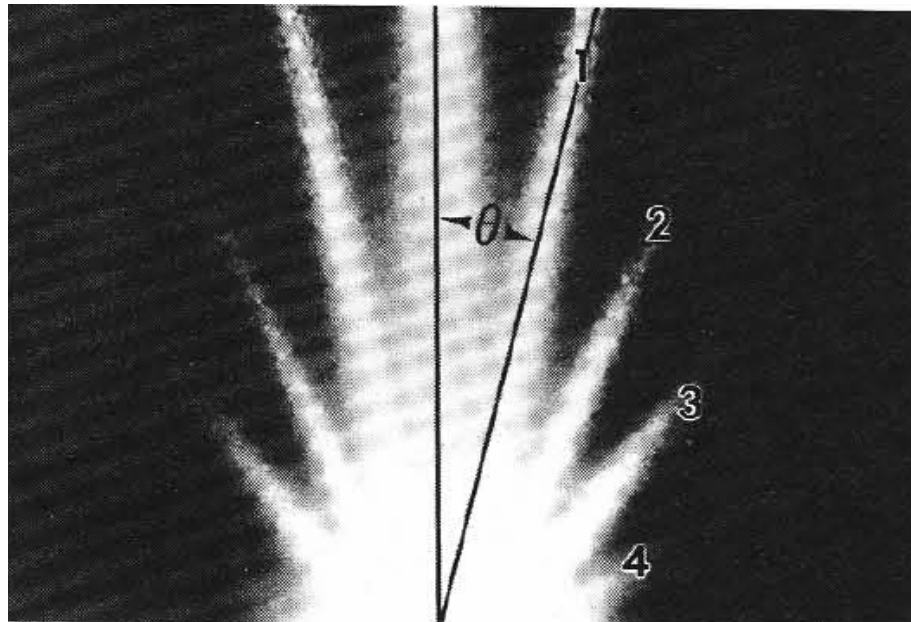


Figure 1.2. Theta diffraction angle can be measured using photodiode array interfaced to a computer. Any four of the diffraction orders (labeled 1,2,3,4) can be used with the known diffraction angle and laser wavelength to calculate sarcomere length (Lieber et al., 1990).

Sarcomere length can be determined by the diffraction pattern using the following equation:

$$n\lambda = d \cdot \sin\theta$$

where n is the diffraction order, λ is the laser wavelength, d is the grating spacing which equals SL, and θ is the diffraction angle which is measured by a photodiode array interfaced with a computer (Lieber et al., 1982). It is important to measure sarcomere length so that it can be used to normalize fiber length measurements to a reference sarcomere length. By normalizing fiber lengths, we can account for differences in muscle fiber lengths that may occur due to fixation at longer or shorter sarcomere lengths (Lieber, 2010).

After the measured architectural parameters are determined, the PCSA of a muscle can be calculated by the following equation:

$$PCSA = \frac{m \cdot \cos\theta}{\rho \cdot FL}$$

where m is mass, θ is the surface pennation angle, ρ is muscle density, and FL is muscle fiber length. The PCSA is significant because it is directly proportional to the maximum tetanic tension that the muscle can generate (Powell et al., 1984). The PCSA provides a numerical representation of the sum of cross-sectional areas of all the fibers within a muscle. The pennation angle term is included in the PCSA equation to account for the loss of muscle force relative to the same muscle if it had a zero pennation angle. However this term does not significantly influence PCSA because pennation angles typically only range from 0 to 30 degrees. The PCSA equation has been previously verified by testing estimated maximum muscle tetanic tension to measured maximum tetanic tension (Powell et al., 1984). However, the limitation with the PCSA equation is that it assumes a constant pennation angle during muscle contraction. Studies have shown pennation angles can vary during contraction which may decrease a PCSA's ability to accurately predict force (Zuurbier and Huijing, 1992).

Through muscle architectural studies, we have learned that the human body is organized in such a way that it can use sarcomeres to produce a wide variety of movements that range in force and speeds, allowing muscles to be designed for a specific function. The functional significance of muscle architecture is that PCSA is proportional to muscle force and that muscle fiber length is proportional to muscle velocity and excursion (Powell et al., 1984; Edman et al., 1985). If two muscles were identical in all parameters except PCSA, the muscle with the larger PCSA would be able to generate a proportionally higher maximum tetanic tension which shifts isometric length-tension and isotonic force-velocity curves toward higher tension values. If two muscles were identical in all parameters except fiber length, the muscle with longer fibers would have a larger active muscle range and an increase in muscle velocity which would shift isometric length-tension and isotonic force-velocity curves toward higher muscle length and velocity values. There is a longer active range because for a given change in

muscle length, each sarcomere in series lengthens less. The increase in contraction velocity is due to the fact that although each sarcomere within the fiber contracts at the same velocity, a muscle with long fibers has more sarcomeres in series so that the overall muscle velocity is greater. The functional differences between muscles with differing PCSA or FL are shown in Figure 1.3. Muscle architecture reveals how much force the muscle generates, how fast it contracts (velocity), and its active range of contraction (excursion). In general, muscles that have a large PCSA and short fibers are designed to produce force and muscles with a small PCSA and long fibers are designed for excursion (Lieber and Bodine-Fowler, 1993).

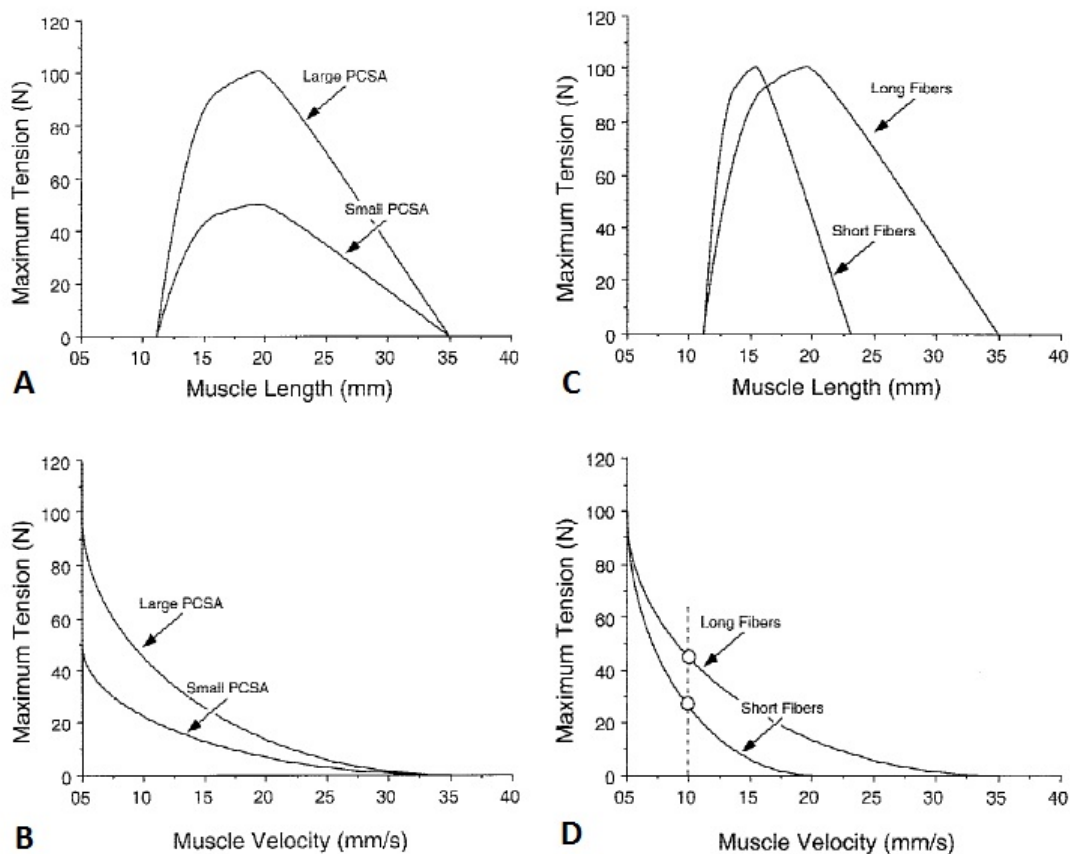


Figure 1.3. A,B: Schematics showing the effect of PCSA on isometric-length tension (A) and isotonic force-velocity (B) muscle properties. C,D: Schematics showing the effect of FL on isometric-length tension (C) and isotonic force-velocity (D) muscle properties. (Graphs from Lieber and Friden, 2000.)

In discussing the general aspects of muscle architecture, it is important to consider several other factors. It should be understood that muscle architectural properties affect the extrinsic muscle properties which are properties that vary with absolute muscle size like mass or PCSA and do not influence intrinsic properties like fiber length/muscle length ratio (Lieber et al., 2010). Additionally, the generalizations that PCSA corresponds to maximal tetanic force and longer FL correspond to greater excursions or velocities may not necessarily apply to muscle-joint systems. While longer fibers have a longer functional range compared to shorter fibers, it does not necessarily indicate that muscles with longer fibers have larger range of motion values due to the influence of muscle moment arms. Additionally, tendon may affect architectural functional predictions because of their high compliancy at low loads and low compliancy at higher loads which causes a rightward shift and increased operating range in the intrinsic shape of the muscle length- tension curve (Lieber et al., 2010). Although the discussion of joints and tendons is not relevant to the pelvic floor muscles as they do not cross any joints and have no tendon, it is worth mentioning that moment arm and tendon properties are additional factors that may affect functional predictions from architectural measurements for muscles in general. Therefore, even though muscle architecture may predict isometric properties of a muscle which is useful for the study of the pelvic floors as it is assumed that muscle lengths of the muscle remain constant, architectural studies of other muscles may require additional studies in the effects of joint kinematics and tendon content on predicting the muscle's *in vivo* function.

1.3 Motivations for Characterizing Female Pelvic Floor Muscle Architecture

The pelvic floor is an understudied muscle region in the human body that provides support to the bladder, uterus, and rectum. Because the PF muscles provide support to all of these organs, any weakness in or injury to these muscles can result in impaired function of these

structures. Investigating the relationship between the PF muscle that is damaged or injured and its corresponding symptoms is critical to advancing treatments for patients suffering by a pelvic floor disorder. Before we can begin to discover this relationship, we must first have a comprehensive understanding of the pelvic floor muscles. This requires studying and defining the muscle architecture of the pelvic floor muscles.

PF dysfunctions are debilitating problems that prevent women from enjoying a full and active life. It is estimated that up to 37% of women are affected by a PF disorder (Lukacz et al., 2006). The most common pelvic floor disorders include urinary incontinence and pelvic organ prolapse. The prevalence of urinary incontinence in women has been estimated to be between 13.1% - 49.6% with the rate depending on the definition of urinary incontinence assumed in the particular study (Sung and Hampton, 2009). It's also important to note that prevalence rates are also dependent on the severity of the symptoms, the type of incontinence (stress incontinence vs. urge incontinence) and how a woman is impacted by the symptoms (Sung and Hampton, 2009). The costs associated with urinary incontinence in the United States in 2000 have been estimated to be approximately \$19.5 billion (Hu et al., 2004). Considering that this cost only accounts for just one of the many pelvic floor disorders, it's evident that PF dysfunction has an economic impact on patients and is a field worth investigating so that we can hopefully develop more effective and affordable treatments. Pelvic organ prolapse refers to the "loss of fibromuscular support of the pelvic viscera that results in vaginal protrusion" (Laycock and Haslam, 2002). It has been reported that an estimated 50% of parous women have some degree of genital prolapse, only 10-20% seeks evaluation and treatment for the condition, and nulliparous women account for only 2% of prolapse cases in North America (Laycock and Haslam, 2002). Like other PF disorders, the incidence/prevalence of prolapse depends on the type of prolapse (cytocele, uterine, rectocele) and other factors like age.

There are several treatment options for those suffering from a PF condition. A low risk intervention involves specific pelvic floor muscle re-education exercises. However, physical therapy rehabilitation requires a person who has the ability to contract the correct muscle and follow a rigid exercise regime (Laycock and Haslam, 2002). These requirements may prevent some people, like older patients or those suffering from other health conditions, from benefiting from this treatment plan. The more invasive and less conservative option of surgery is also available to suffering patients. It has been reported that over 200,000 American women have surgery for pelvic floor dysfunction each year and at least 11% of women will require a surgical pelvic floor repair in their lifetime (DeLancey et al., 2007, Lukacz et al., 2006). Of the women that receive surgery, one in every four requires a second operation (Janda et al., 2003). Despite the prevalence of pelvic floor dysfunction in women, there is limited knowledge on the skeletal muscle architecture of the pelvic floor.

Studies have found that stretching, tearing, and denervation injury from childbirth or lower back trauma can result in PF damage (Davila et al., 2006). While the causes of PF damage have been investigated, the effect of the damage on muscle structure or the subsequent changes in muscle properties remains unknown. Before we can begin to discover such effects, we must first develop a fundamental understanding of the native pelvic floor muscle architecture. Considering that muscle function is strongly determined by its muscle architecture, expanding on the currently limited information of PF muscle architecture is of great interest (Burkholder et al., 1994). By studying pelvic floor architecture, we can gain insight on the fundamental relationship between its architectural design and its function. Our results can also lead to advancements in computer modeling of the pelvic floor. There have been studies that have attempted to model the pelvic floor muscles in a living patient using magnetic resonance imaging (MRI) scans (Fielding et al., 1999; Lien et al., 2004). However, this method only provides morphological parameters. Cadaveric studies provide access to important parameters

like optimal muscle length and fiber orientation which cannot be obtained by MRI studies (Janda et al., 2003). Additionally, our research can lead into further investigation of the muscular changes associated with parity and pelvic floor disorders. Discovering this information may help improve treatment decisions made in the clinical field and advance rehabilitation strategies for patients suffering from pelvic floor dysfunction. There is clearly a need to discover the architectural properties of the pelvic floor muscles so that we can better understand its muscle function in the human body. Thus, the overall objective of our research is to characterize female pelvic floor muscle architecture.

1.4 Pelvic Floor Muscle Anatomy

The pelvic floor consists of endopelvic fasciae that attach the pelvic organs (bladder, uterus, rectum) to the pelvic walls. Although these ligaments suspend the organs, withstanding constant forces is not a main function of ligaments (Laycock and Haslam, 2001). It is more likely that the pelvic floor muscles, rather than the ligaments, bear most of the pelvic load and holds the organs in place. The pelvic floor muscles include the coccygeus and the levator ani muscles (Figure 1.4). The CM is triangular in shape and lies posterior to the LA. This muscle originates from the ischial spine and the sacrospinous ligament, and inserts into the lateral aspect of the coccyx. The coccygeus is innervated by the ventral primary rami of spinal nerves S3-S4 and is perfused by the inferior gluteal artery (Gest and Schlesinger, 1995).

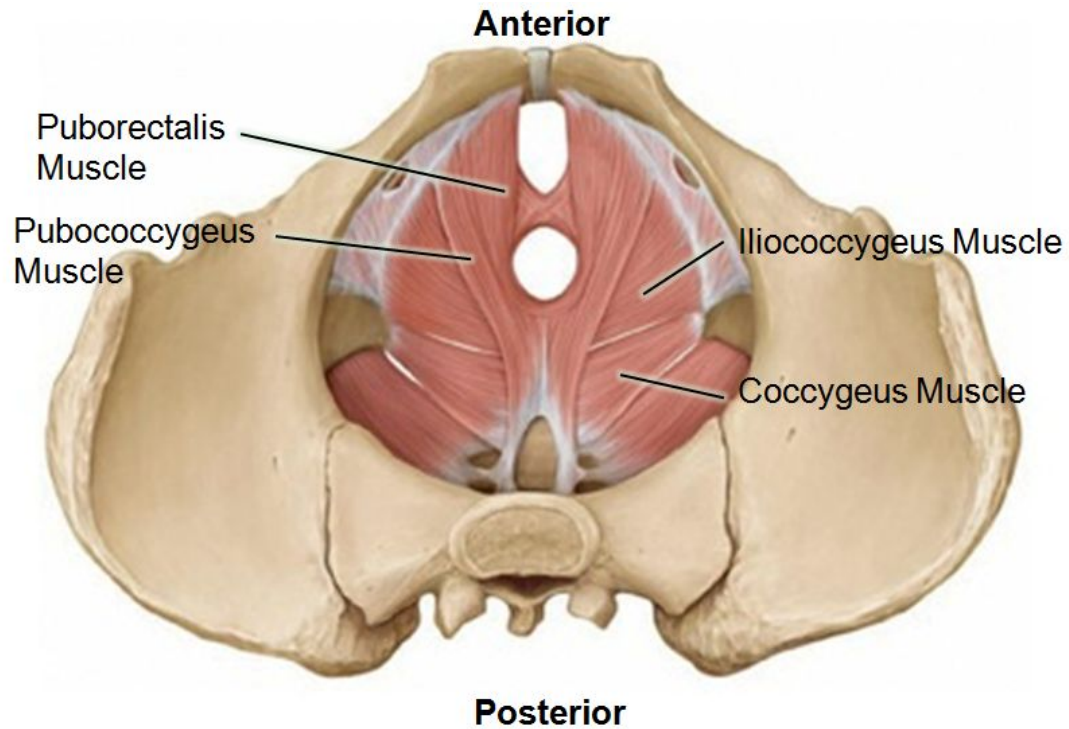


Figure 1.4. Illustration showing the pelvic floor muscles from a superior view. The iliococcygeus, pubococcygeus, and puborectalis muscles are collectively called the levator ani. (Original unlabeled photo taken from Schünke et al., 2007)

The levator ani complex is defined by several muscles that lie anterior to the coccygeus. A literature review has shown that while the origin and insertion pairs of LA muscles are fairly consistent, there is confusion relating to LA muscle terminology and description (Kearney et al., 2004).. Therefore, we present the terminology stated in Terminologia Anatomica (TA), the international standard on human anatomic terminology. The levator ani muscles include the iliococcygeus, the pubococcygeus, and the puborectalis. Table 1.1 summarizes the system used by TA and also includes the origins, insertions, and function as reported by Kearney et al., 2004.

Table 1.1. Muscles of the Levator Ani (Modified table reproduced from Kearney et al., 2004).

Terminologia Anatomica	Origin/Insertion	Function
Iliococcygeus	Tendinous arch of the levator ani/ the 2 sides fuse in the iliococcygeal raphe	The 2 sides form a supportive diaphragm that spans the pelvic canal
“Pubococcygeus” (recommended pubovisceral)		
Puboperinealis	Pubis/perineal body	Tonic activity pulls perineal body ventrally toward pubis
Pubovaginalis	Pubis/vaginal wall at the level of the mid-urethra	Elevates vagina in region of mid-urethra
Puboanalis	Pubis/intersphincteric groove between internal and external anal sphincter to end in the anal skin	Inserts into the intersphincteric groove to elevate the anus and its attached anoderm
Puborectalis	Pubis/forms sling behind rectum	Forms a sling behind the rectum, creating the anorectal angles and closing the pelvic floor

The iliococcygeus muscle is a fan-shaped muscle that originates from the arcus tendineus levator ani, a fibrous band in the pelvic wall, and inserts in the iliococcygeal raphe. It has also been referenced to additionally insert into the lower coccyx. This muscle forms a sheet spanning the pelvis that provides a surface for the organs to rest. The iliococcygeus is located anterior to the coccygeus as seen in Figure 1.5. Like the other pelvic floor muscles, this muscle is innervated by the ventral primary rami of spinal nerves S3-S4 and perfused by the inferior gluteal artery (Gest and Schlesinger, 1995).

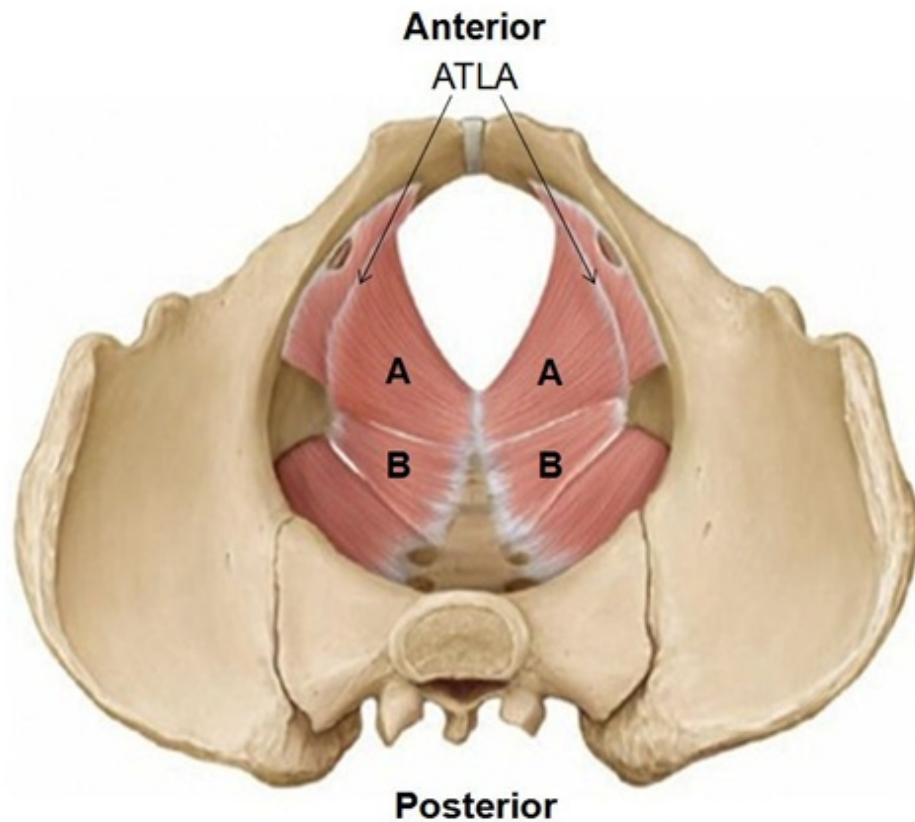


Figure 1.5. Illustration showing the superior view of the A) the iliococcygeus muscles and B) the coccygeus muscles (pubococcygeus and puborectalis muscles have been removed). The iliococcygeus originates from the arcus tendineus of the levator ani (ATLA). (Original unlabelled photo taken from Schünke et al., 2007)

The pubococcygeus has been referenced to originate from the internal surface of superior pubic ramus, and insert onto the coccyx, lower sacrum, and the iliococcygeal (anococcygeal) raphe. There have also been reports of fibers inserting into perineal body, the vagina wall, and the anal sphincter which is why the TA further divides the pubococcygeus into the puboperinealis, pubovaginalis, and puboanalis muscles based on the different insertion locations (Kearney et al., 2004). Although this muscle is made of several components, clinicians typically do not subdivide and simply refer this muscle region in its entirety as the pubococcygeus muscle, also known as the pubovisceral muscle (Laycock and Haslam, 2002). The puborectalis muscle originates from the superior pubic ramus on both sides of the pubic

symphysis and has no bony insertion. Rather, the puborectalis fibers join from the right and left sides to form a U-shaped sling behind the rectum. Similar to the other pelvic floor muscles, both the pubococcygeus and puborectalis muscles are innervated by the ventral primary rami of spinal nerves S3-S4 and perfused by the inferior gluteal artery (Gest and Schlesinger, 1995).

Approximately two-thirds of the muscle fibers are type 1 (slow-twitch) which are cited to be responsible for the resting tonus of muscle and one-third of the fibers are type 2 (fast-twitch) which have been suggested to be used when a quick powerful contraction is required (Laycock and Haslam, 2002). The opening of the levator ani in which the urethra and vagina pass is named the urogenital hiatus. The rectum also passes through this opening, but it is not included in the name since the levator ani muscle directly attaches to the rectum. The hiatus is kept closed by the normal tonic activity of the levator ani muscles. The levator ani muscles constantly contract and close the vagina lumen, eliminating any opening through which prolapse could occur. (Laycock and Haslam, 2002)

The anatomy and function of the pelvic floor components has been compared to a ship in a berth held by ropes floating on top of water (Paramore, 1918). The organs reference the ship which rests on the pelvic floor muscles that represent the water. The organs are attached to the pelvis by ligaments symbolically representing the ship being anchored to the berth by ropes. Similar to lowering the water level and forcing the ropes to suspend the ship, when the pelvic floor muscles lower due to damage the ligaments are required to support the organs and hold everything in place. With time, the connective tissue fails due to overload and pelvic floor discomfort or dysfunction occurs (Laycock and Haslam, 2002).

When depicting the pelvic floor muscles within the human body, these muscles have been classically described as having the shape of a basin because of necropsy observations. Interestingly, the assumption of a basin-shaped pelvic floor is inaccurate for its description *in*

vivo. Magnetic resonance imaging technology has now revealed that the pelvic floor shape in living human patients is actually a dome shape when muscular tonus is present (Hugosson et al., 1991). Furthermore, multiple studies agree that the the levator ani muscle is dome-shaped at rest, becomes more horizontal as the muscle straightens during voluntary pelvic contractions, and exhibits the basin-shape as the muscle descends during bearing down (Hugosson et al., 1991, Hjartardóttir et al., 1997). It has been hypothesized that a possible reason for why the pelvic floor becomes basin shape during bearing down occurs is because of decreased tonus and increased intra-abdominal pressure (Hugosson et al., 1991). The shape dependence on tonus theory may account for the basin-shape found in cadaveric bodies since the tonic activity of muscles has been completely lost.

1.5 Pelvic Floor Muscle Architecture of Other Investigators

To our knowledge, there is only one study that has attempted to fully characterize the architectural properties of the female pelvic floor (Janda et al., 2003). Although this study's objective was to measure pelvic floor morphological parameters for finite element modeling purposes, the investigators were able to measure key architectural elements such as muscle length, fiber length, and sarcomere length, and able to calculate the PCSA of the pelvic floor muscles.

While many of the methods done by Janda et al. were for modeling purposes, the architectural methods they used were standard (See 1.2 Muscle Architecture). They completely removed the levator ani complex and cleared it from its surrounding tissue. In their muscle definitions, they chose to divide the levator ani muscle complex into pubococcygeus, iliococcygeus, and coccygeus components. The investigators defined how many elements each muscle should be divided into based on the locations of muscle bundles that were measured with the palpator, an instrument used to measure positions and geometry of the pelvic floor. The

study chose to divide the pelvic muscles into eight muscle parts to represent the pubococcygeus, iliococcygeus, and coccygeus muscles of the right and left sides. For modeling purposes, they then further divided these muscle parts into 22 muscle elements (Fig 1.6).

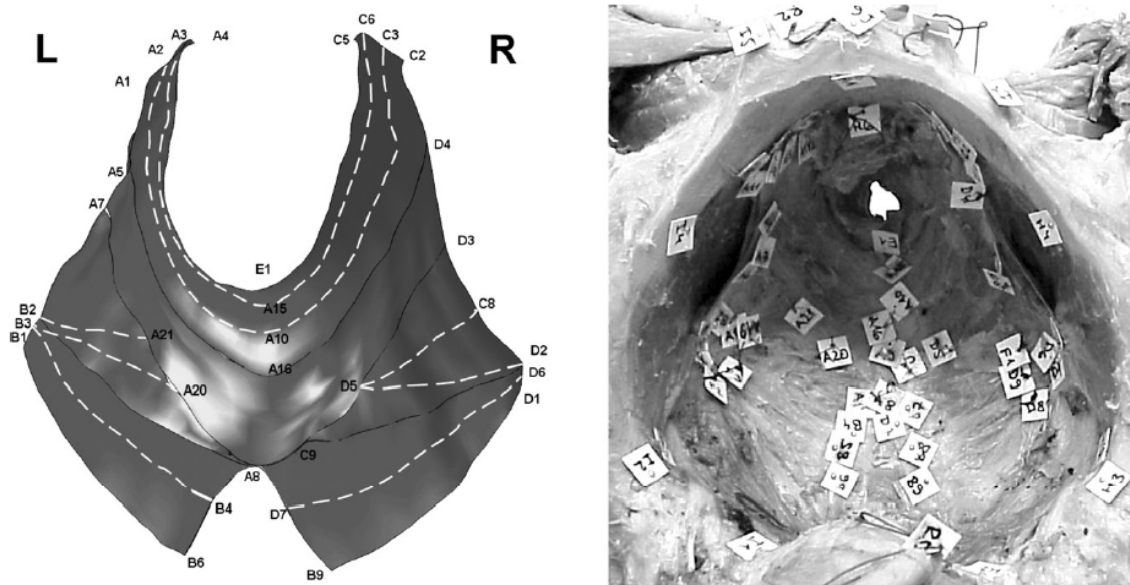


Figure 1.6. On the left side: Schema showing pelvic floor muscle divisions into 22 elements (L-left, R-Right). On the right side: Photo showing the labeled origins and insertions of fiber elements. (Janda et al., 2003).

Stating that pelvic floor muscles have no tendon, this study set muscle fiber length equal to the muscle element length. They determined such lengths by copying the length between the origin and insertion of an element to a string and then measuring with a ruler. A digital scale was used to mass each muscle element. This study considered pennation angles to be negligible and used laser diffraction to directly measure three sarcomere lengths at the three representative locations along the fiber bundle. Similar to our own methods, optimum or normalized fiber length was calculated by dividing the measured fiber bundle length by the mean sarcomere length in that fiber bundle, and then multiplying by an optimum *in vivo* sarcomere length of 2.7 μm . PCSA for each muscle element was calculated as mass divided by density and then subsequently divided by the optimal fiber length of that element. They used a

specific density of skeletal muscle as 1.057 g/cm^3 because these samples were soaked during weighing.

The results of the Janda et al. study showed the pelvic floor muscles to be near optimal muscle lengths. The fiber bundle lengths varied slightly within muscle groups and were reported to be due to the trapezoidal shape of the levator ani complex. Their measured sarcomere lengths were between $2.0 \text{ }\mu\text{m}$ and $3.2 \text{ }\mu\text{m}$. They found that some sarcomere measurements could not be measured in a few muscle elements. It was assumed that these fibers were damaged and subsequently attributed them to be artifacts that could be ignored. The mean sarcomere lengths for the muscle elements ranged between $2.2 \text{ }\mu\text{m}$ and $2.9 \text{ }\mu\text{m}$. Their results suggest that after simulated contractions, the muscle fibers will be below the optimal length on the ascending limb of the force-length curve. They also mentioned that measuring sarcomere length in the coccygeus muscle was very difficult because of the large atrophy of the muscle tissue that they presumed to be a result of the age of cadaveric subject (72 years old).

With only one study that has reported findings on the architectural properties of pelvic floor muscles, there is limited data on this subject and it is clear that further investigation is needed to acquire a better understanding of the pelvic floor muscle architecture. Additionally, the results and conclusions of this study should be read with an understanding of its limitations. Aside from the use of cadaveric and aged tissue, the greatest drawback is the use of a single cadaveric human subject. With data based on a single pelvic floor specimen, it would be difficult to justify generalizations of pelvic floor muscle properties to the female population. Therefore, our study intends to expand on the currently limited pelvic floor architectural knowledge by investigating the pelvic floor muscles of five female cadaveric human subjects.

1.6 Thesis Organization

This thesis provides a summary of preliminary research towards characterizing the female pelvic floor skeletal muscle architecture. The details of our methods and results are outlined in Chapter II. The clinical relevance of our research is also discussed at the end of the chapter. Chapter III summarizes our conclusions, discloses the limitations of our work, and suggests possible directions for future investigation. The end of the thesis lists references and includes an appendix with additional data tables and figures.

CHAPTER II

MUSCLE ARCHITECTURE OF THE FEMALE PELVIC FLOOR

2.1. Introduction

To understand muscle design and functional performance, we need to have an accurate knowledge of the skeletal muscle architecture (Gans, 1982; Sacks and Roy, 1982). Therefore it is important to characterize the architecture of the pelvic floor muscles if we want to completely understand the function of these muscles in the human body. The two most important architectural parameters are physiological cross-sectional area (PCSA) and fiber length (FL) because these parameters have functional significance. PCSA corresponds to maximum tetanic tension, and FL predicts maximum contraction velocity and the excursion range (Powell et al., 1984; Edman et al., 1985). Knowing these architectural parameters will allow us to predict the functional properties of the pelvic floor muscles. Furthermore, studying these architectural properties will help guide further research that may translate into clinical changes, aiming to better prevent and treat pelvic disorders as well as to improve outcomes of pelvic floor surgical repairs. The purpose of this project was to quantify female pelvic floor muscle architecture and better understand their function within the human body.

2.2 Materials and Methods

2.2.1 Donor Information

All measurements were performed on five formaldehyde-fixed human female cadavers obtained for scientific research from the Anatomy Bequest Program at the University of Minnesota. The specimens were selected for having no known pelvic floor dysfunction or pathology to the pelvic floor. Table 2.1 summarizes the donor history including age, height,

weight, cause of death, and the number of reported pregnancies/births. Anatomical pelvic dimensions of diameter transversa (Dt) and diameter conjugata (Dc) were also measured for each cadaver and the dimensions are reported in the last column of Table 2.1. The mean age \pm standard deviation of the cadavers was 74 ± 19 years old.

Table 2.1. Summary of donor history and anatomical pelvic dimensions.

Donor	Age (years)	Height	Weight (lbs)	Cause of Death	Pregnancies / births	Pelvic Dimensions Dt (cm) X Dc (cm)
1	87	5'4	108	Pneumonia	1	11.9 X 11.4
2	69	5'4	101.4	Aspiration pneumonia; Right heart failure	NA	15.3 X 13.5
3	99	5'8	105	Complications of right femur fracture; Fall	NA	13.8 X 12.8
4	55	5'6	112	Metastatic breast cancer	2	14.2 X 14.1
5	60	5'5	120	Metastatic lung cancer (non small cell)	5	13.8 X 12.1

2.2.2 Muscle Divisions of the Pelvic Floor

When the pelvic floors were harvested from donors, they were basin-shaped and still attached to part of the coccyx to maintain frame of reference. To facilitate cleaning and dissection of the muscles, we cut the specimens anteriorly from the pubis symphysis down the midline, through the urogenital hiatus, and inferiorly into the anal opening which resulted in a trapezoidal shape of the pelvic floor muscles. The pelvic floor generally did not have distinct muscle boundaries. To help us better visualize the individual muscles of the pelvic floor, we took high resolution photographs of each pelvic floor specimen from the superior and inferior view (Figure 2.1). To show the thinness of the muscles, particularly the iliococcygeus muscle, we also took high resolution photos of each specimen illuminated with a light box.

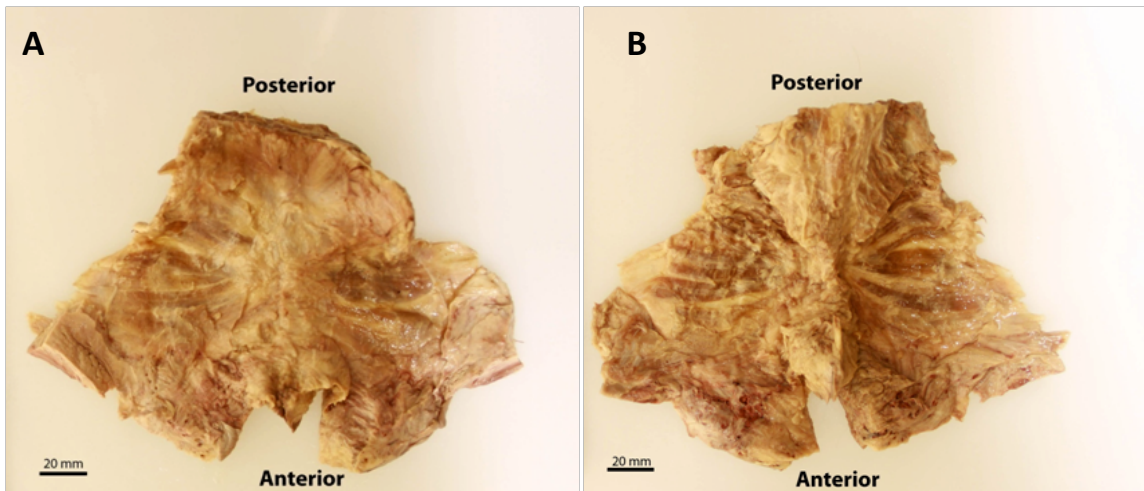


Figure 2.1. Photograph of Donor's 1 pelvic floor from the A) superior and B) inferior view.

We attempted to isolate the coccygeus, iliococcygeus, pubococcygeus, and puborectalis muscles from each other by following fibers from the origin and insertion points as outlined by Kearney et al., 2004 and displayed in Table 2.1. An urogynecologist was consulted to confirm general muscle locations within the pelvic floor specimens. All final divisions were made only after two investigators agreed on the boundaries. We were able to identify the coccygeus muscle and iliococcygeus muscle by the TA origin-insertion pairs. When the CM was isolated from the ICM, it was also cut from the attached coccyx. Due to difficulty in separating pubococcygeus muscles from the puborectalis, we decided to analyze the muscle architecture of the pubococcygeus and puborectalis muscles combined together which we refer to as the pubococcygeus/rectalis muscle (PC/RM). This decision was supported by an urogynecologist. Figure 2.2 shows a schematic of our muscle divisions and Figure 2.3 shows the outline of the muscle boundaries on a high resolution photograph of a pelvic floor.

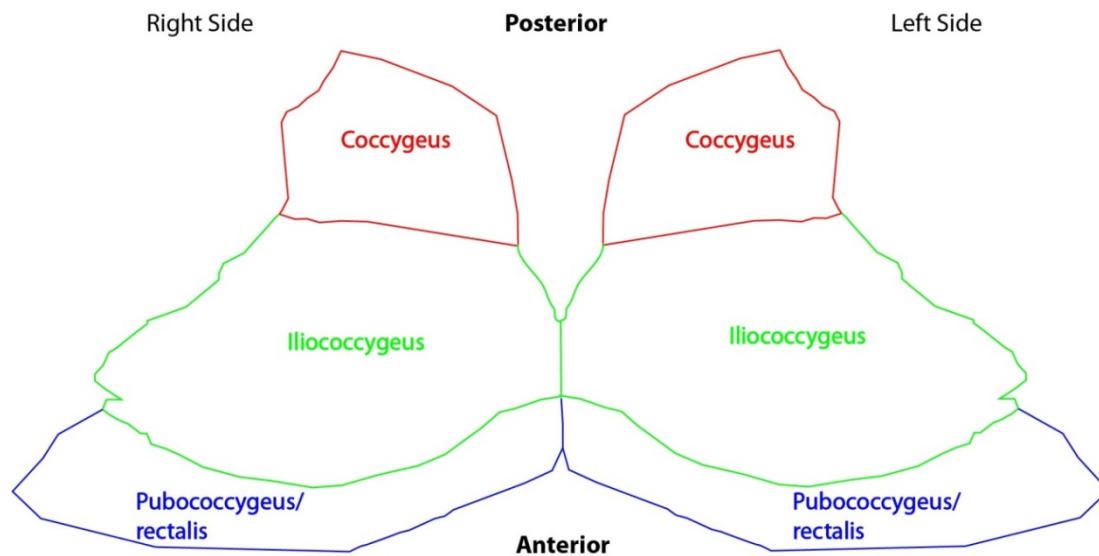


Figure 2.2. Schema showing pelvic floor muscle divisions into 6 sections with 3 muscles (coccygeus, iliococcygeus, pubococcygeus/rectalis) on each of the right and left sides.

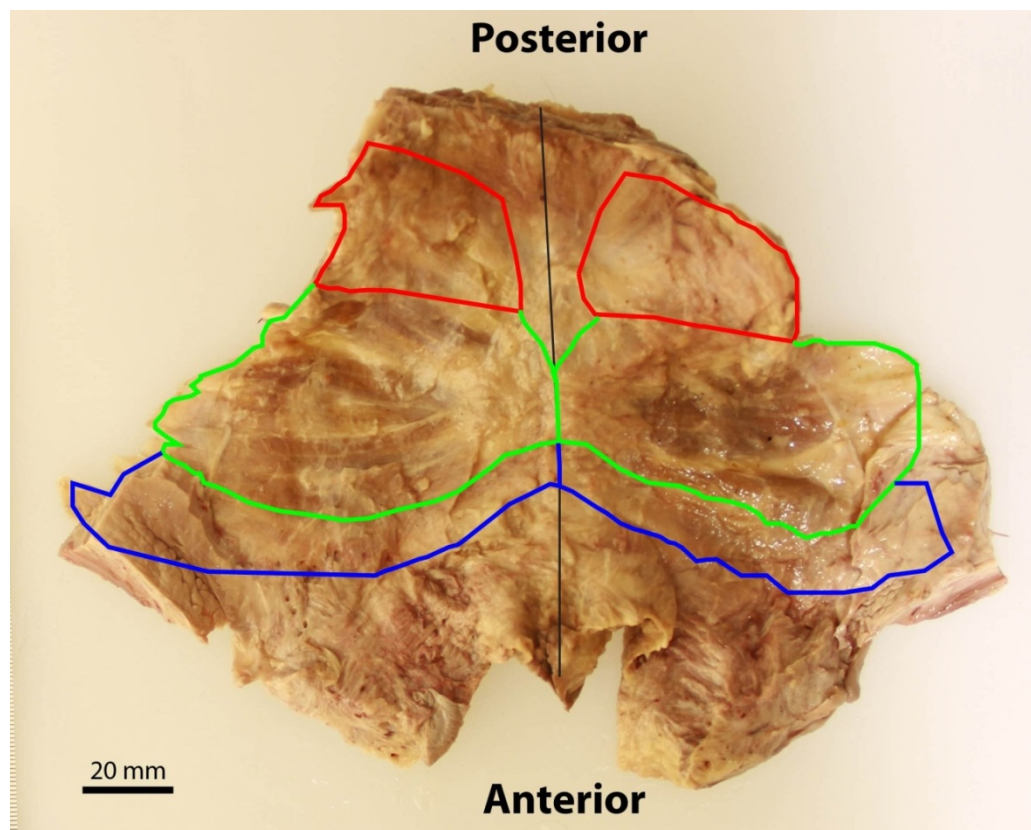


Figure 2.3. Photograph of a pelvic floor with superimposed boundaries of the coccygeus (red), iliococcygeus (green), and pubococcygeus/rectalis (blue) muscles.

2.2.3 Measurements of Muscle Parameters

Muscle architecture was characterized according to the method originally developed by Sacks and Roy, 1982 and previously described by Lieber et al., 1990. Once the pelvic floors were harvested and cut anteriorly/inferiorly starting from the pubis region through the anal opening, the muscles remained in 1X phosphate buffer solution (PBS) at room temperature until further dissection. The pelvic floor muscle complex was bisected and individual muscles were divided into anatomical right and left sections as discussed in the previous section. After removing excess fluid, each muscle section was weighed individually for mass using a digital scale. Similar to the Janda et al. study, pennation angle for all muscles were considered negligible. Each muscle section was visually divided into three regions (Anterior, Mid, Posterior) and three fascicles (bundles of fibers) were dissected from each region for architectural measurements. Figure 2.4 shows a schematic of the approximate regions (Anterior, Mid, Posterior) within each muscle from which we took 3 fascicles per region for architectural measurements.

With this design, we measured 9 samples per muscle section and as there are 6 muscle sections per pelvic floor (3 muscles, right vs. left), we had a total of 54 sample measurements for each donor (with the exception of one donor where we could only obtain a total of 52 measurement*). Fascicles were measured using electronic digital calipers to the nearest 0.01 mm and it was assumed that all of the fibers within a bundle were of equal length so fascicle length equals fiber length. If fiber lengths were not oriented in a way that could accurately be measured with a caliper, such as in curved fibers, suture was used to follow the fiber lengths and the suture length was measured by the caliper. After each fascicle was dissected, fascicles

* Donor 4 did not have sufficient right pubococcygeus/rectalis muscle in the Mid and Anterior regions to permit measurement of 3 fiber lengths so only 2 fiber lengths were obtained for each of these regions.

were immersed in a 15% sulfuric acid solution for at least 30 minutes to soften and partially digest the connective tissue. Fascicles that did not undergo immediate dissection for sarcomere length measurements were stored in 1X PBS at room temperature until time of dissection.

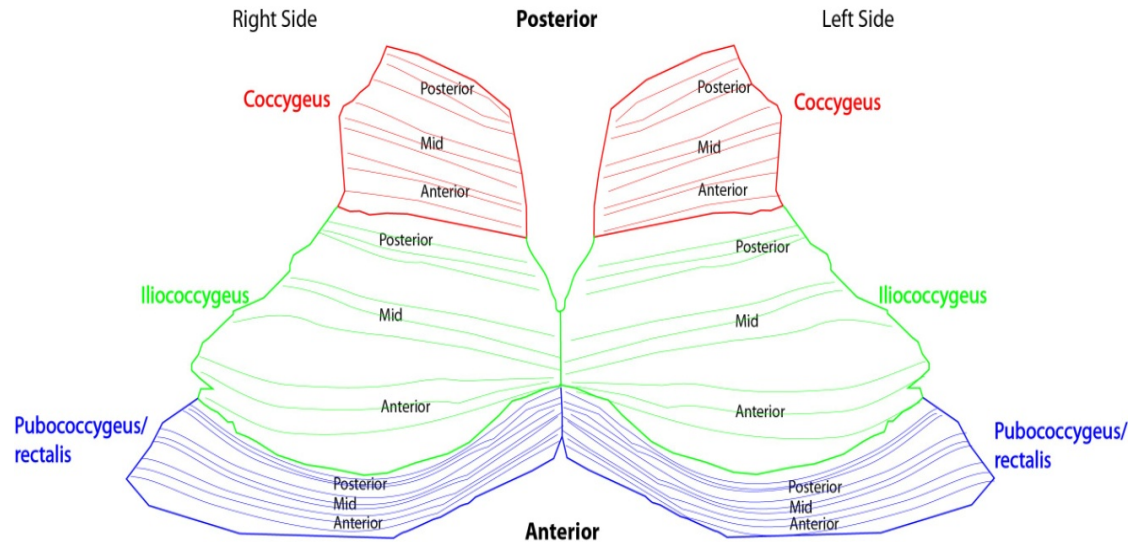


Figure 2.4. Schematic showing our measurement design of sampling 3 fibers within each region (Anterior, Mid, Posterior) for each of the pelvic floor muscles on the right and left sides.

Under a binocular dissecting scope and with the use of surgical forceps, smaller bundles of fibers (approximately 10-20 fibers) were teased apart from the larger bundle and placed on a glass slide for SL measurements. Because of the striations on skeletal muscles, SL of isolated fiber bundles were able to be measured by laser diffraction according to the method described by Lieber et al, 1984. A He-Ne laser was used to emit a laser diffraction pattern from a muscle fiber bundle (Figure 1.2). SL can be determined from the diffraction angle using the grating equation:

$$n\lambda = d \cdot \sin\theta$$

where n is the diffraction order, λ is the laser wavelength, d is the grating spacing which equals SL, and θ is the diffraction angle. For our experimental setup, $\lambda = 0.632 \mu\text{m}$ and the diffraction angle was measured by a photodiode array interfaced with a computer (Lieber et al., 1984). SL can be calculated using different diffraction orders but in this study, only the zero to first or first

to first orders were used. Fibers were only used if at least three useable sarcomere lengths were obtained.

Fiber lengths were normalized by dividing the measured fiber length by its measured sarcomere length, and then multiplying by a standard sarcomere length of 2.7 μm which is the optimal sarcomere length *in vivo* (Janda et al., 2003).

We also calculated the physiological cross-sectional area for each muscle section. PCSA was calculated using the following formula:

$$PCSA = \frac{m \cdot \cos\theta}{\rho \cdot FL}$$

where m is mass, θ is the surface pennation angle, ρ is muscle density, and FL is muscle fiber length. We used the specific density of muscle as 1.057 g/cm^3 since muscles were hydrated during weighing (Lieber et al., 1992). Table 2.1 summarizes the mean measured and calculated parameters among the five donors.

2.2.4 Statistical Analysis

Right and left sections of a muscle were treated as independent samples. This allowed us to analyze each pelvic floor muscle with a sample size of 10 ($n=10$). One-way ANOVA was used to compare mean PCSA, fiber length, and sarcomere length between the different muscles with *post-hoc* t-tests as appropriate. Significance was set at $\alpha=0.05$. Results are presented as means \pm SEM except where noted.

2.3 Results

Table 2.2 displays the mean data for each muscle's sarcomere length, normalized fiber length, and PCSA as well as the data within each of the muscle regions. We compared muscle sarcomere length, normalized fiber length, and PCSA between pelvic floor muscles to find which muscles were significantly different than others.

Table 3.2. Mean architectural parameters \pm SEM for each muscle.

	PC/RM	ICM	CM
Sarcomere Length (μm)	2.07 \pm 0.02	1.97 \pm 0.04	2.01 \pm 0.04
Normalized Fiber length (cm)*	11.29 \pm 1.22	8.38 \pm 0.75	5.25 \pm 0.55
PCSA (cm^2)	0.60 \pm 0.07	0.64 \pm 0.05	0.81 \pm 0.08

*significant $p < 0.01$

One-way ANOVA was done for each architectural parameter to compare means between muscle groups. Figure 2.5 shows a graph of the mean sarcomere lengths for each muscle. No significant sarcomere length differences were found ($p > 0.1$). The One-way ANOVA on fiber lengths between muscles revealed a significant difference ($p < 0.01$). A Tukey *post-hoc* test showed significant differences between the coccygeus and pubococcygeus/rectalis muscles, and between the coccygeus and iliococcygeus muscle which is graphically represented in Figure 2.6. The one-way ANOVA on PCSA showed that the PCSA differences were not significant ($p > 0.05$). Figure 2.7 shows a graphical representation of the mean PCSA for each muscle.

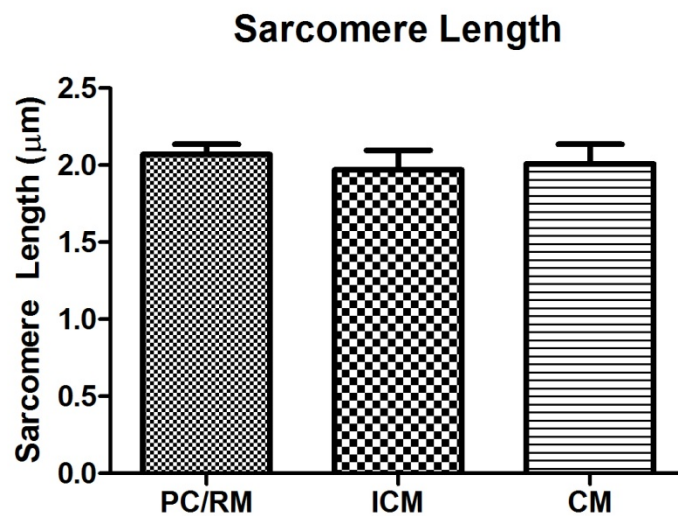


Figure 2.5. Mean sarcomere lengths for each pelvic floor muscle. PC/RM- pubococcygeus/rectalis muscle, ICM- iliococcygeus muscle, CM- coccygeus muscle

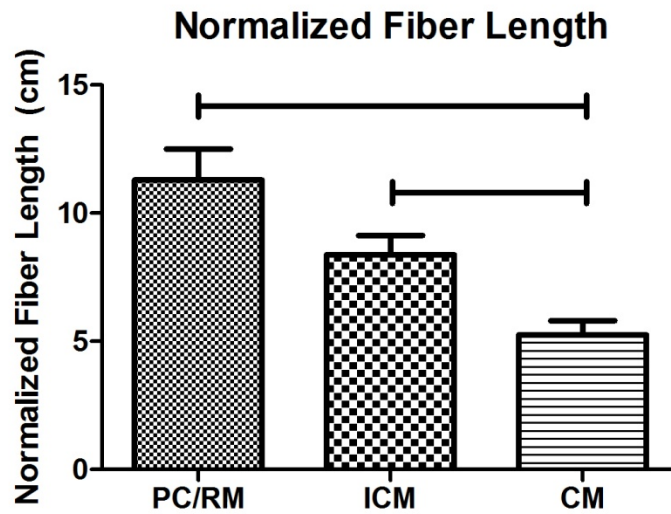


Figure 2.6. Mean normalized fiber lengths for each pelvic floor muscle. PC/RM- pubococcygeus/rectalis muscle, ICM- ilioococcygeus muscle, CM- coccygeus muscle

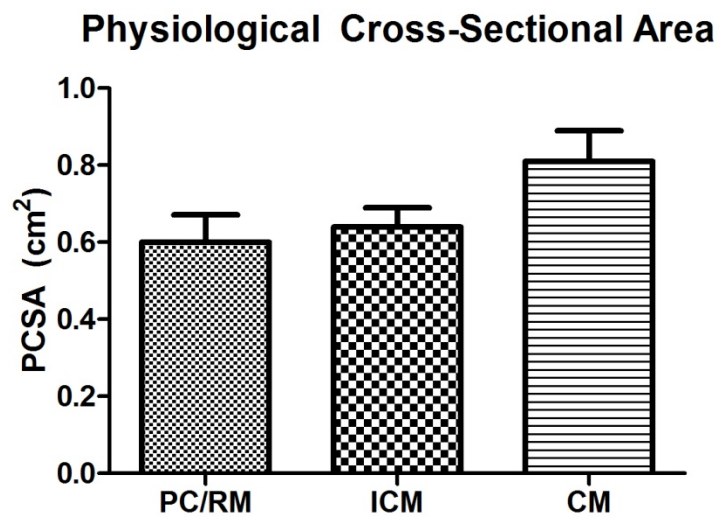


Figure 2.7. Mean physiological cross-sectional area (PCSA) for each pelvic floor muscle. PC/RM- pubococcygeus/rectalis muscle, ICM- ilioococcygeus muscle, CM- coccygeus muscle

Physiological cross-sectional area was also plotted against fiber length for each muscle on a scatter graph as seen in Figure 2.8.

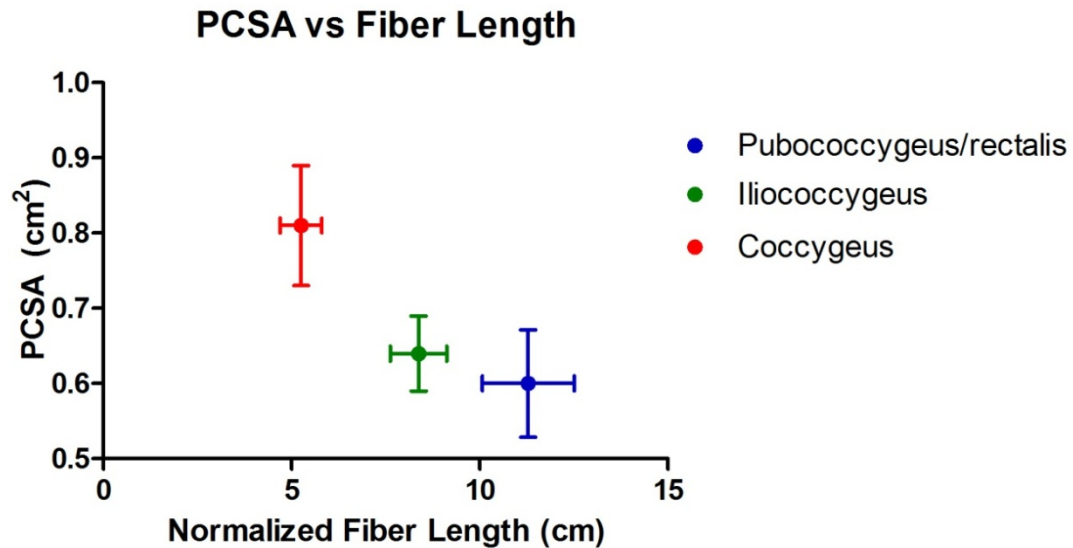


Figure 2.8. Scatter graph of the normalized fiber length and physiological cross-sectional areas of the pelvic floor muscles.

2.4 Discussion

Characterizing the muscle architecture of the pelvic floor muscle was challenging for several reasons. This muscle was highly covered and interspersed with connective tissue and substantial amounts of fat which required extensive cleaning and dissection. Isolating the individual muscles from each other was also not trivial since there were no distinctive boundaries between muscles. Additionally, pelvic floor specimens were highly variable in geometry and dimensions which made it harder to compare muscle divisions between specimens (Figure 2.9). Furthermore, there was very little muscle to characterize due to the thinness of the pelvic floor muscle. The iliococcygeus was especially thin as exemplified in Figure 2.10 where a pelvic floor is illuminated by a light box. Taking these factors into consideration, we were able to characterize sarcomere length, fiber length, and PCSA of the coccygeus, iliococcygeus, and combined pubococcygeus/rectalis muscles of the pelvic floor.

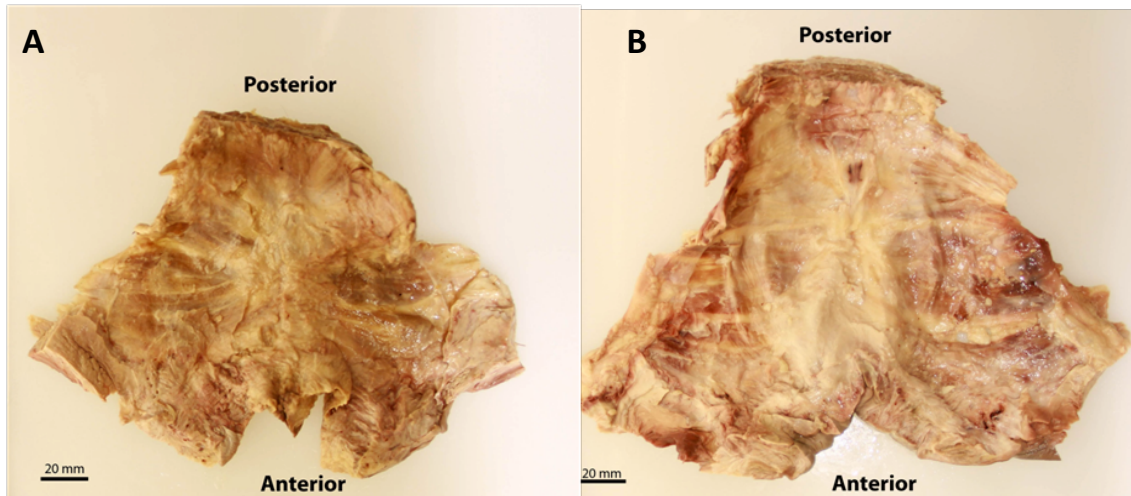


Figure 2.9. The general geometry and size of the pelvic floor muscles is variable among donors as seen in these photographs. A) Photograph of Donor 1's pelvic floor muscles. B) Photograph of Donor 5's pelvic floor muscles. (Scale bar 20 mm)

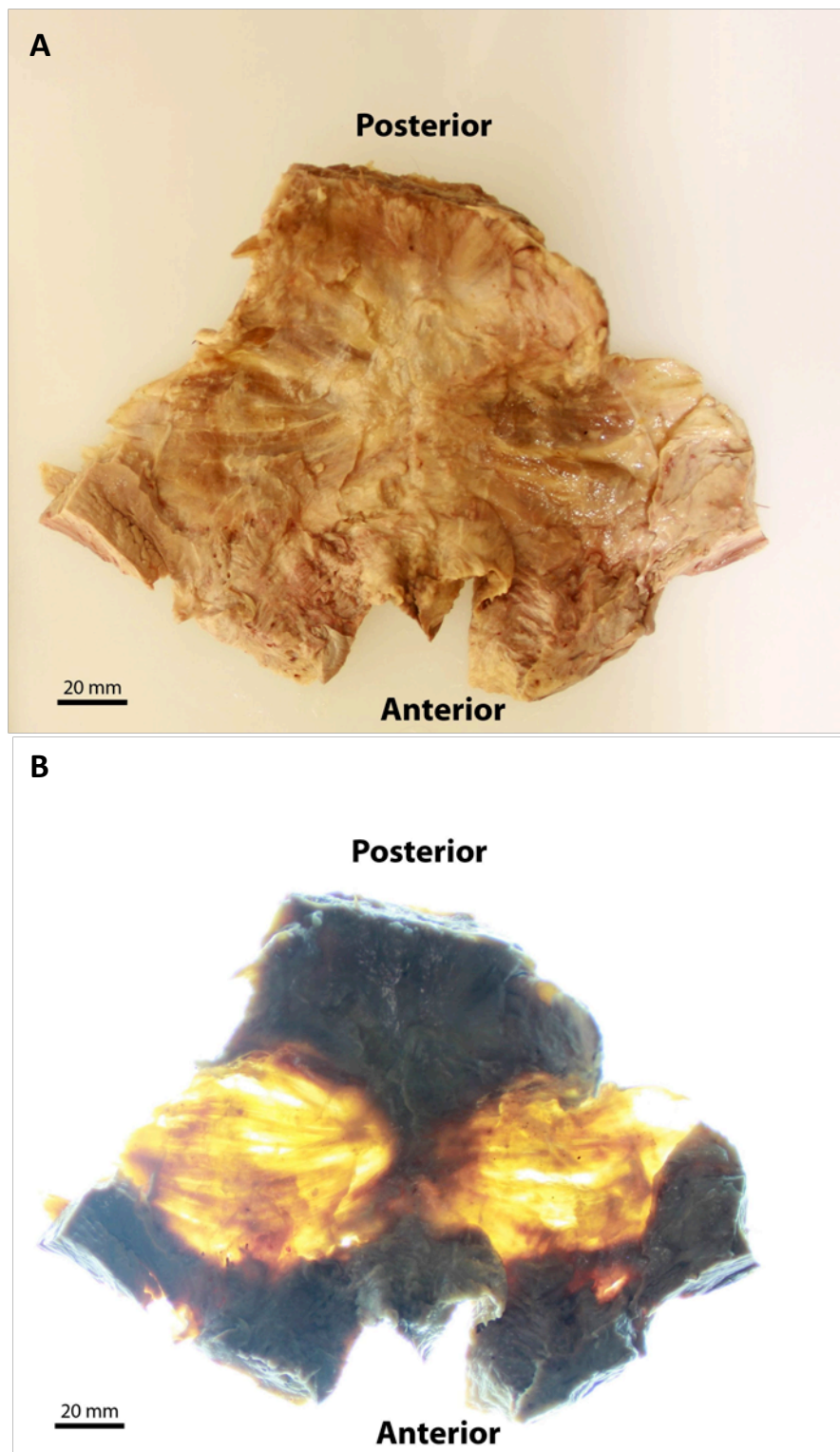


Figure 2.10. Pelvic floor muscles are thin as seen by a photograph of Donor 1's pelvic floor specimen (A) compared to its image when illuminated from behind by a light box (B). (Scale bar 20 mm)

Muscle Divisions

While we attempted to isolate all the pelvic floor muscles from each other according to each muscle's origin/insertion pairs as outlined by Kearney et al., 2004, some muscles could not be confidently separated. In general, the division between the coccygeus and iliococcygeus was relatively easy to identify because the coccygeus was substantially thicker than the thin iliococcygeus and this division was usually identifiable by a region of connective tissue. From our specimens, it was very difficult to discern the muscle boundary between the pubococcygeus and the puborectalis muscle since the majority of the fibers originated from the same anatomical region (the pubis) on one side of the body and either joined with the fibers of the corresponding muscle on the opposite side or inserted into other tissues like the external anal sphincter. After consulting an expert urogynecologist and coming to an agreement that these muscles could not be confidently separated, we decided to analyze the muscle architecture of the pubococcygeus and puborectalis as a combined muscle. To decrease subjectivity of the muscle divisions, all final decisions on muscle boundaries were agreed upon by 2 investigators.

Muscle Architecture

The two most important functional predictors of muscle are FL and PCSA. FL predicts maximum contraction velocity and the excursion range, and PCSA corresponds to maximum tetanic tension (Edman et al., 1985; Powell et al., 1984). We were able to characterize these architectural parameters for the coccygeus, iliococcygeus, and combined pubococcygeus/puborectalis muscles of the pelvic floor.

To account for natural variation in fiber lengths that occurs within muscles, between humans, and due to fixation, measured fiber lengths must be normalized for comparison. This is done by dividing a measured fiber length by its measured sarcomere length, and subsequently multiplying by a standard sarcomere length. The use of sarcomere length to normalize fiber

length in architectural studies has been validated by Felder et al., 2005. Therefore, it is important to measure SL in muscle architectural studies. In our study, sarcomere lengths were measured for each pelvic floor muscle by laser diffraction. The average sarcomere length for the pubococcygeus/rectalis muscle was $2.07 \pm 0.02 \mu\text{m}$ which was not significantly different from the iliococcygeus muscle (1.97 ± 0.04) or the coccygeus (2.01 ± 0.04) as seen in Figure 2.5. While these sarcomere lengths were not significantly different from each other, they were all relatively short considering the optimal sarcomere length *in vivo* is $2.7 \mu\text{m}$ (Janda *et al.*, 2003). This suggests that the sarcomere lengths of the pelvic floor muscles lie on the ascending limb of the force-length curve and would be able to produce more force when the muscle is stretched or lengthened. This may be mechanically advantageous since the pelvic floor muscles would likely be under tension or stretched *in vivo* under the weight of the organs.

In our study, fiber lengths for every muscle were normalized using a standard sarcomere length of $2.7 \mu\text{m}$, the optimal *in vivo* sarcomere length. The mean normalized fiber length for the coccygeus was $5.25 \pm 0.55 \text{ cm}$ which was significantly different from the iliococcygeus fiber length ($8.38 \pm 0.75 \text{ cm}$) and the pubococcygeus/rectalis fiber length (11.29 ± 1.22) as seen in Figure 2.6. Because the pubococcygeus/rectalis muscle had the longest fiber lengths, this would suggest that this muscle has a greater excursion and contraction velocity than the other two muscles.

The average coefficient of variation (CV) among the five donors for the coccygeus, iliococcygeus, and pubococcygeus/rectalis fiber lengths was 12%, 21%, and 21% respectively. The CV of the coccygeus fiber length is low compared to the average fiber length CV of other muscle architecture studies (18%) which indicates that a relatively small sample size is required to detect significant coccygeus FL treatment effects (Tuttle et al., 2011). Sample size can be determined using the equation (van Belle, 2008):

$$n = \frac{16(CV)^2}{(\ln(1 - \delta))}$$

where n is sample size, CV is coefficient of variation, and δ is the expected treatment effect. Based on our data, a sample size of $n= 5$ would be required to show a 20% treatment effect in coccygeus fiber length between two different population (i.e. patients without PF dysfunction vs. patients with a PF dysfunction). Contrastingly, the CV of the iliococcygeus and the CV of the pubococcygeus/rectalis fiber lengths are relatively high compared to other architectural studies and would thus require a higher sample size. Our data indicates that for these muscles, a sample size of $n= 14$ is needed for a 20% treatment effect to show significant fiber length differences between 2 populations. As demonstrated, our research is useful in helping to determine the sample size of future studies investigating changes in pelvic floor muscle architectural changes between groups. Additionally, it shows that for an experiment to be sufficiently powered, the sample size must also take into account which pelvic floor muscle is being analyzed since the CV is muscle dependent.

The average PCSA for the coccygeus was $0.81 \pm 0.08 \text{ cm}^2$ which was larger than either of the PCSA values for the iliococcygeus ($0.64 \pm 0.05 \text{ cm}^2$) or the pubococcygeus/puborectalis ($0.60 \pm 0.07 \text{ cm}^2$) as seen in Figure 2.7. This would predict that the coccygeus is designed to generate higher forces than the other two muscles. While the PCSA was not quite significant in the difference among these muscle ($p=0.09$), we anticipate that increasing sample size with the addition of data from more donors will show significant PCSA differences between muscle groups, particularly between the coccygeus and the other two muscles. This is supported by a sample size calculation. Using the PCSA difference and standard deviation between the coccygeus and the pubococcygeus/rectalis, we get an effect size of 0.795. Using G*Power software, this effect size shows that we need an estimated sample size of $n=21$ to show

significant differences with $\alpha=0.05$ and a Power of 0.8 (G*Power 3: Faul et al., 2007). We are currently working on obtaining data from more donors to increase our sample size.

Figure 2.8 shows the scatter graph of the average normalized fiber length and physiological cross-sectional areas of the pelvic floor muscles. Because fiber length is proportional to muscle excursion, and cross-sectional area is proportional to maximum, this graph can be used to compare the relative forces and excursions of muscles within the pelvic floor. Of the PF muscles, the coccygeus is designed with the shortest fibers and the largest PCSA making it an ideal stabilizer with higher force production. The pubococcygeus/rectalis has the longest fiber lengths and the lowest PCSA suggesting that is designed mainly for large excursions. The iliococcygeus muscle is intermediate in both excursion and force production. Muscle injury or surgery that would cause or lead to changes in these muscle's architectural characteristics would alter their functional abilities.

Comparison to Previous Architectural Study

For comparison purposes, we assume that the pubococcygeus muscle measured by Janda et al. is comparable to our combined pubococcygeus/rectalis muscle. The sarcomere lengths that we measured were shorter for all the pelvic floor muscles than those reported by the Janda et al. study. Additionally, the normalized fiber lengths in our data were longer for the pubococcygeus/rectalis and iliococcygeus muscle, and similar in length for the coccygeus muscle compared to their data. For PCSA comparison, Janda et al. reported higher PCSA values for the coccygeus and pubococcygeus values and a slightly lower PCSA value for the iliococcygeus. Figure 2.11 shows a scatter graph of their data and our data for PCSA against fiber length. The muscle to muscle comparisons show differences in absolute values which may be a result of Janda et al. only using a single cadaver in their study. Although we have numerical discrepancies, the general trend of the functional capacities between the pelvic floor muscles is

supported since both studies have demonstrated that the coccygeus muscle has shorter fiber lengths and a larger PCSA, and the pubococcygeus/rectalis has longer fiber lengths and smaller PCSA values.

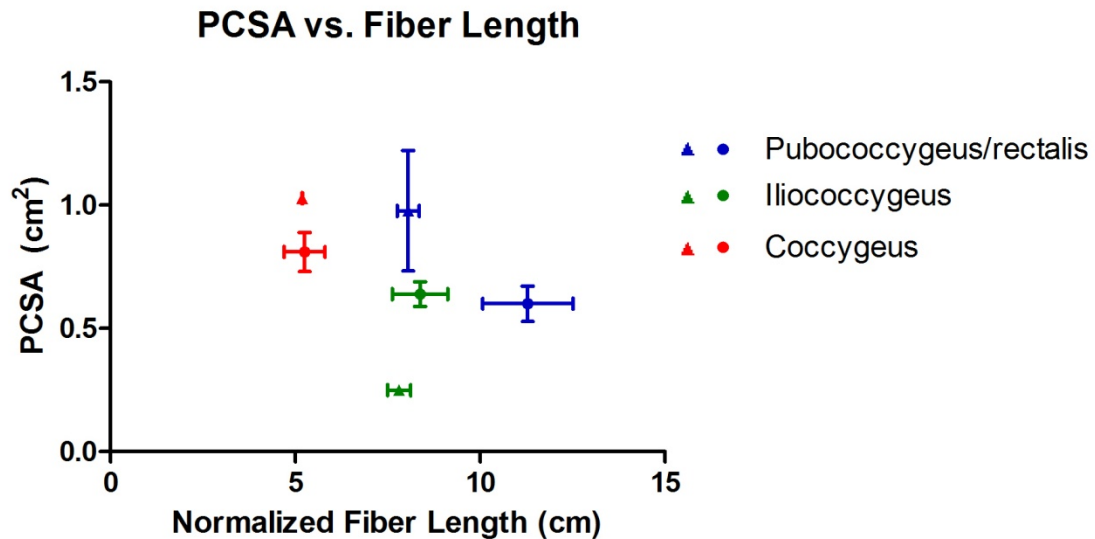


Figure 4.11. Scatter graph of the fiber length and physiological cross-sectional areas of the pelvic floor muscles in our data (●) compared to data reported by Janda et al., 2003 (▲).

2.5 Clinical Relevance

The muscle damage associated with pelvic floor dysfunction has been documented (Hoyte et al., 2001). Before we can begin to study the relationship between pelvic floor muscle damage and its associated symptoms, we must first understand the function of each pelvic floor muscle. Since muscle architecture predicts muscle function, it is important to determine the architecture of the pelvic floor muscles to comprehensively describe muscle function. Therefore, our characterization of the pelvic floor muscle architecture provides insight into their function. Additionally, the characterization of healthy PF architecture is required to investigate architectural changes associated with other factors such as age, parity, or PF disorders. Any change in architectural properties would suggest changes in the muscle's functional abilities.

Additionally, the low CV of fiber lengths in the coccygeus suggests that *in vivo* measurements (i.e. by MRI) of fiber lengths anywhere in this muscle would adequately represent the muscle since FL is relatively uniform within the coccygeus. Contrastingly, the higher CV in the iliococcygeus and pubococcygeus/rectalis suggests that studies conducting *in vivo* measurements of fiber lengths must specify location since FL is variable within these muscles. Understanding and application of the architectural data on these muscles may lead to improved strategies for surgical and nonsurgical rehabilitation of patients suffering from pelvic floor dysfunction.

CHAPTER III

CONCLUSIONS

3.1. Conclusions from Research

In investigating the pelvic floor muscles, we have been able to characterize the architectural parameters of sarcomere length, fiber length, and physiological cross-sectional area for the coccygeus, iliococcygeus, and pubococcygeus/rectalis muscles of the female pelvic floor. The sarcomere lengths were relatively short for all three muscles and no significant differences were found between the muscles. Normalized fiber lengths were longest for the pubococcygeus/rectalis muscle and shortest in the coccygeus muscles. Significant fiber length differences were found between the coccygeus muscle and the other 2 pelvic floor muscles. PCSA was highest in the coccygeus muscle and lowest in the pubococcygeus/rectalis muscle although statistical analysis showed no significant differences between muscle groups. The CV of fiber length for the iliococcygeus was the same as the CV of the pubococcygeus/rectalis, both of which were higher than the coccygeus CV. Overall, the architectural data predicts that the coccygeus is designed for force production while the pubococcygeus/rectalis is designed for excursion, suggesting functional specialization within the pelvic floor muscles.

The advantage of our work compared to the previous pelvic floor muscle architecture study done by Janda et al., 2003 is that our study had five donor subjects instead of a single cadaver. In general, our findings reported shorter sarcomere lengths, longer fiber lengths, and lower PCSA values compared to those reported by Janda et al., 2003. However, pelvic floor muscle trends were consistent between studies showing high coccygeus PCSA and high pubococcygeus/rectalis fiber length.

3.2 Limitations

There are several limitations to our findings. First, our specimens are cadaveric and were fixed by formaldehyde. Therefore, the spatial and topographical appearance of the pelvic floor likely does not correspond to its appearance *in vivo* since it has lost its muscle tone. Although the cadaveric pelvic floors are basin-shaped, it has been shown that the pelvic floor is dome-shaped at rest *in vivo* (Hugosson et al., 1991). However, even though there are differences in the topology between a cadaver and living patients, it has been reported that these differences do not affect muscle architectural parameters like optimal sarcomere lengths (Janda et al., 2003).

Another drawback is that our cadaveric donors were fairly old with a mean age of 74 years. Therefore, our characterization of muscle architecture may not apply to younger populations since it has been shown that aging significantly affects human skeletal muscle architecture (Narici et al., 2003). Also, our analysis included donors who had been pregnant and/or given birth which may have altered our architectural analysis so that our results may not accurately represent the architecture of nulliparous women. Additionally, connective tissue was also an issue in our study because it was impossible to completely remove all the connective tissue during our muscle characterization. Therefore, our results likely overestimate PCSA values since our sample mass is not composed of purely contractile elements and probably includes a substantial amount of collagen content. Another limitation was our inability to separately characterize the pubococcygeus muscle and the puborectalis muscle as individual muscles. Our architectural measurements thus may not accurately describe the functions of these muscles if they act independently *in vivo*.

An additional limitation is that our study consisted of only five pelvic floor specimens and that we treated the muscles of the right and left sides independently to have a sample size of

n=10 in our analysis. It may not be appropriate to treat the right and left muscles independently as there may be inherent differences between the right and left sides of the pelvic floor muscles. Lastly, it's important to also consider that donor history may play a role in the architectural results. Donors may have had other medical conditionals that could have an unknown effect on the health of their pelvic floor muscles.

3.3 Future Directions

We plan to address the effect of connective tissue in our analysis by determining collagen content within our samples and adjusting our PCSA values accordingly. Future investigations can expand on the architectural characterization of pelvic floor muscles and study the changes that occur due to certain factors. It would be clinically relevant to compare the muscle architecture between nulliparous women and parous women since it has been reported that parity is linked to pelvic floor disorders (Lukacz et al., 2006). Additionally, future work can be done in characterizing the PF muscle architecture of younger donors to see if age plays a significant role in pelvic floor muscle architecture. It would also be ideal to compare cadaveric architectural studies to *in vivo* measurements done by MRI, ultrasonography, or tissue biopsies to determine if cadaveric architectural pelvic floor characterization is representative of the architecture in living patients. Such studies can lead to better assessment of PF function and promote more effective rehabilitation therapies for patients suffering from pelvic floor disorders.

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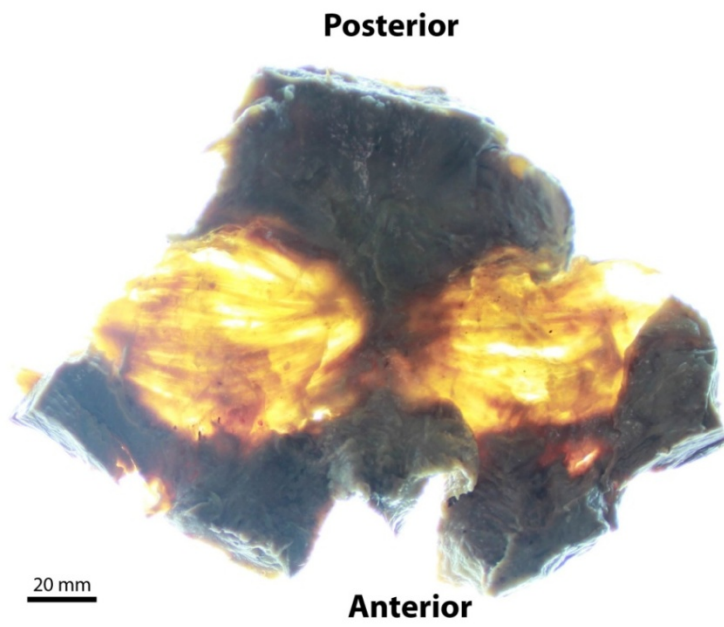
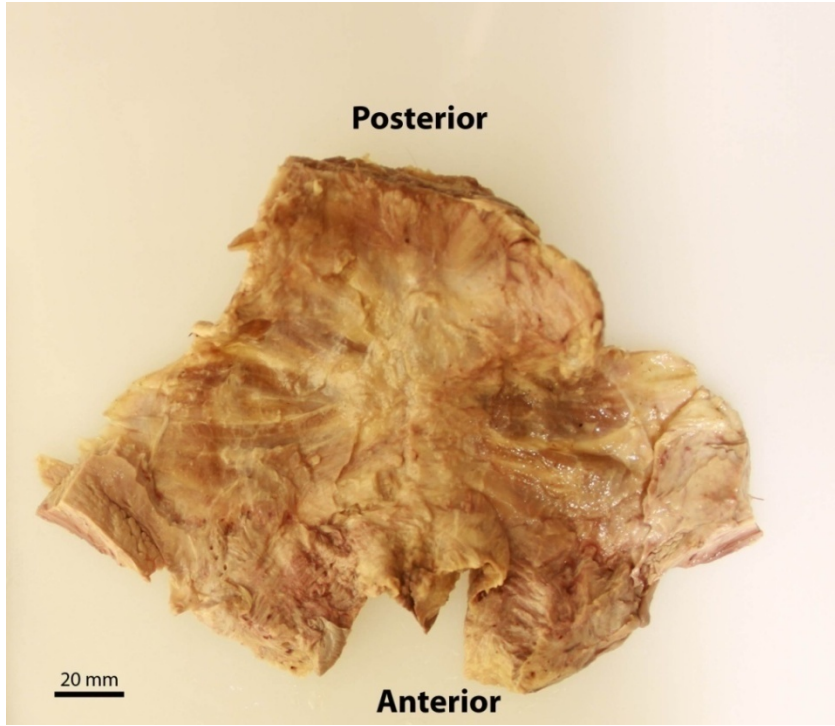
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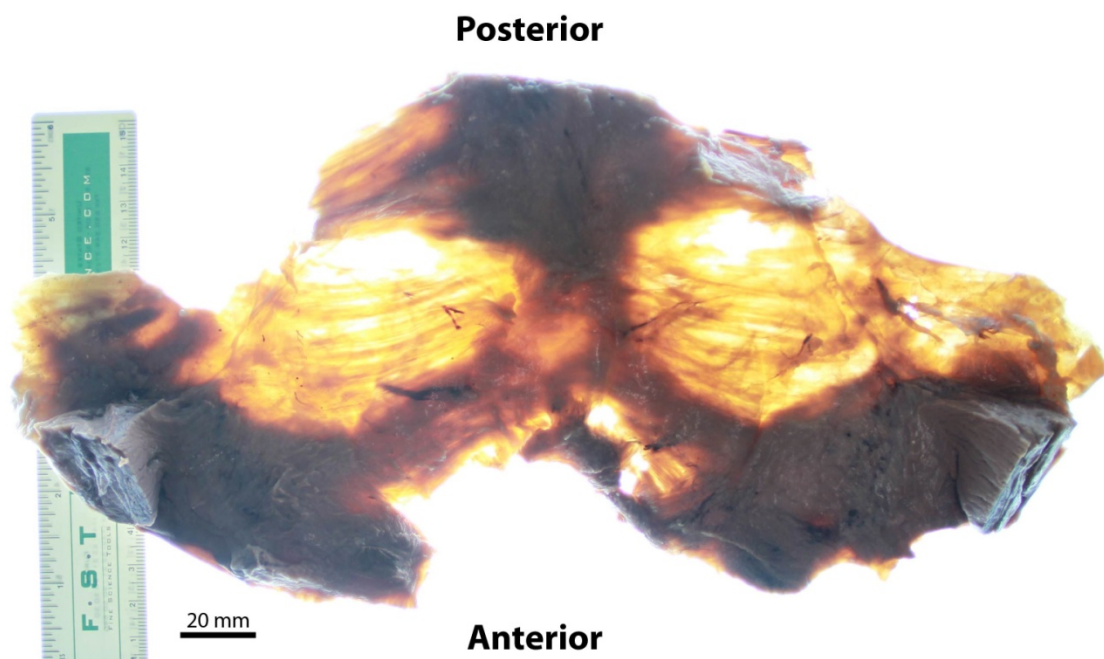
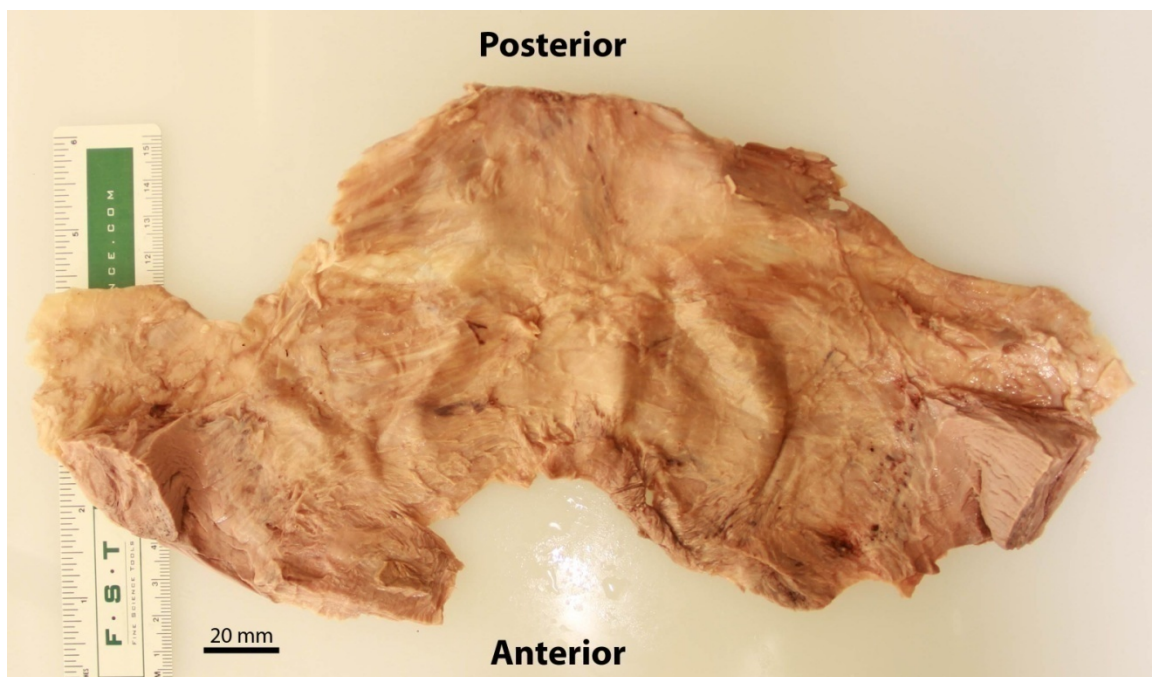
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APPENDIX

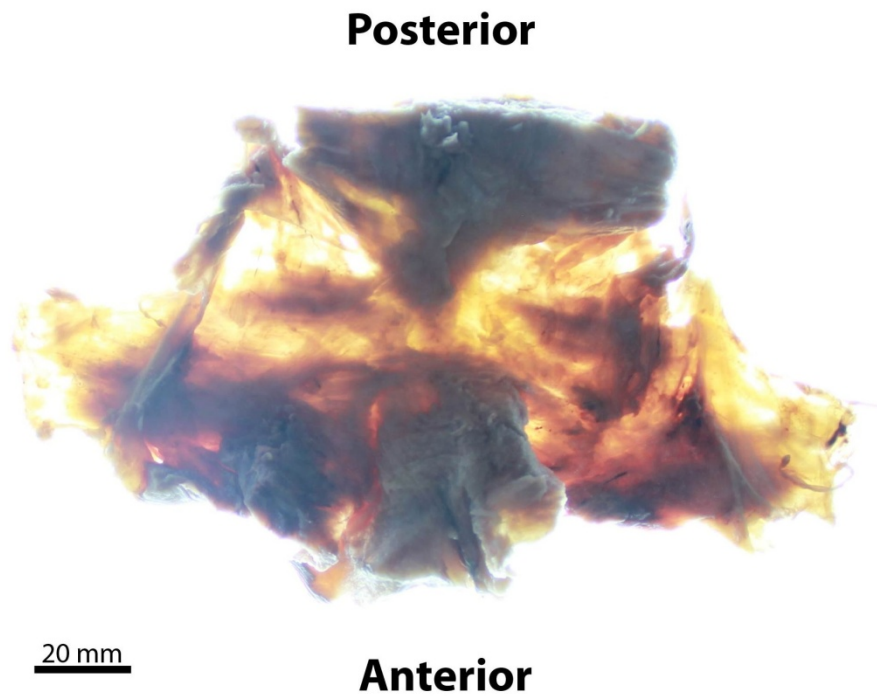
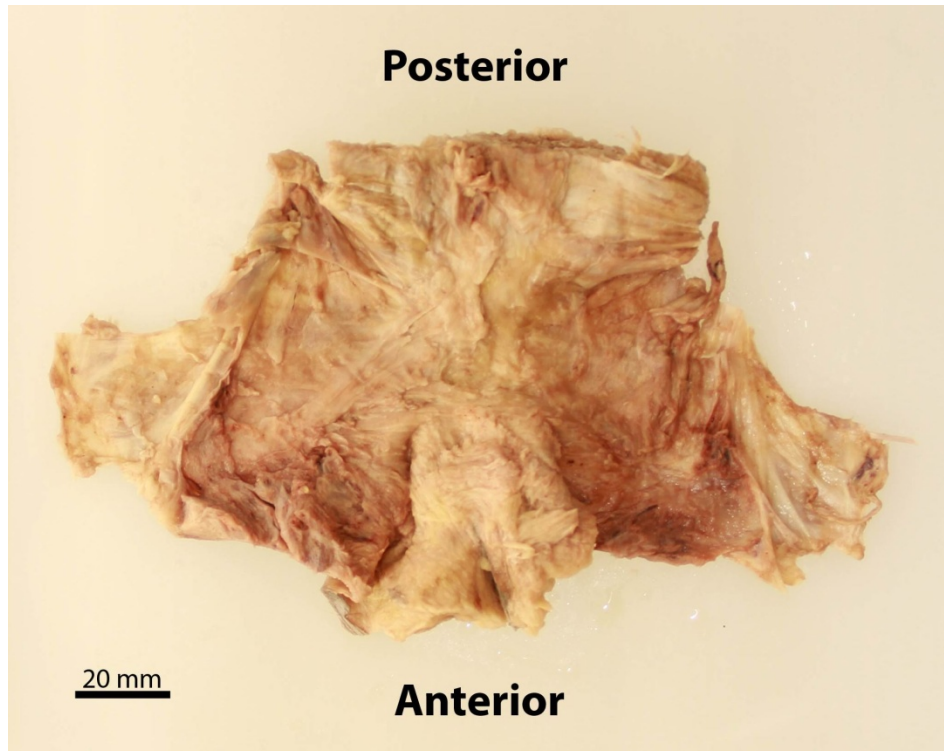
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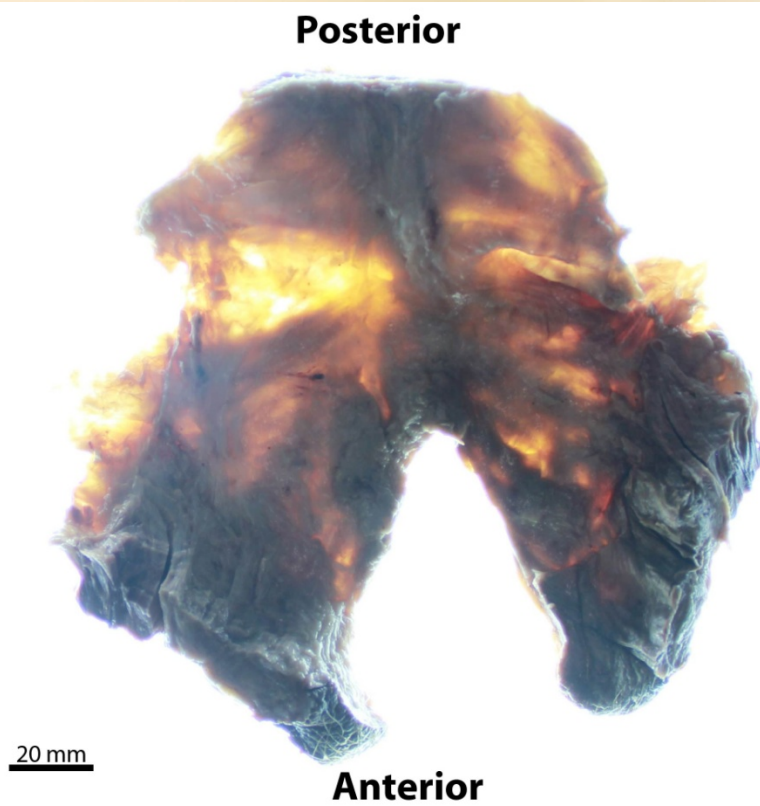
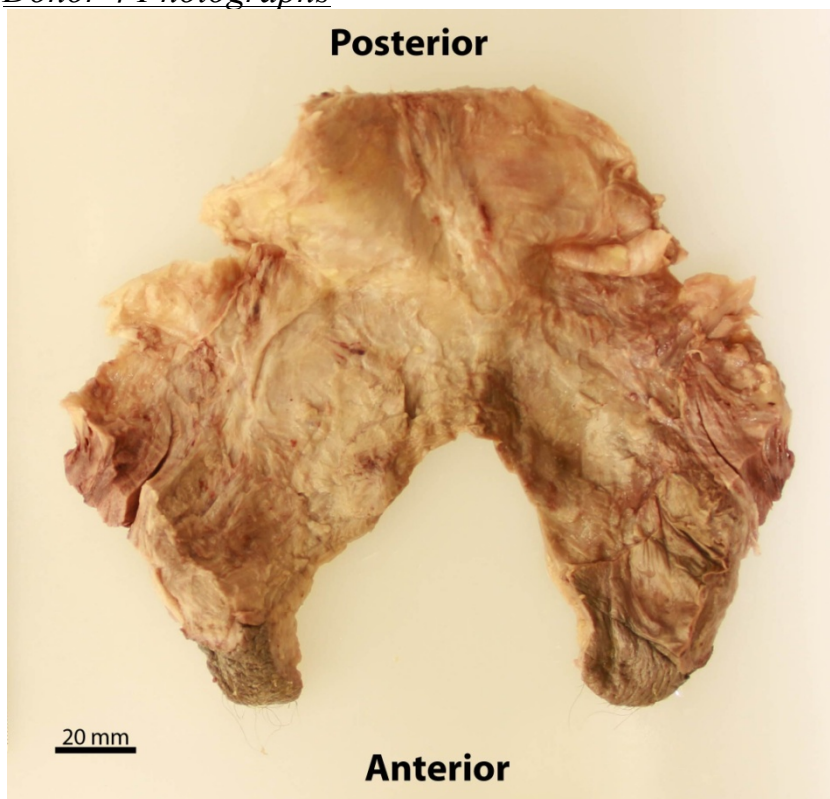
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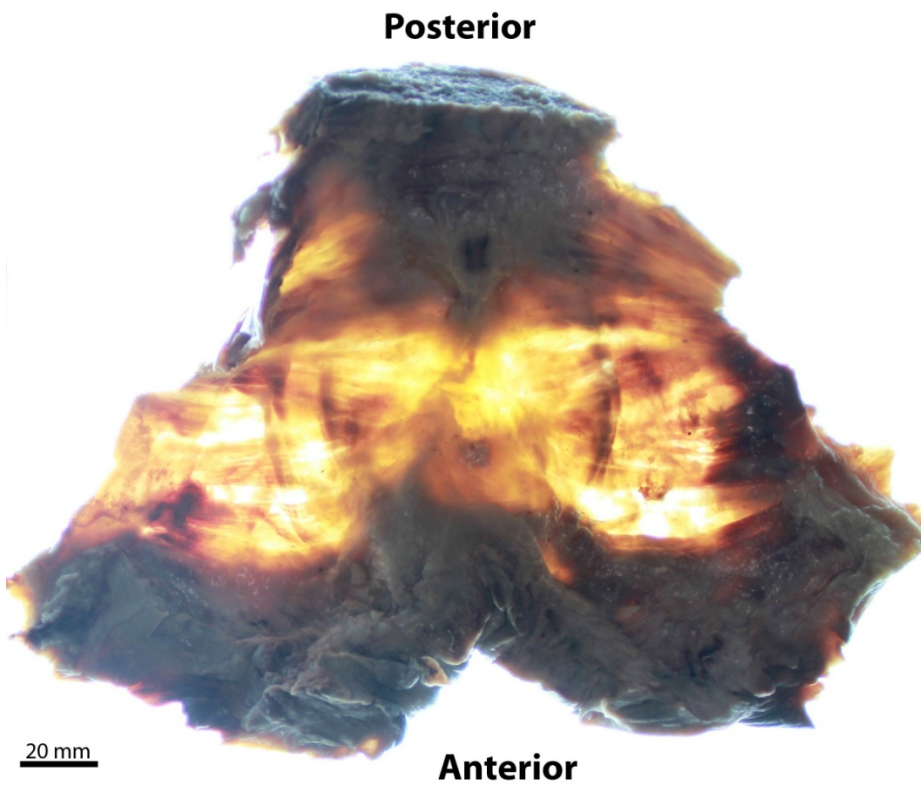
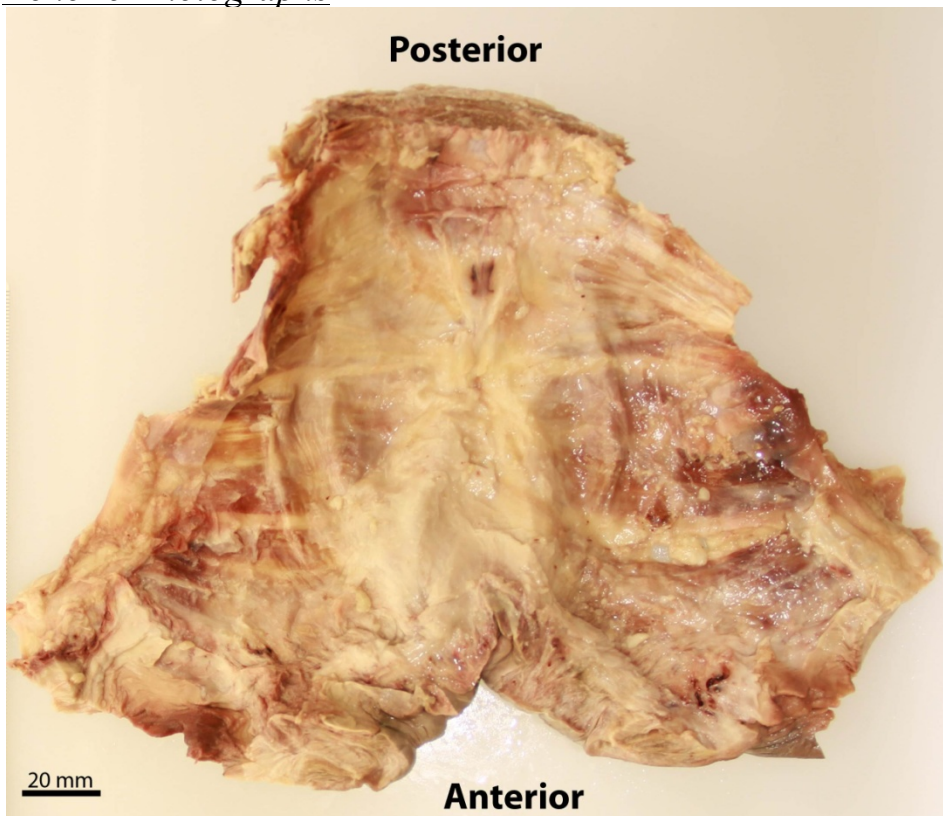
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Donor 4 Photographs



Donor 5 Photographs



Data of Left and Right Muscles

Table Mean sarcomere lengths \pm SEM (μm) of left and right sections of each muscle.

	Pubococcygeus/rectalis	Iliococcygeus	Coccygeus
Left	2.07 \pm 0.02	1.98 \pm 0.01	1.94 \pm 0.05
Right	2.06 \pm 0.04	1.97 \pm 0.05	2.08 \pm 0.03

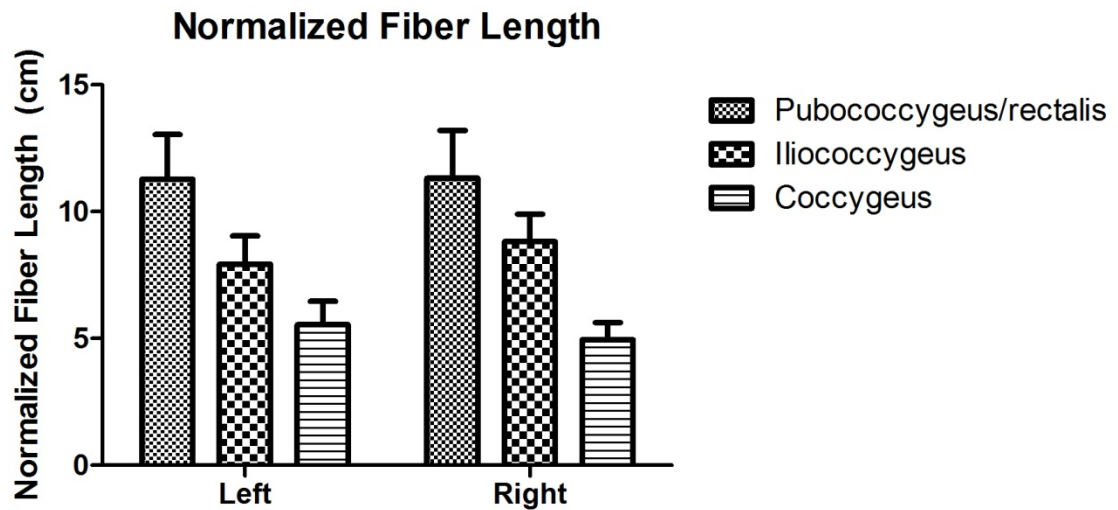


Figure Mean normalized fiber lengths of the left and right sections for each pelvic floor muscle.

Table Mean PCSA \pm SEM (cm^2) of left and right sections of each muscle.

	Pubococcygeus/rectalis	Iliococcygeus	Coccygeus
Left	0.58 \pm 0.07	0.64 \pm 0.07	0.78 \pm 0.09
Right	0.62 \pm 0.13	1.63 \pm 0.09	0.84 \pm 0.15

Data of Regions within Muscles

Table Mean sarcomere lengths \pm SEM (μm) of the three regions within each muscle.

	Pubococcygeus/rectalis	Iliococcygeus	Coccygeus
Anterior	2.06 \pm 0.02	1.99 \pm 0.04	1.99 \pm 0.04
Mid	2.08 \pm 0.03	1.98 \pm 0.06	2.02 \pm 0.05
Posterior	2.08 \pm 0.03	1.96 \pm 0.04	2.01 \pm 0.03

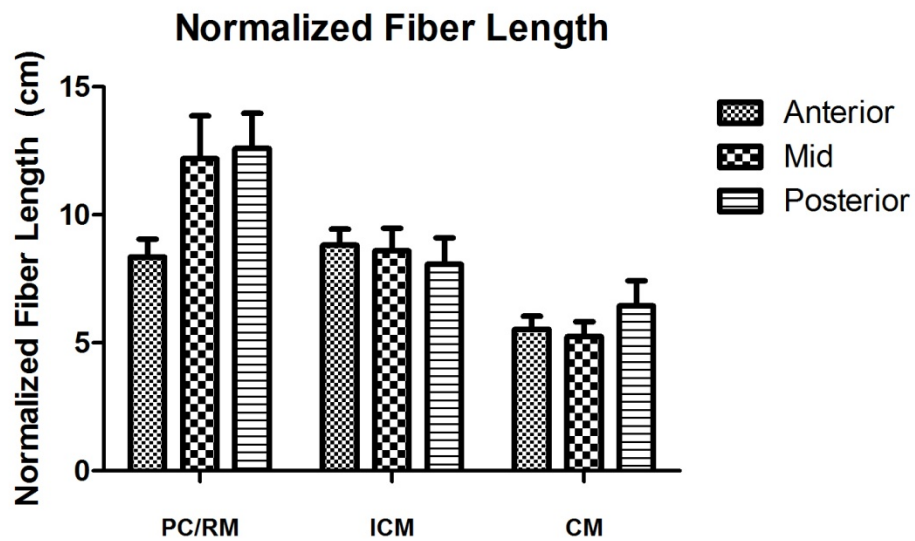


Figure. Regional mean normalized fiber lengths for each pelvic floor muscle. PC/RM- pubococcygeus/rectalis muscle, ICM- iliococcygeus muscle, CM- coccygeus muscle