ARE AGE-RELATED CHANGES EVIDENT IN THE ACTIVE AND/OR PASSIVE COMPONENTS OF PELVIC FLOOR MUSCLE FORCE OUTCOMES IN NULLIPAROUS WOMEN?

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A thesis submitted to the Faculty of Graduate and Postdoctoral Studies in fulfillment of the requirements for:

Masters of Science Degree in Human Kinetics

School of Human Kinetics

Faculty of Health Sciences

University of Ottawa

ABSTRACT

Background: Age-related changes in pelvic floor muscle (PFM) biomechanics may contribute to urinary incontinence in older women; however, empirical evidence is scant.

Purpose: This study aimed to understand the age-related changes in the biomechanical properties of the PFMs in women with no major risk factors for urinary incontinence.

Methods: Thirty-three nulliparous women (20-64 years) were recruited to study active force, rate of force development, endurance, resistance to passive stretch and stiffness properties of the PFMs using an automated dynamometer. Separate regression analyses were performed to investigate the relationship between age and each outcome measure.

Results: No significant relationships were observed between age and any of the outcome measures.

Conclusion: The findings from this study do not support the presence of any age-related changes in PFM mechanics among women aged 20-64. Recruiting women over the age of 65 may be essential to detect age-related changes in PFM biomechanics in nulliparous women.

Key Words: *aging, muscle compliance, muscle contraction, muscle endurance, muscle force, muscle strength dynamometer, muscle stiffness, passive stretch, pelvic floor muscles, pelvic floor dysfunction, urinary incontinence*

List of Abbreviations

1. ANOVA: Analysis of Variance
2. ATP: Adenosine triphosphate
3. BMI: Body Mass Index
4. CSA: Cross sectional area
5. CV: coefficient of variation
6. DOMS: Delayed onset muscle soreness
7. EMG: Electromyography
8. IAP: intra-abdominal pressure
9. ICC: Intra-class Correlation Coefficient
10. ICIQ-B: International Consultation on Incontinence Questionnaire – Bowel Symptoms
11. ICIQ-FLUTS: International Consultation on Incontinence Questionnaire – Female Lower Urinary Tract Symptoms
12. ICIQ-VS: International Consultation on Incontinence Questionnaire – Vaginal Symptoms
13. IVP: Intra-vaginal pressure
14. LA: Levator ani
15. MRI: Magnetic Resonance Imaging
16. MVC: Maximal voluntary contraction
17. PFMs: pelvic floor muscles
18. PFMT: Pelvic floor muscle training
19. PVD: Provoked vestibulodynia
20. QoL: Quality of life
21. SUI: Stress urinary incontinence
22. TVT: Tension Free Vaginal Tape
23. UI: Urinary incontinence
24. USI: Ultrasound Imaging

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Operational Definitions

Active force: the rise in force output, recorded in Newtons (N), observed through neuromuscular activation of a muscle. It can be calculated as an absolute force or as a relative force by subtracting the absolute peak force (total force) from the baseline or resting force.

Muscle Compliance: the change in tissue length per unit of applied force (the inverse of stiffness), which is measured when the muscle is relaxed.

Muscle Endurance: ability to voluntarily produce force either repeatedly against maximal or submaximal external resistance, measured in time or in number of contractions until force falls below a certain threshold, or to sustain a maximal or submaximal force for as long as possible until the force output drops below a certain threshold, measured in seconds.

Intra-abdominal pressure (IAP): pressure within the abdominal cavity. An increase in IAP, generated by abdominal muscle and diaphragmatic co-contraction, is observed during stressful situations such as coughing, sneezing and laughing. IAP can be expressed in mmHg or cmH2O.

Intra-vaginal pressure (IVP): pressure measured from air or fluid contained within a closed system when inserted into the vaginal cavity. It is influenced by IAP and is expressed in mmHg or cmH2O.

Levator Ani (LA) Muscles: the LA muscle is the main muscle group of the pelvic floor, located in the anterior part of the pelvic diaphragm. It consists of the puborectalis, pubococcygeus, and iliococcygeus muscles and its primary function is to provide support to the pelvic organs.

Maximum voluntary contraction (MVC): the peak force produced by a muscle during an effort to generate the strongest voluntary contraction possible. MVC is an active force (defined above) and is expressed in Newtons. A true MVC can only be confirmed when an electrical pulse applied to the motor nerve or the muscle itself does not result in further force output from that muscle, whereas a maximal effort voluntary contraction may not, in fact, generate an MVC.

Pelvic floor dysfunction: term applied to a wide variety of clinical conditions in which the pelvic floor muscles are suspected to be implicated, including urinary incontinence, anal incontinence, pelvic organ prolapse, sensory and emptying abnormalities of the lower urinary tract, sexual dysfunction, and chronic pain syndromes.

Pelvic floor muscle contraction: the lifting and squeezing action in the LA muscles

Pelvic floor muscle tone: resistance of a muscle to lengthening or passive stretching. While PFM tone has been rated on a 7-point scale of -3 to +3, with -3 being hypotonic (very low), +3 being

hypertonic (very high) with 0 representing normal (Reissing et al., 2004), no gold standard currently exists to quantify PFM tone.

Pelvic Organ Prolapse (POP): involves a caudal descent of the bladder, urethra, small intestine, rectum, uterus, or vagina associated with damage or injury to the PFMs and pelvic connective tissues.

Provoked vestibulodynia (PVD): pain associated with a touch or pressure stimulus applied at the entrance of the vagina, also known as the vulvar vestibule.

Rate of force development: the rate at which the contractile elements of the muscle can develop force and is measured in Newtons per second (N/s). For the purposes of the current study, rate of force development was obtained using the steepest linear portion of the force vs time graph

Resistance to passive stretch: the resistance to lengthening a muscle when in its resting state. The resistance to passive stretch can be measured as an absolute force (in N) or a relative force (in N), obtained by subtracting the baseline force from the peak passive force.

Sarcopenia: the preferential loss of type II muscle fibers associated with advanced aging.

Sarcopenia in skeletal muscles is mainly evident after the age of 65.

Muscle Stiffness: the extent to which the tissue can resist deformation. For the purposes of this thesis, muscle stiffness was measured as the slope of the most linear portion of the force vs dynamometer diameter graph, and was expressed as Newtons per millimeters (N/mm)

Stress urinary incontinence: involuntary urine leakage upon effort, exertion, sneezing, or coughing (i.e. stress). Specifically, intra-abdominal pressure is generated through abdominal and diaphragmatic action and is transmitted to the bladder, which can result in urine leakage if the bladder pressure exceeds the closure pressure within the urethra.

Total force: Total force, or the peak force generated during maximal voluntary contraction or a passive elongation, measured by a dynamometer as the sum of active and passive forces generated by the PFMs and their associated connective tissue. It is expressed in Newtons (N)

Urinary incontinence: defined by the International Continence Society as an involuntary loss of urine and is diagnosed primarily through self-report.

Viscoelastic properties: both viscous and elastic properties that co-occur in skeletal muscles. For example, in muscle tissue, the change in muscle length and rate of deformation are directly

proportional to both the applied force and the rate of force application. Two viscoelastic properties of interest include stress relaxation responses and tissue creep. When the relaxed muscle is lengthened and held at constant length, the force required to hold the muscle at that length gradually declines, which is known as stress-relaxation. Conversely, tissue creep describes the change in length of a tissue over time in response to a constant applied force.

Introduction

The pelvic floor region, located at the base of the pelvis, is composed of a sling of muscles and connective tissues that extends from the pubic symphysis to the coccyx (DeLancey, 1994). The pelvic floor muscles (PFMs) and their associated connective tissues help to support the pelvic organs (DeLancey, 1994) and participate in continence (Bump, 1996), sexual (Gordon et al., 1999) and postural control processes (Hodges et al., 2007). The PFMs are organized into superficial and deep layers. The deep layers are comprised of the levator ani (LA) and iliococcygeus muscles, and their associated endopelvic fascia and ligaments (DeLancey, 1994). When functioning optimally, the LA muscles limit strain on the fascia and ligaments that support the pelvic organs. The PFMs can lose their contractile ability due to damage and/or denervation induced by childbirth (DeLancey et al, 2003), repeated stress caused by high impact activities (Howard, 1993) or chronic cough (Miller et al, 2001), accidents (Baessler et al, 2004), and, possibly, aging (Thelen et al, 1996). Dysfunction of the PFMs is thought to result in pelvic organ prolapse (Bump & Norton, 1998), pelvic pain (Howard, 1993), low back pain (Smith et al., 2008), and fecal (Bump et al, 1996) and urinary incontinence (Wallner et al, 2008) in women.

Urinary incontinence (UI), defined as the involuntary leakage of urine, is a major health issue that affects more women than men (Thomas et al., 1980). Many cases of UI are not reported because of embarrassment (Kobashi et al., 2017). Although it is not life-threatening, UI can have a major impact on quality of life. UI affects about 9-72% of women aged 17 to 80 years (BØ and Sherburn, 2005). In Canada, it is estimated that about 50% of women are affected by incontinence (Abrams et al., 2010), making it a highly prevalent condition, and one

that is associated with a significant economic burden. A recent cross-sectional study of women in the United States found that among 6,600 females, 20-30% of women aged 20-30 years, 30-40% of women aged 30-40 years and 30-50% women aged 50 and over regularly experienced urinary incontinence (Altaweel et al., 2012). This rate plateaus up until the age of 70 after which, the prevalence of UI rises again, suggesting that increasing age may be a contributing factor to the development of UI later in life (Hannastad et al., 2000). Such high prevalence rates of UI result in an enormous financial impact on health care systems, with more than 16 billion US dollars being spent in the United States over known cases of UI annually (Wilson et al, 2001). This has placed a greater demand on finding effective treatments for UI, especially since the aging population is on the rise (Borger et al, 2015). It is not understood why UI becomes more prevalent with age in women, but it may be related to the manifestation of the normal age-related changes seen in other skeletal muscles on the striated urethral sphincter and/or the PFMs.

The most common form of UI is stress urinary incontinence (SUI), which is experienced by 50% of all incontinent women (Felicissimo et al., 2010), and is particularly common in older women (Camargo et al., 2009). SUI is defined by involuntary urine leakage upon effort, exertion, sneezing, or coughing (i.e. stress) (Abrams et al., 2002). Specifically, the intra-abdominal pressure generated through abdominal and diaphragmatic action transmits pressure to the bladder, which can result in urine being forced out through the urethra if the bladder pressure exceeds the closure pressure within the urethra. Under normal conditions, when voiding is not desired, the urethral closure pressure remains greater than the bladder pressure both at rest and during increases in intra-abdominal pressure and thus urine leakage is

prevented (Abrams et al., 1988). During activities such as coughing or sneezing, when the bladder pressure exceeds the urethral resting pressure, combined urethral sphincter (BØ and Stein, 1994) and PFM contraction can heighten urethral closure pressure (Rud, 1980) and maintain continence. The urethral sphincter and LA, which are made up of striated muscles, are responsible for approximately one-third of the urethral closure pressure (Rud., 1980).

Urethral profilometry has shown that both maximal and resting urethral closure pressure are lower in women with SUI than in continent women (DeLancey et al, 2008); (BØ et al., 1999), which may be influenced by lower force generation capacity in the PFMs of women with SUI than their age-matched continent counterparts (Chamochumbi et al., 2012). Some studies have shown evidence that women with SUI have lower PFM strength than their continent counterparts (Gunnarsson and Mattiasson, 1999; Hahn et al., 1996; Laycock, 1992); while other studies have not (Morin et al., 2004; BØ et al., 1994). According to Morin et al. (2004), factors other than PFM strength may be involved with SUI pathophysiology. These researchers measured the biomechanics of the PFMs using an intravaginal dynamometer, (Dumoulin et al., 2004) that possesses good test-retest reliability for assessing PFM function (Morin et al., 2007). Morin et al. (2004) showed that women with SUI had lower PFM passive force, absolute endurance and rate of force development during PFM activation than their continent counterparts. Their results suggested that assessment of the PFMs should not be restricted to force generation capacity alone; parameters such as rate of force development, endurance and the passive properties may also provide information to discriminate between incontinent and continent women.

To date, age-related changes in the PFMs remain poorly understood. However, most

studies on other skeletal muscles have demonstrated significant reductions in muscle force generating capacity with increasing age (Frontera et al., 2000) while muscle endurance is generally maintained (Meredith et al., 1989; Hepple & Rice., 2015). Declines in muscle force generating capacity are most evident between the ages of 65-80 (Jubrias et al., 1997; Kyle et al., 2001). The age-related changes are thought to be associated with the selective loss or denervation of type II or fast twitch muscle fibers, termed sarcopenia (Rosenberg, 1997). Indeed, muscle biopsies in elderly men over the age of 70 have shown lower thigh muscle cross sectional area (CSA), defined as the number of muscle fibers in a muscle cross-section, of over 25 to 40% in older men compared to younger men (Klitgaard et al., 1990; Lexell et al., 1988). Lexell et al (1988) also found that the percentage of type II muscle fibers was lower in older men compared to younger men, suggesting that the differences in type II muscle fiber distribution may be responsible for difference in muscle mass and capacity to generate force between the two age groups. Similar results were found by Nilwik et al (2013), who, through muscle biopsy in male quadriceps muscles, showed that older men (aged 70 and over) had 14% lower CSA and 29% fewer type II muscle fibers compared to younger men (22-44) (Nilwik et al., 2013). Muscle biopsy results have also shown that with an increase in age, there is an increase in the proportion of type I muscle fibers within skeletal muscle. Larsson and Karlson analyzed muscle tissue from the quadriceps of healthy male participants and found that men over the age of 60 had 66% type I muscle fibers; while younger men in their 20s had 39% type I muscle fibers (Larsson & Karlson, 1978), which provides evidence for the lower force generating capacity seen in older adults compared to young adults despite the two groups demonstrating similar muscle endurance (Hunter et al., 2005).

Age-related changes are also evident in the connective tissues embedded within and surrounding skeletal muscles. It has been shown that connective tissues generally lose compliance or increase in stiffness with age (Campbell et al, 1973). Muscle compliance, defined as the muscle's ability to be deformed or stretched, is the change in muscle length relative to the force applied (Mirsky and Parmley, 1973). As such, muscles generally increase in their resistance to passive stretch (Vandervoort et al, 1992) and lose their capacity to lengthen (Gajdosik et al, 1999) with age. The reduction in collagen concentration, is associated with an increase in muscle stiffness with age (Timiras, 1994) and aged connective tissue is easily damaged (Enoka, 1994). It can be speculated that increase in tissue stiffness with age can contribute to the active force generation capacity of muscles, as the muscles cannot maximally contract since the cross-bridging cycle of actin and myosin filaments may be impaired in older tissues compared to younger tissues (Evan & Lexall, 1995). However, it is difficult to provide evidence for this claim, since muscle stiffness can only be measured when the muscles are relaxed. Little is known about the impact of aging on the force generation and stiffness properties of the PFMs. Therefore, in order to develop interventions to target the age-related changes in the PFMs, it is important to understand the biomechanics of these muscles and how aging affects their function.

It is unclear whether the PFMs are affected by sarcopenia. There are few reports in the literature on differences in PFM strength and endurance characteristics between older and younger women. Madill and McLean (2010) showed that there was a negative correlation between age and the vaginal wall pressure during coughing (Madill & McLean, 2010). Quartly et al (2010) found that the endurance of the PFMs was maintained with age (Quartly et al., 2010).

To date, there are no reports in the literature comparing the passive tissue characteristics of the PFMs between young and older women.

According to PFM biopsies, the PFMs are composed of approximately 30% type II (fast twitch) and 70% type I (slow twitch) (Miller et al 1994) fibers. Type II fibers are needed for high force generation, such as when a task requires rapid, dynamic closure of the urethra or pelvic outlet to prevent urine/fecal leakage and to support the pelvic organs during high impact activities (Koelbl et al., 1989). Type I fibers are required for sustained, tonic support of the urethra and the pelvic organs (Gosling et al., 1989). Findings from a recent study by Morris et al. (2012) suggest that the PFMs show no age-related declines in CSA. Using magnetic resonance imaging, Morris and colleagues found that, in a small sample of nulliparous women, the CSA of the obturator internus muscle was smaller in the older women compared to younger women but that there were no group differences seen in the LA. Since the PFMs are composed predominantly of type I fibers (Gilpin et al, 1989), and given the relative sparing of type I muscle fibers associated with sarcopenia, it is possible that the PFMs are spared from significant age-related declines in muscle strength. This finding may not hold true for all striated muscles implicated in continence function. Perrucchini et al (2002) found that the number and density of striated muscle fibers in urethral sphincter are fewer in older women compared to younger women, demonstrating the possible loss of urethral striated muscle fibers with advanced age, which may contribute to lower urethral contractile force in older women. In two studies, the maximal urethral closure pressure, which is, in part, due to urethral sphincter and PFM action, has been shown to be lower in older women compared to younger women (DeLancey et al, 2008; Clobes et al, 2008) and therefore may reflect age-related reductions in the strength of

urethral sphincters and not the PFMs.

Even though age-related changes in the connective tissue of skeletal muscles are well documented, it is unclear how changes in connective tissue properties affect the muscle mechanics of the PFMs and to date, no studies have looked at the impact of aging on the stiffness properties of these muscles. The connective tissues provide resistance to lengthening and work together with the PFMs to help support the urethra and the pelvic organs. The connective tissues are primarily composed of collagen and elastin. Collagen is the most abundant protein found in ligaments, fascia and tendons. The amount and type of collagen in the PFMs has an effect on the tissue properties (Goldspink et al., 1994). Bailey et al., found that an increase in age is significantly related to reduction in collagen content in skeletal muscles (Bailey et al., 1971). Reduction in collagen content, and alteration in collagen type ratios have also been observed in vaginal tissue samples of women with pelvic organ prolapse compared to control women (Lei et al., 2007). A lack of collagen and lower number of periurethral vessels are seen in hypotonic urethra, which is an important factor that contributes to low urethral closure pressure in post-menopausal women with UI (Schwenzer et al., 1989). Alternations in connective tissue composition may contribute to increased stiffness in the PFMs of older women (Yong et al., 2003), which may, in turn, impair the force generation capacity of the PFMs, thereby contributing to the pathophysiology of SUI in older women. However, there is no evidence in the literature to support this claim.

There are several confounding factors that may impact the study of age-related changes in the PFMs. For example, there is a clear link between parity and damage to the PFMs (Dietz & Lanzarone, 2005; Dietz et al., 2011). Wesnes and colleagues reported that the incidence and

prevalence of SUI are higher in parous women compared to nulliparous women, with this risk being higher in women who have had vaginal delivery compared to cesarean delivery (Wesnes et al., 2009). Other risk factors such as heavy smoking (>20 cigarettes per day) and diabetes mellitus (Hannestad et al., 2003) may also contribute to UI presumably through alterations in the neural and mechanical function of the PFMs. Hannestad and colleagues studied data from 27,936 women, twenty-five percent of whom had urinary incontinence, and found that there was an association between increase in age and weight and increase in UI prevalence. The mechanism by which body weight contributes to SUI is thought to be due to high gravitational forces induced on the PFMs that may lead to muscle strain. However, there is no evidence supporting this claim. The literature provides contradictory evidence on hysterectomy and pelvic floor surgery as risk factors for SUI (Altman et al., 2007).

As mentioned previously, even though age-related changes are demonstrated in the urethral muscles (Perrucchini et al., 2002; DeLancey et al., 2008), it is still unclear how aging impacts the biomechanics of the PFMs. In order to understand the age-related changes on the force generation and stiffness properties of the PFMs, it is important to consider confounding factors that may impact outcomes. Therefore, a sample of nulliparous women who were not obese (BMI < 30) and had no history of heavy-smoking, diabetes mellitus or urogynecological surgeries was recruited to study age-related changes in PFM function in the absence of known confounding variables.

The purpose of this study was to determine whether age-related changes are evident when studying the in-vivo biomechanical properties of the PFMs in women with no major risk factors for SUI, that is, non-obese, non-smoking, nulliparous women with no history of diabetes

mellitis. This cross-sectional study was designed to investigate the associations between age and maximal force generating capacity, rate of force development, endurance and resistance to passive stretch of the PFMs in nulliparous women. The outcome of this research may help us to understand the normal age-related changes seen in the PFMs in women and help us to develop interventions that prevent or reverse these changes.

Literature Review

The aim of this literature review is to provide information about the PFMs, their function, and their role in maintaining continence in women. This review will also look at published studies that discuss different techniques used to study the force generation and stiffness properties of the pelvic floor muscles, as well as how aging impacts the biomechanics of these muscles. This literature search included four databases (PubMed, PsycINFO, Web of Science and Web of Knowledge) which were searched using the key words *pelvic floor muscle "OR" pelvic floor "AND" muscle force "OR" endurance "OR" muscle stiffness "OR" muscle compliance, pelvic floor "AND" dynamometry, urinary incontinence "OR" pelvic floor dysfunction "AND" aging.* Peer-reviewed journal articles published in English between 1970 and 2017 were included, and the reference lists of retrieved articles were hand searched for other relevant journal articles.

Pelvic Floor Muscle Anatomy and Function in Women

The pelvic floor is organized into superficial and deep muscle layers and includes the connective tissue of the endopelvic fascia and ligaments. The deeper layer of the PFMs (Figure 1a) is the primary component of the pelvic diaphragm, consisting of the LA and ileococcygeus muscles and their associated connective tissues (DeLancey, 1994). The LA muscles form the anterior part of the pelvic diaphragm, while the ileococcygeus muscles form the posterior part (DeLancey, 1994). The LA muscles span from the pubic bone to the anal sphincter and behind the rectum. The LA is composed of three subsections, named based on their attachment points: the puborectalis, pubococcygeus and puboviscerali muscles, with the latter further subdivided

into pubovaginal, puboperineal and puboanal units, which are also named for their origin and insertion sites. MRI results show that the ascent and descent of the pelvic floor, is likely due to the contraction and relaxation of the pubococcygeus, ileococcygeus and ischiococcygeus muscles (DeLancey, 1988).

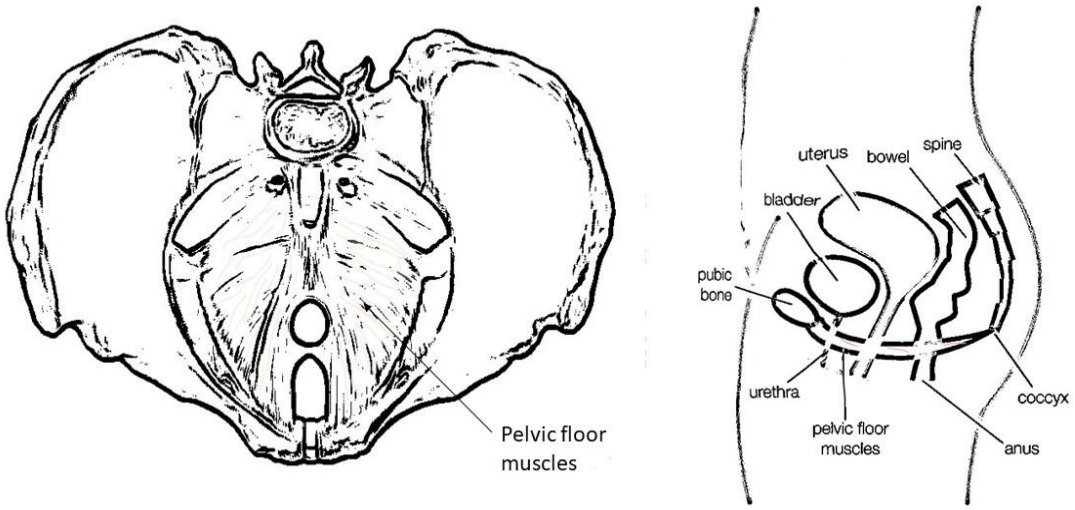

Figure 1.1: Anatomy of the pelvic floor muscles (PFMs). Anterior view of the PFMs adapted from Primal's 3D Human Anatomy and Physiology 2015 (left). Sagittal view of the PFMs and the pelvic organs supported by the muscles of the pelvic floor (right).

The urethra, the vagina and the rectum pass through the urogenital hiatus, a midline gap in PFMs and connective tissue within the pelvic diaphragm, to exit the body. (DeLancey, 1994). The connective tissue within the PFMs, like the other muscles in our body, consists of collagen, fibroblasts, elastin, and has smooth muscle cells and neurovascular and fibrovascular bundles (Norton, 1993) embedded within it. The connective tissues and endopelvic fascia form the dense, fibrous connective tissue layer that surrounds the vagina and attaches to the arcus tendineus fascia pelvis located bilaterally on either side of the urethra and vagina. Each arcus

tendineus fascia pelvis extends from the lower part of the pubic symphysis and attaches to the pubic bone ventrally and to the ischial spine dorsally. The bilateral arcus tendineus fascae pelvis, as well as other ligaments and fascia, provide attachment points and support the urethra, holding it in position along the anterior vaginal wall (DeLancey et al., 1988).

According to Ashton-Miller, the anatomical structures that are important in order to prevent incontinence and prolapse in women include both sphincteric and supportive systems (Ashton-Miller, 2001). The primary function of the pelvic floor is to provide support to the pelvic organs whereby they can retain their shape and relative position in the pelvis regardless of posture or activity (Morgan et al., 2005). The LA plays an important role in preventing any strain on the ligaments and fasciae that support the pelvic organs. The LA and the connective tissues work in synergy. When the LA muscles are relaxed, the surrounding connective tissue provide support to the pelvic organs; when LA are contracted, they relieve the surrounding connective tissues of the stress from supporting the pelvic organs, (DeLancey, 1999). During LA contraction, the distal vagina is under strain, which explains the dense fibers around the region to withstand the tension (DeLaney, 1999). The LA muscles contract simultaneously with the diaphragm and abdominal wall muscles to build intra-abdominal pressure. This LA contraction helps to tense the suburethral fascial layer, thereby compressing the urethra against the supporting endopelvic fascia in order to close it during increases in abdominal pressure to prevent urine leakage (DeLancy, 1994). The PFMs automatically contract before or during increases in intra-abdominal pressure (Constantinou and Govan, 1981) presumably to reinforce pelvic organ support. When the PFMs contract, they shorten anteriorly and cranially to close the levator hiatus. Their contraction creates tension in the endopelvic fascia (Barbic et al, 2003),

which lifts the pelvic organs (DeLancey, 1988) and enhances closure of the urethra (Rud, 1980).

Histochemical analysis has shown that the LA muscles have a larger proportion of type I muscle fibers than type II (Gosling et al., 1981). It is estimated that the LA muscles consist of approximately 66-90%, of type I (slow twitch) muscle fibers (Morris et al, 2012) and about 30% or less type II (fast twitch) muscle fibers (Gilpin et al, 1989). Type I muscle fibers are high in oxidative enzyme and acid-stable myosin ATP-ase and are low in phosphorylase and alkaline-stable myosin ATP-ase, meaning that they are relatively resistant to fatigue (Herbison et al., 1982), whereas type II muscle fibers contain relatively low concentrations of acid-stable myosin ATP-ase, but high levels of alkaline-stable myosin ATP-ase, meaning that they can perform rapid contractions and fatigue quickly (Herbison et al., 1982; Brooke & Kaiser, 1970). The fiber type composition of the PFMs suggest that they should have relatively high fatigue resistance, (Brooke & Kaiser, 1970), which is presumably tied to their role in providing tonic support to the pelvic organs through sustained activation (Gosling et al, 1981; DeLancey & Starr, 1990). The type II fibers of the LA muscles are also important, however, as they are needed to assist the external urethral sphincter in generating high urethral closure force to prevent urine leakage during tasks that produce high intra-abdominal pressure such as coughing and sneezing (Gosling et al, 1981). Consistent with this functional role, the type II (fast) fibers within the PFMs are clustered around the urethral and anal sphincters (Laurberg & Swash, 1989). In addition to the maintenance of continence, the action of the PFMs prevents the endopelvic ligaments and fascia from being over-stretched and damaged through high tensile forces imposed by coughing, sneezing etc. (DeLancey, 1994).

Importance of PFMs in Continence Mechanism

The LA muscles are responsible for closing the urogenital hiatus thus assisting with urinary and fecal continence, and, by total relaxation, allowing defecation and urination to occur. As long as the LA muscles maintain the proper closure of the genital hiatus, the ligaments and fasciae that provide support for the pelvic organs are under minimal tension. Ghafar and colleagues have found that both prolapse and SUI are associated with decreased LA contraction strength (Ghafar et al, 2013) and wider urogenital hiatus. As mentioned previously, the urethral support system consists of connections between the LA muscles and endopelvic fascia (Ashton-Miller et al., 2001). Damage to the connection between LA muscles and the connective tissue or damage to the LA muscles themselves can cause a loss of urethral support and urethral hypermobility (Peschers et al., 1996), which is thought to be associated with the development of SUI (Smith et al., 1989). According to Abrams et al., (1988), that urethral closure pressure must exceed intravesical (or bladder) pressure to prevent urine leakage (Abrams et al., 1988). Since striated muscle fibers in the urethra has been shown to be responsible for one-third of the urethral closure pressure, damage to these muscles are associated with decreased urethral closure pressure, which has been shown to be associated with the development of SUI (Rud et al., 1980).

The rise in intra-abdominal pressure (IAP) during coughing has been shown to cause downward displacement of the urethra and vesical neck that is visible in ultrasound images (Howard et al., 2000). Howard et al (2000) showed that there was less vesical neck mobility when the PFMs are contracted during coughing, than during Valsalva maneuver in continent nulliparous and primiparous women compared to primiparous women with SUI. The

researchers speculated that in continent women, the stretch-resistance properties of the PFMs and connective tissues stabilize the urethra and prevent the downward displacement of the urethra and vesical neck during increase in IAP. Stretch-resistance is measured as stiffness, and is defined as the extent to which a structure can resist deformation (Baugmart, 2000). Several studies have shown that, compared to incontinent women, continent women generate stronger PFM contraction (Hoyte et al., 2001; Braekken et al., 2010; Chamochumbi et al., 2012) and possibly greater PFM passive stiffness (Howard et al., 2000). Hoyte et al (2000) suggested that since continent nulliparous and primiparous women displayed less vesical neck mobility compared to incontinent primiparous women during coughing as observed in ultrasound imaging, continent women must have higher PFM stiffness than incontinent women. However, their claim cannot be supported as PFM passive stiffness was not measured using direct force measurements. It is speculated that in continent women, stiffness of the PFMs may prevent the hypermobility of the bladder neck and compresses the urethra against the supporting endopelvic fascia to close it during increases in IAP. Hence, the PFMs and their surrounding connective tissues are crucial in maintaining continence in women as damage to the endopelvic fascia may change the alignment of the PFMs and the pelvic organs, as well as cause the displacement of the urethra from the anterior vaginal wall, which can contribute to decreased pressure transmission from the bladder neck to the external urethral meatus (Hilton & Stanton, 1983) and delay PFM contraction during increase in IAP, thus causing accidental urine leakage in women (DeLancey & Ashton-Millier, 2004).

Further evidence of the importance of the PFMs in maintaining continence is shown by researchers that studied the effect of PFM strength training on SUI. Strengthening the PFMs

can effectively reduce symptoms of SUI (Berghmans et al, 1998); even in older women (Dumoulin et al, 2011). It is important to understand how age-related changes in the PFMs contribute to the development of SUI in women, as well as to develop optimal PFM training programs to target the specific age-related changes seen in women in order to prevent or optimally manage SUI in an aging population.

Age-related changes are evident in striated muscles (Thelen el al., 1996). Since muscle density has been shown to decrease with age (McNeil et al., 2005), loss of striated urethral muscle density may explain lower urethral closure pressure in older women (DeLancey et al., 2008). Rud and collages reported significantly lower resting urethral closure pressure in older women compared to younger women (Rud et al., 1980). According to data recorded through striated urogenital sphincter muscle biopsies from 34 female cadavers aged 15 to 80, the muscle mass and fiber density of the striated urethral sphincter appears to decrease with age (Perucchini et al., 2002). In addition to age-related loss of muscle fiber and muscle mass, trauma incurred in the PFMs during vaginal delivery can also contribute to the decline of muscle mass. Vaginal delivery causes the LA muscles to undergo mechanical strain, and, when forces are extreme, it can cause levator avulsion. (DeLancey, 1994); (Dietz & Lanzarone, 2005). Interestingly, the prevalence of levator avulsion from the public symphysis is higher in older vs younger mothers, suggesting that the tissues may be stiffer on older compared to younger women (Dietz & Simpson, 2007).

Assessment of Pelvic Floor Muscle Function

Three key ways to evaluate PFM function are to study neuromuscular activation using

electromyography (EMG) (Keshwani & McLean, 2012), force output using dynamometry (Dumoulin et al., 2004; Madill et al., 2013) and dynamic changes in tissue morphology using ultrasound (USI) (McLean et al, 2013) or magnetic resonance imaging (MRI) (Constantinou et al., 2002). Each form of measurement has strengths and drawbacks, as discussed below.

The PFMs can be difficult structures to study using EMG as they are buried deep within the pelvis and therefore traditional adhesive surface electrodes are not useful (Keshwani and McLean, 2015). Hence, specialized electrodes mounted on intravaginal probes are required to record EMG data from the PFMs. Although between-trial reliability using surface EMG has been shown to be acceptable, between-day/test-retest reliability was generally poor when evaluating the peak EMG amplitudes generated during maximum voluntary contractions (MVCs) (Auchincloss & McLean, 2009). These authors noted that EMG should not be used for between subject comparison when measuring PFM functions. The reliability and validity of EMG signal quality is affected by probe geometry, electrode size, electrode configuraiton and measurement site (Keshwani & McLean., 2015); (Voorham-van der Zalm et al., 2006). Many EMG electrode configurations do not produce repeatable data and are likely contaminated by crosstalk and motion artifact (Keshwani and McLean, 2013). The findings of Peschers and colleagues support this claim, suggesting that when PFMs are contracted in a supine position, EMG is unable to distinguish between a PFM contraction and contractions from muscles in proximity to the PFMs, thereby producing crosstalk during recordings (Peschers et al., 2001). Since intra-abdominal pressure has been shown to affect intra-vaginal pressure (Bø & Sherburn., 2005), it cannot be assumed that EMG amplitude is an indication of PFM force (Keshwani & McLean., 2013), especially since the relationship between muscle force and EMG

amplitude is non-linear (Lawrence et al., 1983). In addition, EMG is sensitive to movement, any movement in the PFMs relative to the electrode surface can cause noise on the EMG recordings that can be erroneously interpreted as EMG signal. (Gentilcore-Saulnier et al., 2016). Despite these limitations, PFM EMG activation amplitude may be lower in women with SUI than in continent women during voluntary contractions (Aukee et al., 2003), however this finding was not corroborated by other researchers (Madill and McLean 2010; Smith et al, 2007). Madill & McLean (2010) showed that women with mild SUI actually generate higher EMG activation amplitudes during coughing at supine position than continent women whereas women with moderate and severe SUI generate lower PFM EMG activation. These results may suggest that in women with mild SUI, the PFMs may serve as compensatory mechanisms to counterbalance the deficits in urethral closure (Madill and McLean, 2010). The age-related effects on PFM activation amplitude are difficult to study using EMG because of the large inter-individual and between day variability (Auchincloss and McLean, 2009) and therefore remain unknown.

Ultrasonography (USI) and magnetic resonance imaging (MRI) can be used to visualize dynamic changes during PFM contraction through motion of the anorectal angle, urethra and bladder neck. Dynamic changes in the PFM morphology (Dietz et al) and changes in muscle volume/thickness (Morkved et al., 2004) can be studied using real-time 3D transpareneal ultrasound. This imaging technique has been used to show that the levator hiatus becomes smaller and larger when the puborectalis muscles are contracted and relaxed, respectively (Weinstein et al., 2007). Morkved et al, (2004) used transperineal USI to study the relationship between PFM thickness and vaginal squeeze pressure measured using a vaginal balloon catheter. Their results showed moderate inter-rater reliability (ICC 0.71) and high intra-rater

reliability (ICC 0.98) for ultrasound measurements and that there was a moderate correlation (r=0.703) between vaginal squeeze pressure and PFM thickness in continent and incontinent nulliparous pregnant women (Morkved et al., 2004). Yet it is unclear whether differences in PFM thickness reflect differences in PFM strength or force generation capacity. Transperineal USI has been used to study PFM contractile function through measures of levator plate elevation and bladder neck displacement (Braekken et al., 2009). Using 4D transperineal USI, Morin et al. (2014) compared the bladder neck displacement, levator plate angle and levator hiatus diameter at rest with maximal PFM contraction in a supine position between nulliparous asymptomatic women and nulliparous women with provoked vestibulodynia (PVD). They found that women with PVD had narrower levator hiatus, less bladder neck displacement and lower levator plate angle during maximal contraction compared to asymptomatic control women. They also found that, compared to their results at rest, women with PVD had lower levator plate angle and levator hiatus diameter during maximal contraction, suggesting that women with PVD have lower PFM contractile force than asymptomatic women. However, displacement of pelvic floor landmarks is not a direct measure of force and may not reflect fast or strong PFM contraction, it is rather the result of muscle action and thus these conclusions may not be valid.

It has been shown that strengthening the PFMs in women with SUI resulted in elevation of the levator plate, as seen by MRI analysis, concurrently with muscle hypertrophy and increased stiffness of the PFMs and surrounding connective tissue (Bø, 2004; Hoyte et al., 2001). Using MRI, Tunn et al 2006 observed significant fascial defects in women with SUI, while they did not find any significant relationship between fascial defects and aging. Using MRI in a small sample of women with SUI (n=5), Dumoulin et al., 2007 found that the urogenital hiatus

was significantly smaller following PFM training and suggested that these changes reflected PFM hypertrophy and increase in PFM stiffness (Dumoulin et al., 2007) as good LA stiffness is required for urethral stability during coughing, sneezing etc. (Balmforth et al., 2004). Again, however, changes in morphology do not necessarily reflect changes in PFM force-generating capacity.

When studying the maximal voluntary contraction and stiffness properties of the PFMs, the biggest limitation of using imaging techniques such as MRI and USI is that they are not useful for assessing the mechanical properties of the tissue, as the data cannot be used to quantify the forces generated by the PFMs. The sampling rates for MRI are too slow to visualize the movement of tissues in fast maneuvers like coughing (Peng et al., 2007). The motion in USI show the anatomical displacement of the pelvic structures. However, movement of the organs does not reflect PFM activation and it does not provide useful information about the force dynamics or what caused the change in movement.

Dynamometry used to assess PFM Force Outcomes

Direct PFM strength and resistance to passive stretch measurements are essential to understand the biomechanical properties of the PFMs. Although intravaginal palpation remains the standard in clinical practice (Laycock, 1992), dynamometry has recently become popular as an objective research tool to measure the isometric strength and resistance to passive stretch of the PFMs. It provides objective measurement of PFM strength and it has been found to be reliable, due to its high coefficient of dependability for maximal PFM strength measurements (ICC = 0.88) (Dumoulin et al., 2004); maximal rate of force development (ICC = 0.86); as well as

to possess good test–retest reliability for speed of contraction (ICC = 0.79) and endurance parameters (ICC = 0.81) (Morin et al., 2007). Using an intra-vaginal dynamometer, Verelst et al., (2004) demonstrated good test-retest reliability of PFM active and passive force measured in healthy women when the arms of the dynamometer produced a total anteroposterior diameter of 30 to 40mm (Verelst et al., 2004). A recent study by Ashton-Miller demonstrated that their vaginal dynamometer measured maximum vaginal closure force without evidence of crosstalk from intra-abdominal pressure, while retaining good between-day repeatability (Day 1: 3.41 ± 1.8N; Day 2: 3.42 ± 1.6N) (Ashton-Miller et al., 2014). Like the force generation capacity, quantifying the stiffness properties of the PFMs is a useful measure to understand the state of the muscles. The elastometer developed by Kruger et al., 2011 has also been shown to have high test-retest reliability to measure PFM stiffness (ICC=0.92; ICC=0.86) (Kruger et al., 2011).

The Montreal dynamometer is designed with two metal arms, with a fixed upper arm and movable lower arm that has two pairs of strain gauges glued on each side of the moveable lower arm. The strain gauges are mounted in a Wheatstone bridge configuration, and the device is theoretically designed to ensure force measurement is independent of the location where force is applied on the lower arm. This is an important feature given that the dynamometer may move inside the vagina during a given task and given that the positioning of the dynamometer may differ from one participant to the next. (Dumoulin et al, 2003). The Montreal dynamometer has good reliability with repeated measurements (Morin et al., 2004) and has been used to evaluate the following PFM properties between continent and incontinent women:

1. Rate of force development (N/s): At 19mm dynamometer opening, the participants were instructed to contract their PFMs maximally and relax as fast as possible within a 15-sec duration. The slope of the first contraction was calculated to find the rate of force development. The incontinent group had lower rate of force development than the continent group.
2. Peak resistance to passive stretch (N): The women were instructed to keep their PFMs relaxed for 15 seconds. The incontinent group had lower passive force than the continent group at two different apertures, 19 and 24 mm.
3. Maximal voluntary contraction strength (N): The highest force generated throughout the three trials was used for analysis and baseline force was subtracted. Results indicated that there were no statistically significant differences in force generation capacity between the continent and incontinent women at two apertures, 19 and 24mm.
4. Endurance (N): The participants were instructed to sustain a maximal PFM contraction for 90 seconds. The area under the force curve taken between 10 and 60 seconds was calculated to find endurance, which showed that the incontinent group had lower endurance values than the continent group.

Total force, or the peak force generated during maximal voluntary contraction measured by the dynamometer is the sum of active and passive force from the PFMs and the connective tissue (Verelst et al., 2004; Morin et al., 2004). The active force is obtained by subtracting the passive force generated upon insertion of the device into the vagina from the total force (Verelst et al., 2004). Verelst and colleagues studied the relationship between change in

dynamometer diameter and force development in the PFMs of healthy continent women. They measured the PFM active force and passive stiffness between 30mm to 50mm aperture, increasing the aperture by increments of 5mm to prevent drastic stretching of the muscles. Their results showed that there was a significant increase in force generation capacity and stiffness as the dynamometer aperture was increased (Verelst et al., 2004). The researchers also compared the active and passive properties of the PFMs between continent and incontinent women. They found that although there were no significant differences in passive stiffness between the groups, stiffness did increase as the dynamometer aperture was increased in both groups. The continent group generated higher force than the incontinent group between 30mm to 50mm aperture and the force generated at 50mm was significantly higher than the force generated at 30mm in both groups (Verelst et al., 2007). The researchers found that when the diameter of the probe was changed beyond 40mm, the participants experienced discomfort and pain during PFM contractions (Verelst et al., 2004). Verelst and colleagues suggested that the reliability of their force data increases as the device diameter increases, shown by decreased coefficient of variance (CV) values at higher diameters; they concluded that to detect significant changes in force generation capacity of the PFMs, participants should be tested at diameters ranging from 30mm to 40mm. However, their results do not provide conclusive evidence as CV is not a good measure of reliability.

Like the force generation capacity, quantifying the stiffness properties of the PFMs is a useful measure to understand the state of the muscles. Passive properties of the PFMs are poorly understood and should be assessed with a direct measurement device such as dynamometer or elastometer to detect significant changes in passive force (Gajdosik, 2001).

According to Verelst et al (2007), PFM stiffness, or change in force (DF) divided by change in dynamometer diameter (Dd), expressed as N/mm (Verelst et al., 2007), may be an important factor in maintaining continence. Kruger and colleagues used an elastometer to study the stiffness properties of the PFMs (Kruger et al., 2008), which has been shown to have high test-retest reliability to measure in vivo PFM stiffness in continent women (ICC=0.92; ICC=0.86) (Kruger et al., 2011). They found that at 40mm aperture, there was a significant difference between their groups in terms of PFM stiffness and observed higher PFM stiffness in nulliparous women that had competed in high impact sport over years, compared to nulliparous control participants (Kruger et al., 2008). They hypothesized that muscle hypertrophy contributes to increased muscle stiffness, as well as their capability to generate higher force than their aged matched counterparts when measured at 40mm diameter (Kruger et al., 2008).

Other tools to assess PFM strength include digital assessment, air or water filled catheters and vaginal cones.

Digital assessment yields only moderate inter-rater reliability (Spearman's rho=0.70) (Bo & Finckenhagen, 2001). In clinical settings, several different rating scales are used, among which includes the Brink scale (Brink et al., 1989) and the Modified Oxford scale (Laycock and Jerwood, 2001) as the two most common approaches. These scales are subjective and discrete, quantifying the strength of the PFMs using, in the case of the Modified Oxford Scale, - grades ranging from 0 (no palpable contraction) to 5 (strong contraction). Although digital palpation is practical for teaching proper PFM contractions, these digital measures have limited reliability as the grading system is subjective and is dependent on the physiotherapist performing the

assessments (Ashton-Miller et al, 2014). A recent study by Brazález (2017) suggests that vaginal palpation may be more useful to study PFM morphology and motor control, instead of assessing PFM strength (Brazález et al., 2017).

Vaginal balloon catheters are also used to assess PFM strength. The balloon senses changes in pressure induced by PFM contraction. However, intravaginal balloon catheters are susceptible to increases in intraabdominal pressure and may therefore not accurately reflect forces generated through PFM contraction (Ashton-Miller et al., 2014).

Weighted vaginal cones are another option. Cones of known weights are placed into the vagina above the levator plate, and women are asked to contract their PFMs to lift the cone. Through observation of the perineum or loss of the cone from the vagina, PFM strength can be quantified (Plevnik, 1985). However, weighted cones have also been shown to be unreliable for PFM strength assessment because a heavy cone can still be held in place in the vagina despite a weak PFM contraction (Hahn et al., 1996).

In the practice of obstetrics, urogynecology and physiotherapy, measurements of PFMs maximal voluntary force is considered an important factor to assess when treating SUI in women (Bo et al., 2001) and it has been suggested that other factors such as speed of contraction, stiffness and endurance should be considered as well (Morin et al., 2004); (Morin et al., 2008). It is important to use valid and reliable tools and techniques to evaluate differences in PFM mechanics between older and younger women. Based on the literature reviewed, the biomechanics of the PFMs are best studied using dynamometric force measurements, as this technique directly measures force developed by the LA muscles, is presumably not influenced by increase in IAP and has demonstrated strong reliability metrics.

Age related Changes seen in Human Skeletal Muscles

Although little is known about the impact of increasing age on the PFMs and connective tissues, there is evidence that age-related changes exist in other human skeletal muscles.

The resting length of a skeletal muscle is the length at which it has the maximal ability to generate force when stimulated. The effect of resting fiber length on muscular contraction is referred to as the length-tension relationship (McCully & Faulkner, 1983). Based on this phenomenon, lengthening of skeletal muscles from a maximally shortened position will result in an increase in force generation capacity until the muscle reaches its optimal length, beyond which, there is less overlap between actin and myosin and thus force generating capacity declines (McCully & Faulkner, 1983). The capacity to generate maximal force declines with age and becomes more evident after the age of 65 (Jubrias et al., 1997).

Age-related muscular atrophy, also referred to as sarcopenia, is characterized by the preferential loss (Vergijk et al., 2007) or atrophy (Nilwik et al., 2013) of type II fibers and sparing of type I fibers (Verdijk et al, 2007). The loss in strength is primarily attributed to muscle atrophy due to a decrease in muscle fiber CSA and total number of muscle fibers associated with aging (Brooks et al., 1994). Aging causes the CSA of many striated muscles to decrease by 25–40% from their peak between the ages of 20 and 60 years (Close et al., 2005). Muscle biopsy results from quadriceps muscles of men over the age of 60 showed that type II muscle fiber CSA was significantly smaller than type I muscle fiber CSA. Muscle mass and strength have been shown to decrease with increasing age (Stav et al, 2007; Nilwik et al, 2013), which is consistent

with evidence of loss of individual muscle fibers (McNeil et al., 2005) and preferential denervation of the type II motor units (Verdijk et al, 2007). Biopsy results from the quadriceps of healthy male participants showed that younger men in their twenties had 39% type I muscle fibers, while men over the age of 60 had 66% type I muscle fibers (Larsson & Karlson, 1978). Regression analysis showed that type II muscle fiber content and size were the main predictors for lower muscle strength in older men (Verdijk et al., 2010). With aging, there is also some indication of motor unit remodeling, whereby type II muscle fibers are progressively denervated (Faulkner et al., 2007). The orphaned motor units are reinnervated by the neurons of adjacent type I motor units, essentially converting the muscle fibers to type I, and resulting in "fiber-type-grouping" (Lexell et al, 1986). Changes in connective tissue composition are also evident with advanced aging. During motor unit remodeling, the denervated type II muscle fibers are also replaced with connective tissues (Lexell et al., 1983). Compared to younger adults, older individuals have significantly more fat and connective tissue within their muscle bellies (Kent-Braun et al., 2000). Evidence of motor unit remodeling has been demonstrated in a study by Roos and colleagues, where they observed larger motor unit (MU) potentials during EMG recordings of older adults (Roos et al., 1997), suggesting that the motor unit has been innervated by type I muscle fibers and likely produces greater MU potential due to the increased number of type I muscle fibers. Electrophysiological testing has also shown markedly lower number of excitable motor units in the biceps brachii and brachialis muscles of males and females over the age of 70 (Doherty et al., 1993). Functional evidence of fiber-type-grouping was demonstrated by Hepple & Rice. (2015), who found that compared to older adults (60 and over), younger adults (20-30) performed better in dynamic knee extension tasks where high

force output was required; whereas older adults performed better in endurance-type tasks such as sustained holds or intermittent contractions (Hepple & Rice, 2015). Results from Hepple & Rice indicates that, due to loss of type II muscle fibers, larger motor units cannot be recruited to perform powerful contractions, which results in lower force generation in older adults than younger adults; whereas the older adults tend to perform better in endurance-type exercises due to an increase in type I muscle fibers, which contribute to tonic muscle action. During isokinetic knee extension performance test, 65 to 80-year-old participants generated significantly less force than younger and middle-aged (aged 20 and over) participants (Jubrias et al., 1997). Concentric (muscles are shortened) muscle force has been shown to be affected by age-related changes. Gajdosik and colleagues compared three groups of women, young (20-39 years), middle aged (40-59 years) and older (60-84 years) during concentric exercises. They showed that the older group had lower active and passive force generation capacity in their calf muscles compared to younger and middle-aged women (Gajdosik et al., 1999). In comparison to concentric muscle force, eccentric muscle force (muscles are lengthened) appears to be less affected by aging (Porter et al., 1997) and muscles in older individuals have been shown to have increased force generating capacity, increased stiffness and reduction in maximum contraction velocity while the participants perform muscle lengthening contractions (Thelen, 2003). These results indicate that the mechanical efficacy of a muscle contraction is affected by age-related fiber-type distribution of the muscle group, as well as the type of contraction being performed.

Like force generation, rate of force development has also been shown to be slower in older adults during concentric exercises (Porter et al., 1997); (Thelen et al., 1996). Rate of force development is the ability to rapidly develop muscular force during muscle contraction

(Aagaard et al., 2002). Larsson et al (1979) showed a decrease in maximum knee extension velocity in participants over the age of 65 compared to younger participants (Larsson et al., 1979). The reduction in maximum contraction velocity is likely due to preferential loss of fast twitch motor units, which results in slower rate of force development in older individuals (Doherty et al., 1993).

Muscles produce both active force and passive resistance in response to stretching. Reflex muscle contraction contributes to active force, while structural support from the skeletal muscles and surrounding connective tissue contribute to passive resistance to stretching (Magid & Law, 1985). As a muscle is passively lengthened, the muscle provides resistance to the passive stretch, eventually it reaches a point where the muscle is at its maximal length; stretching the muscle beyond this point causes damage to the muscles. Resistance to passive stretch, when a muscle is relaxed, is related to stretching stable cross-links between the actin and myosin filaments and deformation of the connective tissue both within and surrounding the muscles (Hill, 1968); (Garett et al., 1988). Quantitative and qualitative changes in the connective tissue associated with aging are thought to influence resistance to passive stretch/stiffness properties in skeletal muscles (James & Parker., 1989). Age-related changes in the properties and composition of connective tissue, particularly collagen, is associated with increased stiffness in the lower limb joint muscles (Hall, 1976). While there are almost no studies regarding intramuscular collagen changes with aging in humans, there is evidence of increase in collagen content in aging animal tissues (Alnaqeeb & Goldspink, 1987). A study by Haus and colleagues showed that there is no difference in collagen concentration in the skeletal muscles between younger and older participants using molecular biology techniques (Haus et

al., 2007). As the aging tissue are less elastic, muscles and embedded connective tissue is less able to stretch and can rupture quicker (Enoka, 1994). The increase in muscle stiffness in aging tissues is thought to be an attribute of increased fibrous connective tissues in the muscle bellies (Timiras, 1994) Stiffness in the skeletal muscles can contribute to force generation capacity, due to the slowing of actin-myosin cross-bridging cycling such as the actin-myosin cross-bridge detachment rate in aging tissue (Larsson et al., 1997). However, it is difficult to evaluate how stiffness affects the contractile forces of the skeletal muscles since stiffness can only be measured when the tissue is relaxed.

Age-related changes in the skeletal muscles can be reversed through strength and flexibility training. Resistance training exercises can improve dynamic strength in older adults. McCartney et al. (1996) compared two groups of older adults (resistance training group vs no training group) aged 60 to 80 years, and found that the group that participated in resistance exercise program for two years showed moderate muscle hypertrophy and gradual increase in muscle strength following the training program (McCartney et al., 1996). Muscle stiffness has been shown to increase after resistance and general strength training in healthy men and women (Whitehead et al., 2001). A possible explanation could be due to increase in collagen amount and its degree of cross-linking, as well as increase in muscle mass, which provides greater structural support and resistance to passive stretch (Folland & Williams, 2007).

Age-related changes due to fiber-type-grouping may affect the female PFMs, whereby with increase in age, women may generate lower PFM force than younger women due to the loss of type II muscle fibers and have higher PFM stiffness than younger women due to changes in connective tissue properties.

Age related changes in the PFMs in Women

There are very few studies that have investigated the impact of age on PFM mechanical properties and there are no studies that examined age-related changes in the PFMs using a longitudinal approach. Only four studies have reported evidence of age-related declines in urethral (Trowbridge et al, 2007; DeLancey et al, 2008; Clobes et al, 2008) or vaginal (Madill & McLean, 2010) closure pressure.

According to DeLancy et al (2008), maximal urethral closure pressure is the factor that most strongly predicts SUI. Some researchers have documented a reduction of maximal urethral closure pressure with age (Pandit et al., 2000; Trowbridge et al., 2007; DeLancey et al, 2008; Valentini et al., 2011); which may be associated with a reduction in the density of urethral circular smooth muscle (Clobes et al 2008) and also with changes in PFM structure and function (Rud, 1980). Perucchini et al. (2002) compared the urethra and surrounding tissue from 25 female cadavers between 15-80 years old and found that the CSA of striated muscle in the ventral wall was significantly smaller in older women than younger women, but the mean muscle fiber diameter was not significantly affected by age (Perucchini et al., 2002). The researchers also observed significantly smaller urethral sphincter muscle fiber CSA in parous women between the ages of 60 to 70 years (Peruccini et. al., 2002).

Age-related changes in urethral striated muscle fiber CSA have also been shown to be present in nulliparous women and therefore are unlikely to be related to muscle or nerve injury associated with childbirth. In a single case, Perucchini and colleagues found that urethral striated muscle fiber count was similar between an older 78-year old nulliparous woman and a

23-year-old nulliparous woman, suggesting that loss of muscle fibers may be associated with parity and not age (Perucchini et al., 2002). This result has, however, yet to be confirmed through a larger study. The striated sphincters have been shown to be comprised of both type I (slow twitch) and type II (fast twitch) muscle fibers (Light et al., 1997). The significant decrease in type II muscle fiber CSA could be a contributing factor to the decrease in urethral closure pressure with age. This hypothesis is supported by Trowbridge et al's (2007) results that found that the maximal urethral closure pressure was 40% less even in nulliparous women between the ages of 60 to 70 years compared to nulliparous women between the ages of 20 to 30 years (Trowbridge et al., 2007). 54% of the loss in closure pressure has been shown to be due to age-related changes (Rud et al., 1980), with an average reported rate of loss of 2% per year (Pandit et al., 2000). The results from Rud et al. (1980), Trowbridge et al. (2007), and Pandit et al. (2000) suggest that the loss of urethral muscle fibers may be related to decreased urethral muscle strength and increased prevalence of SUI in older women (Rud et al., 1980); (Pandit et al., 2000).

Even though age-related changes are evident in the urethral muscles, no studies to date have found evidence of age-related changes in the LA muscles. LA force, measured by vaginal closure force during resting and maximal PFM contraction using an instrumented vaginal speculum showed no difference between older nulliparous and younger nulliparous women (Trowbridge et al., 2007). However, with a sample size of only eleven older nulliparous women, this study may have been underpowered. A recent study using MRI showed that young and older women demonstrated no difference in the CSA of their LA muscles. The same study showed that the nearby obturator internus muscles, which are predominantly composed of

type II muscle fibers, did show evidence of atrophy in the same sample of older women, and thus it was concluded that the LA do not demonstrate age-related muscular atrophy (Morris et al, 2012). However, findings to date do not conclusively demonstrate that the LA are unaffected by sarcopenia. Using ultrasound imaging, researchers have found either negative or weak correlations between age and changes in LA muscle morphology during maximal contraction, strain and Valsalva maneuver ($r = -0.25$, $p < 0.01$) (Weemhoff et al., 2010), ($r = 0.179$, $p = 0.113$) (Quiroz et al., 2013). Constantinou et al (2002) found less bladder neck elevation during PFM contraction in older compared to younger women using MRI, however, whether these differences are due to changes in connective tissue properties or muscle strength/power is unclear. Using EMG, Gunnarsson et al. (1999) reported that incontinent women over the age of 50 had less ability to activate their PFM muscles compared to age-matched continent women. The authors suggested that, in incontinent women, the changes could be due to neuromuscular damage to the PFMs due to delivery, as well as vaginal hormonal changes such as changes in collagen properties associated with aging (Gunnarsson & Mattiasson, 1999).

The effect of aging on the stiffness and connective tissue properties in women is still unknown. Tuttle et al. (2014) measured the amount of connective tissue in the PFMs of older female cadavers (aged 85 and above) and found that the PFMs contained 9-11% collagen, which is significantly higher than the amount of connective tissue found in other skeletal muscles in the body (Tuttle et al., 2014). According to the authors, these connective tissues contribute to passive tension in the PFMs. At relatively short sarcomere length, stiffness in the PFMs contribute to higher muscle force generation than active properties alone (Tuttle et al., 2014). This study provides evidence that stiffness in PFMs contributes to the force generation

capacity; however, a limitation of this study is that the researchers only assessed the PFMs of older female cadavers, and, it is unknown whether the amount of connective tissue is also higher in the PFMs of younger female cadavers.

Biochemical analysis of vaginal tissue between premenopausal and postmenopausal women reveals that lower vaginal estrogen is associated with higher tissue stiffness (Goh et al., 2002), with the greatest tissue stiffness observed in postmenopausal women with pelvic organ prolapse (Lei et al., 2007). However, differences in tissue stiffness may not be due to reduction in collagen content due to aging. Cervical tissue analysis showed that collagen content, which is estrogen dependent, was lower in women with prolapse and SUI (Trutnovsky et al., 2014) regardless of age and menopausal status (Wong et al., 2003). Large scale longitudinal studies such as Study of Women's Health Across the Nation (Weitje et al., 2011) and the British prospective cohort study (Mishra et al., 2010) found no association between estrogen deficiency after menopause and the development of urinary incontinence (Trutnovsky et al., 2014). It has instead been suggested that abnormalities in the connective tissue may predispose women to prolapse and incontinence. With advanced aging, denervation, devascularization as well as collagen degradation of the connective tissue may lead to changes in the passive and active properties of the PFMS such as increased stiffness and decreased force generation capacity (Yong et al., 2003).

Comparing the contractile strength of women from two different studies that used the same dynamometer and methods, a sample of 17 women aged 60 and over, demonstrated maximal PFM contractile strength of 4.12N ± 3.48 (Madill et al., 2013), whereas a sample 30

women aged 21-44 demonstrated maximal PFM contractile strength of 5.9N ± 2.8 (Morin et al., 2004). Further, using a perineometer, Quartly et al (2010) showed that increase in age was positively correlated with greater PFM endurance. Taken together, these studies suggest that age-related changes in the PFMs may exist, and may contribute to declines in continence function with increasing age. Age-related changes may be better understood when studied using direct measures of muscle function in-vivo through intravaginal dynamometry.

Age related changes in the PFMs, like other skeletal muscles, can be reversed using resistance training. In a recent study, Madill et al. (2013) assessed PFM biomechanics using a dynamometer in women with SUI over the age of 60 and showed that pelvic floor muscle training (PFMT) can reverse age related changes in the PFMs. Following a 12-week PFMT program, although the researchers did not find differences in force generating capacity between older women in the training group compared to the non-training group, they found that the training group demonstrated improvement in the number of rapid-repeated contractions the women could perform in a 10s period: 8.2 ±2.7 in the training group vs 5.6±2.4 in the non-training group, suggesting that the training group could generate faster contractions and, possibly, more rapid relaxation (Madill et al., 2013). Their results indicated that training the PFMs can improve SUI symptoms even among older women. PFMT can also improve pelvic organ support in older women as MRI analysis showed that the levator plate angle was much smaller and the anorectal angle was much narrower in older women following the training (Madill et al., 2013). Some women in the training group reported discomfort when the dynamometer was inserted intravaginally (Madill et al., 2013) suggesting that PFMT may cause elevation of the levator plate and stiffness in the vaginal connective tissues, which would

contribute to better pelvic organ support (Bo, 2004; Dumoulin & Hay-Smith, 2010). Madill and McLean (2010) used air-filled catheters embedded within fixed regions within a solid intravaginal probe to measure how increase in age affects intravaginal pressure generated during MVC and coughing in continent women, women with mild SUI and women with severe SUI. Their results did not show significant age-related declines in anterior and posterior vaginal wall pressures generated through maximum voluntary contractions (Madill and McLean, 2010).

The PFMs are thought to be important to continence function and pelvic organ support. Although age related changes have been observed in other skeletal muscles, very little is known about the age-related changes that are seen in the PFMs of women. Such knowledge would help us to understand the mechanisms underlying SUI and POP, particularly among aging women and may be useful in the development of muscle rehabilitation programs to prevent or improve symptoms of SUI, POP, or other pelvic floor disorders among aging women.

Research Question

Our goal was to investigate the natural changes in PFMs mechanics that occur with age. Specifically, we aimed to answer the following questions:

1. Is the capacity to generate maximum force during a PFM contraction lower in older women?
2. Is PFM active force lower in older women?
3. Is rate of force development during maximal voluntary PFM contraction lower in older women?
4. Is resistance to passive stretch of the PFMs higher in older women?

5. Is the stiffness of the PFMs and paravaginal tissues higher in older women?

6. Is PFM endurance higher in older women?

METHODS

This study received approval from the University of Ottawa Health Sciences and Science Research Ethics Board (see Appendix A).

Participants and Recruitment

Nulliparous women between the ages of 18 and 64 years old who had basic understanding of English were recruited for the study. Women who were smokers at the time of recruitment or who had previously smoked 20 or more cigarettes a day (Hannestad et al., 2007), with obesity, with BMI of 30.0kg/m^2 or higher, with self-reported diabetes mellitus (Hannestad et al., 2007), who had a history of urogynacological surgery (e.g. colposuspension, TVT, TVT-O, hysterectomy), pelvic irradiation, and/or major trauma that affected their mobility were excluded as these conditions may affect a woman's ability to contract her PFMs. Participants were recruited through advertisements posted on social media and printed flyers posted on bulletin boards at the University of Ottawa and within local community centres, hospitals, clinics, nursing homes and residential buildings with permission (see Appendix B). Additionally, advertisements to recruit older participants were posted in senior's centres, distributed through senior aqua-fit classes and emailed to senior organizations and senior female faculty and staff members at the University of Ottawa (see Appendix C). Interested potential participants were screened via telephone interview and/or email correspondence (see Appendix D), and eligible women were scheduled to attend two sessions in the Motor Function Measurement Laboratory at the University of Ottawa. The participants completed three on-line modules of the International Consultation on Incontinence Questionnaire, the Female Lower

Urinary Tract Symptoms (ICIQ-FLUTS), the vaginal Symptoms (ICIQ-VS) and the Bowel symptoms (ICIQ-B) modules (Abrams et al., 2006) before their familiarization session. At their first visit, the participants first signed the study Information and Consent Form (ICF; see Appendix E) and were familiarized with the environment, equipment and study procedures. The dynamometry data were collected in a subsequent session scheduled approximately one week after the first session.

Equipment

Dynamometry: A computer-operated intravaginal dynamometer was used to measure active and passive forces generated by the PFMs (Figure 1.2). This custom-built dynamometer is based on published designs (Dumoulin, et al 2004; Miller et al, 2007; Kruger et al, 2015) and consists of two arms (147mmX60mmX20mm; See Figure 1.3), one of which transfers approximation forces to four strain gauges in a Wheatstone-bridge configuration, which results in force measurements (in Newtons) that are relatively insensitive to the location of the force on the arms. Opening and closing of the arms was achieved through servo-control. Maximal opening (mm), rate of opening (mm/s), and hold times were set through the computer software operated via LabView^{TM} (v15.0f2, National Instruments).

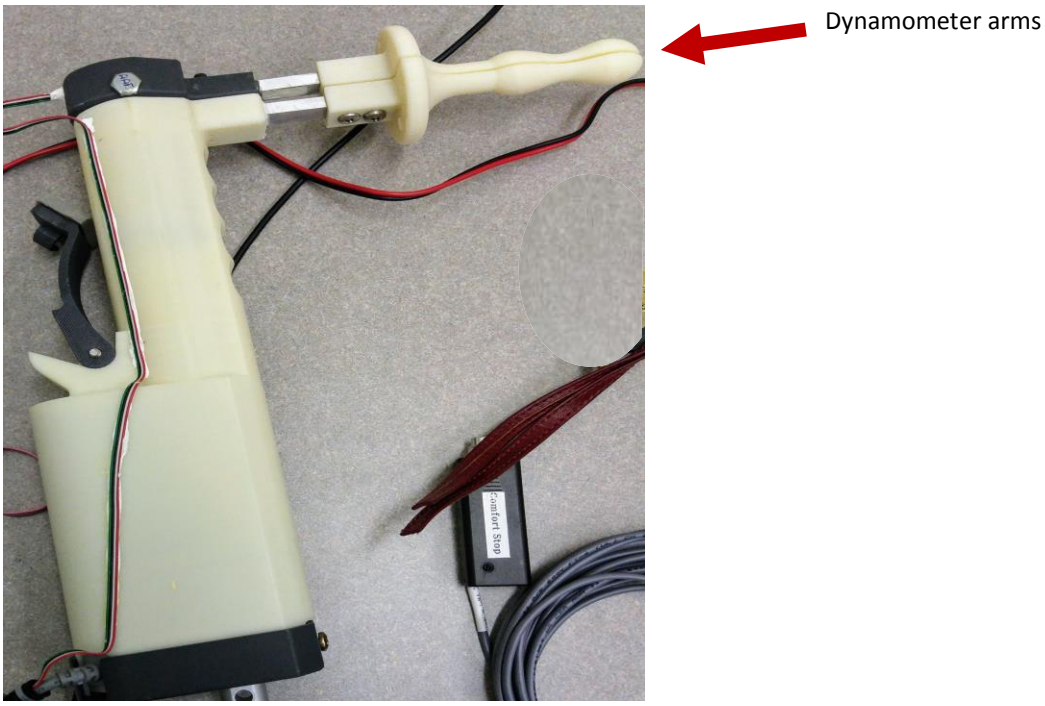

Figure 1.2: Intravaginal dynamometer used to assess the active and passive biomechanical properties of the pelvic floor muscles.

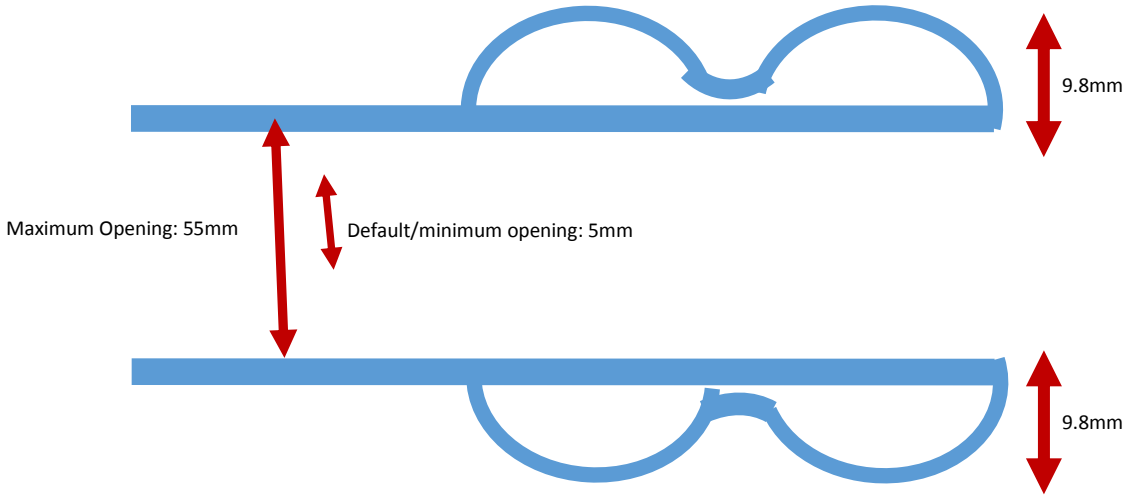

Figure 1.3: Dynamometer arm dimensions measured using precision calipers

For safety, hardware limits were set such that the total anteroposterior diameter of the

dynamometer arms could not exceed more than 55mm to minimize any potential for tissue damage. Once inserted into the vagina and opened, the arms could not close to less than 5mm to avoid pinching of vaginal wall tissue. The dynamometer had two constant speed settings; at the fast speed, the dynamometer arms opened at 50mm/s and at the slow speed, arms opened at 25mm/s. There was a hardware-implemented automatic shutoff that was activated by pressing a switch, which would cause the dynamometer arms to immediately close to the default setting of 5mm. This switch was placed in the research participants' hand during dynamometer testing – and the participant was instructed to push the button to close the arms of the dynamometer if they experienced discomfort. The arms of the dynamometer were single participant use, printed in biocompatible ABS plastic using a 3D printer. The plastic arms were attached to the main body of the dynamometer using screws. The main body of the dynamometer was made of medical grade stainless steel. Once the arms were attached to the main body, the arms were covered using Durex® lubricated condoms. All dynamometer data were recorded in Newtons (N) throughout all tasks at a sampling rate of 100Hz. The between-trial reliability of the dynamometer used in this study was previously confirmed, where all outcome measures used in this study demonstrated intra-class correlation coefficients ranging from very good to excellent (Berube et al., 2018).

Procedure

All study procedures took place at the Motor Function Measurement Lab at the University of Ottawa, Lees Campus, and all assessment procedures were performed by a physiotherapist who was registered with the College of Physiotherapists of Ontario and

rostered as a pelvic floor therapist, such that all study procedures fell within his scope of practice. The physiotherapist was accompanied by the researcher to ensure that, for safety, security and efficiency, there would be three people present in the room during the evaluation sessions.

At the familiarization session, the participant was taken to a private assessment room where she was shown the dynamometer that would be used to assess her PFMs. Once she saw the dynamometer and had all her questions answered regarding the device, she was then left alone in the private assessment room and asked to remove her clothes from the waist down, lie supine on an assessment plinth and cover her lower body with a sheet. Once the participant was ready, she invited the physiotherapist and researcher back into the assessment room.

The researcher first recorded the participant's age, height and weight through self-report. Next, PFM strength and tone were assessed by the physiotherapist using standard manual digital palpation techniques (Laycock et al., 1994). If the participant was unable to perform a proper PFM contraction, defined through palpation as having both a lifting and squeezing action in the LA muscles, she received instruction and tactile feedback from the physiotherapist in order to ensure that a proper contraction was performed prior to any data collection. Once a correct PFM contraction was performed, the physiotherapist scored the PFM strength using the Modified Oxford Grading Scale (Laycock et al., 1994). The PFM tone was rated on a 7-point scale of -3 to +3, with -3 being hypotonic (very low), +3 being hypertonic (very high) while 0 represented a normal and healthy PFM tone (Reissing et al., 2004). The participant was instructed on how to insert the condom-covered arms of the dynamometer into her vagina. Once the dynamometer arms were inserted, the physiotherapist verified the correct

placement of the dynamometer, and held the dynamometer while the research assistant provided verbal instructions to the participant to perform the following study tasks:

- ***Maximal voluntary contraction at 35mm and 25mm anteroposterior diameter***. The arms of the dynamometer were opened to a pre-set diameter. Once the position was achieved and held for at least 5 seconds to allow the tissues to adjust to the lengthened position, the participant was asked to contract her PFMs as strongly as possible in order to generate maximal PFM force, and to hold the contraction for 3-5 seconds, after which the device closed automatically. The researcher provided the following standardized verbal instruction/encouragement: "The arms of the dynamometer will open to pre-set diameter when I press the 'move to start' button and you will feel a gentle stretch on your pelvic floor muscles. Ready? I will press the button now." - [The researcher pressed the button and the dynamometer arms were opened to the pre-set diameter]. - "Keep your PFMs as relaxed as you can. When I say go, squeeze your pelvic floor muscles around the device as quickly and as hard as you can and hold the contraction until I let you know when to let go of the contraction." - [The research assistant pressed the 'start data collection' button and gave the participant approximately one second of relaxation time before giving the command to perform a maximal voluntary contraction]. Ready, relax, relax, relax and go, go, go…and relax…". Encouragements of "go, go, go…" were given loudly. After three repetitions, the task was repeated with the dynamometer arms open to 25mm. This muscle length was chosen based on Dumoulin et al (2004) who found that women generated maximal PFM forces at 24mm length.

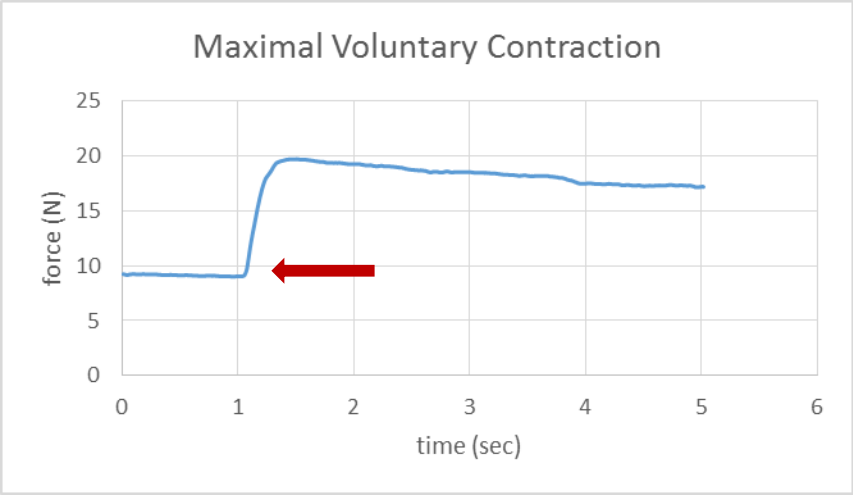

Figure 2.1: Representative Maximal Voluntary Contraction data. In the first second, the pelvic floor muscles are relaxed. The timing of the contraction is indicated at the solid (red) arrow. The contraction was held, with encouragement, until the participant received the command to relax. In this figure, the relaxation phase is not apparent as data collection was terminated after 5 seconds.

- ***Resistance to passive stretch at fast and slow speeds*** – the arms of the dynamometer were opened passively to a pre-set aperture (40mm of anteroposterior diameter) at a pre-determined fast (50mm/s) or slow (25mm/s) speed, while the participants kept her PFMs as relaxed as possible. Once the arms reached the maximum aperture (40mm), they remained in that position for five seconds during which time, the participant was instructed to continue to keep her PFMs relaxed. All participants were given the following instructions: "when I press start on the computer, you will feel the arms gradually stretch your pelvic floor muscles to a fixed but safe length. Try to keep your pelvic floor muscles relaxed during this stretch. If you feel great discomfort with the stretch, push the safety button in your hand to immediately stop and reverse the stretch. Ready, now relax. Now the arms will start to open… remember to keep your pelvic floor muscles relaxed". Trials were repeated if there was any evidence of PFM

activation during the passive stretch as determined through visual inspection of the force vs time graphs generated on the real-time display (See Figure 3.6).

The passive resistance task was repeated with the arms of the dynamometer opening at a slower rate of 25mm/s (slow speed). Participants received the same instruction and encouragement but were told that this time the arms would open much slower.

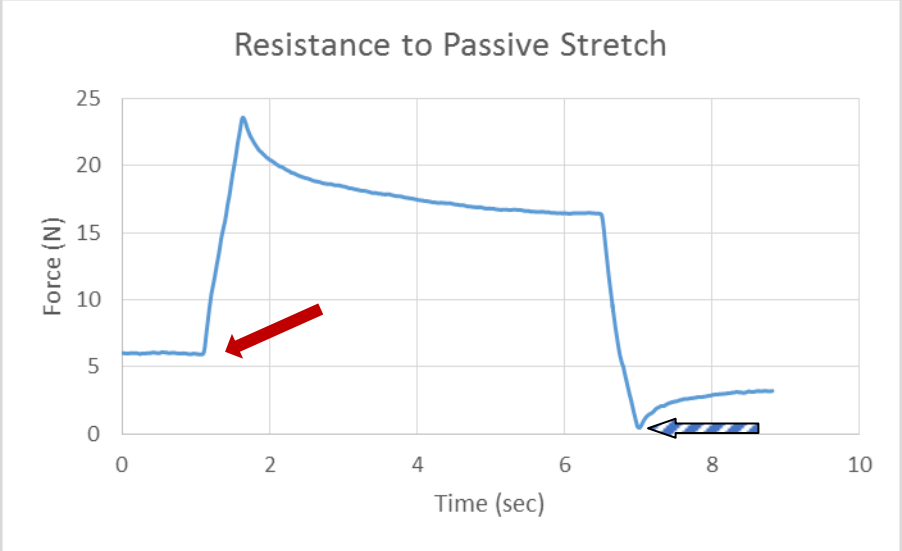

Figure 2.2: Representative resistance to passive stretch data. Once the arms of the dynamometer were opened to 40mm, the participants kept their PFMs as relaxed as possible according to the instructions they received. The opening of the dynamometer arms is indicated by the solid (red) arrow. Visual inspection of the graph shows no evidence of PFM muscle activation while the dynamometer provided passive stretch to the PFMs for five seconds, after which, the dynamometer closed automatically. The relaxation phase is shown in the graph, indicated by the diagonal patterned arrow.

- ***Pelvic floor muscle endurance*** – The dynamometer arms were opened to 25mm. Participants were instructed to keep their PFMs relaxed for five seconds, then to perform a maximal voluntary contraction and hold the contraction for five seconds. This process was repeated over three minutes. The researcher used a timer for this procedure and provided the following instructions "I will tell you when to relax and when to contract your PFMs. I will press 'start data collection' button now, start by relaxing your muscles for five seconds…" Repeated Encouragements of "relax, relax, and

squeeze, squeeze, hold it, hold it" were given (loudly)

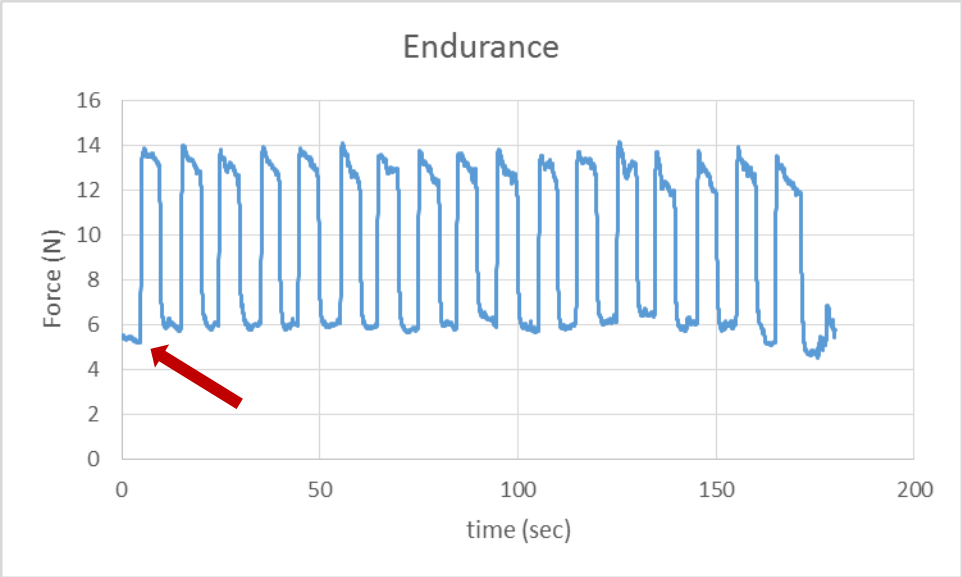

Figure 2.3: Representative endurance graph. The dynamometer arms were opened to 25mm and the participants were instructed to contract their PFMs for 5 seconds and relax for 5 seconds. This process was repeated over three minutes. At the beginning of the task, participants were given 5 seconds of relaxation time before the instruction to maximally contract their PFMs were given. The beginning of the first contraction is indicated by the solid (red) arrow. Note that in this instance, there is no evidence of fatigue.

All procedures, except for the endurance task, were repeated over three trials and a rest of two minutes was given between trials and tasks to allow the muscles to recover from fatigue (Edwards et al., 1977). The same instructions were repeated before the repetition of each task.

This concluded the familiarization session. The participant was left alone in a private room to remove the dynamometer arms and to get dressed. The dynamometer arms were cleaned and disinfected, labelled and stored for use at the subsequent testing session. All participants were asked to practice three sets of 10 PFM MVCs per day over the next week before returning for the scheduled data collection session. Participants also received daily email reminders from the researcher to do their PFM contractions and were given an adherence form (Appendix F) to fill out, which they were asked to bring with them to their next scheduled

assessment. At the data collection session, the same procedures were employed as in the familiarization session. At the completion of the data collection session, the dynamometer arms were discarded and the participants were thanked for their support of the research. The participants were then asked if they were willing and available to return the following week to repeat the data collection protocol in order to participate in a separate study that was beyond the scope of this thesis.

Data Processing

All data for this thesis were analyzed from the participants' second visit (data collection session). The data files were first visually inspected to ensure that there was no evidence of contamination by PFM contraction when the PFMs were supposed to be relaxed, or other artifacts.

Outcome measures identified through data processing:

1. Total force during maximum voluntary contraction force (in Newtons) at 35mm and at 25mm: Total force was defined as the sum of active and passive forces generated during a maximal PFM voluntary contraction, and was determined for both the 25mm and 35mm settings. The peak force (N) (Figure 3.1) was the highest force value, generated during each maximum voluntary PFM contraction trial at each muscle length (i.e. dynamometer arms opened to 35mm and to 25 mm).
2. Active force (N) at 35mm and at 25mm: The active force within the maximum voluntary PFM contraction was recorded in Newtons (N). To calculate the baseline force, first, the initial rise in force value was identified from the data file. Moving up ten values from the

initial rise, the baseline resting force was determined by taking the mean of ten data points prior to the initiation of the PFM contraction (Figure 3.1). The active force was calculated by subtracting the peak force (total force) from the mean baseline resting force value at each dynamometer arm opening (35mm and 25mm).

3. Rate of force development (N/s) at 35mm and at 25mm: Rate of force development was determined at each dynamometer setting (i.e. 35mm opening and 25mm opening) using the maximum voluntary contraction data. In this case, the representative steepest points of the slope of force vs time curve (in N/s) was used, by visually identifying the widest range of time while the rise was linear (Figure 3.1). Where the rise in force was linear, the slope was computed over the 20th to 80th percentile of total force. Where there were areas of non-linearity, the slope was determined through identifying the range through which the slope was linear and computing the slope over that range of values.

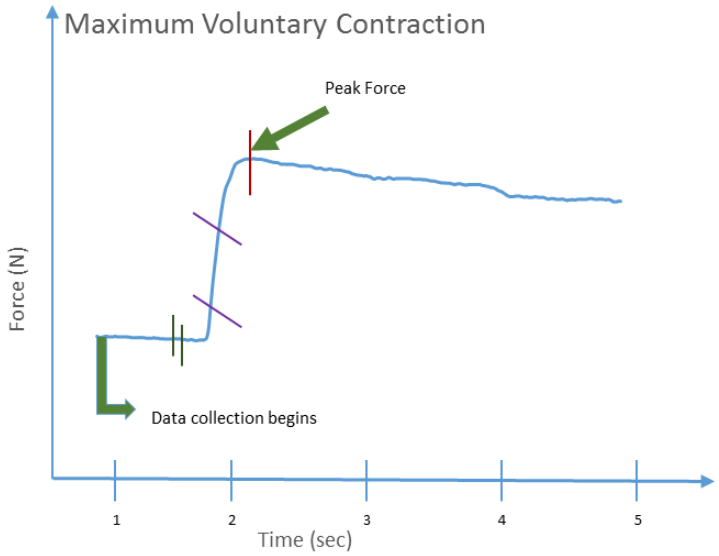

Figure 3.1: Data processing for maximal voluntary contraction – After the dynamometer was inserted and opened to 35mm and 25mm, the PFMs were allowed to relax before data collection began. Once data collection began, the baseline force was determined as the region of stable passive force (green bars - two bars side-by-side) before the participant performed a maximal PFM voluntary contraction, (1) Total force or the peak force (N) shown by red bar (top vertical bar) was the highest force value generated by PFM contraction. (2) Active force (N) was calculated by subtracting the mean of the baseline value, shown by green bars, from the peak force value. (3) Rate of force development (N/s) was calculated using the steepest points of the slope, shown by purple bars (two slanted bars), and dividing the force values to the corresponding time values.

All MVC data files were visually inspected to ensure that that the baseline, slope of the graph and the peak force were visible in the graph. Figure 3.2 illustrates an example of an acceptable trial where the baseline, slope and peak force are visible. Trials where participants contracted their PFMs before receiving the command (Figure 3.3) were excluded from analysis since in these trials, a baseline could not be established before generating maximal force in the PFMs and data was recollected again to ensure that a baseline is established before the participants performed a maximal PFM contraction. Visual inspection of the force vs time graphs of each trial for each task was performed to verify the steepest part of the curve, the peak force value, and the mean baseline value.

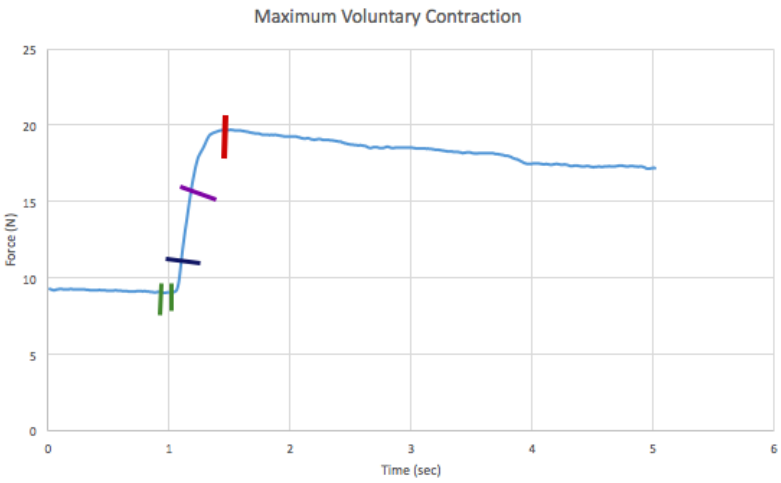

Figure 3.2: Example of data processing of maximum voluntary contraction (MVC) data, where the period over which the baseline force was computed is indicated by the green bars (two vertical bars side-by-side), the start (blue) and end (purple) of the steepest part of the slope where rise in force was linear was used to compute the rate of force development, and peak force was determined (top vertical red bar).

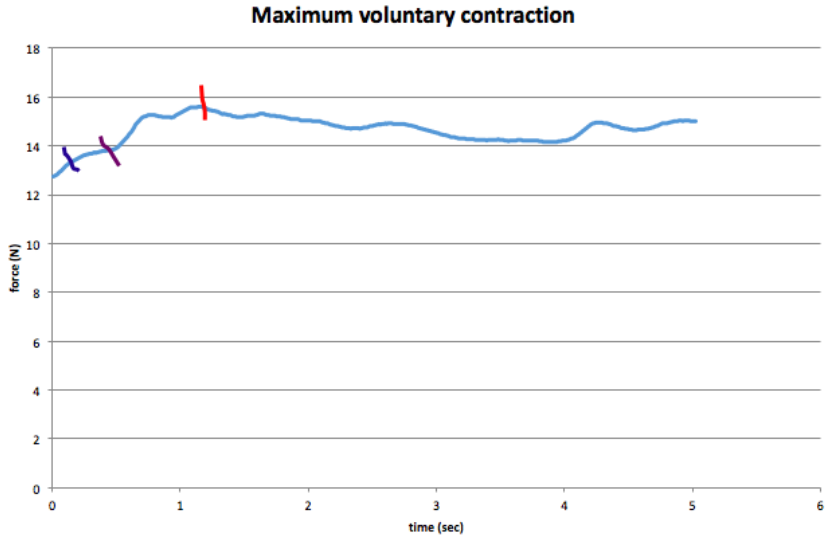

Figure 3.3: Example of a maximum voluntary contraction (MVC) trial that was excluded because the participant performed a PFM contraction before receiving the command and thus neither the baseline force nor the rate of force development could be established.

4. Relative Peak Passive Resistance (N) at 50mm/s (fast speed) and at 25mm/s (slow speed): Peak passive resistance was identified as the highest value generated when the dynamometer arms fully opened to 40mm at both at fast and slow speeds less the

baseline passive force. The mean baseline force was determined by taking the mean of ten data points on a stable point on the curve prior to the opening of the dynamometer arms (Figure 3.4). The relative peak passive resistance, recorded in Newtons (N), was calculated by subtracting the peak passive resistance value from the mean baseline force value at each speed (fast and slow) settings.

5. Stiffness (N/mm) at 50mm/s and at 25mm/s: To find PFM stiffness, the most linear part of the curve was identified using visual inspection. The slope was computed over that range of values, resulting in an estimate of stiffness value recorded in N/mm (figure 3.4).

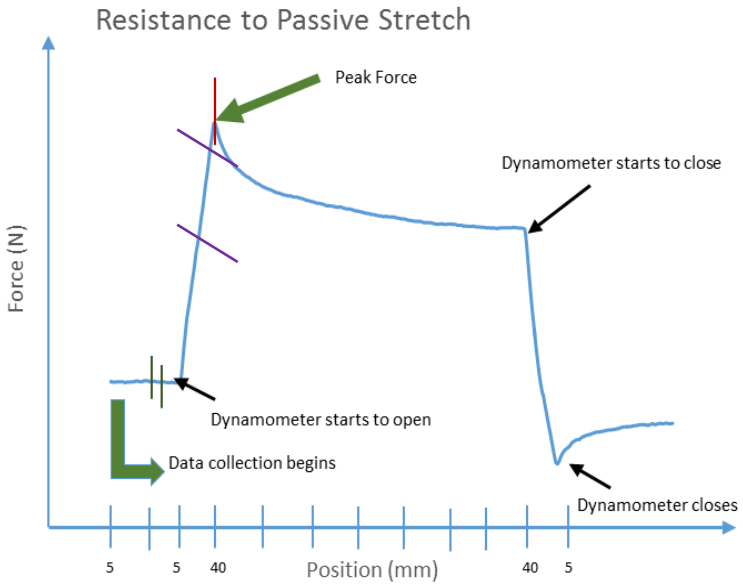

Figure 3.4: Data processing to determine values related to the resistance to passive stretch – After the dynamometer was inserted, the participant was instructed to keep their PFMs relaxed throughout the entire task. (1) The relative peak passive force (N) was calculated by subtracting the peak passive force (red bars, top vertical bar) from the mean baseline value (green bars, two vertical bars side-by-side). (2) Stiffness (N/mm) was calculated by using the most linear points of the slope (purple bars – two slanted bars) and dividing the force values with the corresponding position values.

The data were carefully inspected to ensure that the participants kept their PFMs relaxed during data collection. Trials were excluded from analysis if the participants contracted

their PFMs before or during the opening of the dynamometer arms, which was visible in monitorial time (see Figure 3.6). An example of an acceptable trial is illustrated in Figure 3.5, where the participant kept her PFMs relaxed and the baseline, slope of the graph and peak force is visible in the graph. Visual inspection of the force versus position graphs of each trial for each task was performed to verify the steepest part of the curve, the peak value and the mean baseline value.

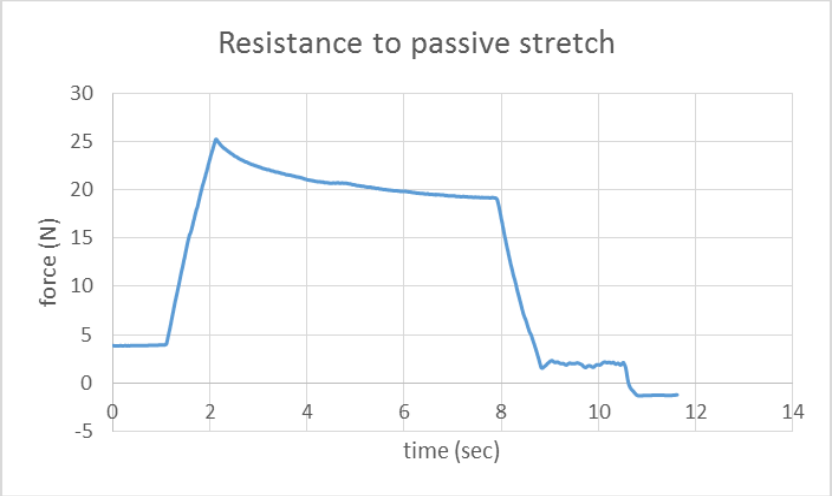

Figure 3.5: An example of a trial where the participant kept her PFMs completely relaxed during data collection

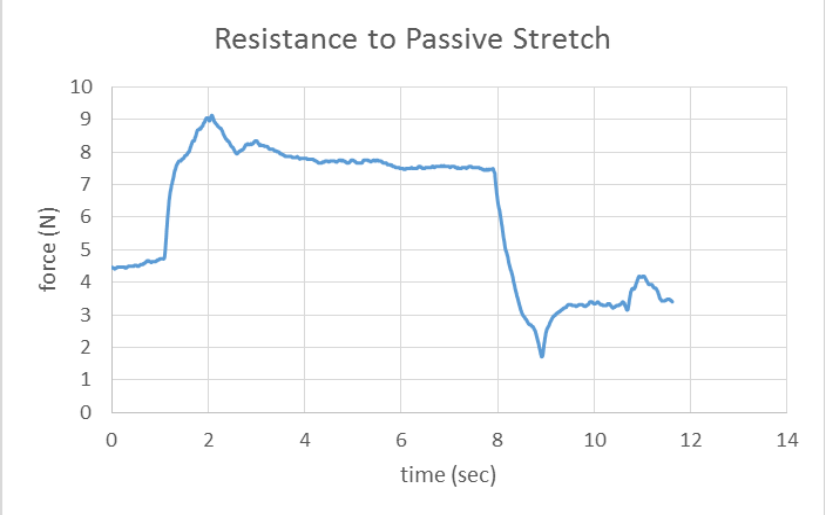

Figure 3.6: An example of a trial for resistance to passive stretch, where the participant did not keep their PFMs relaxed during data collection

6. Endurance (N): Endurance was expressed as percent reduction in force (%). To find the percent reduction in force, the average of the first three contractions were subtracted from the average of the last three contractions divided by the average of the first three contractions. Participants that did not reach at least 80% of their MVC in their first three consecutive trials were excluded from analysis, as these participants were not expected to demonstrate fatigue during the task (Figure 3.7).

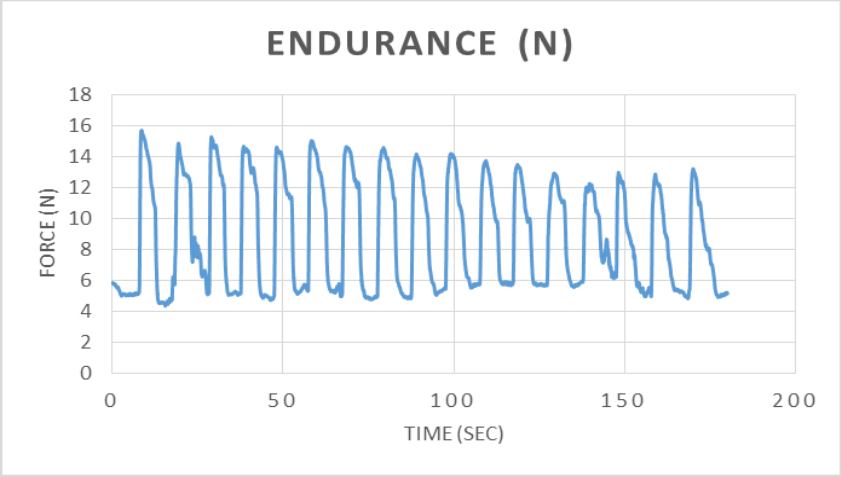

Figure 3.7: Percent reduction in force was determined by subtracting the average of first three contractions from the average of last three contractions

Statistical Analysis

Data analysis was performed using IBM SPSS Statistics 23 software (IBM SPSS Inc., Chicago, IL, USA). The outcome measures were first tested for normality using the Shapiro-Wilk test. Rate of force development at 25mm, relative peak passive resistance at slow speed (25mm/s) and endurance data were not normally distributed. Therefore, they were transformed using a base 10 logarithm to achieve normal distributions. An alpha level of 0.05 was used for statistical significance in all analyses.

One-way repeated measures analysis of variance (ANOVA) models were used, to investigate the impact of trial as a main effect for each outcome. Significant trials effects were found for total force at 35mm ($p < 0.05$) and total force at 25mm ($p < 0.05$), where the participants generated higher MVC values in one trial compared to the other two trials. For these outcomes, the highest force value from each of the trials was used in the subsequent analysis. For the outcome measures that did not have trial effects, an average of all three trials were used in the subsequent data analysis.

Between-trial Reliability:

Intraclass Correlation Coefficients (ICCs) were used to determine consistency between the three trials of each outcome measures using two-way mixed model (3,1) (Shrout & Fleiss, 1979) as reflected by Equation (1):

$$ICC\ (3,1) = \frac{BMS - EMS}{BMS + (k - 1)\ EMS} \qquad (1)$$

where k represents the number of trials that were performed for each task. (BMS = Between Subject Mean Square; EMS = Error Subject Mean Square). The average measure ICC values for each outcome measures were computed. ICC > 0.8 was considered highly reliable.

The impact of age on study outcome variables

In order to determine whether age-related changes were evident in the study outcome measures, linear regression analyses were performed. The correlation coefficient, defined as the strength of the relationship between two variables (Weber & Lamb, 1970), was used to

determine the strength of the association between age and each outcome measures, and the r^2 value was used to determine how much of the total variance in each outcome measures was related to differences in age. The *F*-test was used to assess the overall significance of the model, where, p values > 0.05 indicated that age related effects were not evident in the PFMs.

Demographic and questionnaire data

Descriptive statistics were used on the ICIQ-FLUTS, ICIQ-B and ICIQ-VS scores to summarize the data. The ICIQ-FLUTS questionnaire results were divided into three subscales – the urinary incontinence (UI) symptoms subscale was scored out of 20; filling symptoms subscale was scored out of 15 and voiding symptoms subscale was scored out of 12 (Brookes et al., 2004). The ICIQ-B questionnaire also had three subscales – bowel pattern that had a score range between 1-21, bowel control, with a score range between 0-28 and impact on quality of life (QoL), with a score range between 0-26 (Cotterill et al., 2011). The ICIQ-VS questionnaire consisted of three subscales - vaginal symptoms subscales, that was given a score between 0-53 points, sexual matters subscale, scored between 0-58 points, and impact on quality of life subscale, which was between 0-10 points (Price et al., 2006). Higher ICIQ scores indicated increased severity of incontinence symptoms in women (Brookes et al., 2004). Analyses of variance were used to test for differences in demographic and questionnaire results among participants who were divided into three age cohorts:

Sample size and power calculations

Before beginning recruitment, where we initially planned to recruit women into three age cohorts (younger, middle-aged and older), we performed a power calculation (α=0.05; β=0.80) using G*Power v3.1.9.2 (Dusseldorf University, Germany) for ANOVA, which suggested that a minimum sample size of 12 participants per group would be needed to detect differences in all force outcomes among these age cohorts. The power calculations were performed using data from two different studies where the same intra-vaginal dynamometer and methods were used; a sample of 17 women aged 60 and over demonstrated maximal PFM contractile strength of 4.12N ± 3.48 (Madill et al., 2013), while a sample 30 women aged 21-44 demonstrated maximal PFM contractile strength of 5.9N ± 2.8 (Morin et al., 2004). The participants from the Morin et al. (2004) study generated 3.7N ± 1.4 passive force, since there were no estimates of passive force data for older women reported in the literature, active force data from older women were used to estimate the sample size required to detect significant age-related differences in resistance to passive stretch. Because the researcher did not recruit the required number of participants into each cohort over the course of completing this thesis, regression analyses were instead performed to study the age-related changes in the PFMs.

RESULTS

Participants

Thirty-three nulliparous women between the ages of 20 to 64 years (mean ± standard deviation, 38 ± 16.02 years) participated in the study – eighteen younger women (20-39 years) (25 ± 5.13), ten middle-aged women (40-59 years) (50 ± 6.33), and five older women (>60 years) (63 ± 1.14). Sample demographic characteristics are presented in Table 1.

The low mean scores ± SD for the ICIQ questionnaires indicate that most participants did not experience symptoms of urinary or anal incontinence and did not report any vaginal symptoms associated with pelvic pain, prolapse or urinary and fecal incontinence with the exception of two women, one aged 23 years old (ICIQ-FLUTS-overall: 17; ICIQ-FLUTS-filling: 3; ICIQ-FLUTS-voiding: 4; ICIQ-B-overall: 15; ICIQ-B-bowel control: 15; ICIQ-B-QoL: 13; ICIQ-VS: 17; ICIQ-VS-sexual matters: 5; ICIQ-VS-QoL: 10), and the other 64 years old (ICIQ-FLUTS-overall: 19; ICIQ-FLUTS-filling: 3; ICIQ-FLUTS-voiding: 2; ICIQ-B-overall: 17; ICIQ-B-bowel control: 24; ICIQ-B-QoL: 17; ICIQ-VS: 16; ICIQ-VS-sexual matters: 8; ICIQ-VS-QoL: 6), who reported symptoms on the ICIQ questionnaires. The total force, active force, rate of force development, and endurance values for the older woman (64 years) fell within the 60^{th} and 80^{th} percentile, and the resistance to passive stretch and stiffness values fell within the 70^{th} and 90^{th} percentile; while the total force, active force, rate of force development, and endurance values for the younger woman (23 years) fell within the 20^{th} and 40^{th} percentile, and the resistance to passive stretch and stiffness values fell within the 40^{th} and 60^{th} percentile of the regression plots. The force values and the percentiles in the regression plots for the two women are shown in Appendix G.

Demographic variable n = 33	Mean	Standard deviation (SD)
Height (cm) – overall (20-64 years)	**166.94**	**1.07**
- young (20-39 years) (n=18)	167.51	6.07
- middle-aged (40-59 years) (n=10)	166.37	7.85
- older (60-64 years) (n=5)	166.1	2.24
Weight (kg) – overall (20-64 years)	**63.241**	**1.94**
- young (20-39 years)	62.07	10.24
- middle-aged (40-59 years)	65.09	14.99
- older (60-64 years)	63.77	5.20
BMI (kg/cm) – overall (20-64 years)	**22.64**	**0.59**
- young (20-39 years)	22.07	3.05
- middle-aged (40-59 years)	23.28	4.48
- older (60-64 years)	23.42	2.12
Age (years) – overall (20-64 years)	**38.40**	**16.021**
- young (20-39 years)	25.06	5.13
- middle-aged (40-59 years)	50.1	6.33
- older (60-64 years)	62.4	1.14
ICIQ-FLUTS (/20) overall (20-64 years)	**4.82**	**4.81**
- young (20-39 years)	4.94	4.60
- middle-aged (40-59 years)	3.3	2.87
- older (60-64 years)	7.4	7.92
ICIQ-FLUTS Filling score (/15) overall (20-64 years)	**2.23**	**2.49**
- young (20-39 years)	2.29	2.54
- middle-aged (40-59 years)	2.16	2.33
- older (60-64 years)	2.44	2.71
ICIQ-FLUTS - Voiding score (/12) overall (20-64 years)	**1.28**	**1.78**
- young (20-39 years)	1.5	2.25
- middle-aged (40-59 years)	0.9	1.10
- older (60-64 years)	1	0
ICIQ-B(/21) overall – (20-64 years)	5.45	2.04
- young (20-39 years)	5.69	2.97
- middle-aged (40-59 years)	5.23	3.10
- older (60-64 years)	5.51	3.21
ICIQ-B – bowel control – out of 28 – (20-64 years)	**2.76**	**3.59**
- young (20-39 years)	1.91	2.78
- middle-aged (40-59 years)	2.02	3.23
- older (60-64 years)	2.88	3.87
ICIQ-B – QoL – out of 26 – (20-64 years)	**3.66**	**5.71**

- young (20-39 years)	2.88	5.09
- middle-aged (40-59 years)	1.8	2.39
- older (60-64years)	4.2	5.67
ICIQ-VS – vaginal symptoms – out of 53 – (20-64 years)	**3.64**	**3.82**
- young (20-39 years)	5	6.35
- middle-aged (40-59 years)	2.1	2.33
- older (60-64 years)	4.4	3.78
ICIQ-VS sexual matters – out of 58 – (20-64 years)	**5.29**	**12.01**
- young (20-39 years)	4.99	11.28
- middle-aged (40-59 years)	5.73	7.01
- older (60-64 years)	4.91	6.88
ICIQ-VS QoL – out of 10 – (20-64 years)	**0.69**	**1.1**
- young (20-39 years)	0.66	0.90
- middle-aged (40-59 years)	0.2	0.63
- older (60-64 years)	0.6	1.34

Table 1: Sample demographic information and questionnaire scores. FLUTS refers to female lower urinary tract symptoms, B refers to bowel, VS refers to vaginal symptoms, QoL refers to quality of life

Data cleaning

Data were first visually inspected to ensure quality. Out of the 198 trials collected from thirty-three participants for MVC, only two trials were discarded, and this was because the participants either contracted their PFMs before the command was given to them (n=1) or they kept their PFMs relaxed when they should have been contracting their PFMs (n=1). For the endurance task, one trial was collected from each participant; data from two participants were discarded since the participants either contracted to a level of less than 80% of their MVC (n=1) or their baseline and peak force could not be calculated from their trial (n=1). For the passive stretching task, again 198 trials were collected; only one trial was excluded from analysis since the participant contracted her PFMs during the stretch.

Trial Effects

Significant trials effects were found for total force at 35mm ($F(1.89, 58.62) = 7.30$, $p = .002$) and total force at 25mm ($F(1.89, 58.47) = 3.33$, $p = .045$). For these outcomes, the highest force value from each participant at each dynamometer diameter was used in the subsequent analysis. For the outcome measures that did not have trial effects, an average of all three trials was used in the subsequent data analysis.

Between Trial Reliability

Participant performance was very consistent for both the MVC and resistance to passive stretch tasks; representative data are presented in Figure 4.1 and 4.2. The between trial reliability for all outcome measures was calculated using Intraclass Coefficients (ICCs) mixed-model (3,1) and single measures outcome (Portney & Watkins, 2000). The between trial reliability of the outcome measures was deemed very good to excellent (ICC=0.79-0.97). The ICC values for all outcome measures are summarized in Table 2.

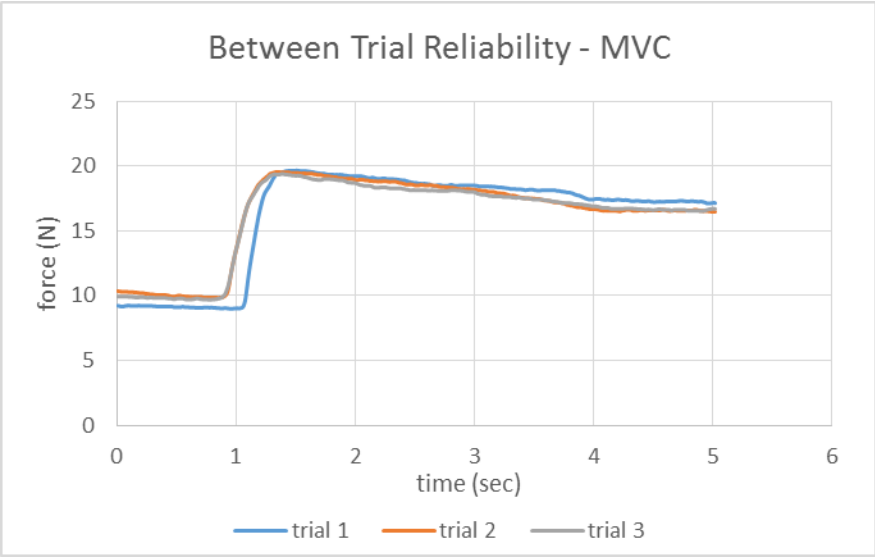

Figure 4.1: An example of three trials of one participant's maximum voluntary contraction force.

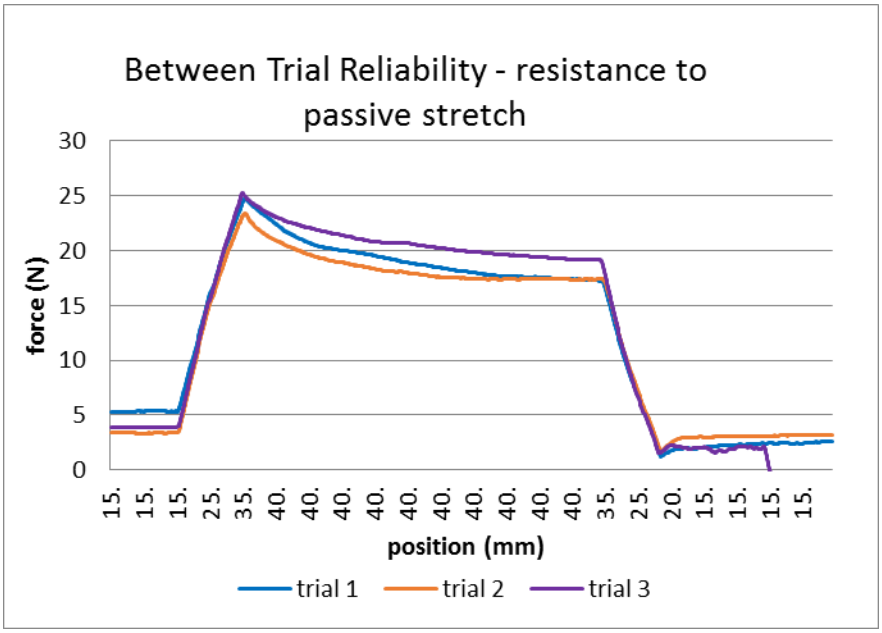

Figure 4.2: Sample data for resistance to passive stretch of the pelvic floor muscles. Three trials are plotted from a single participant as the dynamometer arms were opened to produce an overall anteroposterior diameter from 15mm to a maximum of 40mm diameter at the slow speed (25mm/s).

Between Trial Reliability Metrics

Outcome Measures	**Mean ± Standard Deviation (n= 33)**	**ICC (95% CI) for Single Measure test)**
Total force at 35mm (N)	21.15 ± 3.78	.921 [.863 ± .958]
Total force at 25mm (N)	13.77 ± 2.26	.954 [.919 ± .976]
Active force at 35mm (N)	7.88 ± 3.10	.925 [.869 ± .960]
Active force at 25mm (N)	4.75 ± 2.05	.951 [.914 ± .974]
Rate of force at 35mm (N/s)	24.67 ± 12.62	.904 [.834 ± .949]
Rate of force at 25mm (N/s)	16.49 ± 8.47	.830 [.719 ± .906]
Resistance at fast speed (N)	15.25 ± 4.21	.969 [.945 ± .984]
Resistance at slow speed (N)	13.07 ± 3.99	.973 [.953 ± .986]

Stiffness at fast speed (N/mm)	1.02 ± 0.21	.791 [.523 ± .821]
Stiffness at slow speed (N/mm)	0.82 ± 0.22	.818 [.704 ± .898]

Table 2: Mean values for outcome measures and between trial reliability intraclass correlation coefficients (ICC) with confidence intervals (CI) for each outcome.

Regression Analysis: Relationship between Age and Outcome Measures

The linear regression analyses showed that there was no significant relationship between age and any of the outcome measures. Power calculations for correlation analysis were performed for all dynamometry outcomes as there is no power analysis for regression reported in the literature. Using α=of 0.05, β=0.80 and the r value derived from the individual linear regression analyses performed for each dynamometry outcome measure, based on Faul et al. (2008) estimated sample sizes ranged from n=168 for rate of force development during voluntary PFM contraction (35mm) to n=2896 for the endurance task. Based on these results, it is highly unlikely that significant age-related effects would have been seen even if recruitment had reached our target of 15 per group. It remains possible, however, that recruiting women older than age 65 may generate significant findings.

Total Force (N) at 35mm and 25mm

The regression analyses showed no significant relationship between total force (N) generated by the PFMs and age at the 35mm diameter ($F(1,31) = 0.046$, $p= 0.83$) ($r = 0.038$); nor at the 25mm diameter ($F(1,31) = 0.023$, $p= 0.88$) ($r = 0.027$) setting. The models did not fit the data well, with coefficient of determination (R^2) of .001 for total force at 35mm and at 25mm, and only 0.1% of the variance explained by the models (see Figure 5.1).

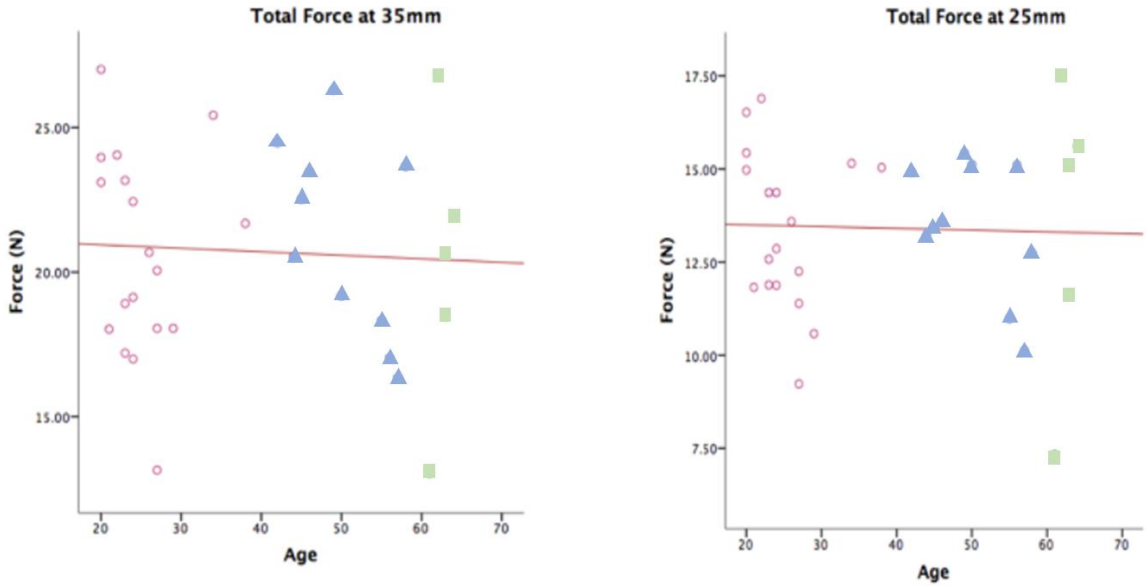

Figure 5.1: Plots showing correlation between age and Total force (N) or peak force generated during MVC at 35mm [(p =.83, r =0 .038, R^2 =0 .001, y = 21.19 + (-0.01)x] (left); and at 25mm [(p=0 .88, r =0 .027, R^2 = 0.001, y = 13.59 + (-4.72^{E-3})x] (right). The pink circles are young women (20-39 years), blue triangles are middle-aged women (40-59 years) and green rectangles are older women (60-64 years)

Active Force (N) at 35mm and 25mm

There was no significant effect of age on the active force (N) generated by the PFMs at 35mm diameter (F(1,31) = .310, p= .58, r = .099) where the regression had coefficient of determination (R^2) of .010; nor at 25mm diameter (F(1,31) = .200, p= .66) (r = .08) where the regression had a coefficient of determination (R^2) of .006. The regression models did not fit the data well and accounted for 1% and 0.6% of the variance in the data (see Figure 5.2).

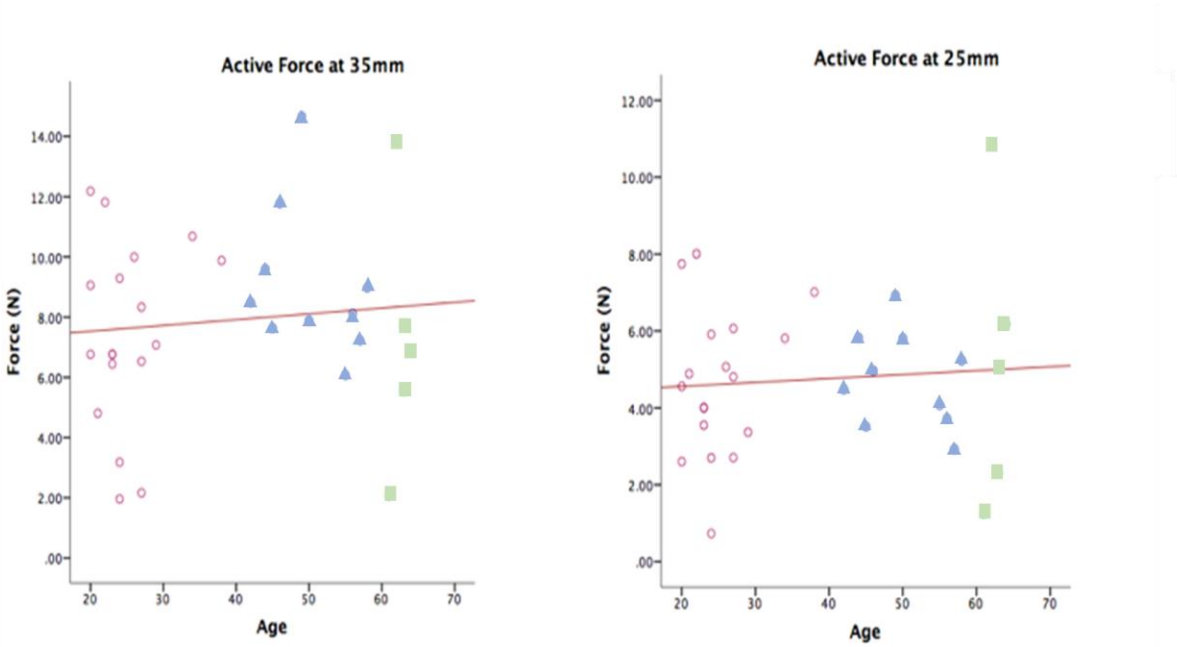

Figure 5.2: Plots showing correlation between age and Active force (N) calculated from MVC at 35mm (p= 0.58, r =0 .099, R^2 =0 .010, y = 7.15 + 0.02x) (left); and at 25mm (p= 0.66, r = 0.08, R^2 = 0.006, y = 4.35 + 0.01x) (right).

Rate of force development (N/s) at 35mm and 25mm

The rate of force development (N/s) during maximal voluntary contraction was not linearly related to age at either the 35mm diameter (F(1,31) = 1.169, p= 0.28) (r = 0.191) with a coefficient of determination (R^2) =0.036; or the 25mm diameter (F(1,31) = 0.278, p= 0.60) (r = 0.094) with a coefficient of determination (R^2) =0.009. The models did not fit the data well, and only 3.6% and 0.9% of the variance explained by the models, respectively (see Figure 5.3).

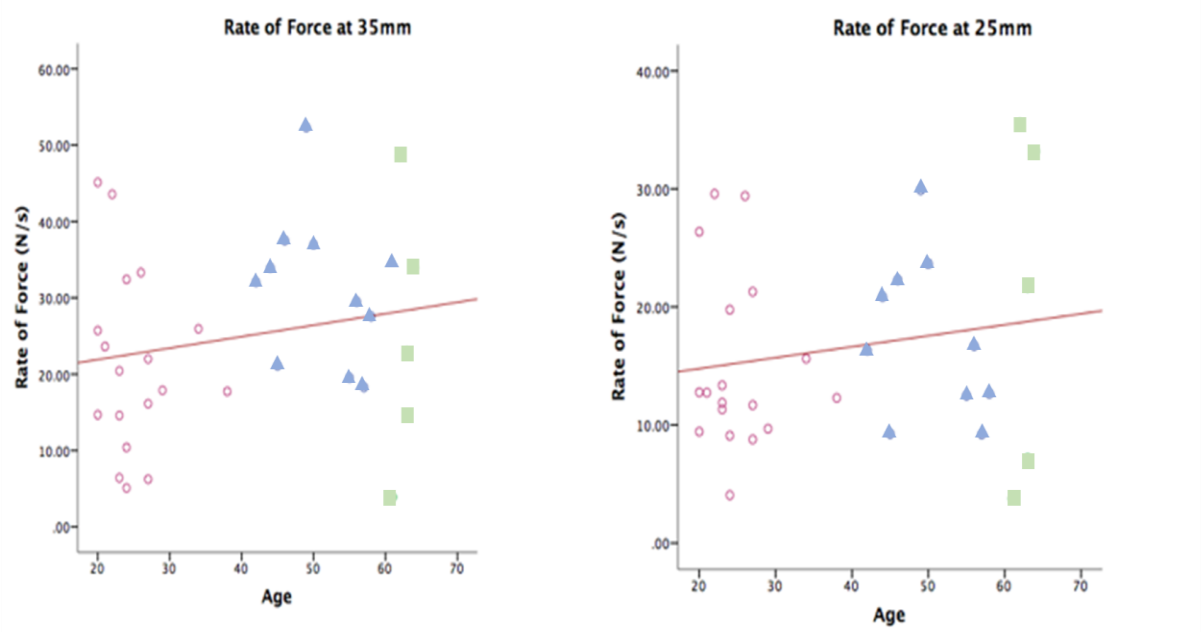

Figure 5.3: Plots showing the correlation between age and rate of force development (N/s) during MVC at 35mm (p= 0.28, r =0 .191, R^2 = 0.036, y = 18.91 + 0.15x) (left); and at 25mm (p= 0.60, r = 0.094, R^2 = 0.009, y = 12.92 + 0.09x) (right).

Relative peak resistance (N) at fast (50mm/s) and slow (25mm/s) speeds of opening

There was no significant relationship observed between age and relative peak resistance (N) measured during resistance to passive stretch at the fast speed of opening (50mm/s) (F(1,31) = 0.155, p= 0.69) (r = 0.07) with a coefficient of determination (R^2) =.005; nor at the slow speed of opening (25mm/s) (F(1,31) = 0.555, p= 0.46) (r = 0.133) with a coefficient of determination (R^2) =0.018. The models did not fit the data well, with only 0.5% of the variance for resistance at fast speed, and 1.8% of the variance for resistance at slow speed explained by the models, respectively (see Figure 5.4).

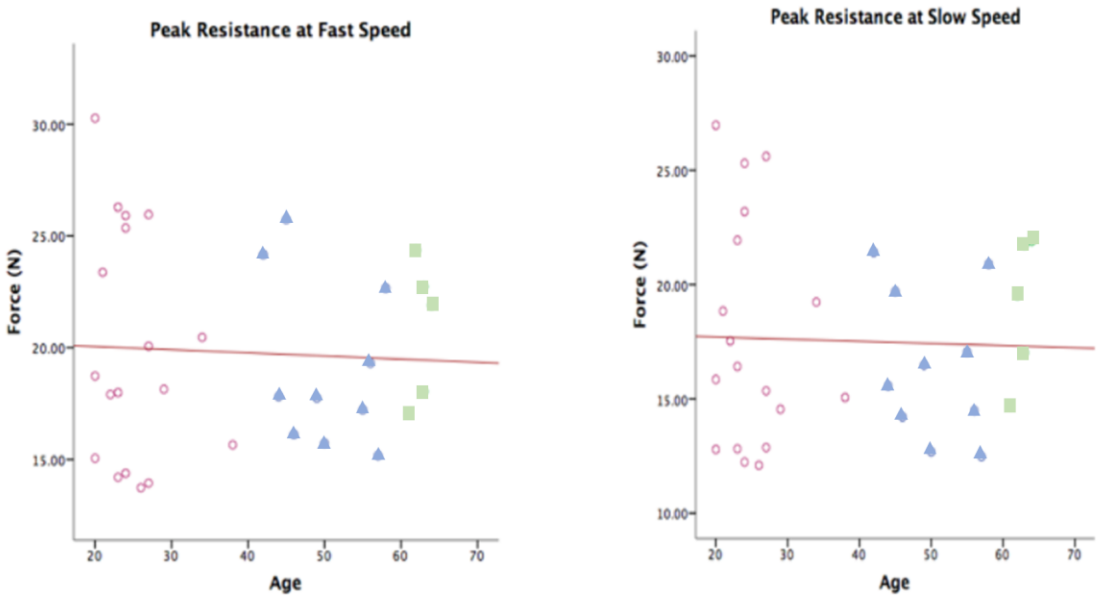

Figure 5.4: Plots showing the correlation between age and Relative peak resistance to passive stretch (N) at fast speed (50mm/s) (p= 0.69, r =0 .07, R^2 = 0.005, y = 20.33 + (-0.01)x) (left); and at slow speed (25mm/s) (p=0 .46, r =0 .133, R^2 =0 .018, y = 17.89 + (-9.35 $^{E-3}$)x) (right).

Stiffness at fast (50mm/s) and slow (25mm/s) speeds of opening

The regression analysis showed that there were no significant age-related changes observed in the stiffness properties of the PFMs at fast speed of opening (50mm/s) (F(1,31) = 0.161, p= 0.69) (r = 0.072), with a coefficient of determination (R^2) $^{=}$=0.005; and at slow speed of opening (25mm/s) (F(1,31) = 0.052, p= 0.82) (r = 0.041) with a coefficient of determination (R^2) =0.002. The model was not a good fit for the data as 0.5% and 0.2% of the variance is explained by the regression model (see Figure 5.5).

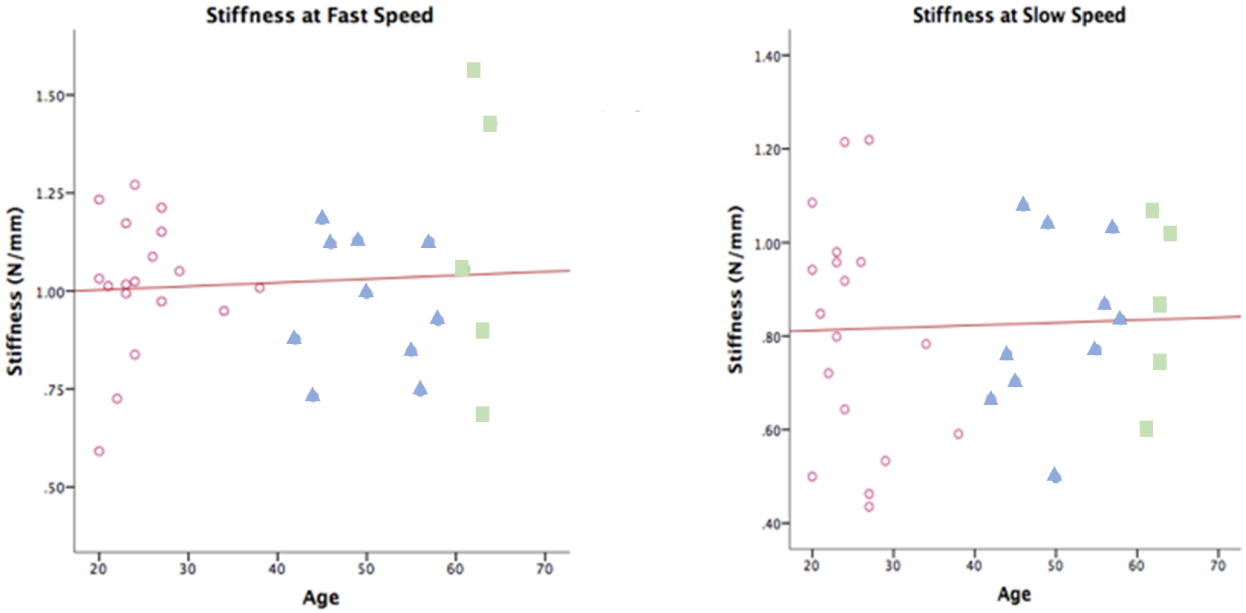

Figure 5.5: Plots showing the correlation between age and stiffness of the PFMs (N/mm) at fast speed (50mm/s) (p= 0.69, r =0 .072, R^2 =0 .005, y = 0.98 + 9.3 $^{E-4}$x) (left); and at slow speed (25mm/s) (p= 0.82, r =0 .041, R^2 =0 .002, y = 0.80 + 5.54 $^{E-4}$x) (right).

Endurance

The mean ± SD percent reduction in force (N) from MVC at 25mm for endurance task was 18.59 ± 21.79%. There was no significant relationship observed between endurance task and age (F(1, 28) = 0.17, p= 0.89) (r = 0.025). The models did not fit the data well, with a coefficient of determination (R^2) =0.001 and only 0.1% of the variance explained by the regression model (see Figure 5.6).

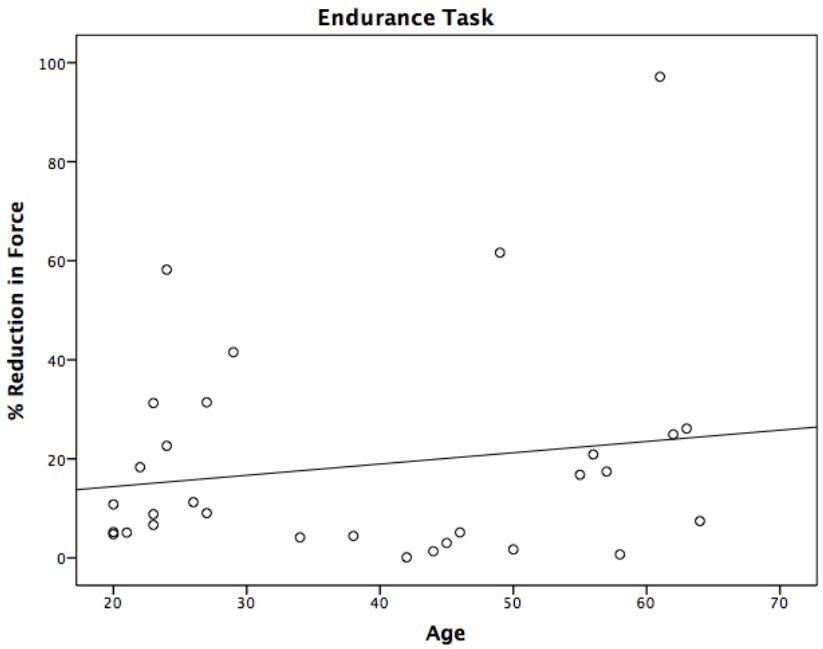

Figure 5.8: Plot showing the correlation between age and percent reduction in force during MVC at 25mm (p= 0.89. r =0 .025, R^2 = 0.001, y = 9.8 + 0.23x).

Discussion

The regression results showed that there were no statistically significant age-related changes seen in the PFM biomechanical properties in young, middle-aged and older nulliparous women including, total and active force generation capacity, rate of force development, endurance, resistance to passive stretch or stiffness. Note however that this study lacked participants over the age of 65. The power calculation suggested that an estimated sample sizes ranging from n=168 for rate of force development during voluntary PFM contraction (35mm) to n=2896 for the endurance task may have shown significant changes in MVC, endurance, and passive properties of the PFMs. However, since the participants in the current study were under the age of 65, recruiting women in the upper age limit may generate significant findings.

Participant Characteristics

The participants in this study were nulliparous, non-smoking, non-obese women between the ages of 20 to 64. The participants had no history of urogynecological surgery and/or physical disability that would impair their ability to perform the PFM contractions. According to the ICIQ questionnaires modules participants did not report any incidence of urinary incontinence, with the exception of two women, one aged 23 years and the other 64 years old, who reported incontinence on the ICIQ-FLUTS. Overall, the sample reported minimal lower urinary tract symptoms (ICIQ-FLUTS-overall: 4.82 ± 4.81; ICIQ-FLUTS-filling: 2.23 ± 2.49; ICIQ-voiding: 1.28 ± 1.78), and moderate bowel (ICIQ-B-overall: 5.45 ± 2.04; ICIQ-B-bowel control: 2.76 ± 3.59; ICIQ-B-QoL: 3.66 ± 5.71) and vaginal (ICIQ-VS: 3.64 ± 3.82; ICIQ-VS-sexual matter: 5.29 ± 12.01; ICIQ-VS-QoL: 0.69 ± 1.1) symptoms. The two women who reported

urinary incontinence did not demonstrate unusual dynamometry outcomes. Maximal voluntary force values generated by the older woman were within the 40^{th} and 60^{th} percentile, and the passive force values were within the 70^{th} and 90^{th} percentile; while the maximal voluntary force values generated by the younger woman were within the 20^{th} and 40^{th} percentile, and the passive force values were within 40^{th} and 60^{th} percentile of the data. Overall, there was no evidence of any association between the dynamometric outcomes and the ICIQ scores.

A sample of healthy nulliparous women was specifically chosen to study the age-related changes in the PFMs, while minimizing other factors such as parity and obesity that may play a role in developing urinary incontinence in women (Hannastead et al., 2007). Since non-obese women were recruited for this study, the average BMI score for the women in this study was relatively low, 22.64kg/cm^2 ± 1.94, which is considered to be in the healthy range but which is low relative to the general population. According to Statistics Canada, about 44% of the adult female population (aged 18 and over) have normal BMI (Orpana et al., 2011). With the small sample of younger (n=18), middle aged (n=10) and older (n=5) women recruited for this study, the results cannot be considered conclusive and cannot be generalized to the Canadian female population.

Age-related changes in the Force Generation Capacity of PFMs

Based on the findings of Morin et al (2004) and Madill et al (2013), we expected to see evidence of age-related differences in the force generation capacity in women. Morin and colleagues studied 30 women between the ages of 21 to 44 and measured the differences in PFM force generation capacity between parous continent women and parous women with SUI. They did

not find any significant differences in force generation capacity at 19mm opening (continent: 4.5N ± 2.3N; SUI: 3.7N ± 1.8N, p = 0.23) or 24mm opening (continent: 5.9N ± 2.8N; SUI: 5.6N ± 3.2N, p = 0.67) between the two groups (Morin et al., 2004). In a later study, Morin and colleagues found similar active force values in 32 women with SUI between the ages of 45 to 75 (3.39N ± 2.4N) (Morin et al., 2008). Madill and colleagues also found similar active force values in 17 women with SUI aged 60 and over. After a 12-week pelvic floor exercise program, they did not find significant differences in force generation capacity between pre-exercise program and post-exercise program (pre: 4.12N ± 3.48N; post: 3.09N ± 3.36N, p = 0.92) (Madill et al., 2013). The active force values from these three studies generated at 24mm opening using the Montreal dynamometer (Morin et al (2004) - 5.9N ± 2.8N; Morin et al (2008) - 3.39N ± 2.4N; Madill et al. (2013) - 4.12N ± 3.48) are consistent with the active force values in our study generated at 25mm dynamometer opening (4.75N ± 2.05).

The older participants (n=5) in this study were between the ages of 60 and 64 years. According to the literature, age related changes in other skeletal muscles are mainly evident after the age of 65 due to the effects of sarcopenia (Jubrias et al., 1997). In this study, the lack of age-related changes seen in the PFM force generation and stiffness properties may be because the older participants were all below the age of 65. The findings of this study are consistent with findings reported by Quartly et al (2010), who found intravaginal pressure, measured using a perineometer, was not correlated with age (r=−0.31, P=0.107) in women between the ages of 19 to 58 with no history of incontinence (Quartly et al., 2010). Intra-vaginal pressure is influenced by intra-abdominal pressure during a maximal voluntary PFM contraction (Bø et al., 1990), which, however, may have affected their results. In the current study, an

intravaginal dynamometer was used to measure PFM force generation capacity in nulliparous women, and the methods were selected to minimize the influence of intra-abdominal pressure during maximal PFM contraction. It is interesting to note that even though age-related effects were not evident in the force generated during voluntary contractions (Quartly et al., 2010), during voluntary coughing, which generates a reflex PFM contraction (Bø & Stein, 1994), Madill and McLean found a negative correlation between age and pressure measured at the anterior and posterior vaginal walls in women between the ages of 30 to 70 years old (Madill & McLean, 2010).

According to Fukanaga and colleagues, muscle volume has been found to be a good predictor of maximal muscle force (Fukanaga et al., 2001). The LA muscles have been shown to be composed predominantly of type I muscle fibers and, unlike type II muscle fibers, they are relatively unaffected by sarcopenia. Hence, it is possible that we did not see significant differences in maximal PFM force generation capacity associated with age because the PFMs are not affected by sarcopenia. This lack of age-related effect on the force generation capacity of the PFMs is supported by the findings of Morris et al (2012) who found no significant difference in LA CSA or muscle volume in older nulliparous women (aged 63 and over) compared to younger women (aged 20-25) (Morris et al., 2012). Even though CSA is not a direct measure of force generation capacity, the findings from Morin et al (2004) provides functional evidence that corroborates Morris et al's (2012) results. Similar to our study, Morin and colleagues did not report age-related differences in force generation capacity of the PFMs (Morin et al., 2004). Likewise, Trowbridge et al (2007) did not observe age-related changes in vaginal closure pressure in a sample of eleven nulliparous women aged 60 and over compared

to younger nulliparous women. The sample size in the Trowbridge study was only eleven women, and as such, like the current study, the results from this study maybe not be conclusive (Trowbridge et al., 2007).

In addition to maximum force generation capacity, we did not observe statistically significant correlations between rate of force development (N/s) and age. In comparison, Morin et al (2004) reported significant differences in age and parity when comparing PFM rate of force development (Morin et al., 2004). Due to the selective loss of type II muscle fibers associated with sarcopenia, it is plausible that maximal rate of force generation would be affected by advanced age (Barry et al., 2005); motor unit discharge rates from the motor neurons are lower in older individuals and this may contribute to slower rates of force development in older adults compared to younger adults (Klass et al., 2008).

Levator avulsions are present in the PFMs of parous women compared to nulliparous women (Delancey et al., 2003). Concurrent damage to the motor nerves may affect motor neuron discharge rate and motor unit recruitment during voluntary contractions. These factors could explain the effect of age and parity on PFM rate of force development reported by Morin et al (2004). Their study included a sample size of 30 women who were parous and therefore may have sustained damage to neuromotor control through pregnancy and/or delivery.

Two studies have reported age-related decreases in PFM activity measured using surface EMG (Hahn et al., 1996); (Gunnarsson & Mattiasson, 1999). Gunnarsson & Mattiasson found a decrease in EMG activity with increase in age ($p<0.001$) in incontinent women but did not report any differences in continent women. As noted above, they observed age-related loss in muscle mass and levator avulsions associated with childbirth, which may have resulted in

lower muscle activity recorded by EMG in the incontinent group compared to the continent group. The researchers reported that age-related changes in PFMs were greater in incontinent women compared to continent women (Gunnarsson & Mattiasson, 1999). However, surface EMG may record muscle activity of nearby muscles and lower EMG activity in the PFMs may not reflect differences in PFM force generation capacity.

Even if they are not evident in the PFMs, age-related changes may be evident in striated urethral muscles. Perrucchini et al (2002) demonstrated a loss of urethral muscle fibers with advanced age, which could contribute to lower urethral contractile force in older women (Perrucchini et al., 2002). Two studies have shown that urethral closure pressure is lower in older women compared to younger women (Delancey et al., 2008; Clobes et al., 2008) and this result may not be related to childbirth, as Trowbridge et al (2007) reported that nulliparous women between the ages of 60 to 70 years had lower maximal urethral closure pressure than nulliparous women between the ages of 20 to 30 years (Trowbridge et al., 2007). Since the urethral muscles have type II muscle fibers clustered around them and these type II muscle fibers are required to generate maximal urethral closure force when there is an increase in IAP during coughing or sneezing, the striated urethral sphincter may be affected by sarcopenia.

Age-related changes in PFM Endurance

Based on studies on skeletal muscle endurance in older individuals, we did not expect to see any age-related changes in PFM endurance in our sample. Consistent with this, our results showed no correlation between age and PFM endurance. The participants performed the endurance task with 5 seconds of contraction and 5 seconds of relaxation for 180 seconds. The

muscle was considered fatigued if their relative peak force was 80% less than their MVC at 25mm. The percent reduction in force was 18.59 ± 21.79%, which showed that the PFMs were fatigued after 180 seconds. However, there was no evidence of age-related effects on the extent of fatigue observed.

There are different ways in which muscle fatigue can be measured. Morin et al (2004) studied PFM endurance by asking their participants to rapidly contract their PFMs and to hold the contraction for 90 seconds while the dynamometer was opened to 19mm, and Quartly et al (2010) had their participants contract their PFMs, while maintaining the force above 60% of their MVC. During the assessment of the PFMs, endurance is expressed as the length of time, up to 10 seconds, that an MVC can be sustained before the strength is reduced by 35% or more, after which, the muscle is fatigued (Laycock & Jerwood, 2001). When sustaining a contraction for a long period, such as 90 seconds, the contraction may become submaximal, whereby motoneurons become less responsive to the synaptic input and the descending motor command becomes suboptimal. The decrease in discharge rate in the motoneurons leads to decrease in motor unit recruitment (Gandevia, 1992). As a result, there is a decrease in voluntary force generation due to the effect of fatigue on the type II muscle fibers, and as the contraction is prolonged, the decrease in motor unit discharge only allows type I muscle fibers to remain activated (Brooke & Kaiser, 1970). Further, if a maximal contraction was not achieved originally, the hold time may be much longer since the type II fibers would not be recruited and thus evidence of them dropping out would not be evident. This may have been the case in the Morin et al (2004) study; some participants may not have generated maximal contractions since the force achieved for the endurance task was not reported. Further, Morin et al (2004)

reported that endurance was difficult to evaluate as they found oscillating pattern of generating maximal force and decrease in force overtime in their participants. Due to the variability in force generation during a sustained contraction, for our study, we chose a different paradigm to see the effects of fatigue by asking the participants to perform intermittent contractions and relaxations for 180 seconds during the endurance task. This task was selected as it is more likely than a sustained hold to reflect the functional capacity of the PFMs to assist with continence function.

To our knowledge, there is only one study that looked at the correlation between age and PFM endurance in women. Using a perineometer, Quartly et al (2010) found a weak correlation between age and PFM endurance in 28 women aged between 19 to 58 ($r = 0.38$, $p = 0.048$). They found that endurance was higher in women over the age of 40 years. The positive correlation between age and endurance may be due to the muscle fiber composition in the PFMs. The LA muscles are mostly composed of fatigue-resistant type I muscle fibers. While no evidence of sarcopenia was evident in the force-generating capacity, Quartly et al.'s result may reflect the effect of fiber-type-grouping and selective retention of type I fibers with advanced age, which has been shown to increase contractile endurance (Glenmark et al., 1992). However, it is unclear whether these age-related changes can be attributed to changes in muscle mechanics in a sample of women under the age of 65, since, as previously mentioned, the effect of aging in skeletal muscles has been shown to evident primarily after the age of 65.

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Age-related changes in Passive Properties of PFMs

The quantitative and qualitative changes in connective tissue associated with aging are thought to influence the passive properties of the skeletal muscles (James & Parker, 1989). It was hypothesized that with increase in age, resistance to passive stretch would increase. According to our results, no age-related changes were observed in the passive properties of the PFMs and paravaginal tissues, which included relative peak resistance to passive stretch and stiffness. Compared to Morin et al's (2004) resistance to passive force value (3.7N ±1.4N), our values were much higher for both 50mm/s (fast) dynamometer opening (15.25N ± 4.21N) and 25mm/s (slow) opening speeds (13.07N ± 3.99N). Morin et al. (2004) measured the passive resistance at 24mm of dynamometer opening (Morin et al., 2004), whereas in our study, the dynamometer arms were opened to 40mm, and, while Morin et al (2004) manually operated their dynamometer opening, and thus the opening speed was slow (roughly 5mm/s) and may not have been constant, our dynamometer was computer operated and opened at a constant pre-selected speed of either 50mm/s or 25mm/s, which could explain why our participants generated higher values for resistance to passive stretch compared to Morin et al (2004). In a 2008 study, Morin et al looked at the resistance to passive stretch in 32 women with SUI aged between 45 to 75 years old and found that the peak passive resistance was 25.37N ± 2.99N, which was much higher than our values. Morin et al (2008) evaluated their participant's PFM passive resistance at a maximal dynamometer aperture that could be tolerated by the participant, which could have been more than 40mm depending on the participant's comfort level, resulting in higher resistance to passive stretch (Morin et al., 2008).

Tuttle and colleagues found that the amount of connective tissue in the PFMs of older female cadavers (aged 85 and above) is about 9 to 11% collagen, which is about three times

higher than the amount of collagen found in other skeletal muscles in the body (Tuttle et al., 2014). Given that fascia and ligaments work in synergy with the PFMs to support the urethra and the pelvic organs, it may be beneficial to have a higher amount of collagen to prevent any strain on the PFMs and therefore aging may not have a limited effect on the tissue properties of the PFMs in women. A limitation of the study was that the mean age of the female cadavers was 85.6 years ± 16.68, and parity was not reported. As such, it is uncertain whether the findings from the older cadavers reported by Tuttle et al. (2014) can be generalized to all older women.

PFMT has been shown to increase stiffness in the vaginal connective tissue, which can contribute to better pelvic organ support (BØ, 2004). Stiffness in the LA muscles is thought to limit the downward movement of the bladder neck during coughing or sneezing. Howard et al (2000) demonstrated that nulliparous continent women had greater pelvic floor stiffness during coughing than continent or incontinent primiparous women (Howard et al., 2000). Balmforth et al (2006) showed that 14 weeks of PFM training in 97 women with SUI (mean age 49.5 years ± 10.6) resulted in significant elevation of the bladder neck position and less displacement of the bladder neck on Valsalva, suggesting increased LA muscle stiffness after training (Balmforth et al., 2006). However, in the current study, data were collected only one week after the familiarization session. Instead, the women were instructed to perform PFM contractions in the week following their familiarization session and prior to the data collection session in order to limit the effect of task familiarization on outcomes, since the impact of familiarization session on PFM outcomes is currently not known. It is highly unlikely that performing PFMT for one

week would cause significant changes in the tissue stiffness properties of the PFMs in the participants.

Measuring PFM mechanics and Tissue Properties using Dynamometry

To our knowledge, this is the first study to investigate the age-related changes in the active and passive properties of the PFMs in healthy nulliparous women. The different dynamometer settings were chosen to study the force generation, as well as changes in stiffness properties of the PFMs with age.

Maximal voluntary contraction was measured using two different dynamometer openings, 25mm and 35mm. 35mm was chosen based on the findings of Verelst et al (2007) that found between 30mm to 40mm to be the optimal range of dynamometer opening to measure force generation capacity. The 25mm opening was chosen based on Dumoulin et al (2004) who showed that women generated maximal voluntary contraction at 24mm dynamometer opening (Dumoulin et al., 2004). The same dynamometer opening of 24mm was also used by Morin et al (2004) but no significant differences were observed between their 19mm dynamometer opening force values and 24mm dynamometer opening force values (Morin et al., 2004). Even though the data in the current study suggests that women generated greater force on contracting their PFMs at 35mm (7.88N±3.10N) than at 25mm (4.75N±2.05N), age-related changes were not observed at either dynamometer opening. Rate of force development (N/s) was calculated using the steepest part of the slope to see whether the older women took longer to generate maximal force during a PFM contraction than the younger women, which would reflect selective loss of type II muscle fibers. There were no significant

correlations observed between rate of force development and age at 35mm opening and at 25mm opening. But the rate of PFM force development appeared to be higher at 35mm (24.67N/s ± 12.62N/s) than at 25mm (16.49N/s ± 8.47N/s). These results are consistent with the findings from two studies that found a positive association between rate of force development and maximal force (Mirkov et al., 2004; Holtermann et al., 2007). Holtermann and colleagues noted that the positive association could be due to neurophysiological changes; the increase in motor unit firing rate contributes to the recruitment of type II muscle fibers to enhance both rate of force development and maximal force generation (Holtermann et al., 2007). The lack of age-related changes seen in the force generation capacity of the PFMs could again indicate that the contractility of the LA muscles is not influenced by advancing age – again bearing in mind the limitation that our sample of women included no participants over the age of 64.

Resistance to passive stretch and stiffness properties of the PFMs were measured using two different dynamometer opening speeds, 50mm/s (fast) and 25mm/s (slow). Skeletal muscles are shown to have viscoelastic properties (Frankel & Burstein, 1970), and are likely to be influenced by the speed of opening of the dynamometer. Viscous properties are the change in shape or deformation due to the application of a stress to the muscles, while elastic properties refer to the muscle's ability to return to its original state after being stretched (Fung, 1972). We tested the passive properties of the PFMs at a slow and fast speed to see if differences were evident at one speed and not the other. The participants generated greater resistance to passive stretch at 50mm/s opening (15.25N ± 4.21N) than at 25mm/s (13.07N ± 3.99N) ($p<.001$), as well as higher stiffness at 50mm/s (1.02N/s ± 0.21N/s) than at 25mm/s

(0.82N/s ± 0.22N/s) ($p<.003$). Taylor et al. (1990) demonstrated that peak tensile force was dependent on the rate of stretch applied. Slower speed stretches in the relaxed muscles allow for a greater degree of stress relaxation to occur, which contributes to a lower peak force and a lower stiffness value than stretches applied at a faster rate (Taylor et al., 1990). This could explain why our results show that at the slower speed of dynamometer opening, the resistance to passive stretch and stiffness values were significantly lower than faster speed of opening.

The effect of Familiarization Session on PFM Motor Control

The participants attended two laboratory sessions, and the data were analyzed from their second visit. As noted above, the first session was employed to help the participants learn how to perform a proper PFM contraction, and, beyond the scope of the current study, to study the effect of a familiarization session on PFM dynamometry outcomes. Miller et al showed that muscles do not gain strength over a week, however, they can gain motor control in less than a week (Miller et al., 1998). They found that women over the age of 60 with mild SUI learned how to contract their PFMs before and during voluntary coughing to reduce urine leakage within one week (Miller et al., 1998). They reported that the significant reduction in urine leakage was not likely due to increase in PFM force generation capacity but rather a motor learning effect. Madill et al (2013) found that the resting PFM force was lower following a 12-week PFMT intervention compared to the initial assessment. The researchers speculated that the women became more aware of their PFMs and subsequently became less anxious and were better able to relax during the testing (Madill et al., 2013). In the current study, we did not measure the effects of anxiety or PFM motor control, but we included the familiarization session in an effort

to mitigate the effects of anxiety or poor motor control. Although untested, through the familiarization session, women may have been less anxious and may have developed better control of their PFMs. Improved relaxation may have affected the force and passive resistance measurements, which could explain why some of our findings, such as, passive force and endurance were different than the findings of Morin et al (2004, 2008), Madill et al (2013) and Quartly et al (2010). The participants in previous studies may have been learning how to perform each task during the actual data collection.

Limitations

The biggest limitation of this study was that the sample size for older women was too small and too young to observe any age-related changes in the PFMs. There were only five older women in this study, and all were under the age of 65. As such, the results of this study cannot be generalized to women over the age of 65, which is when effects due to aging have been reported in the literature (Jubrias et al., 1997).

Using animal models, research on the soleus muscles and vastus lateralis, that, similar to the PFMs are predominantly type I muscle fibers, has shown evidence of age-related reduction of force generation capacity (Thompson & Brown, 1999) and muscle atrophy (Brooks & Faulkner, 1988). Gonzalez and Delbono (2001) studied age-related fatigue in the soleus muscles of rats and found that endurance in the soleus muscles were not significantly affected by age in contrast to the endurance in the extensor digitorium longus muscles, which are mainly type II muscle fibers and demonstrated an increase in endurance with age (González & Delbono, 2001). The literature suggests that both type I and type II muscle fibers show evidence of age-

related reductions in muscle force generating capacity (Lexall et al., 1988), thus the negative findings from this current study may be a function of the sample size being small and too young.

During the resistance to passive stretch task, the participants may have kept their PFMs slightly contracted while the arms of the dynamometer were opening even though they were instructed to keep their PFMs as relaxed as possible. When the arms of the dynamometer started to open automatically, the participants may have contracted their PFMs as a reflex reaction to the fast opening speed, which may have resulted in higher PFM resistance to passive stretch than was observed at the slow speed. While measuring resistance to passive stretch, there was no evidence of PFM activation seen during the passive stretch. Nonetheless, in the future, to ensure participants keep their PFMs relaxed, surface EMG should be recorded concurrently with the dynamometer during the resistance to passive stretch task.

The sample size for this current study was too small and younger than 65 years. Only ten middle-aged and five older women participated in the study. Due to the nature of the study, younger women may have been more inclined to participate in this study than older and middle-aged women, and it was more difficult to recruit older nulliparous women than younger nulliparous women. Strategies used to recruit older nulliparous participants included, visiting aqua fit classes, physiotherapy clinics, senior charity events and local nursing homes where the women were shown videos of dynamometer used to assess the PFMs. Senior's associations such as, Ottawa Senior Pride Network, as well as senior faculty and staff members at the uOttawa were contacted in an attempt to access interested and eligible older women. In the future, to recruit a larger sample of older women, senior organizations should be regularly

visited or contacted, and recruitment posters should be posted on bulletin boards in hospitals, local clinics, nursing homes and fitness centers. Since some of the older participants in this study were recruited through word of mouth, it may be worthwhile to ask the participants if they could recommend a friend or family member that meets the inclusion criteria.

To date, this is the first study that has specifically looked at the age-related changes in PFM mechanics and tissue properties in women with no major risk factors for UI. Quartly et al. (2010) studied the correlation between age and PFM biomechanics, and did not find any significant age-related changes, which may have been the result of a parous sample. By eliminating the confounding factors such as, parity, high BMI and heavy smoking, our study only focused on the normal age-related changes seen in the PFMs of women with no risk factors for urinary incontinence. A larger sample of older women would better help us understand how age-related changes influence strength generation, endurance and tissue properties in these muscles.

Conclusion

The findings from this study corroborates the findings from Quartly et al (2010) that suggests that aging may not have a significant effect on the force generation capacity of the PFMs. Since this study lacked women over the age of 65, efforts are underway to recruit more older participants before the result can be considered conclusive.

Overall, we did not find any significant correlation between age and PFM biomechanics including both active and passive tissue properties in our participants. While the findings of this study cannot be generalized to populations over the age of 65, perhaps like other skeletal muscles, age-related changes may appear in the PFMs after the age of 65. Age-related changes may be more significant in the urethral muscle and the surrounding tissue than in the LA in women. Hence, for future studies, it may be worthwhile to also investigate the urethral sphincters, which has shown age-related decline in functional capacity. Since the PFMs are thought to be important to continence function, by recruiting a larger sample of older nulliparous women, we can begin to understand the normal age-related changes seen in the PFMs of women in order to develop prevention and rehabilitation programs that specifically address age-related changes should they exist.

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Ethics Approval Notice

Health Sciences and Science REB

Principal Investigator / Supervisor / Co-investigator(s) / Student(s)

First Name	Last Name	Affiliation	Role
Linda	McLean	Health Sciences / Others	Supervisor
Mahin	Semmen	Health Sciences / Human Kinetics	Student Researcher

File Number: H10-15-19

Type of Project: Master's Thesis

Title: Are Age Related Changes Evident in Pelvic Floor Muscle Mechanics

Approval Date (mm/dd/yyyy)	Expiry Date (mm/dd/yyyy)	Approval Type
01/07/2016	01/06/2017	Ia

(Ia: Approval, Ib: Approval for initial stage only)

Special Conditions / Comments:
N/A

Bureau d'éthique et d'intégrité de la recherche Office of Research Ethics and Integrity

This is to confirm that the University of Ottawa Research Ethics Board identified above, which operates in accordance with the Tri-Council Policy Statement (2010) and other applicable laws and regulations in Ontario, has examined and approved the ethics application for the above named research project. Ethics approval is valid for the period indicated above and subject to the conditions listed in the section entitled "Special Conditions / Comments".

During the course of the project, the protocol may not be modified without prior written approval from the REB except when necessary to remove participants from immediate endangerment or when the modification(s) pertain to only administrative or logistical components of the project (e.g., change of telephone number). Investigators must also promptly alert the REB of any changes which increase the risk to participant(s), any changes which considerably affect the conduct of the project, all unanticipated and harmful events that occur, and new information that may negatively affect the conduct of the project and safety of the participant(s). Modifications to the project, including consent and recruitment documentation, should be submitted to the Ethics Office for approval using the "Modification to research project" form available at: http://research.uottawa.ca/ethics/submissions-and-reviews.

Please submit an annual report to the Ethics Office four weeks before the above-referenced expiry date to request a renewal of this ethics approval. To close the file, a final report must be submitted. These documents can be found at: http://research.uottawa.ca/ethics/submissions-and-reviews.

If you have any questions, please do not hesitate to contact the Ethics Office at extension 5387 or by e-mail at: ethics@uOttawa.ca.

Signature:

Riana Marcotte
Protocol Officer for Ethics in Research
For Daniel Lagarec, Chair of the Health Sciences and Sciences REB

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Appendix B – Poster for Recruitment (School Bulletin Board)

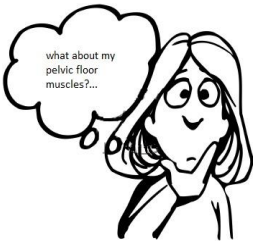
LADIES, EVER WONDER HOW FIT YOUR PELVIC FLOOR MUSCLES ARE?

uOttawa

The pelvic floor muscles are constantly working to support your pelvic organs. Dysfunction in these muscles, which can occur at any age, can lead to a wide range of problems such as sexual pain, pelvic organ prolapse, fecal and urinary leakage.
If you are interested in learning more about your pelvic floor muscles, we are looking for healthy female volunteers to study age-related changes in the pelvic floor muscles.

Study Procedures:

- Telephone screening to determine your eligibility
- Completion of questionnaires
- Attend two pelvic floor muscle assessments at the Motor Function Measurement Lab, University of Ottawa



Eligibility Criteria:

- Cis female - Age 20+
- No history of urogynocological surgery,
- Non heavy smokers – if you have smoked more than half a pack a day for over 10 years
- Cannot be pregnant or have ever given birth
- No pain during, sexual or non-sexual (eg. tampon insertion) vaginal penetration activities

Benefits:

- You will learn about your pelvic floor muscles and receive information sheet about pelvic floor muscle exercises, which, if performed correctly and regularly, can help to **prevent incontinence and improve sexual function**
- You will be taught how to perform a proper pelvic floor muscle contraction by a licensed physiotherapist

Contact: [redacted] MSc Candidate, School of Human Kinetics, University of Ottawa
PI: [redacted], PhD, School of Rehabilitation Science, University of Ottawa

Appendix C – Recruitment Poster for Older Women

LADIES, EVER WONDER ABOUT YOUR PELVIC FLOOR MUSCLES?

uOttawa

These set of muscles are constantly working to support your pelvic organs.
Dysfunction in these muscles can lead to a wide range of problems such as sexual or pelvic pain, pelvic organ prolapse, fecal and urinary leakage, which is mostly seen in older women.

If you are interested in learning more about your pelvic floor muscles, our lab is looking for female volunteers to study age-related changes in the pelvic floor muscles in order to develop proper interventions to target these age-related changes.

Study Procedures:

- Telephone screening to determine your eligibility
- Completion of questionnaires
- Attend pelvic floor muscle assessment at the Motor Function Measurement Lab, University of Ottawa

Benefits:

- You will learn about your pelvic floor muscles and receive information sheet about pelvic floor muscle exercises, which, if performed correctly and regularly, can help to **prevent incontinence and improve sexual function**
- You will be taught how to perform proper pelvic floor muscle contractions by a licensed physiotherapist

Eligibility Criteria:

- Cis-female age 20+
  No history of: urogynocological surgeries
- Non heavy smokers – if you smoke or have smoked more than half a pack a day for the past 10 years
- Cannot be pregnant or have ever given birth
- No pain during sexual or non-sexual (eg. tampon insertion) vaginal penetrative activities

Contact [redacted] MSc Candidate, School of Human Kinetics, University of Ottawa
PI: Dr [redacted], PhD, School of Rehabilitation Science, University of Ottawa
Location – [redacted]

uOttawa

Appendix D – Telephone Information Sheet

Telephone Information Sheet

Hello, my name is [redacted], I am a graduate student researcher at Dr. [redacted] research Laboratory at the University of Ottawa.

Thank you for your interest in the study, "ARE AGE RELATED CHANGES EVIDENT IN PELVIC FLOOR MUSCLE MECHANICS?"

I would like to tell you about the study, and the details of what your participation would entail if you are eligible and choose to participate. If you are still interested after the description of the study, then I will ask you a number of questions to determine your eligibility for the study. This description could take about 10-20 minutes. Is now an appropriate time to complete the interview? Please interrupt me at any time if you have questions.
If no, is there a convenient time for you to call and discuss the study?

The purpose of this study is to understand the age-related changes in the pelvic floor muscles in of women who have no major risk factors for incontinence.

The pelvic floor muscles are a group of muscles that work together to support the pelvic organs, such as the bladder, so that these organs can maintain their relative position within the pelvis regardless of your posture or activity. Dysfunction in these muscles may result in urinary incontinence in women, but researchers are not sure what role of the pelvic floor muscles play in developing urinary incontinence. Aging may contribute to dysfunction in the pelvic floor muscles, but we are still unsure. Therefore, we will assess the pelvic floor muscles among different ages of women to see if there are any differences in the pelvic floor muscles among younger and older women. We will be using a strength measurement device called dynamometer, to assess your pelvic floor muscles. To date, no studies have looked at age-related changes in the pelvic floor muscles. Therefore, the findings from this study will allow us to more completely understand the role of aging on pelvic floor muscles and also help us design interventions that target specific age-related changes in the pelvic floor muscles in women.

Should you choose to participate, your participation in the study would be limited to completing questionnaires and to attending two sessions in the Motor Function Measurement Laboratory. The first session will take approximately 1.25 hours and the second session will take approximately 45 minutes. Are you still interested in hearing more?

If she says yes, then the study procedures will be explained to her.

If she says no, she will be asked if there is a better time to discuss the study further, which will be noted down the telephone log sheet. If the volunteer states she is not interested in participating, she will be asked why and her response will be recorded on log at the end of this form.

This is what will happen if you choose to participate:

You will be scheduled two visits at the research laboratory of Dr. [redacted] at the Lees Avenue Campus of the University of Ottawa. At the first visit you will be asked to read and sign a study information and consent form. Before signing this form you should makes sure that all of your questions and concerns have been adequately addressed. If you consent to participate, you will be will asked to fill out a series of questionnaires about your general health and quality of life, your bladder, bowel, and vaginal symptoms. Once you complete the questionnaires, you will be taken to the examination room for a pelvic floor muscle assessment by the graduate student research assistant. Your height and weight will be measured and recorded. You will be left alone in the room to remove clothes from your waist down and cover yourself with a sheet that will be provided to you. Once you are changed, you will invite the physiotherapist and the graduate student back into the room. The physiotherapist will then perform an assessment of your pelvic floor muscles and teach you how to perform a proper pelvic floor muscle contraction. This will involve the physiotherapist inserting two gloved fingers into your vagina to feel your pelvic floor muscles, and she will resist contraction of your muscles so that you can feel them better. She will provide feedback to you so that you will learn how to perform a proper contraction. After you have learned the pelvic floor muscle contraction, she will instruct you on how to insert a pelvic floor muscle strength testing device into your vagina. This device measures how hard you can contract your pelvic floor muscles, how many contractions you can do and how flexible your pelvic floor muscles are. This is a safe device that does not carry risk of injury to your muscles. However, at any point of the study, if you feel uncomfortable, you may press the safety switch in the device to stop it or ask the physiotherapist to stop the assessment. This part of the assessment will last approximately 20 minutes and will involve you performing three repetitions of muscle contractions, three repetitions of muscle stretching, and a series of repeated contractions until the physiotherapist tells you that your muscles are fatigued.

At the end of the session, you will be asked to schedule a second session in the following week, where the same procedures from the familiarization session will be repeated, making sure to avoid your menstrual period, if applicable. You will be asked to perform ten repetitions of pelvic floor muscle contractions every day for the next seven days, and you will be given a compliance log sheet, where you will record the number of contractions you have completed. The second session will take about 45 minutes.

This is the study in a nutshell. Do you have any questions? Are you interested in seeing if you are eligible for participating in the study? **If no, thank them for their time, and ask them to feel free to call back if they change their mind.**

Exclusion Criteria:

- Currently pregnant or have carried a pregnancy to the end of the third trimester in the past
- Prior urogynecological surgery
- Heavy smokers - currently smoke or have smoked more than half a pack a day for the past ten years
- Known neurological or connective tissue disorders that might affect contractility or connective tissue properties
- Psychiatric disorders that might result in the testing environment or procedures causing undue stress
- Pain during sexual intercourse or tampon insertion

Telephone Screening Form

Name:_____ Date of screening: _____(yyyy-mm-dd)

Home #: ( ) - (best time to call:) leave a message? Y N
Cell #: ( ) - (best time to call:) leave a message? Y N
Work #: ( ) - (best time to call:) leave a message? Y N

Mailing address OR fax # (only if necessary) to send the questionnaires and LOI before the in-lab assessment:

Email address: _____
Recruitment Source: Flyer [1] Social Media [2] Word of Mouth [3]
Interested in finding out if they are eligible? Y [1] N [0] If no, reason_____
Interested in participating? Y [1] N [0] If no, reason_____
Availability _____

**Let them know that someone will contact them a few days before their appointment to let them know the details of where to go and what to bring*:
How do you want to be reminded of you appointment?

- **Email**
- **Phone msg**
- **No reminder**

Call Log:

Date	**Time**	**RA**	**Outcome**
_____	_____	_____	_____
_____	_____	_____	_____
_____	_____	_____	_____
_____	_____	_____	_____

Notes:

Initial telephone screening (not stored with the contact sheet for confidentiality) ID_____

1. What language do you prefer: English or French? E[1] F [2]

   *To determine if you are eligible to participate in the study we will need to ask you some questions about your medical history. Is that okay?

2. How old are you? _____ (* not eligible if under 20) DOB: _____(yyyy-mm)

3. Are you currently pregnant or are you trying to become pregnant? Y *[1] N [2]

4. When was your last pregnancy? _____(yyyy-mm) (* not eligible if parous)

5. Are you currently suffering from any medical (neurological, etc.), pain or psychiatric conditions? Y* [1] N [2] If yes:

a)With what condition(s) have you been diagnosed? (If unsure of eligibility ask questions about how long they've had this diagnosis, severity of condition)

Gynecological (e.g., endometriosis, pelvic inflammatory disease, recurrent yeast infections, etc)
_____

Psychiatric (e.g., depression) _____

Connective tissue disorder * _____

Chronic pain conditions_____
a) Do you experience pain during sexual intercourse or during tampon insertion?
b) Do you think this/these condition(s) prevent you from participating in the study?
Y* [1] N [2] Details: _____

6. Are you currently taking any medication or analgesics for the conditions named above (i.e., pain medication)? Y [1] N [2] if yes, which ones?:
_____
_____
_____
_____
_____

7. Have you ever had surgery (specifically urogynecological)? Y [1] * N [0]

If yes, which ones you have had.

Partial Hysterectomy	O 1	Ovariectomy	O 5
Complete hysterectomy	O 2	Colposuspension:	O 6
TVT	O 3	Others _____	O 7
Tubal Ligation	O 4	None	O 8

8. Do you currently experience episodes of involuntary urine leakage? Y *[1] N [0]
9. In the past, have you experienced episodes of involuntary urine leakage? Y *[1] N [0].
If yes:

a. Have you had any episodes of leakage within the past year Y [1]* N [0]

b. Have you received any medical treatments for these leakage episodes? (such as medications, hormone creams, collagen injections, or other medical treatments) Y [1] * N [0]

c. Have you received any surgical treatment for these leakage episodes? Y [1] * N[0]

10. Do you currently have episodes of fecal incontinence?
Y* [1] N [0] Details: (frequency, duration)_____

11. Are you aware whether you have an overactive bladder (peeing over 15 times a day for small amounts) as diagnosed by a doctor? Y* [1] N [0] **Suspected [2]**

12. Are you aware whether you have a pelvic organ descent? (prolapse) Y* [1] N [0]
If yes, does it bulge out of your vagina if you strain? Y* [1] N [0]

13. Do you have any devices in your abdomen that would prevent you from undergoing ultrasound testing? Y* [1] N [0]

14. Do you smoke? Y [1] N[0]
If so, how often do you smoke?_____

15. How often do you exercise?_____

16. For future reference, would like to be offered to participate in other studies in the lab? Y* [1] N [0]
ELIGIBLE FOR IN-LAB SESSION? Y [1] **N*** [2] (enter info on p.1)

If no:

- Thank them for their interest and their time.

If yes:

- Explain and mail, fax or email their letter of information and consent form, and the three questionnaires
- Make sure they have appropriate contact information
- Book testing and remind them they will receive an email/phone call before the testing to remind them of the appointment

Thank you!

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Appendix E – Letter of Information and Consent Form

Letter of Information

Title of the Study: ARE AGE RELATED CHANGES EVIDENT IN PELVIC FLOOR MUSCLE MECHANICS?

Principal Investigator: Dr. [redacted], PhD, Full Professor, School of Rehabilitation Sciences, University of Ottawa. [redacted]

Graduate Student Researcher: [redacted], MSc candidate, School of Human Kinetics, Faculty of Health Science, University of Ottawa. [redacted]

Background Information
Urinary incontinence, defined as involuntary leakage of urine, is a major health issue among women. The pelvic floor muscles provide support to the pelvic organs and help women to hold urine in their bladder by contracting during tasks that commonly lead to urine loss including coughing, sneezing or laughing. Age related changes in the pelvic floor muscles may contribute to the development of urinary incontinence in women, yet very few studies have looked at the effect of age on the pelvic floor muscles. Researchers have found a decrease in pelvic floor muscle strength in older compared to younger women but little is known about the effect of increasing age on other properties such as endurance and stiffness of these muscles. The findings of this study will allow us to more completely understand the role of aging on pelvic floor muscles and also help us design interventions that target specific age-related changes in the pelvic floor muscles in women.

Graduate student Mahin Semmen will read this consent form with you and answer any questions you may have. The research ethics board from the University of Ottawa has reviewed the study protocol and has found it to be in compliance with ethical standards.

Purposes of the Study
The purpose of this study is to better understand the effect of age on the pelvic floor muscles in healthy women who have no major risk factors for incontinence.

IF you choose to participate in this study, your participation will involve two assessment visits at the Motor Function Measurement Lab at the University of Ottawa, Lees Campus. The first visit will take approximately 1 hour and 25 minutes and the second visit will take approximately 45 minutes:

Questionnaires

You will be given four questionnaires to fill out at your first session. The first questionnaires will ask about your general health and quality of life. The other three questionnaires will ask about bowel, bladder and vaginal symptoms. Once you have completed the questionnaires, you will have your pelvic floor muscles assessed by the study physiotherapist, Kevin Varette.

Physical Assessment and Dynamometry

Mahin Semmen, the graduate student research assistant will measure your height, weight and your waist and hip circumference. You will then be left alone in a private examination room to remove your clothes from your waist down and cover yourself with a sheet that will be provided to you similar to what you do during Pap tests by your doctor. Once you have changed, you will invite the physiotherapist and graduate student back into the room. The physiotherapist will ask you to lie down on the examination table. You will have pillows behind your back with your legs supported. The physiotherapist will l feel your pelvic floor muscles by inserting two gloved fingers into your vagina. He will feel your pelvic floor muscles while she asks you to contract them. If you are unable to contract these muscles properly, which is very common, the physiotherapist will teach you the proper way to contract the muscles by providing you with instruction and feedback on your performance. You may ask to stop or to slow the testing down at any time.

After you have learned to perform a proper pelvic floor muscle contraction, the physiotherapist will instruct you on how to insert a custom-built force-measurement device into your vagina. Once you have done so, the physiotherapist will ensure that the device has been inserted properly. He will also place two sticky dots on the skin near your vagina to record muscle activity from your pelvic floor muscles. Once these devices are in place, the experiment will begin. You may ask to take a break or to slow down the testing at any time. First, you will be asked to contract your pelvic floor muscles in the same way the physiotherapist instructed you to do, and the device will measure how much force you can generate with your contraction. After three attempts, the device will be set to a new position, opened to put some stretch on your pelvic floor muscles, and you will repeat three contractions in this position. The device will be returned to its initial position and then you will be instructed to keep your pelvic floor muscles relaxed while the device opens slowly three times and then more quickly three times. These tasks may be repeated if we find that your pelvic floor muscles are contracting while the device is opening. Finally, you will be asked to perform several repetitions of a pelvic floor muscle contraction at a pace of one contraction every 10 seconds. You will repeat the contraction until the physiotherapist indicates that your muscles are tired, which she will detect using the force measured during your contractions as a guide. This will conclude the first visit. You will be left in the private assessment area to change back into your clothes. Before leaving, you will be asked to schedule a second session in the following week, making sure to avoid your menstrual period, if applicable. You will be asked to perform ten repetitions of pelvic floor muscle contractions every day for the next seven days, and you will be given a log sheet, where you will record the number of contractions you have completed. We will also contact you every day to make sure that you fill out the log. At the second session you will repeat the same data

collection as the first session, however you will not be required to complete the questionnaires at the second visit, which will make it a bit shorter.

Risks
As with any exercise, there is a chance that you will experience muscle soreness in your pelvic floor between 24-48 hours after the testing sessions. This phenomenon, known as delayed onset muscle soreness, is not known to be at all harmful and should disappear within 48 hours. If you continue to have soreness in your pelvic floor after the testing session, please contact the principal investigator, Dr. Linda McLean, at the number provided below.

There are no known risks associated with any of the testing procedures. The muscle strength testing device has limits set to prevent damaging any of your pelvic tissues through over-stretching or through pinching. It also has a safety switch that will be under your control such that you can shut down the device at any time during testing. Once you press the switch, the device will immediately close and turn off its power. The arms of the strength testing device are new, and will be disposed of after your testing session to avoid any risk of infection. Further, the entire device and the examination table are disinfected after each use.

Benefits
Participation in this study has no direct benefits. Through participating, you will learn how to perform a proper pelvic floor muscle contraction, and it is well known that performing such contractions on a regular basis can help to prevent and/or improve symptoms of incontinence and pelvic organ prolapse. If you are interested in learning more, speak with the physiotherapist during testing and she will provide you with a handout and guidance on the performance of a home program of pelvic floor muscle exercises.

Compensation
You are not paid to participate in this research study but you will receive reimbursement for your travel (bus fare) or parking fees related to your participation in this study.

Although there are no identified risks associated with participation in this study, in the event of a research-related injury or illness, you are not waiving your legal rights by agreeing to participate in this study. The study physiotherapist still has his legal and professional responsibilities.

Withdrawal from Study
You have the right to withdraw your participation from this study at any time and without providing any reason.

Confidentiality
You will not be identifiable in any publications or presentations resulting from this study.
All researchers and research assistants have been instructed regarding the protection of personal information and have signed agreements stating that they will respect the confidentiality of research participants. No identifying information will leave the University of

Ottawa. We retain only one electronic file with participant contact information and it is password protected and stored on an encrypted computer. Study documents will not identify you by name. You will be assigned a study participant number that will be used on all forms. The single electronic file that links your name to your participant number will only be accessible by Dr. McLean and her research student, Mahin Semmen, at the University of Ottawa and will also be password protected and saved on an encrypted computer. All electronic records will be stored on a secure computer at the University of Ottawa and protected by a user password, again only accessible by Dr. McLean and Mahin Semmen. All paper records will be stored in a locked file cabinet in the research laboratory. All files will be kept for a period of 10 years after the study has been completed. At the end of the retention period, all paper records will be disposed of in confidential waste or shredded, and all electronic records will be permanently deleted.

Participant Rights

Your participation in this study is voluntary. You may withdraw from this study at any time. You are free to choose not to answer any questions you are asked through the questionnaires or to refuse any components of the assessment without giving any reason. The researchers may withdraw you from the study for scientific reasons at any time. In this case, they would give you a clear and valid reason. You have the right to obtain copies of any study forms that contain your personal information.

As a participant in this study, you have the right to an environment that you feel comfortable in. You are welcome to bring a friend or family member with you to the evaluation session.

If at any time, you have further questions or problems you can contact the study investigators:

Dr. [redacted]

Or the graduate student researcher, [redacted] at the Motor Function Measurement Laboratory at [redacted]

The University of Ottawa: Sciences and Health Sciences Research Ethics Board has reviewed this protocol. This ethics board considers the ethical aspects of research studies involving human subjects at The University of Ottawa. If you have any questions about your rights as a research subject, you may contact the Protocol Officer for Ethics in Research at the University of Ottawa, at (613) 562-5387 or ethics@uottawa.ca

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Consent to Participate in Research

I understand that I am being asked to participate in a research study that is

This study has been explained to me by [redacted]

I have read this 5 page Patient Information Sheet and Consent Form or have had this document read to me. All my questions have been answered to my satisfaction. If I decide at a later stage in the study that I would like to withdraw my consent, I may do so at any time.
I voluntarily agree to participate in this study.
A copy of the signed Information Sheet and/or Consent Form will be provided to me.

Signatures

_____
Participant's Name (Please Print)

_____ _____
Participant's Signature Date

Investigator Statement (or Person Explaining the Consent)

I have carefully explained to the research participant the nature of the above research study. To the best of my knowledge, the research participant signing this consent form understands the nature, demands, risks and benefits involved in participating in this study. I acknowledge my responsibility for the care and well-being of the above research participant, to respect the rights and wishes of the research participant, and to conduct the study according to applicable Good Clinical Practice guidelines and regulations.

_____
Name of Investigator/Delegate (Please Print)

_____ _____
Investigator/Delegate Date

Appendix F – Compliance Log Sheet for PFM Exercise

Name:
Date:
Date of second visit:

Pelvic Floor Muscle Exercise Compliance Log Sheet

1. Day 1
   Number of contractions –
   Number of sets –
   Time of contractions (circle ones that apply) – morning noon afternoon evening sunset night midnight

2. Day 2
   Number of contractions –
   Number of sets –
   Time of contractions (circle ones that apply) – morning noon afternoon evening sunset night midnight

3. Day 3
   Number of contractions –
   Number of sets –
   Time of contractions (circle ones that apply) – morning noon afternoon evening sunset night midnight

4. Day 4
   Number of contractions –
   Number of sets –
   Time of contractions (circle ones that apply) – morning noon afternoon evening sunset night midnight

5. Day 5
   Number of contractions –
   Number of sets –
   Time of contractions (circle ones that apply) – morning noon afternoon evening sunset night midnight
6. Day 6
   Number of contractions –

Number of sets –
Time of contractions (circle ones that apply) – morning noon afternoon evening sunset night midnight

7. Day 7
   Number of contractions –
   Number of sets –
   Time of contractions (circle ones that apply) – morning noon afternoon evening sunset night midnight

Notes:

Please indicate if you performed more than the recommended number of pelvic floor muscles exercises (10 contractions per day) throughout the week:

Appendix G

Maximal PFM Voluntary Force and Passive Resistance values:

Young woman – 23 years (n=1)			Older woman – 64 years (n=1)			Mean±SD of all force values (n=33)
Outcome Measures	Force values	Percentile	Outcome Measures	Force values	Percentile	
Total force (N) – 35mm	18.91	30th	Total force (N) – 35mm	21.96	38th	21.72 ± 3.62
Total force (N) – 25mm	12.57	35.5th	Total force (N) – 25mm	14.81	81.5th	13.41 ± 2.30
Active force (N) – 35mm	6.76	35th	Active force (N) – 35mm	6.86	75th	7.88 ± 3.10
Active force (N) – 25mm	4.02	40.5th	Active force (N) – 25mm	6.07	79th	4.75 ± 2.04
Rate of force (N/s) – 35mm	14.59	25th	Rate of force (N/s) – 35mm	31.94	82nd	24.67 ± 12.62
Rate of force (N/s) – 25mm	11.88	35th	Rate of force (N/s) – 25mm	29.31	84.5th	16.48 ± 8.47
Resistance to Passive Stretch (N) – 50mm/s	17.31	35th	Resistance to Passive Stretch (N) – 50mm/s	19.56	55th	15.25 ± 4.43
Resistance to Passive Stretch (N) – 25mm/s	13.43	30th	Resistance to Passive Stretch (N) – 25mm/s	16.28	60th	13.07 ± 4.23
Stiffness (N/mm) – 50mm/s	1.13	65.5th	Stiffness (N/mm) – 50mm/s	1.41	90th	1.02 ± 0.21
Stiffness (N/mm) – 25mm/s	0.97	75th	Stiffness (N/mm) – 50mm/s	1.02	81st	0.82 ± 0.22

Appendix H: Correlation Power Calculation

Outcome Measures	R value	Total Sample Size required
Total force (N) at 35mm	0.038	1876
Total force (N) at 25mm	0.027	2742
Active force (N) at 35mm	0.099	629
Active force (N) at 25mm	0.08	964
Rate of force development (N/s) at 35mm	0.191	168
Rate of force development (N/s) at 25mm	0.094	698
Resistance to passive stretch (N) at fast speed (50mm/s)	0.07	1260
Resistance to passive stretch (N) at slow speed (25mm/s)	0.133	348
Stiffness (N/mm) at fast speed (50mm/s)	0.072	1191
Stiffness (N/mm) at slow speed (25mm/s)	0.041	1674
Endurance (N) at 25mm	0.025	2896

α=of 0.05; β=0.80